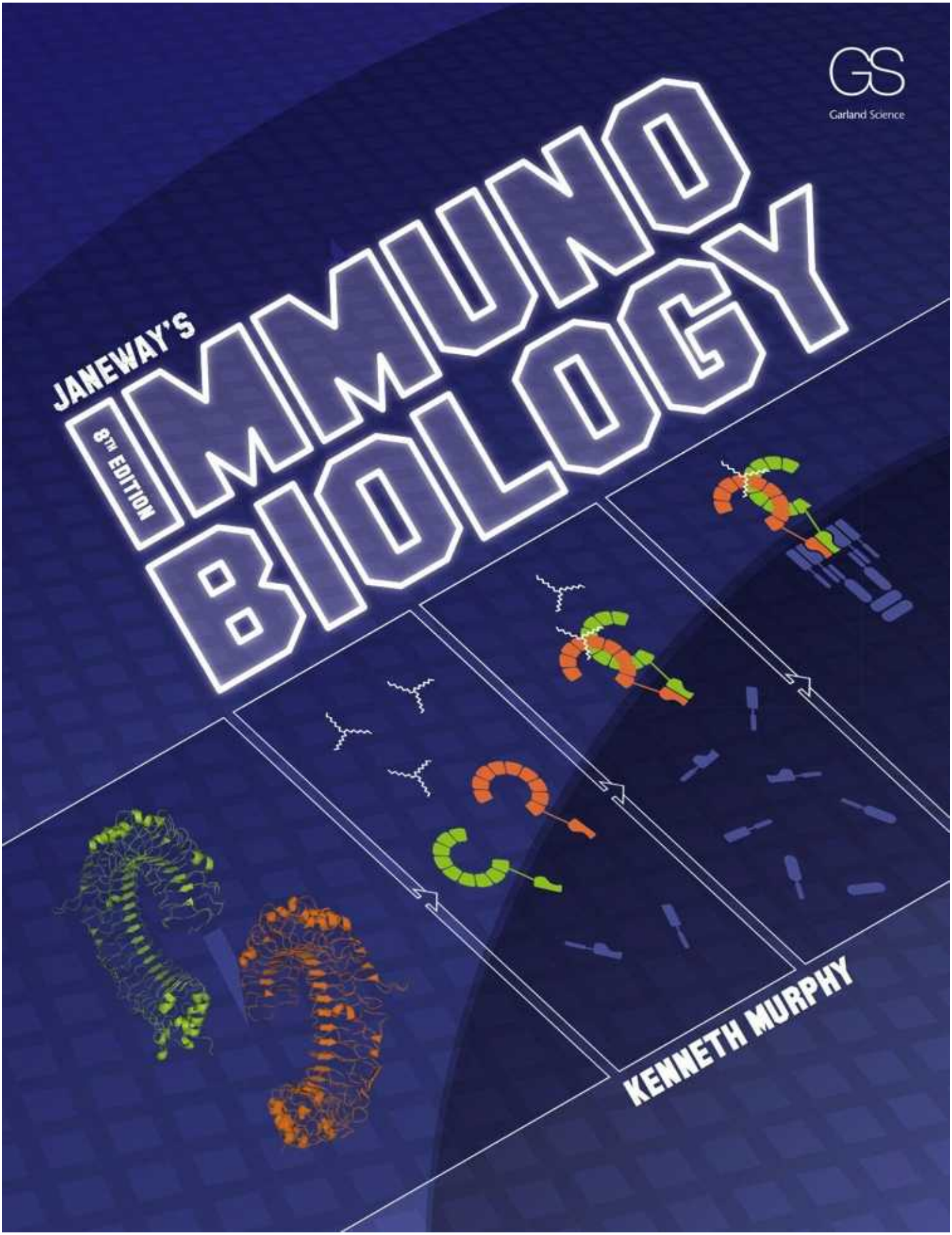
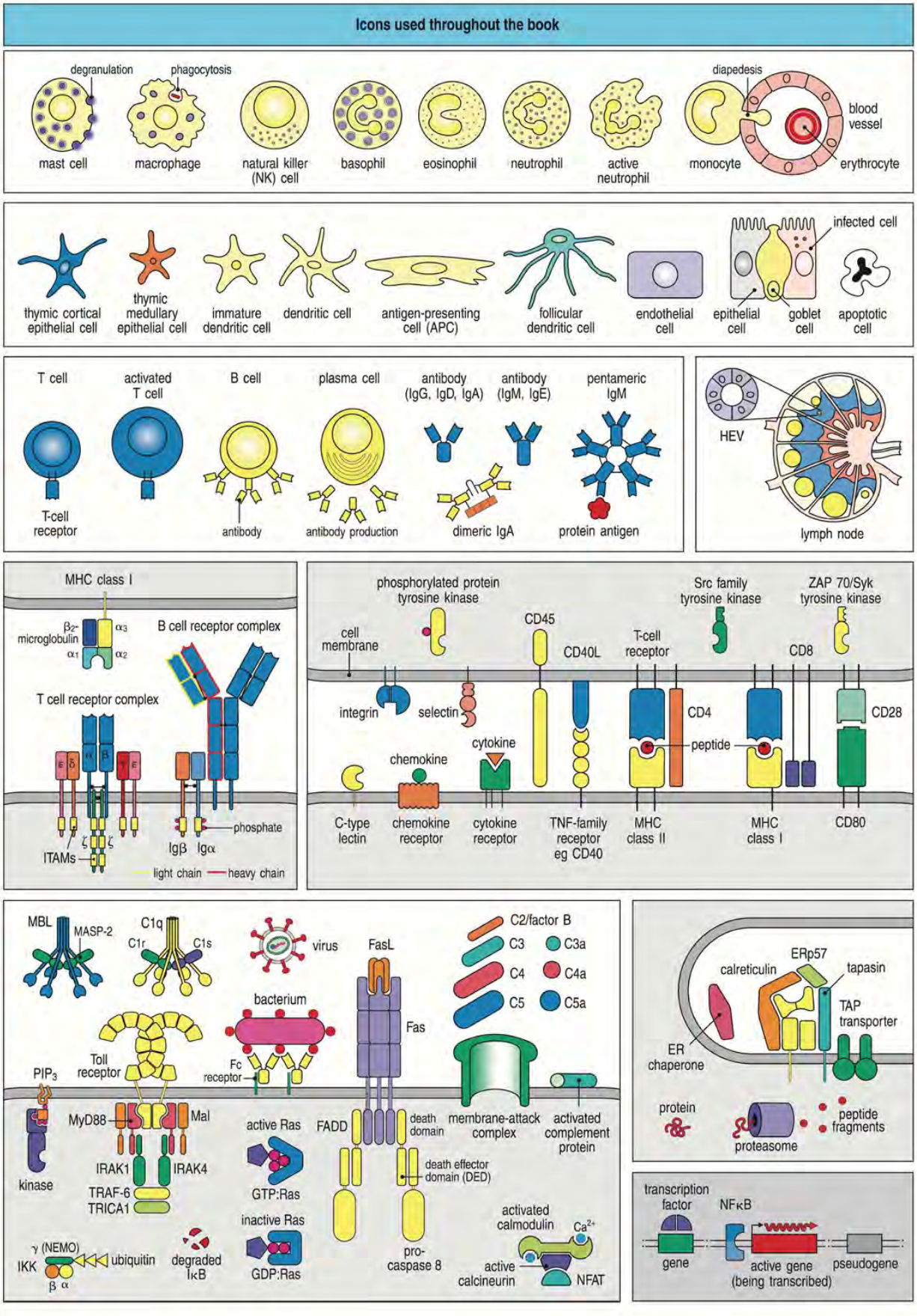


Petitioner Merck, Ex. 1170, p. 1
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003



Petitioner Merck, Ex. 1170, p. 2
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

JANEWAY'S

IMMUNOBIOLOGY

8TH EDITION

Kenneth Murphy
Washington University School of Medicine, St. Louis

With acknowledgment to:

Charles A. Janeway Jr.

Paul Travers
MRC Centre for Regenerative Medicine, Edinburgh

Mark Walport
The Wellcome Trust, London

With contributions by:

Allan Mowat
University of Glasgow

Casey T. Weaver
University of Alabama at Birmingham

Garland Science
Taylor & Francis Group
LONDON AND NEW YORK

Petitioner Merck, Ex. 1170, p. 3
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Vice President: Denise Schanck
Assistant Editor: Janete Scobie
Text Editor: Eleanor Lawrence
Production Editor: Ioana Moldovan
Typesetter and Senior Production Editor: Georgina Lucas
Copy Editor: Bruce Goatly
Proofreader: Sally Huish
Illustrations and Design; Animations Programming: Matthew McClements, Blink Studio, Ltd.
Permissions Coordinator: Becky Hainz-Baxter
Indexer: Medical Indexing Ltd.
Director of Digital Publishing: Michael Morales
Associate Editor: Monica Toledo

© 2012 by Garland Science, Taylor & Francis Group, LLC

This book contains information obtained from authentic and highly regarded sources. Reprinted material is quoted with permission, and sources are indicated. A wide variety of references are listed. Reasonable efforts have been made to publish reliable data and information, but the author and the publisher cannot assume responsibility for the validity of all materials or for the consequences of their use. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means—graphic, electronic, or mechanical, including photocopying, recording, taping, or information storage and retrieval systems—without permission of the copyright holder.

ISBN 978-0-8153-4243-4

Library of Congress Cataloging-in-Publication Data

Murphy, Kenneth P.
Janeway's immunobiology / Kenneth Murphy with acknowledgment to Paul Travers, Mark Walport ; with contributions by Allan Mowat, Casey T. Weaver.
p. cm.
ISBN 978-0-8153-4243-4 (alk. paper)
1. Immunology. I. Travers, Paul, 1956- II. Walport, Mark. III. Janeway, Charles. IV. Title.
QR181.J37 2011
616.07'9--dc23

2011023486

Published by Garland Science, Taylor & Francis Group, LLC, an informa business,
711 Third Avenue, 8th floor, New York, NY 10017, USA,
and 2 Park Square, Milton Park, Abingdon, OX14 4RN, UK.

Printed in the United States of America

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

Garland Science
Taylor & Francis Group

Visit our website at http://www.garlandscience.com

Contents

PART I	**AN INTRODUCTION TO IMMUNOBIOLOGY AND INNATE IMMUNITY**	
Chapter 1	Basic Concepts in Immunology	1
Chapter 2	Innate Immunity: The First Lines of Defense	37
Chapter 3	The Induced Responses of Innate Immunity	75
PART II	**THE RECOGNITION OF ANTIGEN**	
Chapter 4	Antigen Recognition by B-cell and T-cell Receptors	127
Chapter 5	The Generation of Lymphocyte Antigen Receptors	157
Chapter 6	Antigen Presentation to T Lymphocytes	201
PART III	**THE DEVELOPMENT OF MATURE LYMPHOCYTE RECEPTOR REPERTOIRES**	
Chapter 7	Signaling Through Immune-System Receptors	239
Chapter 8	The Development and Survival of Lymphocytes	275
PART IV	**THE ADAPTIVE IMMUNE RESPONSE**	
Chapter 9	T Cell-Mediated Immunity	335
Chapter 10	The Humoral Immune Response	387
Chapter 11	Dynamics of Adaptive Immunity	429
Chapter 12	The Mucosal Immune System	465
PART V	**THE IMMUNE SYSTEM IN HEALTH AND DISEASE**	
Chapter 13	Failures of Host Defense Mechanisms	509
Chapter 14	Allergy and Allergic Diseases	571
Chapter 15	Autoimmunity and Transplantation	611
Chapter 16	Manipulation of the Immune Response	669
Appendix I	Immunologist's Toolbox with Immunological Constants	717
Appendix II	CD Antigens	763
Appendix III	Cytokines and Their Receptors	779
Appendix IV	Chemokines and Their Receptors	782
Biographies		784
Glossary		786
Index		823

Petitioner Merck, Ex. 1170, p. 5
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Basic Concepts in Immunology

1

Immunology is the study of the body's defense against infection. We live surrounded by microorganisms, many of which cause disease. Yet despite this continual exposure we become ill only rarely. How does the body defend itself? When infection does occur, how does the body eliminate the invader and cure itself? And why do we develop long-lasting immunity to many infectious diseases encountered once and overcome? These are the questions addressed by immunology, which we study to understand our body's defenses against infection at the cellular and molecular levels.

Immunology is a relatively new science. Its origin is usually attributed to **Edward Jenner** (Fig. 1.1), who observed in the late 18th century that the relatively mild disease of cowpox, or vaccinia, seemed to confer protection against the often fatal disease of smallpox. In 1796, Jenner demonstrated that inoculation with cowpox could protect against smallpox. He called the procedure **vaccination**, and this term is still used to describe the inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease. Although Jenner's bold experiment was successful, it took almost two centuries for smallpox vaccination to become universal, an advance that enabled the World Health Organization to announce in 1979 that smallpox had been eradicated (Fig. 1.2), arguably the greatest triumph of modern medicine.

When Jenner introduced vaccination he knew nothing of the infectious agents that cause disease: it was not until late in the 19th century that **Robert Koch** proved that infectious diseases are caused by microorganisms, each one responsible for a particular disease. We now recognize four broad categories of disease-causing microorganisms, or **pathogens**: viruses, bacteria, fungi, and the unicellular and multicellular eukaryotic organisms collectively termed parasites.

The discoveries of Koch and other great 19th-century microbiologists extended Jenner's strategy of vaccination to other diseases. In the 1880s, **Louis Pasteur** devised a vaccine against cholera in chickens, and developed a rabies vaccine that proved a spectacular success upon its first trial in a boy bitten by a rabid dog. These practical triumphs led to a search for the mechanism of protection and to the development of the science of immunology. In the



Fig. 1.1 Edward Jenner. Portrait by John Raphael Smith. Reproduced courtesy of Yale University, Harvey Cushing/John Hay Whitney Medical Library.

Petitioner Merck, Ex. 1170, p. 6
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

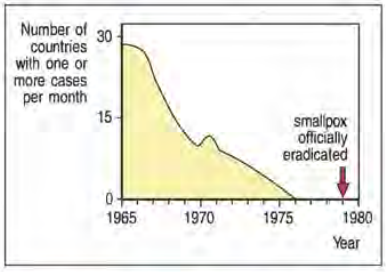




Fig. 1.2 The eradication of smallpox by vaccination. After a period of 3 years in which no cases of smallpox were recorded, the World Health Organization was able to announce in 1979 that smallpox had been eradicated, and vaccination stopped (upper panel). A few laboratory stocks have been retained, however, and some fear that these are a source from which the virus might reemerge. Ali Maow Maalin (lower panel) contracted and survived the last case of smallpox in Somalia in 1978. Photograph courtesy of Dr. Jason Weisfeld.

early 1890s, **Emil von Behring** and **Shibasaburo Kitasato** discovered that the serum of animals immune to diphtheria or tetanus contained a specific 'antitoxic activity' that could confer short-lived protection against the effects of diphtheria or tetanus toxins in people. This activity was due to the proteins we now call **antibodies**, which bound specifically to the toxins and neutralized their activity.

The responses we make against infection by potential pathogens are known as **immune responses**. A specific immune response, such as the production of antibodies against a particular pathogen or its products, is known as an **adaptive immune response** because it is developed during the lifetime of an individual as an adaptation to infection with that pathogen. In many cases, an adaptive immune response also results in the phenomenon known as immunological memory, which confers lifelong **protective immunity** to reinfection with the same pathogen. This is just one of the features that distinguish an adaptive immune response from the **innate immune response**, or innate immunity, which is always immediately available to combat a wide range of pathogens but does not lead to lasting immunity and is not specific for any individual pathogen. At the time that von Behring was developing serum therapy for diphtheria, innate immunity was known chiefly through the work of the great Russian immunologist **Elie Metchnikoff**, who discovered that many microorganisms could be engulfed and digested by phagocytic cells, which he called 'macrophages.' These cells are always present and ready to act, and are a front-line component of innate immune responses. In contrast, an adaptive immune response takes time to develop and is highly specific; antibodies against the influenza virus, for example, will not protect against poliovirus.

It quickly became clear that antibodies could be induced against a vast range of substances. Such substances were called **antigens** because they could stimulate *anti*body *gen*eration. Much later, it was discovered that antibody production is not the only function of adaptive immune responses, and the term antigen is now used to describe any substance that can be recognized and responded to by the adaptive immune system. The proteins, glycoproteins, and polysaccharides of pathogens are the antigens normally responded to by the immune system, but it can recognize and make a response to a much wider range of chemical structures—hence its ability to produce allergic immune responses to metals such as nickel, drugs such as penicillin, and organic chemicals in the leaves of poison ivy. The innate and adaptive immune responses together provide a remarkably effective defense system. Many infections are handled successfully by innate immunity and cause no disease; in those that cannot be resolved, the activities of the innate immune system trigger an adaptive immune response. If the disease is overcome, the adaptive immune response is often followed by lasting immunological memory, which prevents disease if reinfection occurs.

This book describes the mechanisms of both innate and adaptive immunity and illustrates how they are integrated into an effective overall system of defense against invasion by pathogens. Although the white blood cells known as **lymphocytes** possess the most powerful ability to recognize and target pathogenic microorganisms, they need the participation of the innate immune system to initiate and to mount their offensive. Indeed, the adaptive immune response and innate immunity use many of the same destructive mechanisms to finally destroy invading microorganisms.

In this chapter we first introduce the principles of innate and adaptive immunity, the cells of the immune system, the tissues in which they develop, and the tissues through which they circulate. We then outline the specialized functions of the different types of cells and the mechanisms by which they eliminate infection.

Petitioner Merck, Ex. 1170, p. 7
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Principles of innate and adaptive immunity.

The body is protected from infectious agents and the damage they cause, and from other harmful substances such as insect toxins, by a variety of effector cells and molecules that together make up the **immune system**. In this part of the chapter we discuss the main principles underlying immune responses and introduce the cells and tissues of the immune system on which an immune response depends.

1-1 The immune system recognizes infection and induces protective responses.

To protect the individual effectively against disease, the immune system must fulfill four main tasks. The first is **immunological recognition**: the presence of an infection must be detected. This task is carried out both by the white blood cells of the innate immune system, which provide an immediate response, and by the lymphocytes of the adaptive immune system. The second task is to contain the infection and if possible eliminate it completely, which brings into play **immune effector functions** such as the complement system of blood proteins, the antibodies produced by some lymphocytes, and the destructive capacities of lymphocytes and the other white blood cells. At the same time the immune response must be kept under control so that it does not itself do damage to the body. **Immune regulation**, or the ability of the immune system to self-regulate, is thus an important feature of immune responses, and failure of such regulation contributes to conditions such as allergy and autoimmune disease. The fourth task is to protect the individual against recurring disease due to the same pathogen. A unique feature of the adaptive immune system is that it is capable of generating **immunological memory**, so that having been exposed once to an infectious agent, a person will make an immediate and stronger response against any subsequent exposure to it; that is, they will have protective immunity against it. Finding ways of generating long-lasting immunity to pathogens that do not naturally provoke it is one of the greatest challenges facing immunologists today.

When an individual first encounters an infectious agent, the initial defenses against infection are physical and chemical barriers, such as antimicrobial proteins secreted at mucosal surfaces, that prevent microbes from entering the body. If these barriers are overcome or evaded, other components of the immune system come into play. The complement system can immediately recognize and destroy foreign organisms, and phagocytic white blood cells, such as macrophages and neutrophils of the innate immune system, can ingest and kill microbes by producing toxic chemicals and powerful degradative enzymes. Innate immunity is of ancient origin—some form of innate defense against disease is found in all animals and plants. The macrophages of humans and other vertebrates, for example, are presumed to be the direct evolutionary descendants of the phagocytic cells present in simpler animals, such as those that Metchnikoff observed in the invertebrate sea stars.

Innate immune responses occur rapidly on exposure to an infectious organism. In contrast, responses by the adaptive immune system take days rather than hours to develop (summarized in Fig. 1.34). However, the adaptive immune system is capable of eliminating infections more efficiently because of the exquisitely specific recognition functions of lymphocytes. These cells can recognize and respond to individual antigens by means of highly specialized **antigen receptors** on the lymphocyte surface. The billions of lymphocytes present in the body collectively possess a vast repertoire of antigen receptors, which enables the immune system to recognize and respond to

Petitioner Merck, Ex. 1170, p. 8
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

virtually any antigen a person is likely to be exposed to. In this way, adaptive immunity can more effectively focus its resources to overcome pathogens that have evaded or overwhelmed innate immunity. Antibodies and activated lymphocytes produced by an adaptive immune response can also persist after the original infection has been eliminated. They help to prevent immediate reinfection and also provide for long-lasting immunity, allowing a faster and more intense response to a second exposure even when it occurs many years later.

1-2 The cells of the immune system derive from precursors in the bone marrow.

Both innate and adaptive immune responses depend upon the activities of white blood cells or **leukocytes**. These cells all originate in the **bone marrow**, and many of them also develop and mature there. Once mature, they migrate to guard the peripheral tissues: some of them reside within tissues, while others circulate in the bloodstream and in a specialized system of vessels called the **lymphatic system**, which drains extracellular fluid and free cells from tissues, transports them through the body as **lymph**, and eventually empties back into the blood system.

All the cellular elements of blood, including the red blood cells that transport oxygen, the platelets that trigger blood clotting in damaged tissues, and the white blood cells of the immune system, derive from the **hematopoietic stem cells** of the bone marrow. Because these stem cells can give rise to all the different types of blood cells, they are often known as pluripotent hematopoietic stem cells. They give rise to cells of more limited developmental potential, which are the immediate progenitors of red blood cells, platelets, and the two main categories of white blood cells, the **lymphoid** and **myeloid** lineages. The different types of blood cells and their lineage relationships are summarized in Fig. 1.3.

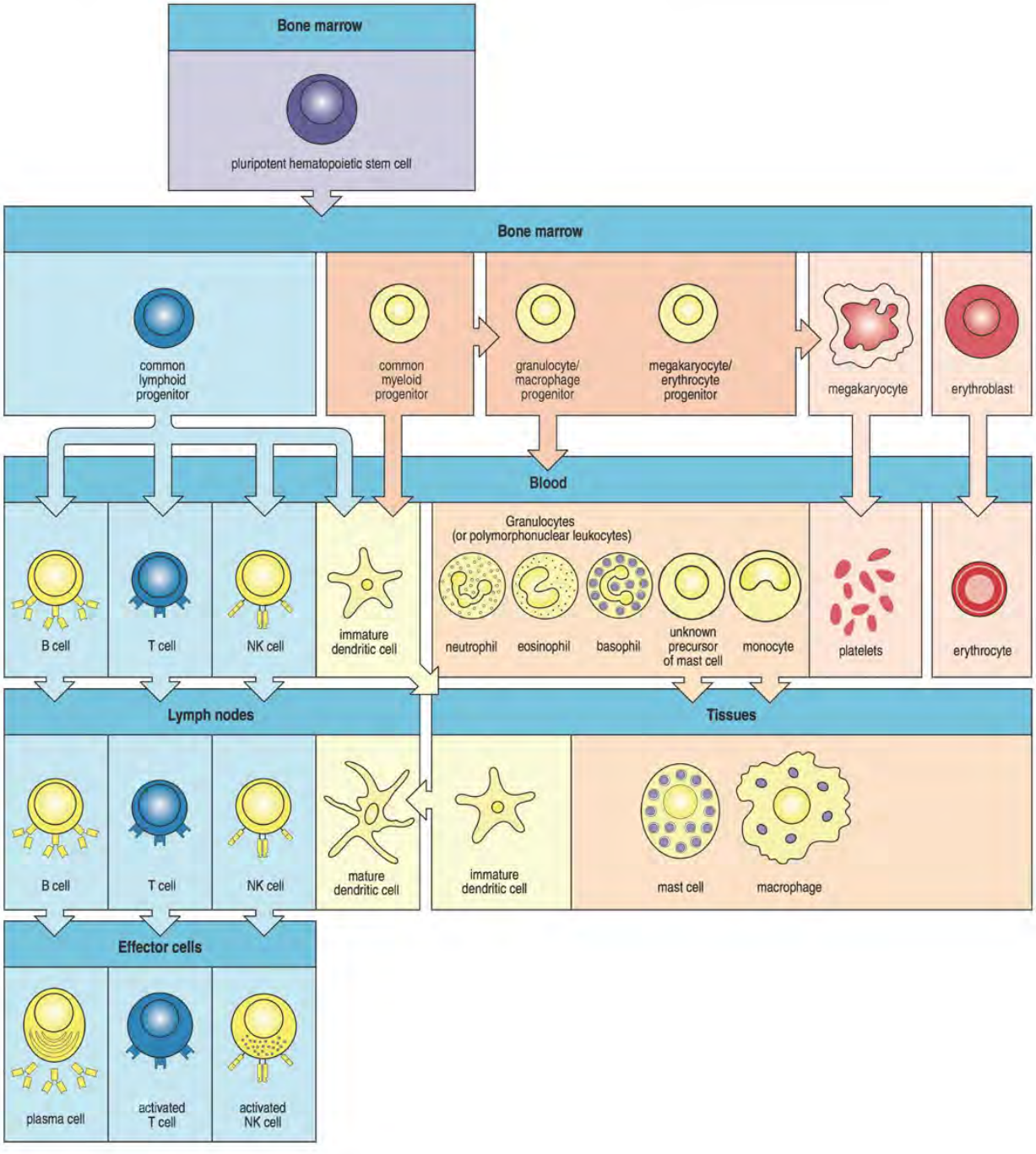
1-3 The myeloid lineage comprises most of the cells of the innate immune system.

The **common myeloid progenitor** is the precursor of the macrophages, granulocytes, mast cells, and dendritic cells of the innate immune system, and also of megakaryocytes and red blood cells, which we will not be concerned with here. The cells of the myeloid lineage are shown in Fig. 1.4.

Fig. 1.3 All the cellular elements of the blood, including the cells of the immune system, arise from pluripotent hematopoietic stem cells in the bone marrow. These pluripotent cells divide to produce two types of stem cells. A common lymphoid progenitor gives rise to the lymphoid lineage (blue background) of white blood cells or leukocytes—the natural killer (NK) cells and the T and B lymphocytes. A common myeloid progenitor gives rise to the myeloid lineage (pink and yellow backgrounds), which comprises the rest of the leukocytes, the erythrocytes (red blood cells), and the megakaryocytes that produce platelets important in blood clotting. T and B lymphocytes are distinguished from the other leukocytes by the possession of antigen receptors, and from each other by their sites of differentiation—the thymus and bone marrow, respectively. After encounter with antigen, B cells differentiate into antibody-secreting plasma cells, while T cells differentiate into effector T cells with a variety of functions. Unlike T and B cells, NK cells lack antigen specificity. The remaining leukocytes are the monocytes, the dendritic cells, and the neutrophils, eosinophils, and basophils. The last three of these circulate in the blood and are termed granulocytes, because of the cytoplasmic granules whose staining gives these cells a distinctive appearance in blood smears, or polymorphonuclear leukocytes, because of their irregularly shaped nuclei. Immature dendritic cells (yellow background) are phagocytic cells that enter the tissues; they mature after they have encountered a potential pathogen. The common lymphoid progenitor also gives rise to a minor subpopulation of dendritic cells, but for simplicity this developmental pathway has not been illustrated. However, as there are more common myeloid progenitor cells than there are common lymphoid progenitors, the majority of the dendritic cells in the body develop from common myeloid progenitors. Monocytes enter tissues, where they differentiate into phagocytic macrophages. The precursor cell that gives rise to mast cells is still unknown. Mast cells also enter tissues and complete their maturation there.

Petitioner Merck, Ex. 1170, p. 9
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Macrophages are resident in almost all tissues and are the mature form of **monocytes**, which circulate in the blood and continually migrate into tissues, where they differentiate. Together, monocytes and macrophages make up one of the three types of phagocytes in the immune system: the others are the granulocytes (the collective term for the white blood cells called neutrophils, eosinophils, and basophils) and the dendritic cells. Macrophages are relatively long-lived cells and perform several different functions throughout



the innate immune response and the subsequent adaptive immune response. One is to engulf and kill invading microorganisms. In this phagocytic role they are an important first defense in innate immunity and also dispose of pathogens and infected cells targeted by an adaptive immune response. Both monocytes and macrophages are phagocytic, but most infections occur in the tissues, and so it is primarily macrophages that perform this important protective function. An additional and crucial role of macrophages is to

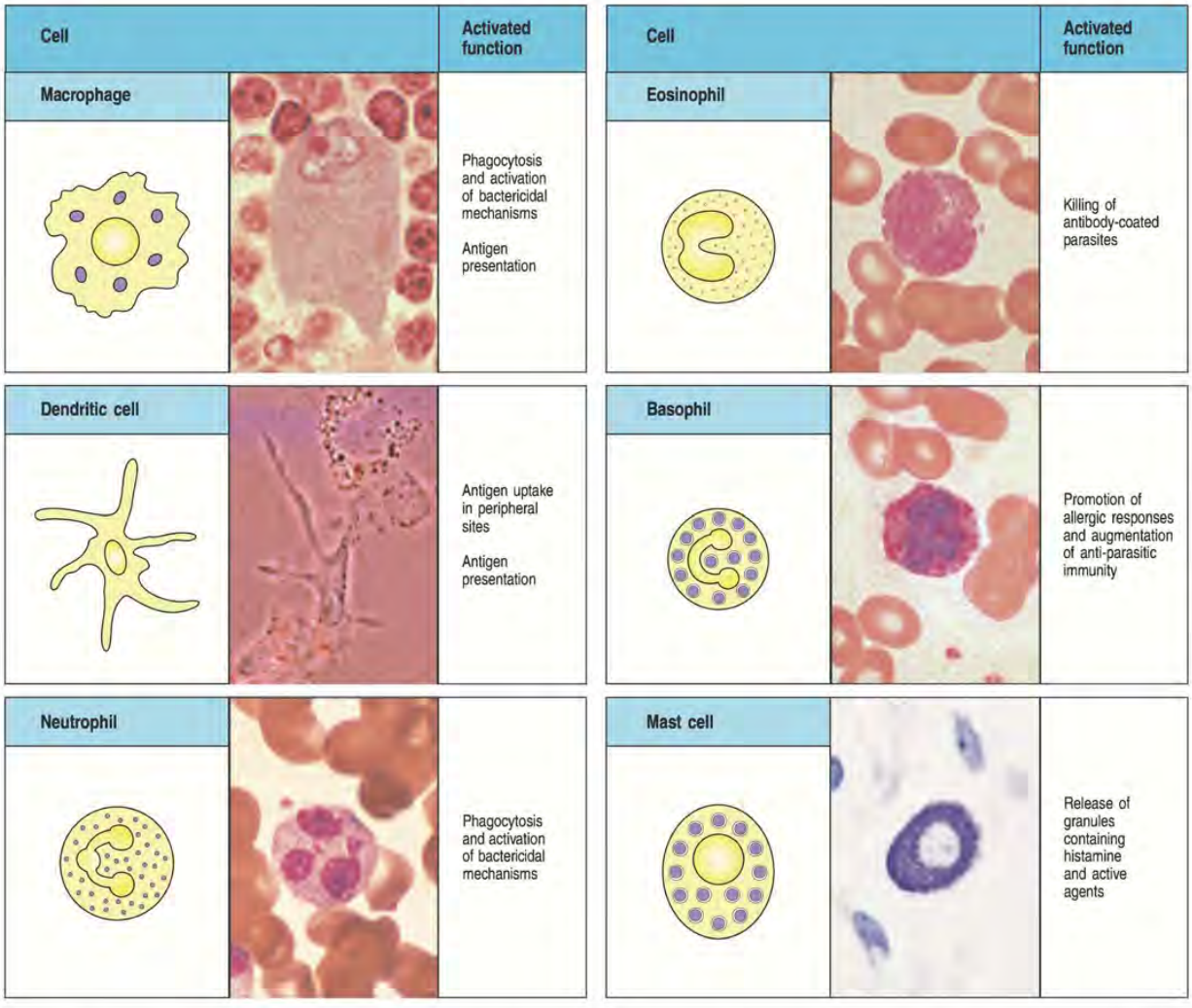


Fig. 1.4 Myeloid cells in innate and adaptive immunity. Cells of the myeloid lineage perform various important functions in the immune response. In the rest of the book, these cells will be represented in the schematic form shown on the left. A photomicrograph of each cell type is shown in the center panels. Macrophages and neutrophils are primarily phagocytic cells that engulf pathogens and destroy them in intracellular vesicles, a function they perform in both innate and adaptive immune responses. Dendritic cells are phagocytic when they are immature and can take up pathogens; after maturing, they function as specialized cells that present pathogen antigens to T lymphocytes in a form they can recognize, thus activating T lymphocytes and initiating adaptive immune responses. Macrophages can also present antigens to T lymphocytes and can activate them. The other myeloid cells are primarily secretory cells that release the contents of their prominent granules upon activation via antibody during an adaptive immune response. Eosinophils are thought to be involved in attacking large antibody-coated parasites such as worms; basophils are also thought to be involved in anti-parasite immunity. Mast cells are tissue cells that trigger a local inflammatory response to antigen by releasing substances that act on local blood vessels. Mast cells, eosinophils, and basophils are also important in allergic responses. Photographs courtesy of N. Rooney, R. Steinman, and D. Friend.

Petitioner Merck, Ex. 1170, p. 11
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

orchestrate immune responses: they help induce inflammation, which, as we shall see, is a prerequisite to a successful immune response, and they secrete signaling proteins that activate other immune-system cells and recruit them into an immune response. In addition to their specialized role in the immune system, macrophages act as general scavenger cells in the body, clearing dead cells and cell debris.

The **granulocytes** are so called because they have densely staining granules in their cytoplasm; they are also called **polymorphonuclear leukocytes** because of their oddly shaped nuclei. There are three types of granulocytes—neutrophils, eosinophils, and basophils—which are distinguished by the different staining properties of the granules. In comparison with macrophages they are all relatively short-lived, surviving for only a few days, and are produced in increased numbers during immune responses, when they leave the blood to migrate to sites of infection or inflammation. The phagocytic **neutrophils** are the most numerous and most important cells in innate immune responses: they take up a variety of microorganisms by phagocytosis and efficiently destroy them in intracellular vesicles using degradative enzymes and other antimicrobial substances stored in their cytoplasmic granules. Their role is discussed in more detail in Chapter 3. Hereditary deficiencies in neutrophil function lead to overwhelming bacterial infection, which is fatal if untreated.

Eosinophils and **basophils** are less abundant than neutrophils, but like neutrophils they have granules containing a variety of enzymes and toxic proteins, which are released when the cells are activated. Eosinophils and basophils are thought to be important chiefly in defense against parasites, which are too large to be ingested by macrophages or neutrophils. They can also contribute to allergic inflammatory reactions, in which their effects are damaging rather than protective. We discuss the functions of these cells in Chapter 10 and their role in allergic inflammation in Chapter 14.

Mast cells, whose blood-borne precursors are not well defined, differentiate in the tissues. Although best known for their role in orchestrating allergic responses, which is discussed in Chapter 14, they are believed to play a part in protecting the internal surfaces of the body against pathogens, and are involved in the response to parasitic worms. They have large granules in their cytoplasm that are released when the mast cell is activated; these help induce inflammation.

There are several kinds of **dendritic cells**, which form the third class of phagocytic cell of the immune system. Most dendritic cells have long finger-like processes, like the dendrites of nerve cells, which give them their name. Immature dendritic cells migrate through the bloodstream from the bone marrow to enter tissues. They take up particulate matter by phagocytosis and also continually ingest large amounts of the extracellular fluid and its contents by a process known as **macropinocytosis**. Like macrophages and neutrophils, they degrade the pathogens they take up, but their main role in the immune system is not the clearance of microorganisms. Instead, the encounter with a pathogen stimulates dendritic cells to mature into cells that can activate a particular class of lymphocytes—the T lymphocytes—which are described in Section 1-4. Mature dendritic cells activate T lymphocytes by displaying antigens derived from the pathogen on their surface in a way that activates the antigen receptor of a T lymphocyte. They also provide other signals that are necessary to activate T lymphocytes that are encountering their specific antigen for the first time, and for this reason dendritic cells are also called **antigen-presenting cells** (**APCs**). As such, dendritic cells form a crucial link between the innate immune response and the adaptive immune response (Fig. 1.5). In certain situations, macrophages can also act as antigen-presenting cells, but dendritic cells are the cells that are specialized in initiating adaptive immune responses. We describe the types and functions of dendritic cells in Chapters 6, 9, 11, and 12.

Petitioner Merck, Ex. 1170, p. 12
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

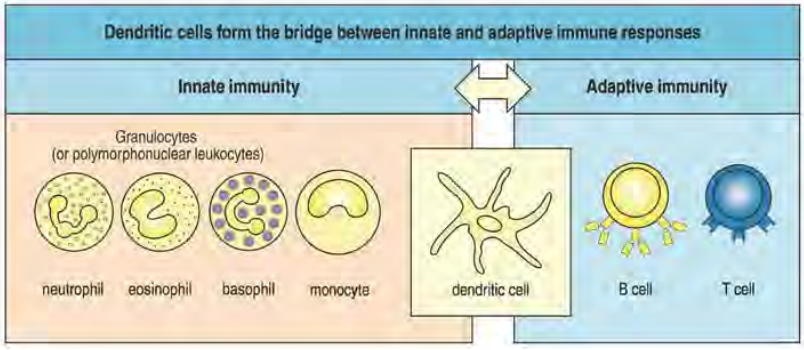


Fig. 1.5 Dendritic cells form a key link between the innate immune system and the adaptive immune system. Like the other cells of innate immunity, dendritic cells recognize pathogens via invariant cell-surface receptors for pathogen molecules, and are activated by these stimuli early in an infection. Dendritic cells in the tissues are phagocytic and are specialized to ingest a wide range of pathogens and to display their antigens at the dendritic cell surface in a form that can be recognized by T cells. As described later in the chapter, activated dendritic cells also produce molecules that enable T cells to be activated by antigen.

1-4 The lymphoid lineage comprises the lymphocytes of the adaptive immune system and the natural killer cells of innate immunity.

The **common lymphoid progenitor** in the bone marrow gives rise to the antigen-specific lymphocytes of the adaptive immune system and also to a type of lymphocyte that responds to the presence of infection but is not specific for antigen, and is thus considered to be part of the innate immune system. This latter is a large cell with a distinctive granular cytoplasm and is called a **natural killer cell** (**NK cell**) (Fig. 1.6). These cells can recognize and kill some abnormal cells, for example some tumor cells and cells infected with herpesviruses, and are thought to be important in holding viral infections in check before the adaptive immune response kicks in. Their functions in innate immunity are described in Chapter 3.

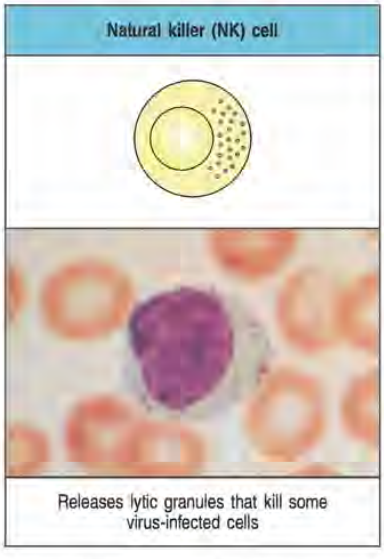


Fig. 1.6 Natural killer (NK) cells. These are large granular lymphoid-like cells with important functions in innate immunity, especially against intracellular infections, being able to kill other cells. Unlike lymphocytes, they lack antigen-specific receptors. Photograph courtesy of B. Smith.

We come finally to the key components of adaptive immunity, the antigen-specific lymphocytes. Unless indicated otherwise, we shall use the term lymphocyte from now on to refer to the antigen-specific lymphocytes only. The immune system must be able to mount an immune response against any of the wide variety of different pathogens a person is likely to encounter during their lifetime. Lymphocytes collectively make this possible through the highly variable antigen receptors on their surface, by which they recognize and bind antigens. Each lymphocyte matures bearing a unique variant of a prototype antigen receptor, so that the population of lymphocytes expresses a huge repertoire of receptors that are highly diverse in their antigen-binding sites. Among the billion or so lymphocytes circulating in the body at any one time there will always be some that can recognize a given foreign antigen.

In the absence of an infection, most lymphocytes circulating in the body are small, featureless cells with few cytoplasmic organelles and much of the nuclear chromatin inactive, as shown by its condensed state (Fig. 1.7). This appearance is typical of inactive cells. It is hardly surprising that until the 1960s, textbooks described these cells, now the central focus of immunology, as having no known function. Indeed, these small lymphocytes have no functional activity until they encounter their specific antigen. Lymphocytes that have not yet been activated by antigen are known as naive lymphocytes; those that have met their antigen, become activated, and have differentiated further into fully functional lymphocytes are known as **effector lymphocytes**.

There are two types of lymphocytes—**B lymphocytes** (**B cells**) and **T lymphocytes** (**T cells**)—each with quite different roles in the immune system and distinct types of antigen receptors. After antigen binds to a **B-cell antigen receptor**, or **B-cell receptor** (**BCR**), on the B-cell surface, the lymphocyte will proliferate and differentiate into **plasma cells**. These are the effector form of B lymphocytes and they produce antibodies, which are a secreted form of the B-cell receptor and have an identical antigen specificity. Thus the antigen that

Petitioner Merck, Ex. 1170, p. 13
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

activates a given B cell becomes the target of the antibodies produced by that cell's progeny. Antibody molecules as a class are known as **immunoglobulins** (**Ig**), and so the antigen receptor of B lymphocytes is also known as **membrane immunoglobulin** (**mIg**) or **surface immunoglobulin** (**sIg**).

The **T-cell antigen receptor**, or **T-cell receptor** (**TCR**), is related to immunoglobulin but is quite distinct in its structure and recognition properties. After a T cell has been activated by its first encounter with antigen, it proliferates and differentiates into one of several different functional types of **effector T lymphocytes**. Effector T-cell functions fall into three broad classes: killing, activation, and regulation. **Cytotoxic T cells** kill cells that are infected with viruses or other intracellular pathogens. **Helper T cells** provide essential additional signals that influence the behavior and activity of other cells. Helper T cells provide signals to antigen-stimulated B cells that influence their production of antibody, and to macrophages that allow them to become more efficient at killing engulfed pathogens. We return to the functions of cytotoxic and helper T cells later in this chapter, and their actions are described in detail in Chapters 9 and 11. **Regulatory T cells** suppress the activity of other lymphocytes and help to control immune responses; they are discussed in Chapters 9, 11, 12, and 15.

During the course of an immune response, some of the B cells and T cells activated by antigen differentiate into **memory cells**, the lymphocytes that are responsible for the long-lasting immunity that can follow exposure to disease or vaccination. Memory cells will readily differentiate into effector cells on a second exposure to their specific antigen. Immunological memory is described in Chapter 11.

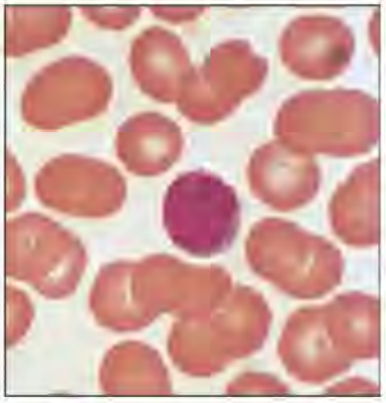


Fig. 1.7 Lymphocytes are mostly small and inactive cells. The upper panel shows a light micrograph of a small lymphocyte in which the nucleus has been stained purple by the hematoxylin and eosin dye, surrounded by red blood cells (which have no nuclei). Note the darker purple patches of condensed chromatin of the lymphocyte nucleus, indicating little transcriptional activity, the relative absence of cytoplasm, and the small size. The lower panel shows a transmission electron micrograph of a small lymphocyte. Again, note the evidence of functional inactivity: the condensed chromatin, the scanty cytoplasm, and the absence of rough endoplasmic reticulum. Photographs courtesy of N. Rooney.

1-5 Lymphocytes mature in the bone marrow or the thymus and then congregate in lymphoid tissues throughout the body.

Lymphocytes circulate in the blood and the lymph and are also found in large numbers in **lymphoid tissues** or **lymphoid organs**, which are organized aggregates of lymphocytes in a framework of nonlymphoid cells. Lymphoid organs can be divided broadly into the **central** or **primary lymphoid organs**, where lymphocytes are generated, and the **peripheral** or **secondary lymphoid organs**, where mature naive lymphocytes are maintained and adaptive immune responses are initiated. The central lymphoid organs are the bone marrow and the **thymus**, an organ in the upper chest. The peripheral lymphoid organs comprise the **lymph nodes**, the **spleen**, and the **mucosal lymphoid tissues** of the gut, the nasal and respiratory tract, the urogenital tract, and other mucosa. The location of the main lymphoid tissues is shown schematically in Fig. 1.8, and we describe the individual peripheral lymphoid organs in more detail later in the chapter. Lymph nodes are interconnected by a system of lymphatic vessels, which drain extracellular fluid from tissues, through the lymph nodes, and back into the blood.

Both B and T lymphocytes originate in the bone marrow, but only the B lymphocytes mature there. The precursor T lymphocytes migrate to the thymus, from which they get their name, and mature there. The 'B' in B lymphocytes originally stood for the **bursa of Fabricius**, a lymphoid organ in young chicks in which lymphocytes mature; fortunately, it can stand equally well for bone marrow derived. Once they have completed maturation, both types of lymphocytes enter the bloodstream as mature naive lymphocytes. They circulate through the peripheral lymphoid tissues, in which an adaptive immune response is initiated if a lymphocyte meets its corresponding antigen. Before this, however, an innate immune response to the infection has usually occurred, and we now look at how this alerts the rest of the immune system to the presence of a pathogen.

Petitioner Merck, Ex. 1170, p. 14
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

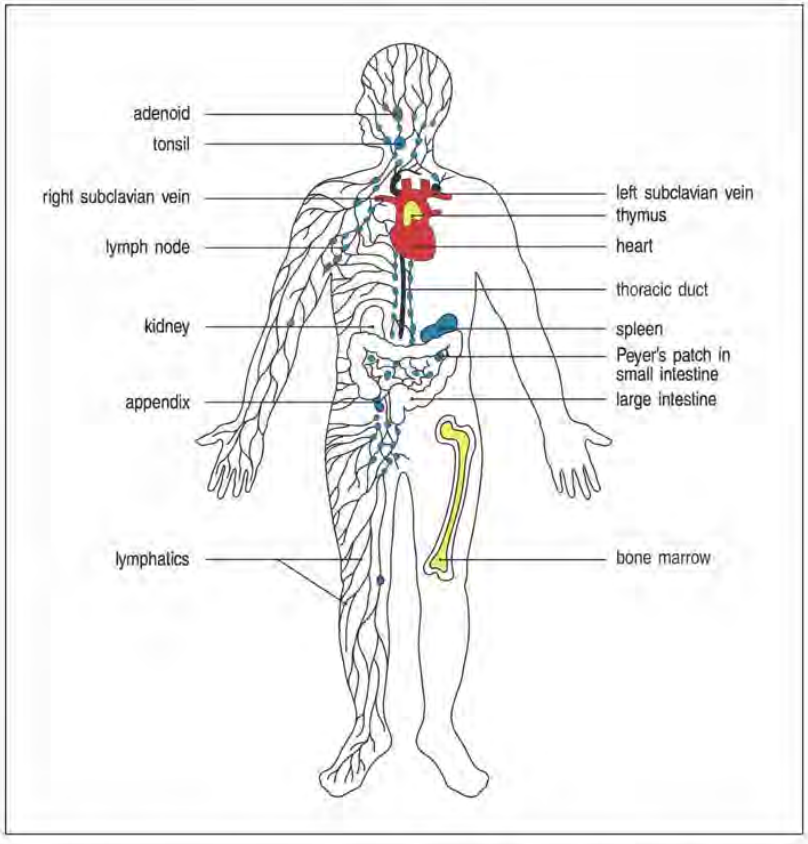


Fig. 1.8 The distribution of lymphoid tissues in the body. Lymphocytes arise from stem cells in bone marrow and differentiate in the central lymphoid organs (yellow)—B cells in the bone marrow and T cells in the thymus. They migrate from these tissues and are carried in the bloodstream to the peripheral lymphoid organs (blue). These include lymph nodes, spleen, and lymphoid tissues associated with mucosa, such as the gut-associated tonsils, Peyer's patches, and appendix. The peripheral lymphoid organs are the sites of lymphocyte activation by antigen, and lymphocytes recirculate between the blood and these organs until they encounter their specific antigen. Lymphatics drain extracellular fluid from the peripheral tissues, through the lymph nodes and into the thoracic duct, which empties into the left subclavian vein. This fluid, known as lymph, carries antigen taken up by dendritic cells and macrophages to the lymph nodes, as well as recirculating lymphocytes from the lymph nodes back into the blood. Lymphoid tissue is also associated with other mucosa such as the bronchial linings (not shown).

1-6 Most infectious agents activate the innate immune system and induce an inflammatory response.

The skin and the mucosal epithelia lining the airways and gut are the first defense against invading pathogens, forming a physical and chemical barrier against infection. Microorganisms that breach these defenses are met by cells and molecules that mount an immediate innate immune response. Macrophages resident in the tissues, for example, can recognize bacteria by means of receptors that bind common constituents of many bacterial surfaces. Engagement of these receptors triggers the macrophage both to engulf the bacterium and degrade it internally, and to secrete proteins called cytokines and chemokines that convey important signals to other immune cells. Similar responses occur to viruses, fungi, and parasites. **Cytokine** is a general name for any protein that is secreted by cells and affects the behavior of nearby cells bearing appropriate receptors. **Chemokines** are secreted proteins that act as chemoattractants (hence the name 'chemokine'), attracting cells bearing chemokine receptors, such as neutrophils and monocytes, out of the bloodstream and into infected tissue (Fig. 1.9). The cytokines and chemokines released by activated macrophages initiate the process known as **inflammation**. Inflammation is beneficial to combating infection by recruiting proteins and cells from the blood into infected tissues that help to directly destroy the pathogen. In addition, inflammation increases the flow of lymph carrying microbes and antigen-presenting cells from the infected tissue to nearby lymphoid tissues, where they activate lymphocytes and initiate the adaptive immune response. Once adaptive immunity has been triggered, inflammation also recruits the effector components of the adaptive immune system—antibody molecules and effector T cells—to the site of infection.

Petitioner Merck, Ex. 1170, p. 15
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

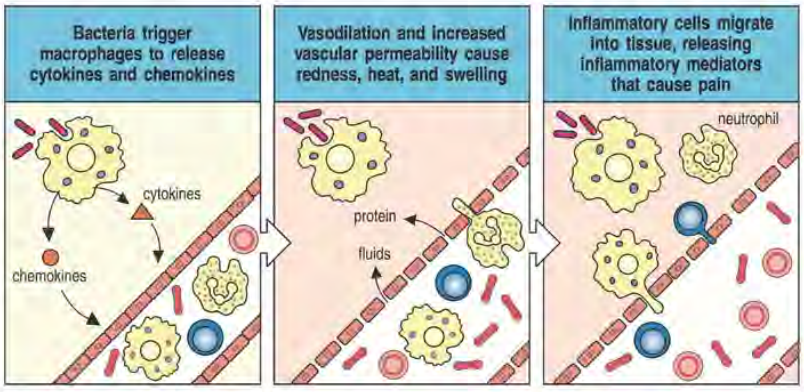


Fig. 1.9 Infection triggers an inflammatory response. Macrophages encountering bacteria or other types of microorganisms in tissues are triggered to release cytokines that increase the permeability of blood vessels, allowing fluid and proteins to pass into the tissues. They also produce chemokines, which direct the migration of neutrophils to the site of infection. The stickiness of the endothelial cells of the blood vessel wall is also changed, so that cells adhere to the wall and are able to crawl through it; first neutrophils and then monocytes are shown entering the tissue from a blood vessel. The accumulation of fluid and cells at the site of infection causes the redness, swelling, heat, and pain known collectively as inflammation. Neutrophils and macrophages are the principal inflammatory cells. Later in an immune response, activated lymphocytes can also contribute to inflammation.

Local inflammation and the phagocytosis of invading bacteria can also be triggered as a result of the activation of a group of plasma proteins known collectively as **complement**. Activation of the complement system by bacterial surfaces leads to a cascade of proteolytic reactions that coats microbes, but not the body's own cells, with complement fragments. Complement-coated microbes are recognized and bound by specific **complement receptors** on macrophages, taken up by phagocytosis, and destroyed.

Inflammation is described clinically by the Latin words *calor*, *dolor*, *rubor*, and *tumor*, meaning heat, pain, redness, and swelling. Each of these features reflects an effect of cytokines or other inflammatory mediators on the local blood vessels. Heat, redness, and swelling result from the dilation and increased permeability of blood vessels during inflammation, leading to increased local blood flow and leakage of fluid and blood proteins into the tissues. Cytokines and complement fragments have important effects on the **endothelium** that lines blood vessels; the **endothelial cells** themselves also produce cytokines in response to infection. The pro-inflammatory cytokines produce changes in the adhesive properties of the endothelial cells, in turn causing circulating leukocytes to stick to the endothelial cells and migrate between them into the site of infection, to which they are attracted by chemokines. The migration of cells into the tissue and their local actions account for the pain.

The main cell types seen in the initial phase of an inflammatory response are macrophages and neutrophils, the latter being recruited into the inflamed, infected tissue in large numbers. Macrophages and neutrophils are thus also known as **inflammatory cells**. Like macrophages, neutrophils have surface receptors for common bacterial constituents and for complement, and they are the principal cells that engulf and destroy the invading microorganisms. The influx of neutrophils is followed a short time later by the increased entry of monocytes, which rapidly differentiate into macrophages, thus reinforcing and sustaining the innate immune response. Later, if the inflammation continues, eosinophils also migrate into inflamed tissues and contribute to the destruction of the invading microorganisms.

1-7 Pattern recognition receptors of the innate immune system provide an initial discrimination between self and nonself.

The defense systems of innate immunity are effective in combating many pathogens, but rely on a limited number of invariant receptors that recognize microorganisms. The pathogen-recognition receptors of macrophages, neutrophils, and dendritic cells recognize simple molecules and regular patterns of molecular structure known as **pathogen-associated molecular patterns** (**PAMPs**) that are present on many microorganisms but not on the

Petitioner Merck, Ex. 1170, p. 16
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

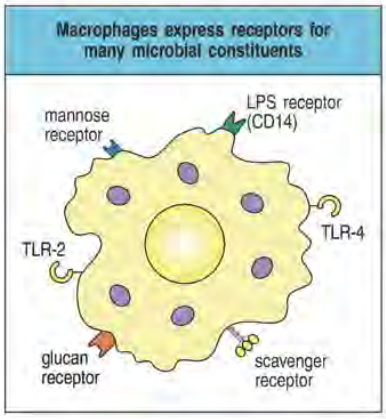


Fig. 1.10 Macrophages express a number of receptors that allow them to recognize different pathogens. Macrophages express a variety of receptors, each of which is able to recognize specific components of microbes. Some, like the mannose and glucan receptors and the scavenger receptor, bind cell-wall carbohydrates of bacteria, yeast, and fungi. The Toll-like receptors (TLRs) are an important family of pattern recognition receptors present on macrophages and other immune cells, and they are able to bind different microbial components; for example, TLR-2 binds cell-wall components of Gram-negative bacteria, whereas TLR-4 binds cell-wall components of Gram-positive bacteria. LPS, lipopolysaccharide.

body's own cells. The receptors that recognize PAMPs are known generally as **pattern recognition receptors** (**PRRs**), and they recognize structures such as mannose-rich oligosaccharides, peptidoglycans, and lipopolysaccharides in the bacterial cell wall, and unmethylated CpG DNA, which are common to many pathogens and have been conserved during evolution, making them excellent targets for recognition because they do not change (Fig. 1.10). We consider this system of innate recognition in detail in Chapter 3. Much of our knowledge of innate recognition has emerged only within the past 10 years, and this is one of the most exciting areas in modern immunology.

The pattern recognition receptors allow the innate immune system to distinguish self (the body) from nonself (pathogen). Detection of nonself activates innate cells and initiates adaptive immunity: macrophages are triggered to engulf microbes; immature dendritic cells are triggered to activate naive T lymphocytes. The molecules recognized by pattern recognition receptors are quite distinct from the individual pathogen-specific antigens recognized by lymphocytes. The fact that microbial constituents were needed to stimulate immune responses against purified proteins highlights the requirement that an innate response must precede the initiation of an adaptive response (see Appendix I, Sections A-1–A-4). This requirement was recognized long before the discovery of dendritic cells and their mode of activation. It was known that purified antigens such as proteins often did not evoke an immune response in an experimental immunization—that is, they were not **immunogenic**. To obtain adaptive immune responses to purified antigens, killed bacteria or bacterial extracts had to be mixed with the antigen. This additional material was termed an **adjuvant**, because it helped the response to the immunizing antigen (*adjuvare* is Latin for 'to help'). We know now that adjuvants are needed, at least in part, to activate dendritic cells to full antigen-presenting status in the absence of an infection. Finding suitable adjuvants is still an important part of vaccine preparation, as we discuss in Chapter 16.

1-8 Adaptive immune responses are initiated by antigen and antigen-presenting cells in secondary lymphoid tissues.

Adaptive immune responses are initiated when antigens or antigen-presenting cells, particularly dendritic cells bearing antigens picked up at sites of infection, reach the secondary lymphoid organs. Like the neutrophils and macrophages described earlier, dendritic cells have pattern recognition receptors that recognize molecular patterns common to microorganisms, such as bacterial lipopolysaccharide. Microbial components binding to these receptors stimulate the immature dendritic cell to engulf the pathogen and degrade it intracellularly. Immature dendritic cells also take up extracellular material, including virus particles and bacteria, by receptor-independent macropinocytosis, and thus internalize and degrade pathogens that their cell-surface receptors do not detect. In addition to the display of antigens that activates the antigen-receptors of lymphocytes, mature dendritic cells also express cell-surface proteins called **co-stimulatory molecules**, which provide signals that act together with antigen to stimulate the T lymphocyte to proliferate and differentiate into its final fully functional form (Fig. 1.11). Free antigens can also stimulate the antigen receptors of B cells, but most B cells require 'help' from activated helper T cells for optimal antibody responses. The activation of naive T lymphocytes is therefore an essential first stage in virtually all adaptive immune responses.

Movie 1.1

1-9 Lymphocytes activated by antigen give rise to clones of antigen-specific effector cells that mediate adaptive immunity.

Cells of the innate immune system express many different pattern recognition receptors, each recognizing a different feature shared by many pathogens.

Petitioner Merck, Ex. 1170, p. 17
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

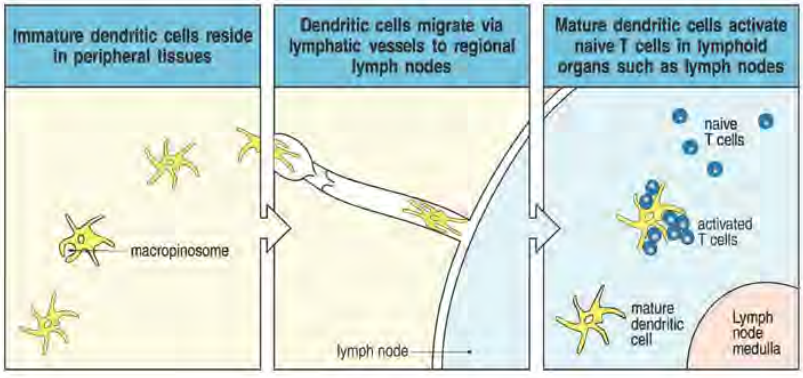


Fig. 1.11 Dendritic cells initiate adaptive immune responses. Immature dendritic cells resident in a tissue take up pathogens and their antigens by macropinocytosis and by receptor-mediated endocytosis. They are stimulated by recognition of the presence of pathogens to migrate through the lymphatics to regional lymph nodes, where they arrive as fully mature non-phagocytic dendritic cells that express both antigen and the co-stimulatory molecules necessary to activate a naive T cell that recognizes the antigen, stimulating lymphocyte proliferation and differentiation.

In contrast, lymphocyte antigen receptor expression is 'clonal'—in other words, each mature lymphocyte emerging from the central lymphoid organs differs from the others in the specificity of its antigen receptor. When that lymphocyte proliferates it forms a **clone** of identical cells bearing identical antigen receptors. The diversity in antigen receptors is generated by a unique genetic mechanism that operates during lymphocyte development in the bone marrow and the thymus to generate millions of different variants of the genes encoding the receptor molecules. This ensures that the lymphocytes in the body collectively carry millions of different antigen receptor specificities—the **lymphocyte receptor repertoire** of the individual.

Lymphocytes are continually undergoing a process akin to natural selection; only those lymphocytes that encounter an antigen to which their receptor binds will be activated to proliferate and differentiate into effector cells.

This selective mechanism was first proposed in the 1950s by **Macfarlane Burnet** to explain why a person produces antibodies against only those antigens to which he or she has been exposed. Burnet postulated the preexistence in the body of many different potential antibody-producing cells, each having the ability to make antibody of a different specificity and displaying on its surface a membrane-bound version of the antibody: this serves as a receptor for the antigen. On binding antigen, the cell is activated to divide and to produce many identical progeny, a process known as **clonal expansion**; this clone of identical cells can now secrete **clonotypic** antibodies with a specificity identical to that of the surface receptor that first triggered activation and clonal expansion (Fig. 1.12). Burnet called this the **clonal selection theory** of antibody production.

1-10 Clonal selection of lymphocytes is the central principle of adaptive immunity.

Remarkably, at the time that Burnet formulated his theory, nothing was known of the antigen receptors of lymphocytes; indeed, the function of lymphocytes themselves was still obscure. Lymphocytes did not take center stage until the early 1960s, when **James Gowans** discovered that removal of the small lymphocytes from rats resulted in the loss of all known adaptive immune responses. These immune responses were restored when the small lymphocytes were replaced. This led to the realization that lymphocytes must be the units of clonal selection, and their biology became the focus of the new field of **cellular immunology**.

Clonal selection of lymphocytes with diverse receptors elegantly explained adaptive immunity, but it raised one significant conceptual problem. If the antigen receptors of lymphocytes are generated randomly during the lifetime of an individual, how are lymphocytes prevented from recognizing antigens

Petitioner Merck, Ex. 1170, p. 18
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

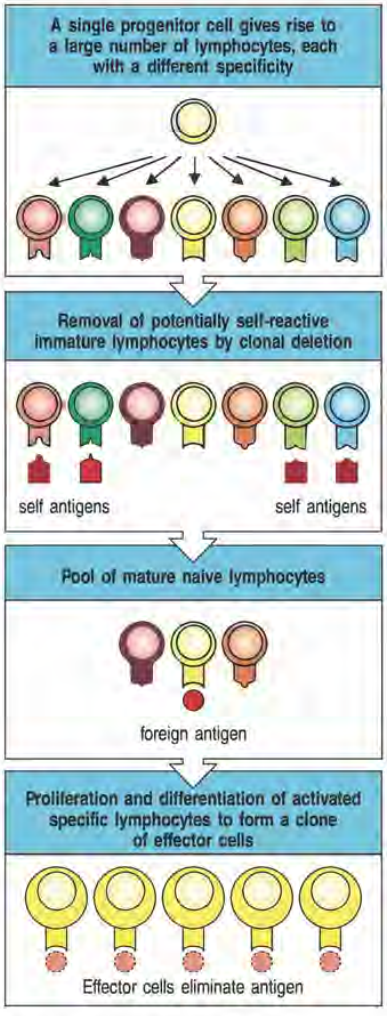


Fig. 1.12 Clonal selection. Each lymphoid progenitor gives rise to a large number of lymphocytes, each bearing a distinct antigen receptor. Lymphocytes with receptors that bind ubiquitous self antigens are eliminated before they become fully mature, ensuring tolerance to such self antigens. When a foreign antigen interacts with the receptor on a mature naive lymphocyte, that cell is activated and starts to divide. It gives rise to a clone of identical progeny, all of whose receptors bind the same antigen. Antigen specificity is thus maintained as the progeny proliferate and differentiate into effector cells. Once antigen has been eliminated by these effector cells, the immune response ceases, although some lymphocytes are retained to mediate immunological memory.

on the tissues of the body and attacking them? **Ray Owen** had shown in the late 1940s that genetically different twin calves with a common placenta, and thus a shared placental blood circulation, were immunologically unresponsive, or **tolerant**, to one another's tissues: they did not make an immune response against each other. **Peter Medawar** then showed in 1953 that exposure to foreign tissues during embryonic development caused mice to become immunologically tolerant to these tissues. Burnet proposed that developing lymphocytes that are potentially self-reactive are removed before they can mature, a process known as **clonal deletion**. He has since been proved right in this too, although the mechanisms of **immunological tolerance** are still being worked out, as we shall see when we discuss the development of lymphocytes in Chapter 8, and some of the situations in which tolerance breaks down in Chapters 14 and 15.

Clonal selection of lymphocytes is the single most important principle in adaptive immunity. Its four basic postulates are listed in Fig. 1.13. The last of the problems posed by the clonal selection theory—how the diversity of lymphocyte antigen receptors is generated—was solved in the 1970s, when advances in molecular biology made it possible to clone the genes encoding antibody molecules.

1-11 The structure of the antibody molecule illustrates the central puzzle of adaptive immunity.

As discussed above, antibodies are the secreted form of the B cell's antigen receptor. Because they are produced in very large quantities in response to antigen, antibodies can be studied by traditional biochemical techniques; indeed, their structure was understood long before recombinant DNA technology made it possible to study the membrane-bound antigen receptors of B cells. The startling feature that emerged from the biochemical studies was that antibody molecules are composed of two distinct regions. One is a **constant region** that takes one of only four or five biochemically distinguishable forms; the other is a **variable region** that can be composed of a seemingly infinite variety of different amino acid sequences, forming subtly different structures that allow antibodies to bind specifically to an equally vast variety of antigens. This division is illustrated in Fig. 1.14, where the antibody is depicted as a Y-shaped molecule. The variable region determines the antigen-binding specificity of the antibody. There are two identical variable regions in an antibody molecule, and it thus has two identical **antigen-binding sites**. The constant region determines the effector function of the antibody: that is, how the antibody will interact with various immune cells to dispose of antigen once it is bound.

Each antibody molecule has a two-fold axis of symmetry and is composed of two identical **heavy chains** and two identical **light chains** (see Fig. 1.14). Heavy and light chains each have variable and constant regions; the variable regions of a heavy chain and a light chain combine to form an antigen-binding site, so that both chains contribute to the antigen-binding specificity of the antibody molecule. The functional properties of antibodies conferred by their constant regions are considered in Chapters 5 and 10.

The T-cell receptor for antigen shows many similarities to the B-cell antigen receptor, and the two molecules are clearly related to each other evolutionarily. There are, however, important differences between the two that, as we shall see, relate to their different roles within the immune system. The T-cell receptor, as shown in Fig. 1.14, is composed of two chains of roughly equal size, called the T-cell receptor α and β chains, each of which spans the T-cell membrane. Each chain has a variable region and a constant region, and the combination of the α- and β-chain variable regions creates a single site for binding antigen. The structures of both antibodies and T-cell receptors are

Petitioner Merck, Ex. 1170, p. 19
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

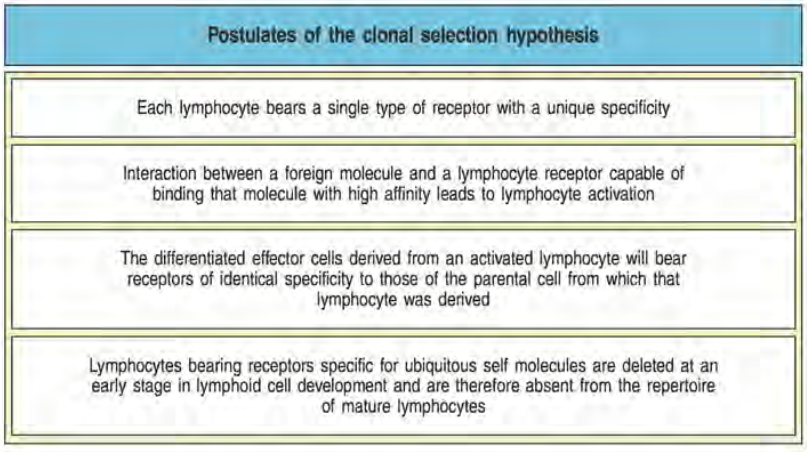


Fig. 1.13 The four basic principles of clonal selection.

described in detail in Chapter 4, and how the diversity of the antigen-receptor repertoire is generated is detailed in Chapter 5.

There is, however, a crucial difference in the way in which the B-cell and T-cell receptors bind antigens: the T-cell receptor does not bind antigen molecules directly but instead recognizes fragments of antigens bound on the surface of other cells. The exact nature of the antigen recognized by T cells, and how the antigens are fragmented and carried to cell surfaces, is the subject of Chapter 6. A further difference from the antibody molecule is that there is no secreted form of the T-cell receptor; the function of the receptor is solely to signal to the T cell that it has bound its antigen, and the subsequent immunological effects depend on the actions of the T cells themselves, as we describe in Chapter 9.

1-12 Each developing lymphocyte generates a unique antigen receptor by rearranging its receptor gene segments.

How are antigen receptors with an almost infinite range of specificities encoded by a finite number of genes? This question was answered in 1976, when **Susumu Tonegawa** discovered that the genes for immunoglobulin variable regions are inherited as sets of **gene segments**, each encoding a part of the variable region of one of the immunoglobulin polypeptide chains. During B-cell development in the bone marrow, these gene segments are irreversibly joined by DNA recombination to form a stretch of DNA encoding a complete variable region. How the complete antigen receptors are assembled from incomplete gene segments is the topic of Chapter 5.

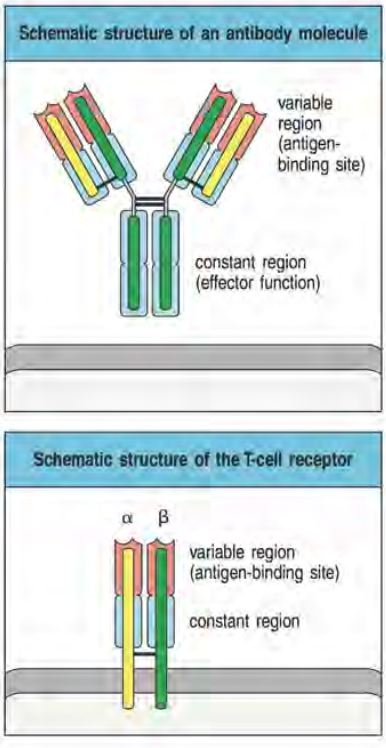


Fig. 1.14 Schematic structure of antigen receptors. Upper panel: an antibody molecule, which is secreted by activated B cells as an antigen-binding effector molecule. A membrane-bound version of this molecule acts as the B-cell antigen receptor (not shown). An antibody is composed of two identical heavy chains (green) and two identical light chains (yellow). Each chain has a constant part (shaded blue) and a variable part (shaded red). Each arm of the antibody molecule is formed by a light chain and a heavy chain such that the variable parts of the two chains come together, creating a variable region that contains the antigen-binding site. The stem is formed from the constant parts of the heavy chains and takes a limited number of forms. This constant region is involved in the elimination of the bound antigen. Lower panel: a T-cell antigen receptor. This is also composed of two chains, an α chain (yellow) and a β chain (green), each of which has a variable and a constant part. As with the antibody molecule, the variable parts of the two chains create a variable region, which forms the antigen-binding site. The T-cell receptor is not produced in a secreted form.

Petitioner Merck, Ex. 1170, p. 20
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Here, we need only mention two features of these mechanisms. First, it is the combinatorial assembly of a large number of different gene segments that makes possible the enormous size of the antigen receptor repertoire. This means that a finite number of gene segments can generate a vast number of different proteins. Second, the assembly process is regulated in a manner that ensures that each lymphocyte expresses only one receptor specificity. Third, because gene segment rearrangement involves an irreversible change in a cell's DNA, all the progeny of that cell will inherit genes encoding the same receptor specificity. This general scheme was later also confirmed for the genes encoding the antigen receptor on T cells.

The potential diversity of lymphocyte receptors generated in this way is enormous. Just a few hundred different gene segments can combine in different ways to generate thousands of different receptor chains. The diversity of lymphocyte receptors is further amplified by junctional diversity, created by adding or subtracting nucleotides in the process of joining the gene segments, and by the fact that each receptor is made by pairing two different variable chains, each encoded by distinct sets of gene segments. A thousand different chains of each type could thus generate 10^6 distinct antigen receptors through this combinatorial diversity. In this way, a small amount of genetic material can encode a truly staggering diversity of receptors. Only a subset of these randomly generated receptor specificities survive the selective processes that shape the peripheral lymphocyte repertoire; nevertheless, there are lymphocytes of at least 10^8 different specificities in an individual human at any one time. These provide the raw material on which clonal selection acts.

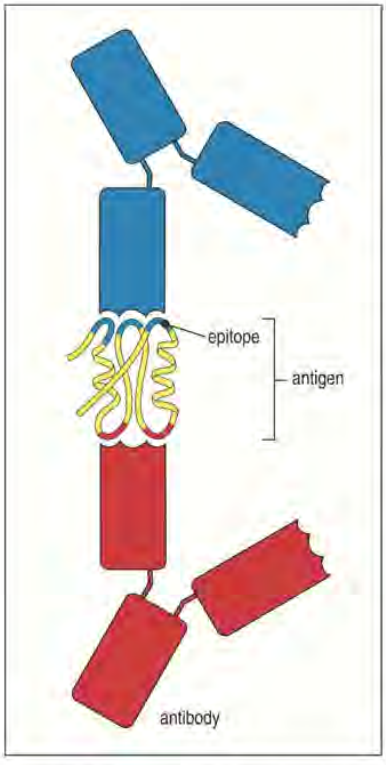


Fig. 1.15 Antigens are the molecules recognized by the immune response, while epitopes are sites within antigens to which antigen receptors bind. Antigens can be complex macromolecules such as proteins, as shown in yellow. Most antigens are larger than the sites on the antibody or antigen receptor to which they bind, and the actual portion of the antigen that is bound is known as the antigenic determinant, or epitope, for that receptor. Large antigens such as proteins can contain more than one epitope (indicated in red and blue), and thus may bind different antibodies. Antibodies generally recognize epitopes on the surface of the antigen.

1-13 Immunoglobulins bind a wide variety of chemical structures, whereas the T-cell receptor is specialized to recognize foreign antigens as peptide fragments bound to proteins of the major histocompatibility complex.

In principle, almost any chemical structure can be recognized by the adaptive immune system as an antigen, but the usual antigens encountered in an infection are the proteins, glycoproteins, and polysaccharides of pathogens. An individual antigen receptor or antibody recognizes a small part of the molecular structure of an antigenic molecule, which is known as an **antigenic determinant** or **epitope** (Fig. 1.15). Macromolecular antigens such as proteins and glycoproteins usually have many different epitopes that can be recognized by different antigen receptors.

The antigen receptors of B cells and T cells recognize antigen in fundamentally different ways. B cells directly recognize the native antigen that either has been secreted by a pathogen or is expressed on its surface (see Fig. 1.15). B cells eventually differentiate into effector plasma cells that secrete antibodies that will bind to and neutralize these antigens and pathogens. In contrast, T-cell receptors do not directly recognize native antigens. Rather, they recognize antigens that have been processed, partly degraded, and displayed as peptides bound to proteins on the surface of antigen-presenting cells (Fig. 1.16). A main source of the antigens recognized by T cells is cells infected with a pathogen, commonly a virus. In this case, the antigen that is recognized by the effector T cells is derived from within the infected cell. Importantly, T-cell receptors will only recognize antigen-derived peptides when these are bound to particular cell-surface glycoproteins called **MHC molecules**, which are encoded in a cluster of genes called the **major histocompatibility complex (MHC)**. The antigen recognized by T-cell receptors is thus a complex of a foreign peptide antigen and an MHC molecule (see Fig. 1.16). We shall see how these compound antigens are recognized by T-cell receptors and how they are generated in Chapters 4 and 6, respectively.

Petitioner Merck, Ex. 1170, p. 21
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

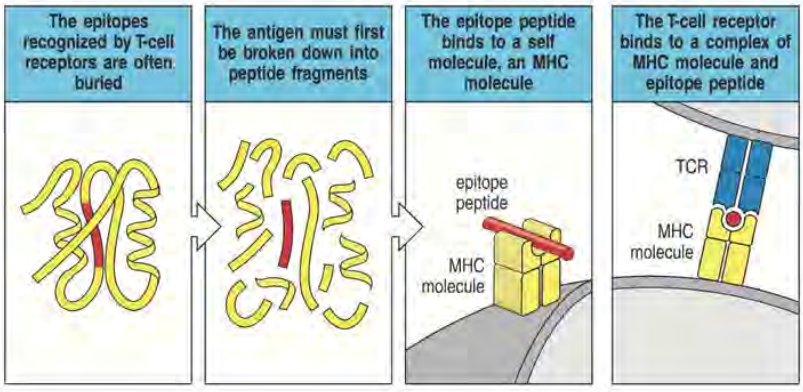


Fig. 1.16 T-cell receptors bind a complex of an antigen fragment and a self molecule. Unlike most antibodies, T-cell receptors can recognize epitopes that are buried within antigens (first panel). These antigens must first be degraded by proteinases (second panel), and the peptide epitope delivered to a self molecule, called an MHC molecule (third panel). It is in this form, as a complex of peptide and MHC molecule, that antigens are recognized by T-cell receptors (fourth panel).

1-14 The development and survival of lymphocytes is determined by signals received through their antigen receptors.

The continuous generation of lymphocytes throughout life creates a problem of keeping total numbers of peripheral lymphocytes relatively constant. In addition, with so many different antigen receptors being generated during lymphocyte development, it is inevitable that potentially dangerous receptors that can react against an individual's own **self antigens** will be produced. Both these problems seem to be solved by making the survival of a lymphocyte dependent on signals received through its antigen receptor. Lymphocytes that react strongly to self antigens during development are removed by clonal deletion, as predicted by Burnet's clonal selection theory, before they mature to a stage at which they could do damage. The complete absence of signals from the antigen receptor during development can also lead to cell death. Lymphocytes that receive either too much or too little signal during development are eliminated by a form of cell suicide called **apoptosis** or **programmed cell death**.

Apoptosis, derived from a Greek word meaning the falling of leaves from the trees, is a general means of regulating the number of cells in the body. It is responsible, for example, for the death and shedding of old skin and intestinal epithelial cells, and the turnover of liver cells. Every day the bone marrow produces millions of new neutrophils, monocytes, red blood cells, and lymphocytes, and this production must be balanced by an equal loss. Most white blood cells are relatively short lived and die by apoptosis. The dying cells are phagocytosed and degraded by specialized macrophages in the liver and spleen.

Lastly, if a lymphocyte's receptor is not used within a relatively short time of its entering the repertoire in the periphery, the cell bearing it dies, making way for new lymphocytes with different receptors. In this way, self-reactive receptors are eliminated, and receptors are tested to ensure that they are potentially functional. The mechanisms that shape and maintain the lymphocyte receptor repertoire are examined in Chapter 8.

1-15 Lymphocytes encounter and respond to antigen in the peripheral lymphoid organs.

Antigen and lymphocytes eventually encounter each other in the peripheral lymphoid organs—the lymph nodes, spleen, and the mucosal lymphoid tissues (see Fig. 1.8). Mature naive lymphocytes are continually recirculating through these tissues, to which pathogen antigens are carried from sites

Petitioner Merck, Ex. 1170, p. 22
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

of infection, primarily by dendritic cells. The peripheral lymphoid organs are specialized to trap antigen-bearing dendritic cells and to facilitate the initiation of adaptive immune responses. Peripheral lymphoid tissues are composed of aggregations of lymphocytes in a framework of nonleukocyte stromal cells, which provide the basic structural organization of the tissue and provide survival signals to help sustain the life of the lymphocytes. Besides lymphocytes, peripheral lymphoid organs also contain resident macrophages and dendritic cells.

When an infection occurs in a tissue such as the skin, free antigen and antigen-bearing dendritic cells travel from the site of infection through the afferent lymphatic vessels into the **draining lymph nodes** (Fig. 1.17), peripheral lymphoid tissues where they activate antigen-specific lymphocytes. The activated lymphocytes then undergo a period of proliferation and differentiation, after which most leave the lymph nodes as effector cells via the efferent lymphatic vessel. This eventually returns them to the bloodstream (see Fig. 1.8), which then carries them to the tissues where they will act. This whole process takes about 4–6 days from the time that the antigen is recognized, which means that an adaptive immune response to an antigen that has not been encountered before does not become effective until about a week after infection (see Fig. 1.34). Naive lymphocytes that do not recognize their antigen also leave through the efferent lymphatic vessel and are returned to the blood, from which they continue to recirculate through lymphoid tissues until they recognize antigen or die.

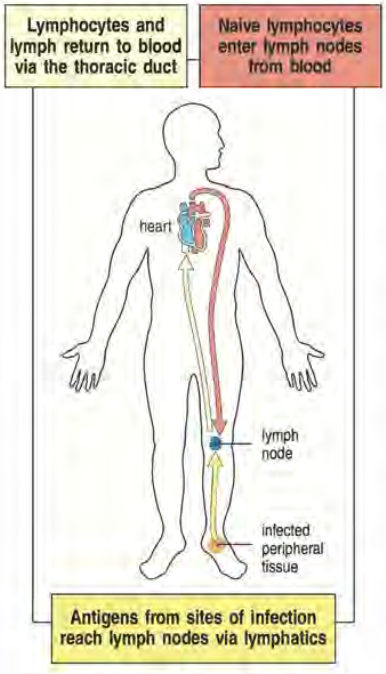


Fig. 1.17 Circulating lymphocytes encounter antigen in peripheral lymphoid organs. Naive lymphocytes recirculate constantly through peripheral lymphoid tissue, here illustrated as a popliteal lymph node—a lymph node situated behind the knee. In the case of an infection in the foot, this will be the draining lymph node, where lymphocytes may encounter their specific antigens and become activated. Both activated and nonactivated lymphocytes are returned to the bloodstream via the lymphatic system.

The **lymph nodes** are highly organized lymphoid organs located at the points of convergence of vessels of the lymphatic system, which is the extensive system that collects extracellular fluid from the tissues and returns it to the blood (see Fig. 1.8). This extracellular fluid is produced continuously by filtration from the blood and is called lymph. Lymph flows away from the peripheral tissues under the pressure exerted by its continual production, and is carried by lymphatic vessels, or **lymphatics**. One-way valves in the lymphatic vessels prevent a reverse flow, and the movements of one part of the body in relation to another are important in driving the lymph along.

Afferent lymphatic vessels drain fluid from the tissues and carry pathogens and antigen-bearing cells from infected tissues to the lymph nodes (Fig. 1.18). Free antigens simply diffuse through the extracellular fluid to the lymph node, while the dendritic cells actively migrate into the lymph node, attracted by chemokines. The same chemokines also attract lymphocytes from the blood, and these enter lymph nodes by squeezing through the walls of specialized blood vessels called **high endothelial venules** (**HEV**). In the lymph nodes, B lymphocytes are localized in **follicles**, which make up the outer **cortex** of the lymph node, with T cells more diffusely distributed in the surrounding **paracortical areas**, also referred to as the deep cortex or **T-cell zones** (see Fig. 1.18). Lymphocytes migrating from the blood into lymph nodes enter the paracortical areas first and, because they are attracted by the same chemokines, antigen-presenting dendritic cells and macrophages also become localized there. Free antigen diffusing through the lymph node can become trapped on these dendritic cells and macrophages. This juxtaposition of antigen, antigen-presenting cells, and naive T cells in the T-cell zone creates an ideal environment in which naive T cells can bind their specific antigen and thus become activated.

As noted earlier, activation of B cells usually requires not only antigen, which binds to the B-cell receptor, but also the cooperation of activated helper T cells, a type of effector T cell (see Section 1-4). The location of B cells and T cells within the lymph node is dynamically regulated by their state of activation. When they become activated, T cells and B cells both move to the border of the follicle and T-cell zone, where T cells can first provide their helper function to B cells. Some of the B-cell follicles include **germinal centers**, where

Petitioner Merck, Ex. 1170, p. 23
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

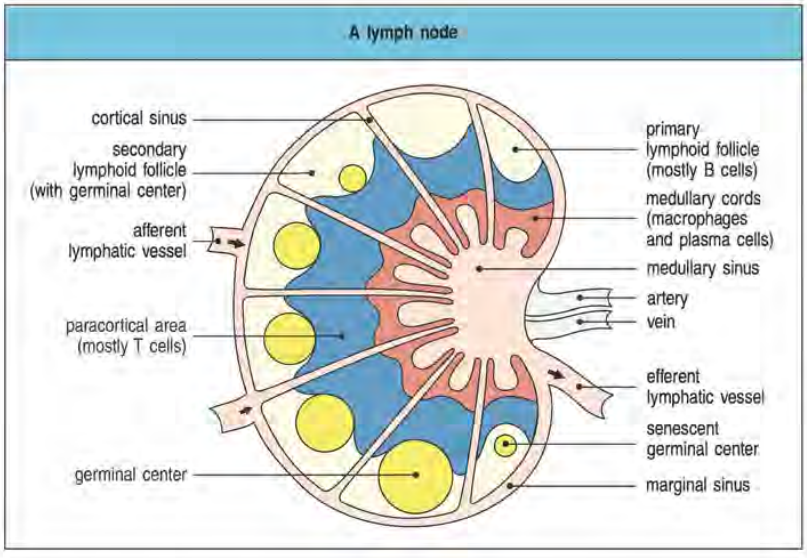


Fig. 1.18 Organization of a lymph node. As shown in the diagram on the left, which shows a lymph node in longitudinal section, a lymph node consists of an outermost cortex and an inner medulla. The cortex is composed of an outer cortex of B cells organized into lymphoid follicles, and deep, or paracortical, areas made up mainly of T cells and dendritic cells. When an immune response is under way, some of the follicles contain central areas of intense B-cell proliferation called germinal centers and are known as secondary lymphoid follicles. These reactions are very dramatic, but eventually die out as senescent germinal centers. Lymph draining from the extracellular spaces of the body carries antigens in phagocytic dendritic cells and phagocytic macrophages from the tissues to the lymph node via the afferent lymphatics. These migrate directly from the sinuses into the cellular parts of the node. Lymph leaves by the efferent lymphatics in the medulla. The medulla consists of strings of macrophages and antibody-secreting plasma cells known as the medullary cords. Naive lymphocytes enter the node from the bloodstream through specialized postcapillary venules (not shown) and leave with the lymph through the efferent lymphatic. The light micrograph shows a transverse section through a lymph node, with prominent follicles containing germinal centers. Magnification ×7. Photograph courtesy of N. Rooney.

activated B cells are undergoing intense proliferation and differentiation into plasma cells. These mechanisms are described in detail in Chapter 10.

In humans, the **spleen** is a fist-sized organ situated just behind the stomach (see Fig. 1.8). It has no direct connection with the lymphatic system; instead, it collects antigen from the blood and is involved in immune responses to blood-borne pathogens. Lymphocytes enter and leave the spleen via blood vessels. The spleen also collects and disposes of senescent red blood cells. Its organization is shown schematically in Fig. 1.19. The bulk of the spleen is composed of **red pulp**, which is the site of red blood cell disposal. The lymphocytes surround the arterioles running through the spleen, forming isolated areas of **white pulp**. The sheath of lymphocytes around an arteriole is called the **periarteriolar lymphoid sheath** (**PALS**) and contains mainly T cells. Lymphoid follicles occur at intervals along it, and these contain mainly B cells. A so-called marginal zone surrounds the follicle; it has few T cells, is rich in macrophages, and has a resident, noncirculating population of B cells known as **marginal zone B cells**, about which little is known; they are discussed in Chapter 8. Blood-borne microbes, soluble antigens, and antigen:antibody complexes are filtered from the blood by macrophages and immature dendritic cells within the marginal zone. Like the migration of immature dendritic cells from peripheral tissues to the T-cell areas of lymph nodes, dendritic cells in the marginal zones in the spleen migrate to the T-cell areas after taking up antigen and becoming activated; here they are able to present the antigens they carry to T cells.

Most pathogens enter the body through mucosal surfaces, and these are also exposed to a vast load of other potential antigens from the air, food, and the natural microbial flora of the body. Mucosal surfaces are protected by an extensive system of lymphoid tissues known generally as the **mucosal immune system** or **mucosa-associated tissues** (**MALT**). Collectively, the mucosal immune system is estimated to contain as many lymphocytes as all the rest of the body, and they form a specialized set of cells obeying somewhat different rules of recirculation from those in the other peripheral lymphoid organs. The **gut-associated lymphoid tissues** (**GALT**) include the **tonsils**, **adenoids**, **appendix**, and specialized structures in the small intestine called **Peyer's patches**, and they collect antigen from the epithelial surfaces of the gastrointestinal tract. In Peyer's patches, which are the most important and highly organized of these tissues, the antigen is collected by specialized epithelial cells called

Petitioner Merck, Ex. 1170, p. 24
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

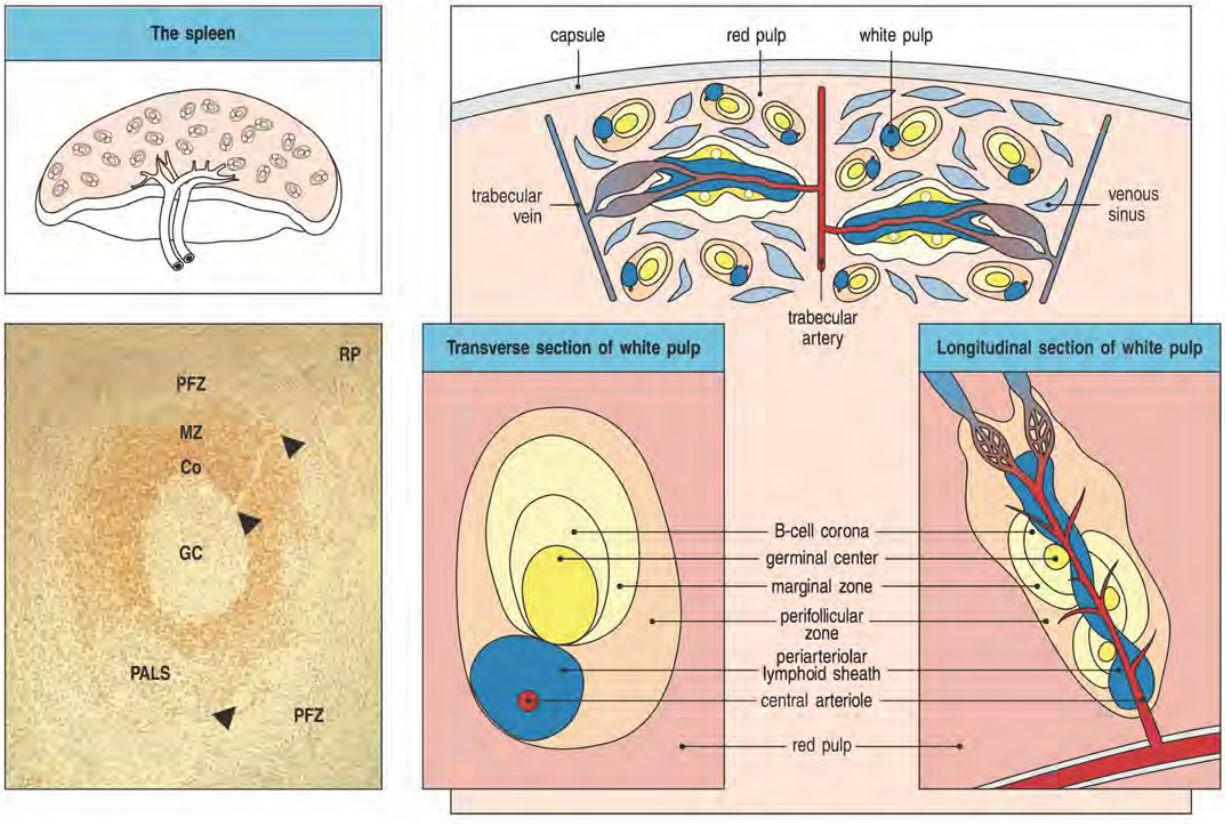


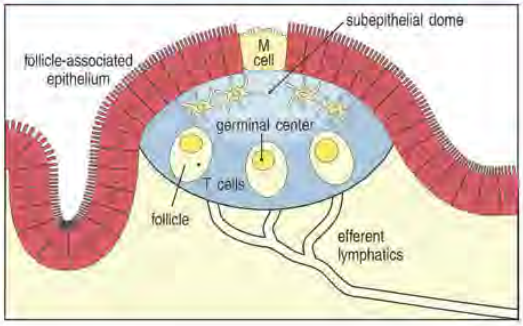
Fig. 1.19 Organization of the lymphoid tissues of the spleen. The schematic at top left shows that the spleen consists of red pulp (pink areas), which is a site of red blood cell destruction, interspersed with the lymphoid white pulp. An enlargement of a small section of a human spleen (top right) shows the arrangement of discrete areas of white pulp (yellow and blue) around central arterioles. Most of the white pulp is shown in transverse section, with two portions in longitudinal section. The bottom two schematics show enlargements of a transverse section (lower center) and longitudinal section (lower right) of white pulp. Surrounding the central arteriole is the periarteriolar lymphoid sheath (PALS), made up of T cells. Lymphocytes and antigen-loaded dendritic cells come together here. The follicles consist mainly of B cells; in secondary follicles a germinal center is surrounded by a B-cell corona. The follicles are surrounded by a so-called marginal zone of lymphocytes. In each area of white pulp, blood carrying both lymphocytes and antigen flows from a trabecular artery into a central arteriole. From this arteriole smaller blood vessels fan out, eventually terminating in a specialized zone in the human spleen called the perifollicular zone (PFZ), which surrounds each marginal zone. Cells and antigen then pass into the white pulp through open blood-filled spaces in the perifollicular zone. The light micrograph at bottom left shows a transverse section of white pulp of human spleen immunostained for mature B cells. Both follicle and PALS are surrounded by the perifollicular zone. The follicular arteriole emerges in the PALS (arrowhead at bottom), traverses the follicle, goes through the marginal zone and opens into the perifollicular zone (upper arrowheads). Co, follicular B-cell corona; GC, germinal center; MZ, marginal zone; RP, red pulp; arrowheads, central arteriole. Photograph courtesy of N.M. Milicevic.

microfold or **M cells** (Fig. 1.20). The lymphocytes form a follicle consisting of a large central dome of B lymphocytes surrounded by smaller numbers of T lymphocytes. Dendritic cells resident within the Peyer's patch present the antigen to T lymphocytes. Lymphocytes enter Peyer's patches from the blood and leave through efferent lymphatics. Effector lymphocytes generated in Peyer's patches travel through the lymphatic system and into the bloodstream, from where they are disseminated back into mucosal tissues to carry out their effector actions.

Similar but more diffuse aggregates of lymphocytes are present in the respiratory tract and other mucosa: **nasal-associated lymphoid tissue** (**NALT**) and **bronchus-associated lymphoid tissue** (**BALT**) are present in the respiratory

Petitioner Merck, Ex. 1170, p. 25
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Peyer's patches are covered by an epithelial layer containing specialized cells called M cells, which have characteristic membrane ruffles



tract. Like the Peyer's patches, these mucosal lymphoid tissues are also overlaid by M cells, through which inhaled microbes and antigens that become trapped in the mucous covering of the respiratory tract can pass. The mucosal immune system is discussed in Chapter 12.

Although very different in appearance, the lymph nodes, spleen, and mucosa-associated lymphoid tissues all share the same basic architecture. They all operate on the same principle, trapping antigens and antigen-presenting cells from sites of infection and enabling them to present antigen to migratory small lymphocytes, thus inducing adaptive immune responses. The peripheral lymphoid tissues also provide sustaining signals to lymphocytes that do not encounter their specific antigen immediately, so that they survive and continue to recirculate.

Because they are involved in initiating adaptive immune responses, the peripheral lymphoid tissues are not static structures but vary quite markedly, depending on whether or not infection is present. The diffuse mucosal lymphoid tissues may appear in response to infection and then disappear, whereas the architecture of the organized tissues changes in a more defined way during an infection. For example, the B-cell follicles of the lymph nodes expand as B lymphocytes proliferate to form germinal centers (see Fig. 1.18), and the entire lymph node enlarges, a phenomenon familiarly known as swollen glands.

Finally, specialized populations of lymphocytes can be found distributed throughout particular sites in the body rather than being found in organized lymphoid tissues. Such sites include the liver and the lamina propria of the gut, as well as the base of the epithelial lining of the gut, reproductive epithelia, and, in mice but not in humans, the epidermis. These lymphocyte populations seem to have an important role in protecting these tissues from infection, and are described further in Chapters 8 and 12.

1-16 Lymphocyte activation requires additional signals beyond those relayed from the antigen receptor when antigen binds.

Peripheral lymphoid tissues promote the interaction between antigen-bearing APCs and lymphocytes, but antigen alone is not sufficient to initiate an adaptive immune response. Lymphocytes require other signals to become activated and to acquire effector functions. These signals are delivered to lymphocytes by another cell through cell-surface molecules known generally as co-stimulatory molecules (see Section 1-8). For naive T cells, an activated dendritic cell usually delivers these signals, but for naive B cells, the second signal is delivered by an activated helper T cell (Fig. 1.21). We discuss the nature of these signals in detail in Chapter 7.

Fig. 1.20 Organization of a Peyer's patch in the gut mucosa. As the diagram on the left shows, a Peyer's patch contains numerous B-cell follicles with germinal centers. T cells occupy the areas between follicles, the T-cell dependent areas. The layer between the surface epithelium and the follicles is known as the subepithelial dome, and is rich in dendritic cells, T cells, and B cells. Peyer's patches have no afferent lymphatics and the antigen enters directly from the gut across a specialized epithelium made up of so-called microfold (M) cells. Although this tissue looks very different from other lymphoid organs, the basic divisions are maintained. As in the lymph nodes, lymphocytes enter Peyer's patches from the blood across the walls of high endothelial venules (not shown), and leave via the efferent lymphatic. The light micrograph in panel a shows a section through a Peyer's patch in the gut wall of the mouse. The Peyer's patch can be seen lying beneath the epithelial tissues. GC, germinal center; TDA, T-cell dependent area. Panel b is a scanning electron micrograph of the follicle-associated epithelium boxed in panel a, showing the M cells, which lack the microvilli and the mucus layer present on normal epithelial cells. Each M cell appears as a sunken area on the epithelial surface. Panel c is a higher-magnification view of the boxed area in panel b, showing the characteristic ruffled surface of an M cell. M cells are the portal of entry for many pathogens and other particles. Panel a, hematoxylin and eosin stain; magnification ×100; panel b, ×5000; panel c, ×23,000. Source: Mowat, A., Viney, J.: *Immunol. Rev*. 1997, **156**:145–166.

Petitioner Merck, Ex. 1170, p. 26
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The induction of co-stimulatory molecules is important in initiating an adaptive immune response because contact with antigen without accompanying co-stimulatory molecules inactivates naive lymphocytes rather than activating them, leading either to clonal deletion or an inactive state known as **anergy**. We return to this topic in Chapter 8. Thus, we need to add a final postulate to the clonal selection theory. A naive lymphocyte can only be activated by cells that bear not only specific antigen but also co-stimulatory molecules, whose expression is regulated by innate immunity.

Macrophages and B cells can also present foreign antigens on their surface and can be induced to express co-stimulatory molecules and thus can activate T cells. These three specialized antigen-presenting cells of the immune system are illustrated in Fig. 1.22. Dendritic cells are the most important of the three in initiating the adaptive immune response, whereas the others function as antigen-presenting cells at later stages, when T cells have acquired particular effector activities. These circumstances are discussed in Chapters 9 and 10.

1-17 Lymphocytes activated by antigen proliferate in the peripheral lymphoid organs, generating effector cells and immunological memory.

The great diversity of lymphocyte receptors means that there will usually be at least a few that can bind to a given foreign antigen. However, this number will be very small, certainly not enough to mount a response against a pathogen. To generate sufficient antigen-specific effector lymphocytes to fight an infection, a lymphocyte with an appropriate receptor specificity is activated first to proliferate. Only when a large clone of identical cells has been produced do these finally differentiate into effector cells. On recognizing its specific antigen on an activated antigen-presenting cell, a naive lymphocyte stops migrating, the volume of the nucleus and cytoplasm increases, and new mRNAs and new proteins are synthesized. Within a few hours, the cell looks completely different and is known as a **lymphoblast**.

Dividing lymphoblasts are able to duplicate themselves two to four times every 24 hours for 3–5 days, so that a single naive lymphocyte can produce a clone of around 1000 daughter cells of identical specificity. These then differentiate into effector cells. In the case of B cells, the differentiated effector cells are the **plasma cells**, which secrete antibody; in the case of T cells, the effector cells are **cytotoxic T cells** able to destroy infected cells, or **helper T cells** that activate other cells of the immune system. Effector lymphocytes do not recirculate like naive lymphocytes. Some effector T cells detect sites of

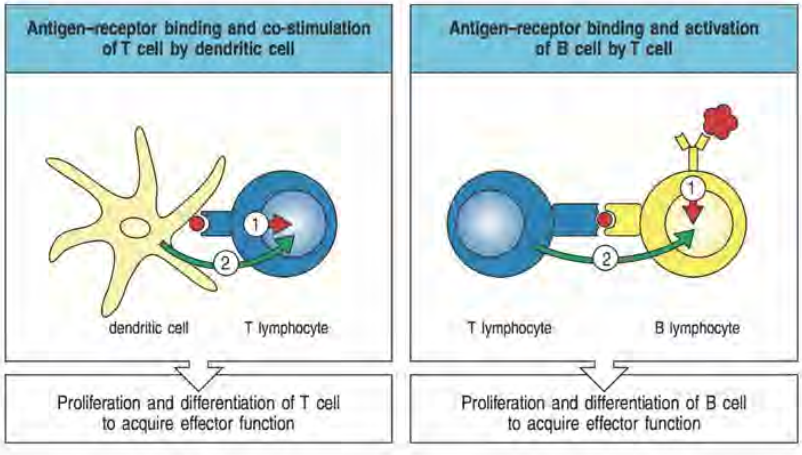


Fig. 1.21 Two signals are required for lymphocyte activation. In addition to receiving a signal through their antigen receptor (signal 1), mature naive lymphocytes must also receive a second signal (signal 2) to become activated. For T cells (left panel), this second signal is delivered by an antigen-presenting cell such as the dendritic cell shown here. For B cells (right panel), the second signal is usually delivered by an activated T cell, which recognizes antigenic peptides taken up, processed, and presented by the B cell on its surface.

Petitioner Merck, Ex. 1170, p. 27
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

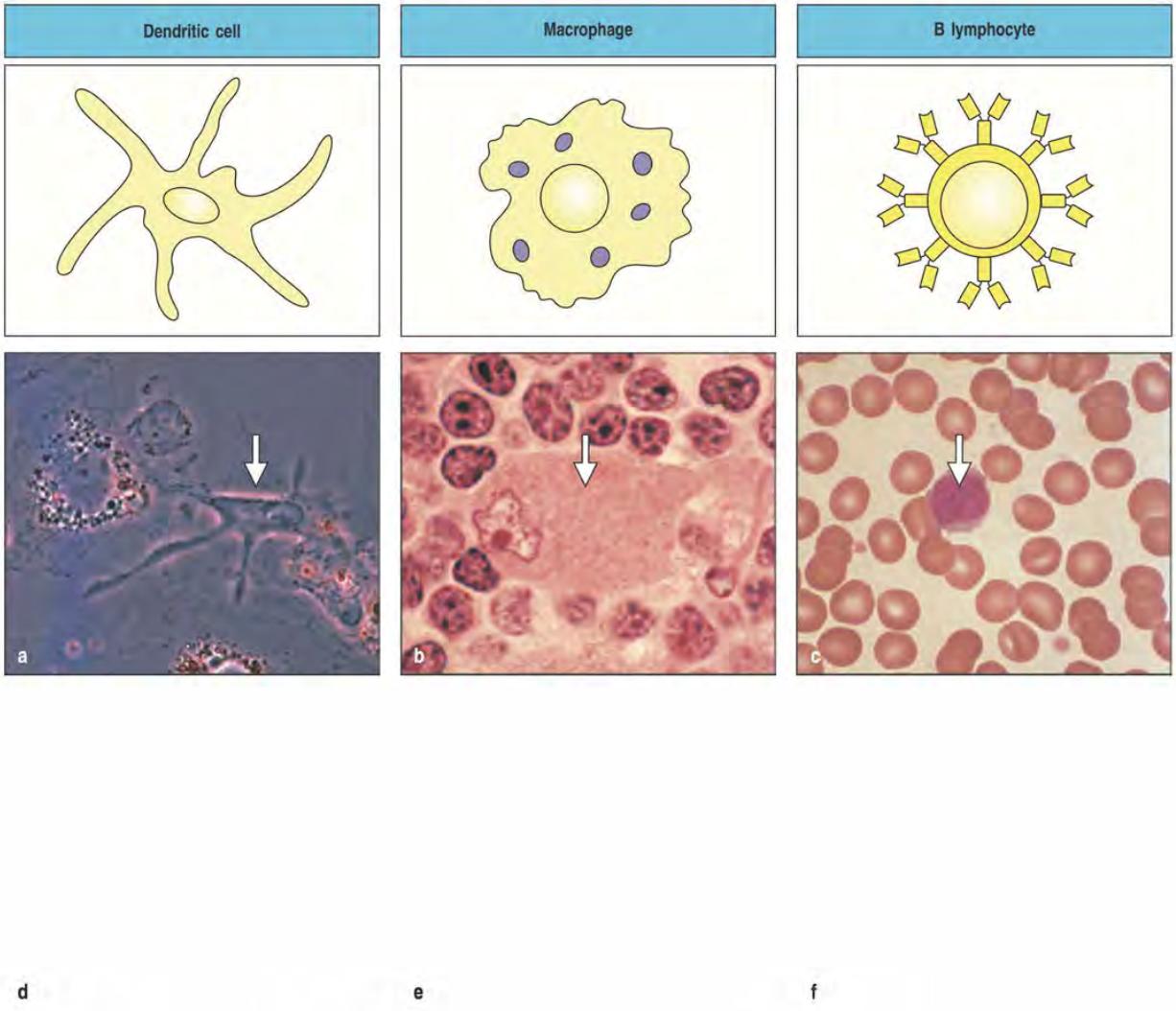


Fig. 1.22 The antigen-presenting cells. The three types of antigen-presenting cells are shown in the form in which they are depicted throughout this book (top row), as they appear in the light microscope (second row; the relevant cell is indicated by an arrow), by transmission electron microscopy (third row) and by scanning electron microscopy (bottom row). Mature dendritic cells are found in lymphoid tissues and are derived from immature tissue dendritic cells that interact with many distinct types of pathogens. Macrophages are specialized to internalize extracellular pathogens, especially after they have been coated with antibody, and to present their antigens. B cells have antigen-specific receptors that enable them to internalize large amounts of specific antigen, process it, and present it. Photographs courtesy of R.M. Steinman (a), N. Rooney (b, c, e, f), S. Knight (d, g), and P.F. Heap (h, i).

Petitioner Merck, Ex. 1170, p. 28
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

infection and migrate into them from the blood; others stay in the lymphoid tissues to activate B cells. Some antibody-secreting plasma cells remain in the peripheral lymphoid organs, but most plasma cells generated in the lymph nodes and spleen migrate to the bone marrow and take up residence there, pouring out antibodies into the blood system. Effector cells generated in the mucosal immune system generally stay within the mucosal tissues.

As noted earlier, after a naive lymphocyte has been activated, it takes 4–5 days before clonal expansion is complete and the lymphocytes have differentiated into effector cells, and so the first adaptive immune response to a pathogen only occurs several days after the infection begins and has been detected by the innate immune system. Most of the lymphocytes generated by the clonal expansion in any given immune response will eventually die. However, a significant number of activated antigen-specific B cells and T cells persist after antigen has been eliminated. These cells are known as **memory cells** and form the basis of immunological memory. They can be reactivated much more quickly than naive lymphocytes, which ensures a more rapid and effective response on a second encounter with a pathogen and thereby usually provides lasting protective immunity.

The characteristics of immunological memory are readily observed by comparing the antibody response of an individual to a first or **primary immunization** with the same response elicited in the same individual by a **secondary** or **booster immunization** with the same antigen. As shown in Fig. 1.23, the secondary antibody response occurs after a shorter lag phase, achieves a markedly higher level, and produces antibodies of higher affinity, or strength of binding, for the antigen. The increased affinity for antigen is called **affinity maturation** and is the result of events that select B-cell receptors, and thus antibodies, for progressively higher affinity for antigen during an immune response. Importantly, T-cell receptors do not undergo affinity maturation, and the lower threshold for activation of memory T cells compared with naive T cells results from a change in the responsiveness of the cell, not from a change in the receptor. We describe the mechanisms of these remarkable changes in Chapters 5 and 10. The cellular basis of immunological memory is the clonal expansion and clonal differentiation of cells specific for the eliciting antigen, and it is therefore entirely antigen specific. It is immunological memory that enables successful vaccination and prevents reinfection with pathogens that have been repelled successfully by

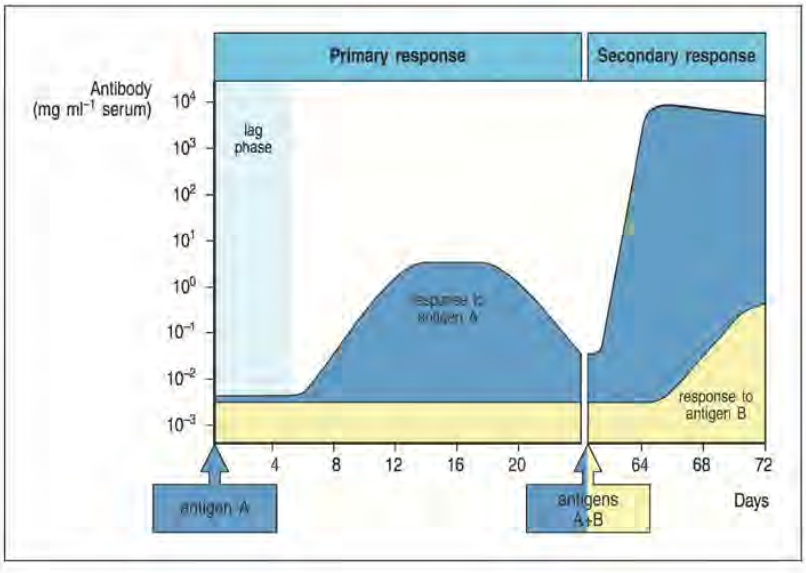


Fig. 1.23 The course of a typical antibody response. The first encounter with an antigen produces a primary response. Antigen A introduced at time zero encounters little specific antibody in the serum. After a lag phase (light blue), antibody against antigen A (dark blue) appears; its concentration rises to a plateau, and then gradually declines. This is typical of a primary response. When the serum is tested for antibody against another antigen, B (yellow), there is little present, demonstrating the specificity of the antibody response. When the animal is later challenged with a mixture of antigens A and B, a very rapid and intense secondary response to A occurs. This illustrates immunological memory, the ability of the immune system to make a second response to the same antigen more efficiently and effectively, providing the host with a specific defense against infection. This is the main reason for giving booster injections after an initial vaccination. Note that the response to B resembles the initial or primary response to A, as this is the first encounter of the host with antigen B.

Petitioner Merck, Ex. 1170, p. 29
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

an adaptive immune response. Immunological memory is the most important biological consequence of adaptive immunity, and its cellular and molecular bases are some of the most active areas of research. The properties of memory cells are described in more detail in Chapter 11.

Summary.

The early innate systems of defense depend on invariant pattern recognition receptors that detect common features of pathogens. Innate defenses are crucially important, but they can be overcome by many pathogens and they do not lead to immunological memory. Recognizing a particular pathogen and providing enhanced protection against reinfection is unique to adaptive immunity. An adaptive immune response involves the selection and amplification of clones of lymphocytes bearing receptors that recognize the foreign antigen. This clonal selection provides the theoretical framework for understanding all the key features of an adaptive immune response. There are two major types of lymphocytes: B lymphocytes, which mature in the bone marrow and are the source of circulating antibodies, and T lymphocytes, which mature in the thymus and recognize peptides from pathogens presented by MHC molecules on infected cells or antigen-presenting cells. Each lymphocyte carries cell-surface receptors of a single antigen specificity. These receptors are generated by the random recombination of variable receptor gene segments and the pairing of distinct variable protein chains: heavy and light chains in immunoglobulins, or the two chains of T-cell receptors. The large antigen receptor repertoire of lymphocytes can recognize virtually any antigen.

Adaptive immunity is initiated when an innate immune response fails to eliminate a new infection, and activated antigen-presenting cells bearing pathogen antigens are delivered to the draining lymphoid tissues. When a recirculating lymphocyte encounters its corresponding antigen in peripheral lymphoid tissues, it is induced to proliferate, and its clonal progeny differentiate into effector T and B lymphocytes that can eliminate the infectious agent. A subset of these proliferating lymphocytes differentiates into memory cells, ready to respond rapidly to the same pathogen if it is encountered again. The details of these processes of recognition, development, and differentiation form the main material of the central three parts of this book.

The effector mechanisms of adaptive immunity.

We have seen in the first part of this chapter how naive lymphocytes are selected by antigen to differentiate into clones of activated effector lymphocytes. We now expand on the mechanisms by which activated effector lymphocytes target different pathogens for destruction in a successful adaptive immune response. The distinct lifestyles of different pathogens (Fig. 1.24) require different responses for both their recognition and their destruction. B-cells recognize native antigens from the extracellular environment and they differentiate into effector plasma cells that secrete antibody back into that environment. T-cells are specialized to detect peptides that have been generated inside the body's cells, whether from extracellular antigens that have been ingested or from proteins produced in situ, and this is reflected in the effector actions of T cells. Some effector T cells directly kill cells infected with intracellular pathogens such as viruses, while others participate in responses against extracellular pathogens by interacting with B cells to help them make antibody.

Petitioner Merck, Ex. 1170, p. 30
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Fig. 1.24 The major types of pathogens confronting the immune system, and some of the diseases they cause.

The immune system protects against four classes of pathogens		
Type of pathogen	**Examples**	**Diseases**
Extracellular bacteria, parasites, fungi	*Streptococcus pneumoniae* *Clostridium tetani* *Trypanosoma brucei* *Pneumocystis jirovecii*	Pneumonia Tetanus Sleeping sickness *Pneumocystis* pneumonia
Intracellular bacteria, parasites	*Mycobacterium leprae* *Leishmania donovani* *Plasmodium falciparum*	Leprosy Leishmaniasis Malaria
Viruses (intracellular)	Variola Influenza Varicella	Smallpox Flu Chickenpox
Parasitic worms (extracellular)	*Ascaris* *Schistosoma*	Ascariasis Schistosomiasis

Most of the other effector mechanisms used by an adaptive immune response to dispose of pathogens are essentially identical to those of innate immunity and involve cells such as macrophages and neutrophils, and proteins such as complement. Indeed, it seems likely that the vertebrate adaptive immune response evolved by the late addition of specific recognition by clonally distributed receptors to innate defense mechanisms already existing in invertebrates. We begin by outlining the effector actions of antibodies, which depend almost entirely on recruiting cells and molecules of the innate immune system.

1-18 Antibodies protect against extracellular pathogens and their toxic products.

Antibodies are found in the fluid component of blood, or plasma, and in extracellular fluids. Because body fluids were once known as humors, immunity mediated by antibodies is known as **humoral immunity**.

As we saw in Fig. 1.14, antibodies are Y-shaped molecules whose arms form two identical antigen-binding sites. These are highly variable from one molecule to another, providing the diversity required for specific antigen recognition. The stem of the Y is far less variable. There are only five major forms of this constant region of an antibody, and these are known as the antibody **classes** or **isotypes**. The constant region determines an antibody's functional properties—how it will engage with the effector mechanisms that dispose of antigen once it is recognized—and each class carries out its particular function by engaging a distinct set of effector mechanisms. We describe the antibody classes and their actions in Chapters 5 and 10.

The first and most direct way in which antibodies can protect against pathogens or their products is by binding to them and thereby blocking their access to cells that they might infect or destroy (Fig. 1.25, left panels). This is known as **neutralization** and is important for protection against viruses, which are prevented from entering cells and replicating, and against bacterial toxins.

For bacteria, however, binding by antibodies is not sufficient to stop their replication. In this case, the function of the antibody is to enable a phagocytic cell such as a macrophage or a neutrophil to ingest and destroy the bacterium. Many bacteria evade the innate immune system because they have an outer coat that is not recognized by the pattern recognition receptors of phagocytes. However, antigens in the coat can be recognized by antibodies, and phagocytes have receptors that bind the stems of the antibodies coating the

Petitioner Merck, Ex. 1170, p. 31
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

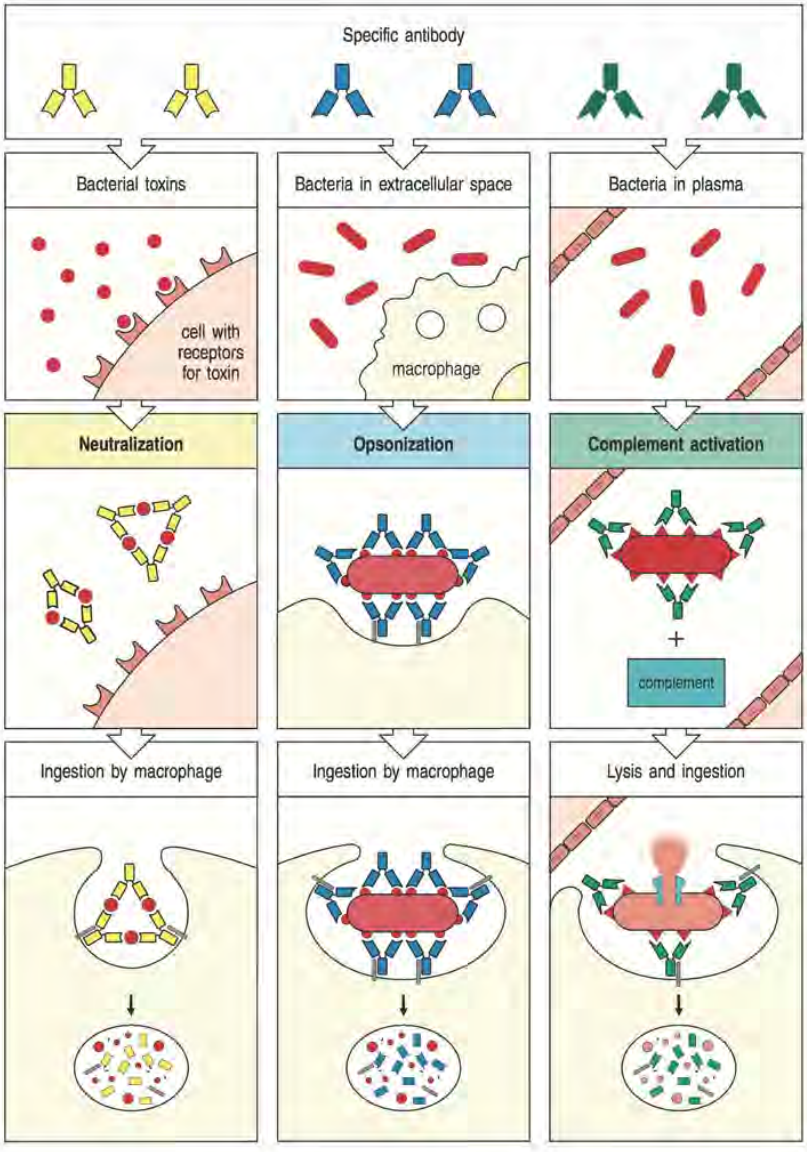


Fig. 1.25 Antibodies can participate in host defense in three main ways. The left panels show antibodies binding to and neutralizing a bacterial toxin, thus preventing it from interacting with host cells and causing pathology. Unbound toxin can react with receptors on the host cell, whereas the toxin:antibody complex cannot. Antibodies also neutralize complete virus particles and bacterial cells by binding and inactivating them. The antigen:antibody complex is eventually scavenged and degraded by macrophages. Antibodies coating an antigen render it recognizable as foreign by phagocytes (macrophages and neutrophils), which then ingest and destroy it; this is called opsonization. The center panels show opsonization and phagocytosis of a bacterial cell. The right panels show activation of the complement system by antibodies coating a bacterial cell. Bound antibodies form a receptor for the first protein of the complement system, which eventually forms a protein complex on the surface of the bacterium that, in some cases, can kill the bacterium directly. More generally, complement coating favors the taking up and destroying of the bacterium by phagocytes. Thus, antibodies target pathogens and their toxic products for disposal by phagocytes.

bacterium, leading to phagocytosis (see Fig. 1.25, center panels). The coating of pathogens and foreign particles in this way is known as **opsonization**.

The third function of antibodies is **complement activation**. Complement, which we discuss in detail in Chapter 2, is first activated in innate immunity by microbial surfaces, without the help of antibodies. But when an antibody binds to a bacterial surface, its constant regions provide a platform to activate the first protein of the complement system. Thus, once antibodies are produced, complement activation against a pathogen can be substantially increased. Complement components that are deposited on a bacterial surface can directly destroy certain bacteria, and this is important in a few bacterial infections (see Fig. 1.25, right panels). The main function of complement, however, is like that of antibodies: it coats the pathogen surface and enables phagocytes to engulf and destroy bacteria that they would not otherwise recognize. Complement also enhances the bactericidal actions of phagocytes; indeed, it is so called because it 'complements' the activities of antibodies.

Petitioner Merck, Ex. 1170, p. 32
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Antibodies of different classes are found in different compartments of the body and differ in the effector mechanisms they recruit, but all pathogens and free molecules bound by antibody are eventually delivered to phagocytes for ingestion, degradation, and removal from the body (see Fig. 1.25, bottom panels). The complement system and the phagocytes that antibodies recruit are not themselves antigen-specific; they depend upon antibody molecules to mark the particles as foreign. Producing antibodies is the sole effector function of B cells. T cells, by contrast, have a variety of effector actions.

1-19 T cells orchestrate cell-mediated immunity and regulate B-cell responses to most antigens.

Antibodies are accessible to pathogens only in the blood and the extracellular spaces. However, some bacteria and parasites, and all viruses, replicate inside cells, where they cannot be detected by antibodies. The destruction of intracellular invaders is the function of the T lymphocytes, which are responsible for the **cell-mediated immune responses** of adaptive immunity. But T lymphocytes participate in responses to a wide variety of pathogens, including extracellular organisms, and so must exert a wide variety of effector activities.

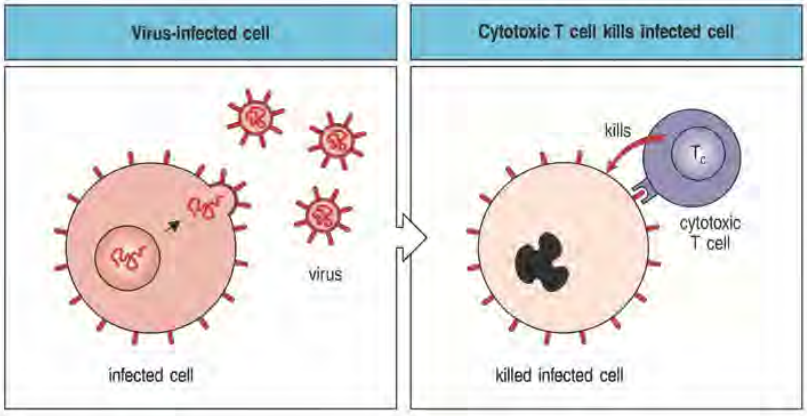
The most direct action of T cells is cytotoxicity. Cytotoxic T cells are effector T cells that act against cells infected with viruses. Antigens derived from the virus multiplying inside the infected cell are displayed on the cell's surface, where they are recognized by the antigen receptors of cytotoxic T cells. These T cells can then control the infection by directly killing the infected cell before viral replication is complete and new viruses are released (Fig. 1.26).

From the end of their development in the thymus, T lymphocytes are composed of two main classes, one of which carries the cell-surface protein called **CD8** on its surface and the other bears a protein called **CD4.** These are not just random markers, but are important for a T cell's function, because they help to determine the interactions between the T cell and other cells. Cytotoxic T cells carry CD8, while the helper T cells involved in activating, rather than killing, the cells that they recognize carry CD4.

CD8 T cells are destined to become cytotoxic T cells by the time they leave the thymus as naive lymphocytes. Naive CD4 T cells, in contrast, can differentiate into several types of effector T cells after their initial activation by antigen. At least four main types of CD4 effector T cells are distinguished, called **T_H1**, **T_H2**, **T_H17**, and **follicular helper T cells,** or **T_{FH} cells**, which are all described in Chapter 9. These effector T-cell subsets promote distinct types of responses aimed at different types of infections. For example, T_H1 cells help to control certain bacteria that take up residence in membrane-enclosed vesicles inside macrophages; the T_H1 cells activate macrophages to increase their intracellular killing power and destroy these bacteria. Important infections that are controlled, at least to some extent, by this function of T_H1 cells are tuberculosis and leprosy, which are caused by the bacteria *Mycobacterium tuberculosis* and *M. leprae*, respectively. Mycobacteria survive intracellularly because they prevent the vesicles they occupy from fusing with lysosomes, which contain a variety of degradative enzymes and antimicrobial substances (Fig. 1.27). However, the infected macrophage presents mycobacteria-derived antigens on its surface that can be recognized by activated antigen-specific T_H1 cells, which in turn secrete particular cytokines that induce the macrophage to overcome the block on vesicle fusion (see Fig. 1.27).

T_H2 cells are specialized in promoting responses at mucosal surfaces and particularly in response to parasitic infections, and again this is largely a result of the distinct set of cytokines that T_H2 cells produce. The effector mechanisms involved are shared with what are commonly known as 'allergic' responses, and are characterized by increased production of a protective barrier of

Petitioner Merck, Ex. 1170, p. 33
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003



a

Fig. 1.26 Mechanism of host defense against intracellular infection by viruses. Cells infected by viruses are recognized by specialized T cells called cytotoxic T cells, which kill the infected cells directly. The killing mechanism involves the activation of enzymes known as caspases, which contain cysteine in their active site and cleave after aspartic acid. These in turn activate a cytosolic nuclease in the infected cell, which cleaves host and viral DNA. Panel a is a transmission electron micrograph showing the plasma membrane of a cultured CHO cell (the Chinese hamster ovary cell line) infected with influenza virus. Many virus particles can be seen budding from the cell surface. Some of these have been labeled with a monoclonal antibody that is specific for a viral protein and is coupled to gold particles, which appear as the solid black dots in the micrograph. Panel b is a transmission electron micrograph of a virus-infected cell (V) surrounded by cytotoxic T lymphocytes. Note the close apposition of the membranes of the virus-infected cell and the T cell (T) in the upper left corner of the micrograph, and the clustering of the cytoplasmic organelles in the T cell between its nucleus and the point of contact with the infected cell. Panel a courtesy of M. Bui and A. Helenius; panel b courtesy of N. Rooney.

mucus at mucosal surfaces, the recruitment of eosinophils, and the production of a particular class of antibody, IgE. Although allergies are usually considered an adverse response to an antigen, this type of immune response has an important protective role against many types of pathogens. Finally, T_H17 cells produce distinct cytokines that help to promote responses rich in the recruitment of neutrophils and which are effective in dealing with extracellular bacteria and fungi.

The term 'helper T cell' was coined well before the various CD4 T-cell subsets were known, to specifically describe T cells that 'help' B cells produce antibody. It is now used more generally in connection with functions described above, rather than being restricted to T cells that help B cells. As we shall see in Chapters 11 and 12, a recently discovered subset of CD4 T cells called T_{FH} cells, which is distinct from the T_H1, T_H2, and T_H17 cells, seems to provide much of the help to B cells; it resides in lymphoid follicles and gives unique signals to B lymphocytes that are required for many aspects of antibody production (discussed in Chapters 9 and 10).

1-20 CD4 and CD8 T cells recognize peptides bound to two different classes of MHC molecules.

The different types of effector T cells must be directed to act against the appropriate target cells. Antigen recognition is obviously crucial, but correct target recognition is also ensured by additional interactions between the CD8 and CD4 molecules on the T cells and the MHC molecules on the target cell.

As we saw in Section 1-13, T cells detect peptides derived from foreign antigens after antigens are degraded within cells, their peptide fragments are captured by MHC molecules, and this complex is displayed at the cell surface (see Fig. 1.16). There are two main types of MHC molecules, called **MHC class I**

Petitioner Merck, Ex. 1170, p. 34
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

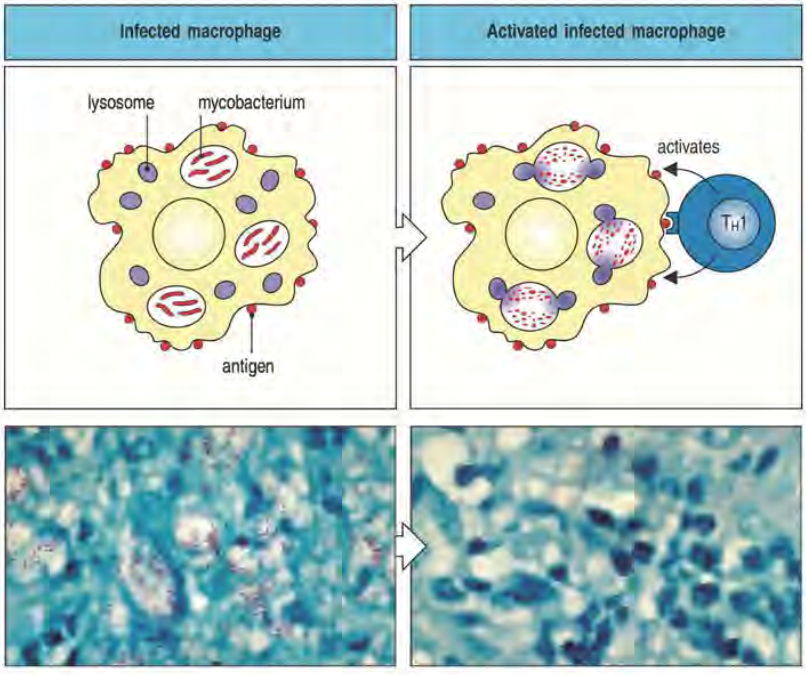


Fig. 1.27 Mechanism of host defense against intracellular infection by mycobacteria. Mycobacteria are engulfed by macrophages but resist being destroyed by preventing the intracellular vesicles in which they reside from fusing with lysosomes containing bactericidal agents. Thus the bacteria are protected from being killed. In resting macrophages, mycobacteria persist and replicate in these vesicles. When the phagocyte is recognized and activated by a T_H1 cell, however, the phagocytic vesicles fuse with lysosomes, and the bacteria can be killed. Macrophage activation is controlled by T_H1 cells, both to avoid tissue damage and to save energy. The light micrographs (bottom row) show resting (left) and activated (right) macrophages infected with mycobacteria. The cells have been stained with an acid-fast red dye to reveal mycobacteria. These are prominent as red-staining rods in the resting macrophages but have been eliminated from the activated macrophages. Photographs courtesy of G. Kaplan.

and **MHC class II**. They have slightly different structures but both have an elongated cleft in the extracellular surface of the molecule, in which a single peptide is trapped during the synthesis and assembly of the MHC molecule inside the cell. The MHC molecule bearing its cargo of peptide is transported to the cell surface, where it displays the peptide to T cells (Fig. 1.28).

There are some important functional differences between the two classes of MHC molecules. First, as noted earlier, the CD8 and CD4 molecules that distinguish different T-cell subsets are not just random markers. These two proteins have the ability to recognize parts of the MHC class I and MHC class II molecules, respectively. CD8 T cells therefore selectively recognize peptides that are bound to MHC class I molecules, and CD4 T cells recognize peptides presented by MHC class II. CD4 and CD8 are known as **co-receptors**, because they are inextricably involved in signaling to the T cell that the receptor has bound the correct antigen. There are also differences in the source of peptides that are processed and eventually bound to the two types of MHC proteins. In most cells, **MHC class I molecules** collect peptides derived from proteins synthesized in the cytosol and are thus able to display fragments of viral proteins on the cell surface (Fig. 1.29). Because MHC class I molecules are expressed on most cells of the body, they serve as an important mechanisms to defend against viral infections. MHC class I molecules bearing viral peptides are recognized by CD8-bearing cytotoxic T cells, which then kill the infected cell (Fig. 1.30). In contrast, **MHC class II molecules** are expressed predominantly by

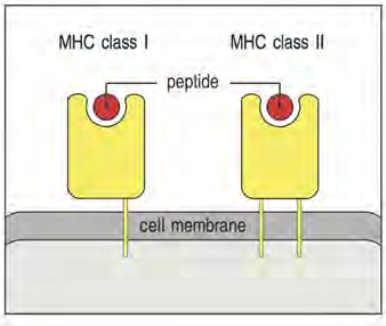


Fig. 1.28 MHC molecules on the cell surface display peptide fragments of antigens. MHC molecules are membrane proteins whose outer extracellular domains form a cleft in which a peptide fragment is bound. These fragments are derived from proteins degraded inside the cell, including both self and foreign protein antigens. The peptides are bound by the newly synthesized MHC molecule before it reaches the cell surface. There are two kinds of MHC molecules—MHC class I and MHC class II—with related but distinct structures and functions. Although not shown here for simplicity, both MHC class I and MHC class II molecules are trimers of two protein chains and the bound self or nonself peptide.

Petitioner Merck, Ex. 1170, p. 35
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

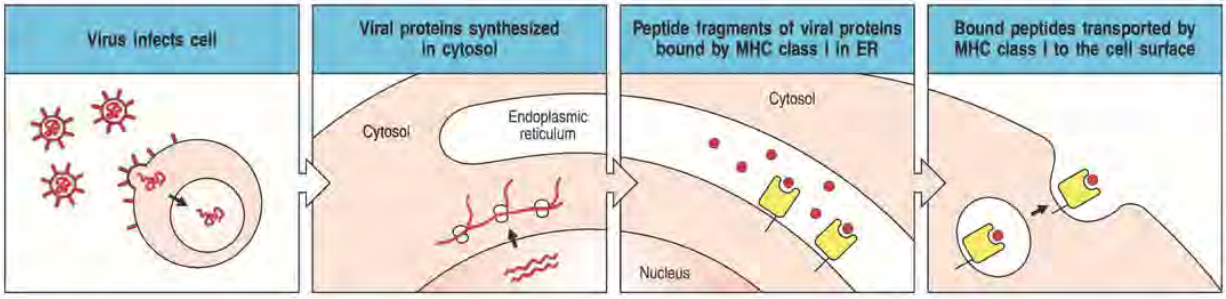


Fig. 1.29 MHC class I molecules present antigen derived from proteins in the cytosol. In cells infected with viruses, viral proteins are synthesized in the cytosol. Peptide fragments of viral proteins are transported into the endoplasmic reticulum (ER), where they are bound by MHC class I molecules, which then deliver the peptides to the cell surface.

antigen-presenting cells (dendritic cells, macrophages, and B cells) and bind peptides derived largely from proteins in intracellular vesicles. These encompass proteins taken up by phagocytosis, proteins derived from pathogens living within macrophage vesicles, or proteins internalized by B cells by endocytosis. This group of cells must either activate, or be activated by, CD4 T cells (Fig. 1.31). We shall see in Chapter 6 exactly how peptides from these different sources are made available to the two types of MHC molecules.

Because the T-cell receptor is specific for a combination of peptide and MHC molecule, any given T-cell receptor will recognize either an MHC class I molecule or an MHC class II molecule. To be useful, T lymphocytes bearing antigen receptors that recognize MHC class I must also express CD8 co-receptors, whereas T lymphocytes bearing receptors specific for MHC class II must express CD4. The matching of a T-cell receptor with a co-receptor of the appropriate type occurs during of process of selection during lymphocyte development, and naive T cells emerge from the central lymphocyte organs bearing the correct combination of receptors and co-receptors. Exactly how this selective process works and how it maximizes the usefulness of the T-cell repertoire are described in Chapter 8.

As we noted earlier, when the various types of effector T cells are stimulated by antigen, they release different sets of effector molecules that affect the target cells or recruit other effector cells in ways we discuss in Chapter 9. These effector molecules include many cytokines, which can influence clonal expansion of lymphocytes, innate immune responses, and effector actions of most immune cells. Because cytokines are central to understanding immune responses, this book has taken the approach of discussing each cytokine individually as their activities are encountered in each aspect of innate and adaptive immunity, rather than treating them arbitrarily together in a single chapter. For convenience, we have summarized their actions in Appendix III.

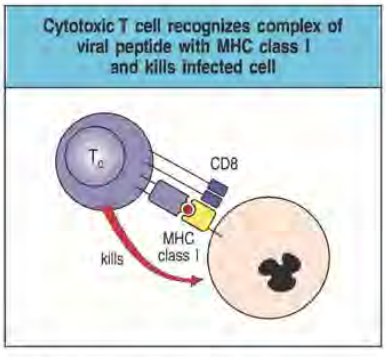


Fig. 1.30 Cytotoxic CD8 T cells recognize antigen presented by MHC class I molecules and kill the cell. The peptide:MHC class I complex on virus-infected cells is detected by antigen-specific cytotoxic T cells. Cytotoxic T cells are preprogrammed to kill the cells they recognize.

1-21 Inherited and acquired defects in the immune system result in increased susceptibility to infection.

We tend to take for granted the ability of our immune systems to free our bodies of infection and prevent its recurrence. In some people, however, parts of the immune system fail. In the most severe of these **immunodeficiency diseases**, adaptive immunity is completely absent, and death occurs in infancy from overwhelming infection unless heroic measures are taken. Other less catastrophic failures lead to recurrent infections with particular types of pathogens, depending on the particular deficiency. Much has been learned about the functions of the different components of the human immune system through the study of these immunodeficiencies, many of which are caused by inherited genetic defects. Because understanding the features of immunodeficiencies requires a detailed knowledge of normal immune mechanisms, we have postponed discussion of most of these diseases until Chapter 13, where they can be considered together.

Petitioner Merck, Ex. 1170, p. 36
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

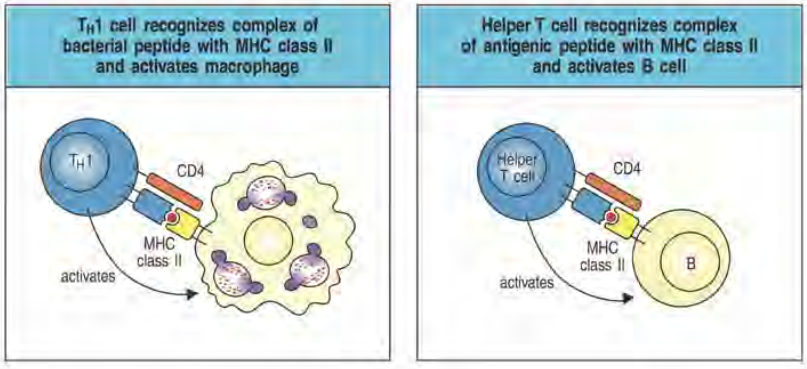


Fig. 1.31 CD4 T cells recognize antigen presented by MHC class II molecules. On recognition of their specific antigen on infected macrophages, T_H1 cells activate the macrophage, leading to the destruction of the intracellular bacteria (left panel). When T_{FH} cells recognize antigen on B cells (right panel), they activate these cells to proliferate and differentiate into antibody-producing plasma cells (not shown).

More than 30 years ago, a devastating form of immunodeficiency appeared, the **acquired immune deficiency syndrome**, or **AIDS**, which is caused by an infectious agent, the human immunodeficiency viruses HIV-1 and HIV-2. This disease destroys T cells, dendritic cells, and macrophages bearing CD4, leading to infections caused by intracellular bacteria and other pathogens normally controlled by such cells. These infections are the major cause of death from this increasingly prevalent immunodeficiency disease, which is discussed fully in Chapter 13 together with the inherited immunodeficiencies.

1-22 Understanding adaptive immune responses is important for the control of allergies, autoimmune disease, and the rejection of transplanted organs.

The main function of our immune system is to protect the human host from infectious agents. However, many medically important diseases are associated with a normal immune response directed against an inappropriate antigen, often in the absence of infectious disease. Immune responses directed at noninfectious antigens occur in **allergy**, in which the antigen is an innocuous foreign substance; in **autoimmune disease**, in which the response is to a self antigen; and in **graft rejection**, in which the antigen is borne by a transplanted foreign cell (both discussed in Chapter 15). The major antigens provoking graft rejection are, in fact, the MHC molecules, as each of these is present in many different versions in the human population—that is, they are highly **polymorphic**—and most unrelated people differ in the set of MHC molecules they express, a property commonly known as their 'tissue type.' The MHC was originally recognized in mice as a gene locus, the **H2 locus**, that controlled the acceptance or rejection of transplanted tissues, whereas the human MHC molecules were first discovered after attempts to use skin grafts from donors to repair badly burned pilots and bomb victims during the Second World War. The patients rejected the grafts, which were recognized by their immune systems as being 'foreign.' What we call a successful immune response or a failure, and whether the response is considered harmful or beneficial to the host, depends not on the response itself but rather on the nature of the antigen and the circumstances in which the response occurs (Fig. 1.32).

Allergic diseases, which include asthma, are an increasingly common cause of disability in the developed world. Autoimmunity is also now recognized as the cause of many important diseases. An autoimmune response directed against pancreatic β cells is the leading cause of diabetes in the young. In allergies and autoimmune diseases, the powerful protective mechanisms of the adaptive immune response cause serious damage to the patient.

Immune responses to harmless antigens, to body tissues, or to organ grafts are, like all other immune responses, highly specific. At present, the usual way

Petitioner Merck, Ex. 1170, p. 37
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

to treat these responses is with immunosuppressive drugs, which inhibit all immune responses, desirable and undesirable alike. If it were possible to suppress only those lymphocyte clones responsible for the unwanted response, the disease could be cured or the grafted organ protected without impeding protective immune responses. At present, antigen-specific immunoregulation is outside the reach of clinical treatment. But as we shall see in Chapter 16, many new drugs, particularly monoclonal antibody therapies, have been developed recently that offer more selective immune suppression to control autoimmune and other unwanted immune responses. We shall discuss the present state of understanding of allergies, autoimmune disease, graft rejection, and immunosuppressive drugs in Chapters 14–16, and we shall see in Chapter 15 how the mechanisms of immune regulation are beginning to emerge from a better understanding of the functional subsets of lymphocytes and the cytokines that control them.

1-23 Vaccination is the most effective means of controlling infectious diseases.

The deliberate stimulation of an immune response by immunization, or vaccination, has achieved many successes in the two centuries since Jenner's pioneering experiment. Mass immunization programs have led to the virtual eradication of several diseases that used to be associated with significant morbidity (illness) and mortality (Fig. 1.33). Immunization is considered so safe and so important that most states in the United States require children to be immunized against up to seven common childhood diseases. Impressive as these accomplishments are, there are still many diseases for which we lack effective vaccines. And even where vaccines for diseases such as measles can be used effectively in developed countries, technical and economic problems can prevent their widespread use in developing countries, where mortality from these diseases is still high.

The tools of modern immunology and molecular biology are being applied to develop new vaccines and improve old ones, and we discuss these advances in Chapter 16. The prospect of controlling these important diseases is tremendously exciting. The guarantee of good health is a critical step toward population control and economic development. At a cost of pennies per person, great hardship and suffering can be alleviated.

Many serious pathogens have resisted efforts to develop vaccines against them, often because they can evade or subvert the protective mechanisms of an adaptive immune response. We examine some of the evasive strategies

Antigen	Effect of response to antigen	
	Normal response	Deficient response
Infectious agent	Protective immunity	Recurrent infection
Innocuous substance	Allergy	No response
Grafted organ	Rejection	Acceptance
Self organ	Autoimmunity	Self tolerance
Tumor	Tumor immunity	Cancer

Fig. 1.32 Immune responses can be beneficial or harmful, depending on the nature of the antigen. Beneficial responses are shown in white, harmful responses in red shaded boxes. Where the response is beneficial, its absence is harmful.

Petitioner Merck, Ex. 1170, p. 38
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

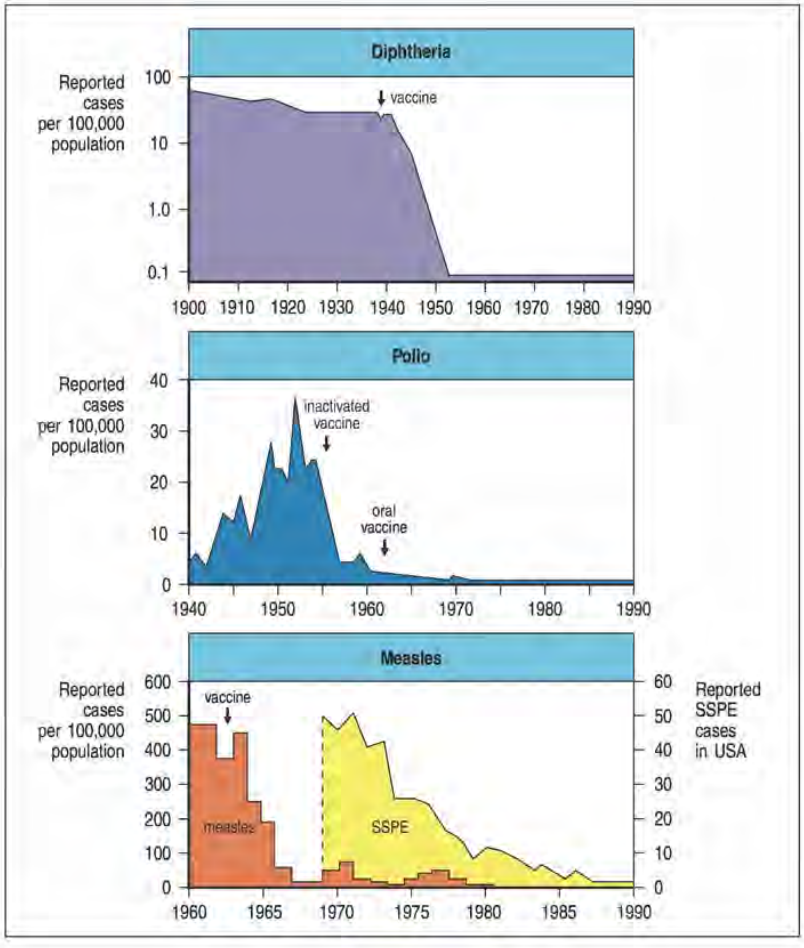


Fig. 1.33 Successful vaccination campaigns. Diphtheria, polio, and measles and their consequences have been virtually eliminated in the United States, as shown in these three graphs. SSPE stands for subacute sclerosing panencephalitis, a brain disease that is a late consequence of measles infection in a few patients. When measles was prevented, SSPE disappeared 15–20 years later. However, because these diseases have not been eradicated worldwide, immunization must be maintained in a very high percentage of the population to prevent their reappearance.

used by successful pathogens in Chapter 13. The conquest of many of the world's leading diseases, including malaria and diarrheal diseases (the leading killers of children) as well as the more recent threat from AIDS, depends on a better understanding of the pathogens that cause them and their interactions with the cells of the immune system.

Summary.

Lymphocytes have two distinct recognition systems specialized for the detection of extracellular and intracellular pathogens. B cells have cell-surface immunoglobulin molecules as receptors for antigen and, upon activation, secrete the immunoglobulin as soluble antibody that provides a defense against pathogens in the extracellular spaces of the body. T cells have receptors that recognize peptide fragments of intracellular pathogens transported to the cell surface by the glycoproteins of the MHC. Two classes of MHC molecules transport peptides from different intracellular compartments to the cell surface to present them to distinct types of effector T cells: cytotoxic CD8 T cells that kill infected target cells, and various types of differentiated CD4 T cells that can activate macrophages, promote allergic responses, mobilize neutrophils, or offer help to B cell for antibody production. Thus, T cells are crucially important for both the humoral and cell-mediated responses of adaptive immunity. The adaptive immune response seems to have grafted

Petitioner Merck, Ex. 1170, p. 39
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

specific antigen recognition by highly diversified receptors onto innate defense systems, which have a central role in the effector actions of both B and T lymphocytes. The vital role of adaptive immunity in fighting infection is illustrated by the immunodeficiency diseases and the problems caused by pathogens that succeed in evading or subverting an adaptive immune response. The antigen-specific suppression of adaptive immune responses is the goal of treatment for important human diseases involving inappropriate activation of lymphocytes, whereas the specific stimulation of an adaptive immune response is the basis of successful vaccination.

Summary to Chapter 1.

The immune system defends the host against infection. Innate immunity serves as a first line of defense but lacks the ability to recognize certain pathogens and to provide the specific protective immunity that prevents reinfection. Adaptive immunity is based on clonal selection from a repertoire of lymphocytes bearing highly diverse antigen-specific receptors that enable the immune system to recognize any foreign antigen. In the adaptive immune response, antigen-specific lymphocytes proliferate and differentiate into clones of effector lymphocytes that eliminate the pathogen. Figure 1.34 summarizes the phases of the immune response and their approximate timings. Host defense requires different recognition systems and a wide variety of effector mechanisms to seek out and destroy the wide variety of pathogens in their various habitats within the body and at its external and internal surfaces.

Phases of the immune response

Response		Typical time after infection to start of response	Duration of response
Innate immune response	Inflammation, complement activation, phagocytosis and destruction of pathogen	Minutes	Days
Adaptive immune response	Interaction between antigen-presenting dendritic cells and antigen-specific T cells: recognition of antigen, adhesion, co-stimulation, T-cell proliferation and differentiation	Hours	Days
	Activation of antigen-specific B cells	Hours	Days
	Formation of effector and memory T cells	Days	Weeks
	Interaction of T cells with B cells, formation of germinal centers. Formation of effector B cells (plasma cells) and memory B cells. Production of antibody	Days	Weeks
	Emigration of effector lymphocytes from peripheral lymphoid organs	A few days	Weeks
	Effector cells and antibodies eliminate the pathogen	A few days	Weeks
Immunological memory	Maintenance of memory B cells and T cells and high serum or mucosal antibody levels. Protection against reinfection	Days to weeks	Can be lifelong

Fig. 1.34 Phases of the immune response.

Petitioner Merck, Ex. 1170, p. 40
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Not only can the adaptive immune response eliminate a pathogen but, in the process, it also generates increased numbers of differentiated memory lymphocytes through clonal selection, and this allows a more rapid and effective response upon reinfection. The regulation of immune responses, whether to suppress them when unwanted or to stimulate them in the prevention of infectious disease, is the major medical goal of research in immunology.

General references.

Historical background

Burnet, F.M.: *The Clonal Selection Theory of Acquired Immunity*. London, Cambridge University Press, 1959.

Gowans, J.L.: **The lymphocyte—a disgraceful gap in medical knowledge.** *Immunol. Today* 1996, **17**:288–291.

Landsteiner, K.: *The Specificity of Serological Reactions*, 3rd ed. Boston, Harvard University Press, 1964.

Metchnikoff, E.: *Immunity in the Infectious Diseases*, 1st ed. New York, Macmillan Press, 1905.

Silverstein, A.M.: *History of Immunology*, 1st ed. London, Academic Press, 1989.

Biological background

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K. and Walter, P.: *Molecular Biology of the Cell*, 5th ed. New York, Garland Publishing, 2007.

Berg, J.M., Stryer, L. and Tymoczko, J.L.: *Biochemistry*, 5th ed. New York, W.H. Freeman, 2002.

Geha, R.S. and Notarangelo, L.D.: *Case Studies in Immunology: A Clinical Companion*, 6th ed. New York, Garland Science, 2012.

Harper, D.R.: *Viruses: Biology, Applications, Control*. New York, Garland Science, 2012.

Kaufmann, S.E., Sher, A. and Ahmed, R. (Eds): *Immunology of Infectious Diseases*. Washington, DC: ASM Press, 2001.

Lodish, H., Berk, A., Kaiser, C.A., Krieger, M., Scott, M.P., Bretscher, A., Ploegh, H., Matsudaira, P.: *Molecular Cell Biology*, 6th ed. New York, W.H. Freeman, 2008.

Lydyard, P., Cole, M., Holton, J., Irving, W., Porakishvili, N., Venkatesan, P., and Ward, K.: *Case Studies in Infectious Disease*. New York, Garland Science, 2009.

Mims, C., Nash, A. and Stephen, J.: *Mims' Pathogenesis of Infectious Disease*, 5th ed. London, Academic Press, 2001.

Ryan, K.J. (ed): *Medical Microbiology*, 3rd ed. East Norwalk, CT, Appleton-Lange, 1994.

Primary journals devoted solely or primarily to immunology

Autoimmunity
Clinical and Experimental Immunology
Comparative and Developmental Immunology
European Journal of Immunology
Immunity
Immunogenetics
Immunology
Infection and Immunity
International Immunology
International Journal of Immunogenetics
Journal of Autoimmunity
Journal of Experimental Medicine
Journal of Immunology
Nature Immunology
Regional Immunology
Thymus

Primary journals with frequent papers in immunology

Cell
Current Biology
EMBO Journal
Journal of Biological Chemistry
Journal of Cell Biology
Journal of Clinical Investigation
Molecular Cell Biology
Nature
Nature Cell Biology
Nature Medicine
Proceedings of the National Academy of Sciences, USA
Science

Review journals in immunology

Advances in Immunology
Annual Reviews in Immunology
Contemporary Topics in Microbiology and Immunology
Current Opinion in Immunology
Immunogenetics Reviews
Immunological Reviews
The Immunologist
Immunology Today
Nature Reviews Immunology
Research in Immunology
Seminars in Immunology

Advanced textbooks in immunology, compendia, etc.

Lachmann, P.J., Peters, D.K., Rosen, F.S., and Walport, M.J. (eds): *Clinical Aspects of Immunology*, 5th ed. Oxford, Blackwell Scientific Publications, 1993.

Mak, T.W., and Saunders, M.E.: *The Immune Response. Basic and Clinical Principles*. Elsevier/Academic Press, 2006.

Mak, T.W., and Simard, J.J.L.: *Handbook of Immune Response Genes*. New York, Plenum Press, 1998.

Paul, W.E. (ed): *Fundamental Immunology*, 6th ed. New York, Lippincott Williams & Wilkins, 2008.

Roitt, I.M., and Delves, P.J. (eds): *Encyclopedia of Immunology*, 2nd ed. (4 vols). London/San Diego, Academic Press, 1998.

Petitioner Merck, Ex. 1170, p. 41
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

4 Antigen Recognition by B-cell and T-cell Receptors

Innate immune responses initially defend the body against infection, but these only work to control pathogens that have certain molecular patterns or that induce interferons and other nonspecific defenses. To effectively fight the wide range of pathogens an individual will encounter, the lymphocytes of the adaptive immune system have evolved to recognize a great variety of different **antigens** from bacteria, viruses, and other disease-causing organisms. An antigen is any molecule or part of a molecule that is specifically recognized by the highly specialized recognition proteins of lymphocytes. On B cells these are the **immunoglobulins** (**Ig**), which are produced by these cells in a vast range of antigen specificities, each B cell producing immunoglobulin of a single specificity (see Sections 1-11 and 1-12). Membrane-bound immunoglobulin on the B-cell surface serves as the cell's receptor for antigen, and is known as the **B-cell receptor** (**BCR**). Immunoglobulin of the same antigen specificity is secreted as **antibody** by terminally differentiated B cells—the plasma cells. The secretion of antibodies, which bind pathogens or their toxic products in the extracellular spaces of the body (see Fig. 1.26), is the main effector function of B cells in adaptive immunity.

Antibodies were the first proteins involved in specific immune recognition to be characterized, and they are still the best understood. The antibody molecule has two separate functions: one is to bind specifically to the pathogen or its products that elicited the immune response; the other is to recruit other cells and molecules to destroy the pathogen once antibody has bound. For example, binding by antibodies neutralizes viruses and marks pathogens for destruction by phagocytes and complement, as described in Section 1-18. Recognition and effector functions are structurally separated in the antibody molecule, one part of which specifically binds to the antigen whereas the other engages the elimination mechanisms. The antigen-binding region varies extensively between antibody molecules and is known as the **variable region** or **V region**. The variability of antibody molecules allows each antibody to bind a different specific antigen, and the total repertoire of antibodies made by a single individual is large enough to ensure that virtually any structure can be recognized. The region of the antibody molecule that engages the effector functions of the immune system does not vary in the same way and is known as the **constant region** or **C region**. It comes in five main forms, each of which is specialized for activating different effector mechanisms. The membrane-bound B-cell receptor does not have these effector functions, because the C region remains inserted in the membrane of the B cell. The function of the B-cell receptor is to recognize and bind antigen via the V regions exposed on the surface of the cell, thus transmitting a signal that activates the B cell, leading to clonal expansion and antibody production. To this end, the B-cell receptor is associated with a set of intracellular signaling proteins, which will be described in Chapter 7.

Petitioner Merck, Ex. 1170, p. 42
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The antigen-recognition molecules of T cells are made solely as membrane-bound proteins, which are associated with an intracellular signaling complex and function only to signal T cells for activation. These **T-cell receptors** (**TCRs**) are related to immunoglobulins both in their protein structure—having both V and C regions—and in the genetic mechanism that produces their great variability, which will be discussed in Chapter 5. The T-cell receptor differs from the B-cell receptor in an important way, however: it does not recognize and bind antigen by itself, but instead recognizes short peptide fragments of protein antigens, which are presented by proteins known as **MHC molecules** on the surfaces of host cells.

The MHC molecules are transmembrane glycoproteins encoded in the large cluster of genes known as the **major histocompatibility complex** (**MHC**). Their most striking structural feature is a cleft in the extracellular face of the molecule, in which peptides can be bound. MHC molecules are highly **polymorphic**—each type of MHC molecule occurs in many different versions within the population. Most people are therefore heterozygous for the MHC molecules: that is, they express two different forms of each type of MHC molecule, which increases the range of pathogen-derived peptides that can be bound. T-cell receptors recognize features of both the peptide antigen and the MHC molecule to which it is bound. This introduces an extra dimension to antigen recognition by T cells, known as **MHC restriction**, because any given T-cell receptor is specific for a unique combination of a particular peptide and a particular MHC molecule. We shall discuss MHC polymorphism and its consequences for T-cell antigen recognition and T-cell development in Chapters 6 and 8, respectively.

In this chapter we focus on the structure and antigen-binding properties of immunoglobulins and T-cell receptors. Although B cells and T cells recog nize foreign molecules in distinct fashions, the receptor molecules they use for this task are very similar in structure. We will see how this basic structure can accommodate great variability in antigen specificity, and how it enables immunoglobulins and T-cell receptors to perform their functions as the antigen-recognition molecules of the adaptive immune response.

The structure of a typical antibody molecule.

Antibodies are the secreted form of the B-cell receptor. Because they are soluble and secreted into the blood in large quantities, antibodies are easily obtainable and easily studied. For this reason, most of what we know about the B-cell receptor comes from the study of antibodies.

Antibody molecules are roughly Y-shaped molecules consisting of three equal-sized portions connected by a flexible tether. Three schematic representations of antibody structure are shown in Fig. 4.1. In this part of the chapter we will explain how this structure is formed and how it allows antibody molecules to perform their dual tasks—binding on the one hand to a wide variety of antigens, and on the other to a limited number of effector molecules and cells that destroy the antigen. Each of these tasks is performed by different parts of the molecule. The ends of the two arms of the Y—the V regions—vary in their detailed structure between different antibody molecules. These are involved in antigen binding. The stem of the Y—the C region—is far less variable and is the part that interacts with effector cells and molecules.

All antibodies are constructed in the same way from paired heavy and light polypeptide chains, and the generic term immunoglobulin is used for all such proteins. Five different **classes** of immunoglobulins—IgM, IgD, IgG, IgA, and

Petitioner Merck, Ex. 1170, p. 43
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

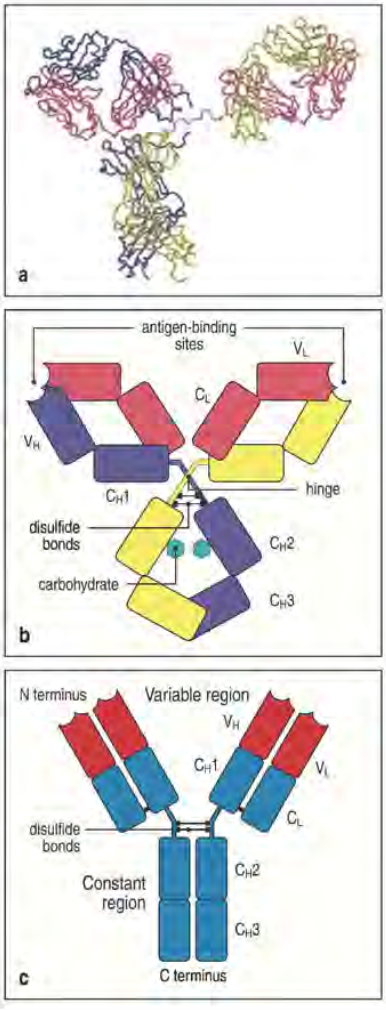


Fig. 4.1 Structure of an antibody molecule. Panel a illustrates a ribbon diagram based on the X-ray crystallographic structure of an IgG antibody, showing the course of the backbones of the polypeptide chains. Three globular regions form a Y shape. The two antigen-binding sites are at the tips of the arms, which are tethered to the trunk of the Y by a flexible hinge region. A schematic representation of the structure in panel a is given in panel b, illustrating the four-chain composition and the separate domains comprising each chain. Panel c shows a simplified schematic representation of an antibody molecule that will be used throughout this book. C terminus, carboxy terminus; N terminus, amino terminus. Structure courtesy of R.L. Stanfield and I.A. Wilson.

IgE—can be distinguished by their C regions. More subtle differences confined to the V region account for the specificity of antigen binding. We will use the IgG antibody molecule as an example to describe the general structural features of immunoglobulins.

4-1 IgG antibodies consist of four polypeptide chains.

IgG antibodies are large molecules with a molecular weight of approximately 150 kDa and are composed of two different kinds of polypeptide chains. One, of approximately 50 kDa, is called the **heavy** or **H chain**, and the other, of 25 kDa, is the **light** or **L chain** (Fig. 4.2). Each IgG molecule consists of two heavy chains and two light chains. The two heavy chains are linked to each other by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide bond. In any given immunoglobulin molecule, the two heavy chains and the two light chains are identical, giving an antibody molecule two identical antigen-binding sites (see Fig. 4.1). This gives it the ability to bind simultaneously to two identical antigens on a surface and hence increase the total strength of the interaction, which is called its **avidity**. The strength of the interaction between a single antigen-binding site and its antigen is called its **affinity**.

Two types of light chains, **lambda** (λ) and **kappa** (κ), are found in antibodies. A given immunoglobulin has either κ chains or λ chains, never one of each. No functional difference has been found between antibodies having λ or κ light chains, and either type of light chain can be found in antibodies of any of the five major classes. The ratio of the two types of light chains varies from species to species. In mice, the average κ to λ ratio is 20:1, whereas in humans it is 2:1 and in cattle it is 1:20. The reason for this variation is unknown. Distortions of this ratio can sometimes be used to detect the abnormal proliferation of a clone of B cells. These will all express the identical light chain, and thus an excess of λ light chains in a person might indicate the presence of a B-cell tumor producing λ chains.

The class, and thus the effector function, of an antibody is defined by the structure of its heavy chain. There are five main heavy-chain classes or **isotypes**, some of which have several subtypes, and these determine the functional

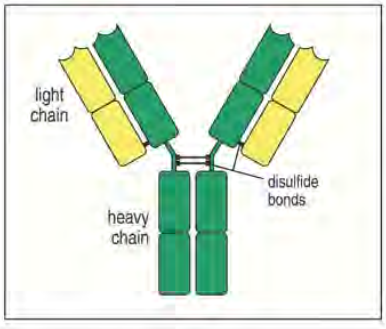


Fig. 4.2 Immunoglobulin molecules are composed of two types of protein chains: heavy chains and light chains. Each immunoglobulin molecule is made up of two heavy chains (green) and two light chains (yellow) joined by disulfide bonds so that each heavy chain is linked to a light chain and the two heavy chains are linked together.

activity of an antibody molecule. The five major classes of immunoglobulin are **immunoglobulin M (IgM)**, **immunoglobulin D (IgD)**, **immunoglobulin G (IgG)**, **immunoglobulin A (IgA)**, and **immunoglobulin E (IgE)**. Their heavy chains are denoted by the corresponding lower-case Greek letter (μ, δ, γ, α, and ε, respectively). IgG is by far the most abundant immunoglobulin and has several subclasses (IgG1, 2, 3, and 4 in humans). The distinctive functional properties of the different classes and subclasses of antibodies are conferred by the carboxy-terminal part of the heavy chain, where it is not associated with the light chain. We will describe the structure and functions of the different heavy-chain isotypes in Chapter 5. The general structural features of all the isotypes are similar, and we will consider IgG, the most abundant isotype in plasma, as a typical antibody molecule.

The structure of a B-cell receptor is identical to that of its corresponding antibody except for a small portion of the carboxy terminus of the heavy-chain C region. In the B-cell receptor, the carboxy terminus is a hydrophobic amino acid sequence that anchors the molecule in the membrane, and in the antibody it is a hydrophilic sequence that allows secretion.

4-2 Immunoglobulin heavy and light chains are composed of constant and variable regions.

The amino acid sequences of many immunoglobulin heavy and light chains have been determined and reveal two important features of antibody molecules. First, each chain consists of a series of similar, although not identical, sequences, each about 110 amino acids long. Each of these repeats corresponds to a discrete, compactly folded region of protein structure known as a protein domain. The light chain is made up of two such **immunoglobulin domains**, whereas the heavy chain of the IgG antibody contains four (see Fig. 4.1a). This suggests that the immunoglobulin chains have evolved by repeated duplication of an ancestral gene corresponding to a single domain.

The second important feature is that the amino-terminal amino acid sequences of the heavy and light chains vary greatly between different antibodies. The variability is limited to approximately the first 110 amino acids, corresponding to the first domain, whereas the remaining domains are constant between immunoglobulin chains of the same isotype. The amino-terminal **variable domains (V domains)** of the heavy and light chains (V_H and V_L, respectively) together make up the V region of the antibody and confer on it the ability to bind specific antigen, whereas the **constant domains (C domains)** of the heavy and light chains (C_H and C_L, respectively) make up the C region (see Fig. 4.1b and c). The multiple heavy-chain C domains are numbered from the amino-terminal end to the carboxy terminus, for example C_H1, C_H2, and so on.

4-3 The antibody molecule can readily be cleaved into functionally distinct fragments.

The protein domains described above associate to form larger globular domains. Thus, when fully folded and assembled, an antibody molecule comprises three equal-sized globular portions joined by a flexible stretch of polypeptide chain known as the **hinge region** (see Fig. 4.1b). Each arm of this Y-shaped structure is formed by the association of a light chain with the amino-terminal half of a heavy chain, whereas the trunk of the Y is formed by the pairing of the carboxy-terminal halves of the two heavy chains. The association of the heavy and light chains is such that the V_H and V_L domains are paired, as are the C_H1 and C_L domains. The C_H3 domains pair with each other but the C_H2 domains do not interact; carbohydrate side chains attached to the C_H2 domains lie between the two heavy chains. The two antigen-binding

Petitioner Merck, Ex. 1170, p. 45
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

sites are formed by the paired V_H and V_L domains at the ends of the two arms of the Y (see Fig. 4.1b).

Proteolytic enzymes (proteases) that cleave polypeptide sequences have been used to dissect the structure of antibody molecules and to determine which parts of the molecule are responsible for its various functions. Limited digestion with the protease papain cleaves antibody molecules into three fragments (Fig. 4.3). Papain cuts the antibody molecule on the amino-terminal side of the disulfide bonds that link the two heavy chains, releasing the two arms of the antibody molecule as two identical fragments that contain the antigen-binding activity. These are called the **Fab fragments**, for **F**ragment **a**ntigen **b**inding. The other fragment contains no antigen-binding activity but was originally observed to crystallize readily, and for this reason it was named the **Fc fragment**, for **F**ragment **c**rystallizable. It corresponds to the paired C_H2 and C_H3 domains and is the part of the antibody molecule that interacts with effector molecules and cells. The functional differences between heavy-chain isotypes lie mainly in the Fc fragment.

Another protease, pepsin, cuts on the carboxy-terminal side of the disulfide bonds (see Fig. 4.3). This produces a fragment, the **$F(ab')_2$ fragment**, in which the two antigen-binding arms of the antibody molecule remain linked. Pepsin cuts the remaining part of the heavy chain into several small fragments. The $F(ab')_2$ fragment has exactly the same antigen-binding characteristics as the original antibody but is unable to interact with any effector molecule. It thus has potential for therapeutic applications.

Many antibody-related molecules can be constructed with the use of genetic engineering techniques. One is a truncated Fab comprising the V domain of a heavy chain linked by a stretch of synthetic peptide to a V domain of a light chain. This is called **single-chain Fv**, named from **F**ragment **v**ariable. Fv molecules could become valuable therapeutic agents because of their small size, which allows them to penetrate tissues easily. For example, Fv molecules specific for tumor antigens and coupled to protein toxins have potential applications in tumor therapy, as discussed in Chapter 16.

4-4 The immunoglobulin molecule is flexible, especially at the hinge region.

The hinge region that links the Fc and Fab portions of the IgG molecule is in reality a flexible tether, allowing independent movement of the two Fab arms. This flexibility is revealed by studies of antibodies bound to small antigens known as **haptens**. These are molecules of various types that are typically about the size of a tyrosine side chain. Although haptens are specifically recognized by antibody, they can only stimulate the production of anti-hapten antibodies when linked to a protein (see Appendix I, Section A-1). Two identical hapten molecules joined by a short flexible region can link two or more anti-hapten antibodies, forming dimers, trimers, tetramers, and so on, which can be seen by electron microscopy (Fig. 4.4). The shapes formed by these complexes show that antibody molecules are flexible at the hinge region. Some flexibility is also found at the junction between the V and C domains, allowing bending

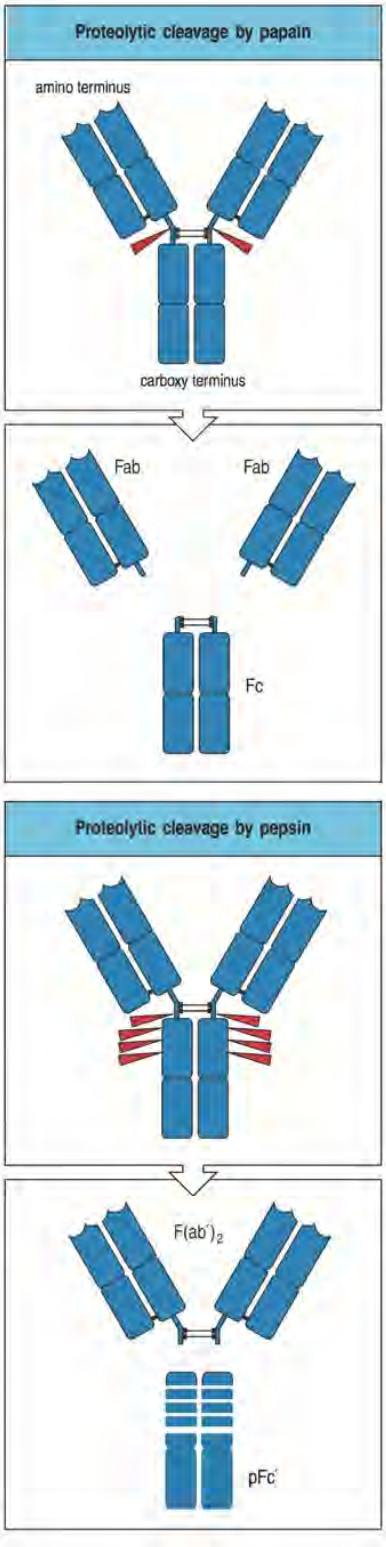


Fig. 4.3 The Y-shaped immunoglobulin molecule can be dissected by partial digestion with proteases. Upper panels: papain cleaves the immunoglobulin molecule into three pieces, two Fab fragments and one Fc fragment. The Fab fragment contains the V regions and binds antigen. The Fc fragment is crystallizable and contains C regions. Lower panels: pepsin cleaves immunoglobulin to yield one $F(ab')_2$ fragment and many small pieces of the Fc fragment, the largest of which is called the pFc′ fragment. $F(ab')_2$ is written with a prime because it contains a few more amino acids than Fab, including the cysteines that form the disulfide bonds.

Petitioner Merck, Ex. 1170, p. 46
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

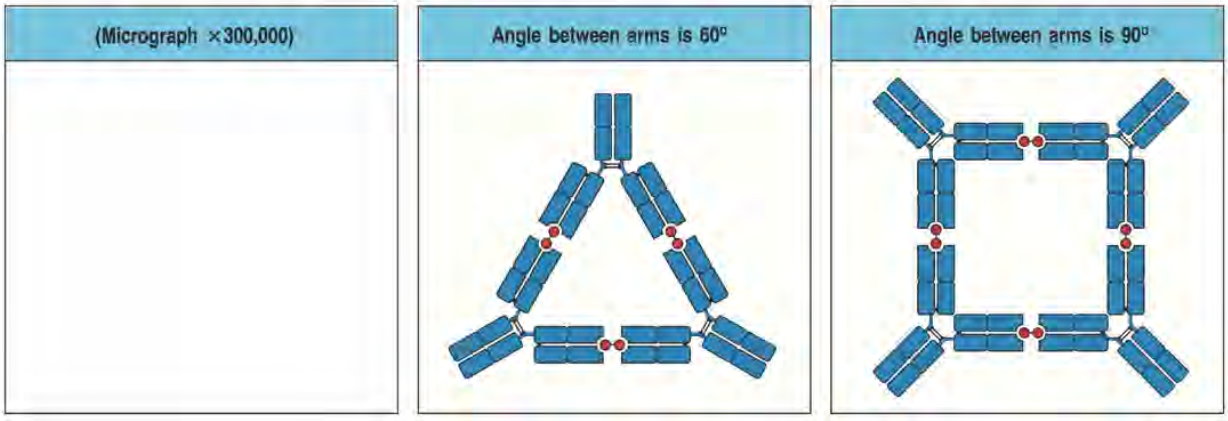


Fig. 4.4 Antibody arms are joined by a flexible hinge. An antigen consisting of two hapten molecules (red balls in diagrams) that can cross-link two antigen-binding sites is used to create antigen:antibody complexes, which can be seen in the electron micrograph. Linear, triangular, and square forms are seen, with short projections or spikes. Limited pepsin digestion removes these spikes (not shown in the figure), which therefore correspond to the Fc portion of the antibody; the $F(ab')_2$ pieces remain cross-linked by antigen. The interpretation of some of the complexes is shown in the diagrams. The angle between the arms of the antibody molecules varies. In the triangular forms, this angle is 60°, whereas it is 90° in the square forms, showing that the connections between the arms are flexible. Photograph (×300,000) courtesy of N.M. Green.

and rotation of the V domain relative to the C domain. For example, in the antibody molecule shown in Fig. 4.1a, not only are the two hinge regions clearly bent differently, but the angle between the V and C domains in each of the two Fab arms is also different. This range of motion has led to the junction between the V and C domains being referred to as a 'molecular ball-and-socket joint.' Flexibility at both the hinge and the V–C junction enables the two arms of an antibody molecule to bind to sites some distance apart, such as the repeating sites on bacterial cell-wall polysaccharides. Flexibility at the hinge also enables antibodies to interact with the antibody-binding proteins that mediate immune effector mechanisms.

4-5 The domains of an immunoglobulin molecule have similar structures.

As we saw in Section 4-2, immunoglobulin heavy and light chains are composed of a series of discrete protein domains, all of which have a similar folded structure. Within this basic structure there are distinct differences between V and C domains. The structural similarities and differences can be seen in the diagram of a light chain in Fig. 4.5. Each domain is constructed from two **β sheets**, which are elements of protein structure made up of strands of the polypeptide chain (β strands) packed together; the sheets are linked by a disulfide bridge and together form a roughly barrel-shaped structure, known as a **β barrel**. The distinctive folded structure of the immunoglobulin protein domain is known as the **immunoglobulin fold**.

Both the essential similarity of V and C domains and the critical differences between them are most clearly seen in the bottom panels of Fig. 4.5, in which the cylindrical domains are opened out to reveal how the polypeptide chain folds to create each of the β sheets and how it forms flexible loops as it changes direction. The main difference between the V and C domains is that the V domain is larger, with an extra loop. The flexible loops of the V domains form the antigen-binding site of the immunoglobulin molecule.

Many of the amino acids that are common to the C and V domains lie in the core of the immunoglobulin fold and are essential for its stability. Other proteins with sequences similar to those of immunoglobulins have been shown to form domains of similar structure, which are called **immunoglobulin-like domains** (**Ig-like domains**). These domains are present in many proteins of the immune system, and in proteins involved in cell–cell recognition and adhesion in the nervous system and other tissues. Together with the immunoglobulins and the T-cell receptors, they make up the extensive **immunoglobulin superfamily**.

Petitioner Merck, Ex. 1170, p. 47
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

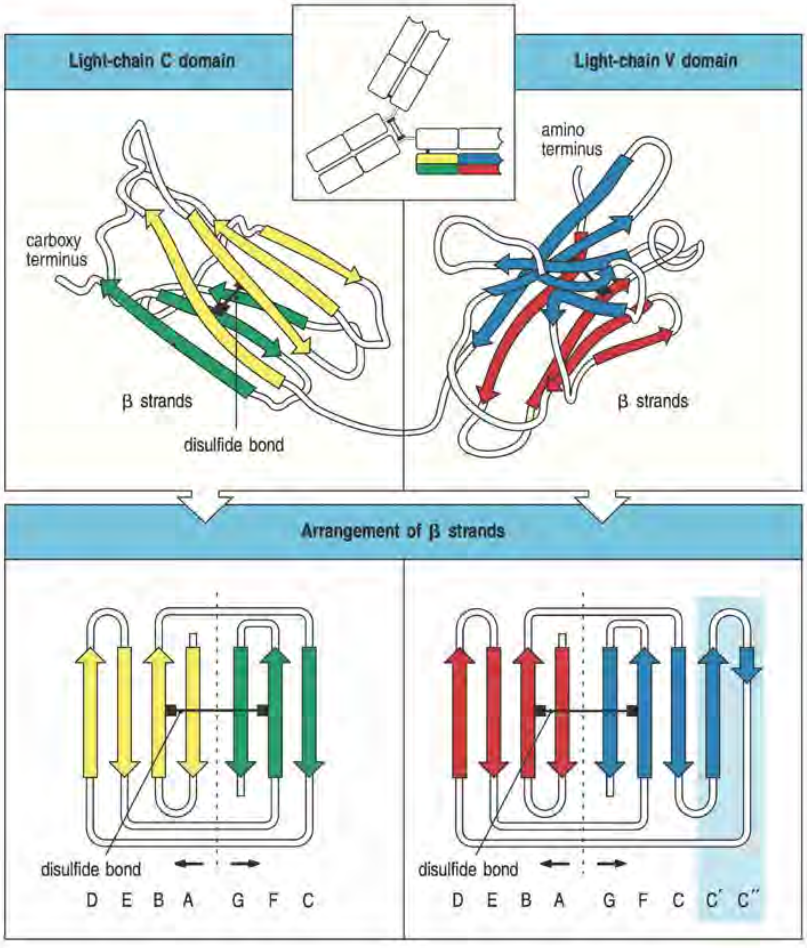


Fig. 4.5 The structure of immunoglobulin constant and variable domains. The upper panels show schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain (β strands) running in opposite directions (antiparallel) pack together to form two β sheets (shown in yellow and green for the C domain and red and blue for the V domain), which are held together by a disulfide bond. The way in which the polypeptide chain folds to give the final structure can be seen more clearly when the sheets are opened out, as shown in the lower panels. The β strands are lettered sequentially with respect to the order of their occurrence in the amino acid sequence of the domains; the order in each β sheet is characteristic of immunoglobulin domains. The β strands C′ and C″ that are found in the V domains but not in the C domains are indicated by a blue shaded background. The characteristic four-strand plus three-strand (C-region type domain) or four-strand plus five-strand (V-region type domain) arrangements are typical immunoglobulin superfamily domain building blocks, found in a whole range of other proteins as well as antibodies and T-cell receptors.

Summary.

The IgG antibody molecule is made up of four polypeptide chains, comprising two identical light chains and two identical heavy chains, and can be thought of as forming a flexible Y-shaped structure. Each of the four chains has a variable (V) region at its amino terminus, which contributes to the antigen-binding site, and a constant (C) region, which determines the isotype. The isotype of the heavy chain determines the functional properties of the antibody. The light chains are bound to the heavy chains by many noncovalent interactions and by disulfide bonds, and the V regions of the heavy and light chains pair in each arm of the Y to generate two identical antigen-binding sites, which lie at the tips of the arms of the Y. The possession of two antigen-binding sites allows antibody molecules to cross-link antigens and to bind them much more stably and with higher avidity. The trunk of the Y, called the Fc fragment, is composed of the carboxy-terminal domains of the heavy chains. Joining the arms of the Y to the trunk are the flexible hinge regions. The Fc fragment and hinge regions differ in antibodies of different isotypes, thus determining their functional properties. However, the overall organization of the domains is similar in all isotypes.

Petitioner Merck, Ex. 1170, p. 48
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The interaction of the antibody molecule with specific antigen.

In this part of the chapter we look at the antigen-binding site of an immunoglobulin molecule in more detail. We discuss the different ways in which antigens can bind to antibody, and address the question of how variation in the sequences of the antibody V domains determines the specificity for antigen.

4-6 Localized regions of hypervariable sequence form the antigen-binding site.

The V regions of any given antibody molecule differ from those of every other. Sequence variability is not, however, distributed evenly throughout the V region but is concentrated in certain segments, as is clearly seen in a **variability plot** (Fig. 4.6), in which the amino acid sequences of many different antibody V regions are compared. Three particularly variable segments can be identified in both the V_H and V_L domains. They are designated **hypervariable regions** and are denoted HV1, HV2, and HV3. In the heavy chains they run roughly from residues 30 to 36, 49 to 65, and 95 to 103, respectively, while in the light chains they are located roughly at residues 28 to 35, 49 to 59, and 92 to 103, respectively. The most variable part of the domain is in the HV3 region. The regions between the hypervariable regions, which comprise the rest of the V domain, show less variability and are termed the **framework regions**. There are four such regions in each V domain, designated FR1, FR2, FR3, and FR4.

The framework regions form the β sheets that provide the structural framework of the domain, whereas the hypervariable sequences correspond to three loops at the outer edge of the β barrel, which are juxtaposed in the folded domain (Fig. 4.7). Thus, not only is diversity concentrated in particular parts of the V domain sequence, but it is also localized to a particular region on the surface of the molecule. When the V_H and V_L immunoglobulin domains are paired in the antibody molecule, the three hypervariable loops

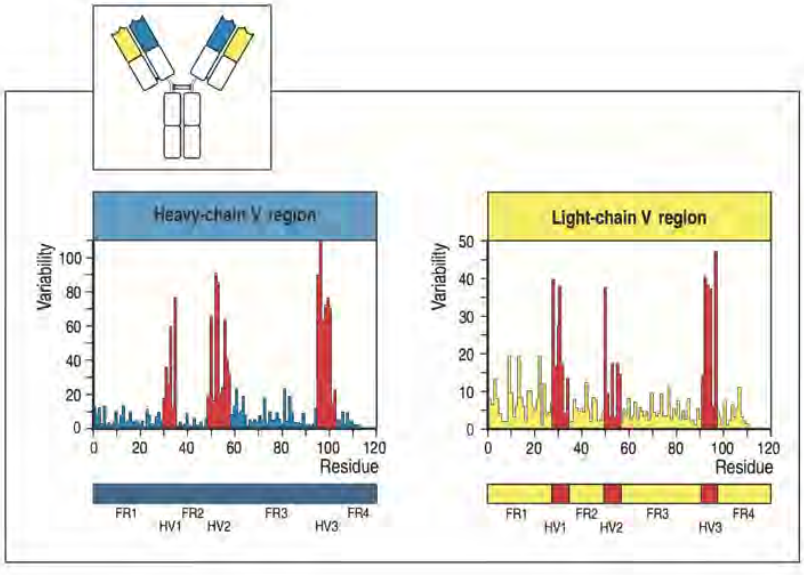


Fig. 4.6 There are discrete regions of hypervariability in V domains.
A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains is shown. At each amino acid position the degree of variability is the ratio of the number of different amino acids seen in all of the sequences together to the frequency of the most common amino acid. Three hypervariable regions (HV1, HV2, and HV3) are indicated in red. They are flanked by less variable framework regions (FR1, FR2, FR3, and FR4, shown in blue or yellow).

Petitioner Merck, Ex. 1170, p. 49
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

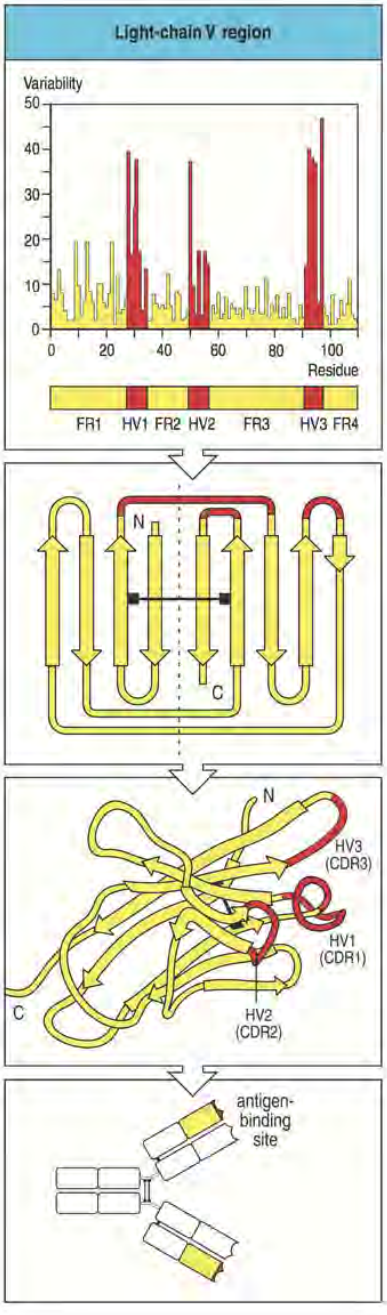


Fig. 4.7 The hypervariable regions lie in discrete loops of the folded structure. When the hypervariable regions are positioned on the structure of a V domain it can be seen that they occur in loops that are brought together in the folded structure. In the antibody molecule, the pairing of a heavy chain and a light chain brings together the hypervariable loops from each chain to create a single hypervariable surface, which forms the antigen-binding site at the tip of each arm. Because they are complementary to the antigen surface, the hypervariable regions are more commonly known as the complementarity-determining regions (CDRs). C, carboxy terminus; N, amino terminus.

from each domain are brought together, creating a single hypervariable site at the tip of each arm of the molecule. This is the **antigen-binding site** or **antibody combining site**, which determines the antigen specificity of the antibody. These six hypervariable loops are more commonly termed the **complementarity-determining regions**, or **CDRs**, because the surface they form is complementary to that of the antigen they bind. There are three CDRs from each of the heavy and light chains, namely CDR1, CDR2, and CDR3. Because CDRs from both V_H and V_L domains contribute to the antigen-binding site, it is the combination of the heavy and the light chain, and not either alone, that determines the final antigen specificity (see Fig. 4.6). Thus, one way in which the immune system is able to generate antibodies of different specificities is by generating different combinations of heavy-chain and light-chain V regions. This is known as **combinatorial diversity**; we will encounter a second form of combinatorial diversity when we consider in Chapter 5 how the genes encoding the heavy-chain and light-chain V regions are created from smaller segments of DNA.

4-7 Antibodies bind antigens via contacts with amino acids in CDRs, but the details of binding depend upon the size and shape of the antigen.

In early investigations of antigen binding to antibodies, the only available sources of large quantities of a single type of antibody molecule were tumors of antibody-secreting cells. The antigen specificities of these antibodies were unknown, so many compounds had to be screened to identify ligands that could be used to study antigen binding. In general, the substances found to bind to these antibodies were haptens (see Section 4-4) such as phosphocholine or vitamin K_1. Structural analysis of complexes of antibodies with their hapten ligands provided the first direct evidence that the hypervariable regions form the antigen-binding site, and demonstrated the structural basis of specificity for the hapten. Subsequently, with the discovery of methods of generating **monoclonal antibodies** (see Appendix I, Section A-12), it became possible to make large amounts of pure antibody specific for a given antigen. This has provided a more general picture of how antibodies interact with their antigens, confirming and extending the view of antibody–antigen interactions derived from the study of haptens.

The surface of the antibody molecule formed by the juxtaposition of the CDRs of the heavy and light chains is the site to which an antigen binds. Clearly, as the amino acid sequences of the CDRs are different in different antibodies, so too are the shapes and properties of the surfaces created by these CDRs. As a general principle, antibodies bind ligands whose surfaces are complementary to that of the antigen-binding site. A small antigen, such as a hapten or a short peptide, generally binds in a pocket or groove lying between the heavy-chain and light-chain V domains (Fig. 4.8a and b). Some antigens, such as proteins, can be the same size as, or larger than, the antibody itself. In these cases, the interface between antigen and antibody is often an extended surface that involves all the CDRs and, in some cases, part of the framework region as well (see Fig. 4.8c). This surface need not be concave but can be flat, undulating, or even convex. In some cases, antibody molecules with elongated CDR3 loops can protrude a 'finger' into recesses in the surface of the antigen, as shown in

Petitioner Merck, Ex. 1170, p. 50
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

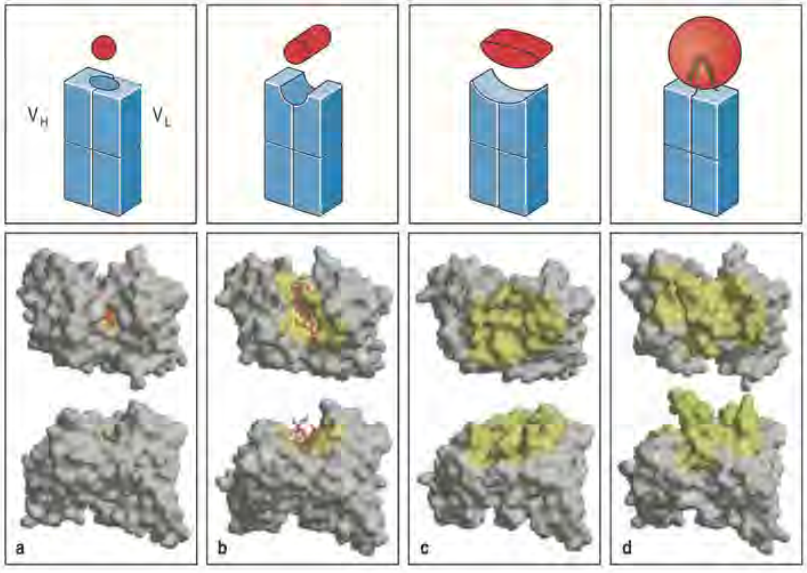


Fig. 4.8 Antigens can bind in pockets, or grooves, or on extended surfaces in the binding sites of antibodies. The panels in the top row show schematic representations of the different types of binding sites in a Fab fragment of an antibody: first panel, pocket; second panel, groove; third panel, extended surface; and fourth panel, protruding surface. Below are examples of each type. Panel a: the top image shows the molecular surface of the interaction of a small hapten with the complementarity-determining regions (CDRs) of a Fab fragment as viewed looking into the antigen-binding site. The ferrocene hapten, shown in green, is bound into the antigen-binding pocket (yellow). In the bottom image (and in those of panels b, c, and d) the molecule has been rotated by about 90° to give a side-on view of the binding site. Panel b: in a complex of an antibody with a peptide from the human immunodeficiency virus (HIV), the peptide (green) binds along a groove (yellow) formed between the heavy-chain and light-chain V domains. Panel c: complex between hen egg-white lysozyme and the Fab fragment of its corresponding antibody (HyHel5). The surface on the antibody that comes into contact with the lysozyme is colored yellow. All six CDRs of the antigen-binding site are involved in the binding. Panel d: an antibody molecule against the HIV gp120 antigen has an elongated CDR3 loop that protrudes into a recess on the side of the antigen. The structure of the complex between this antibody and gp120 has been solved, and it is now known that only the heavy chain interacts with gp120. Structures courtesy of R.L. Stanfield and I.A. Wilson.

Fig. 4.8d, where an antibody binding to the HIV gp120 antigen projects a long loop against its target.

4-8 Antibodies bind to conformational shapes on the surfaces of antigens.

The biological function of antibodies is to bind to pathogens and their products, and to facilitate their removal from the body. An antibody generally recognizes only a small region on the surface of a large molecule such as a polysaccharide or protein. The structure recognized by an antibody is called an **antigenic determinant** or **epitope**. Some of the most important pathogens have polysaccharide coats, and antibodies that recognize epitopes formed by the sugar subunits of these molecules are essential in providing immune protection against such pathogens. In many cases, however, the antigens that provoke an immune response are proteins. For example, protective antibodies against viruses recognize viral coat proteins. In all such cases, the structures recognized by the antibody are located on the surface of the protein. Such sites are likely to be composed of amino acids from different parts of the polypeptide chain that have been brought together by protein folding. Antigenic determinants of this kind are known as **conformational** or **discontinuous epitopes** because the structure recognized is composed of segments of the protein that are discontinuous in the amino acid sequence of the antigen but are brought together in the three-dimensional structure. In contrast, an epitope composed of a single segment of polypeptide chain is termed a **continuous** or **linear epitope**. Although most antibodies raised against intact, fully folded proteins recognize discontinuous epitopes, some will bind to peptide fragments of the protein. Conversely, antibodies raised against peptides of a protein or against synthetic peptides corresponding to part of its sequence are occasionally found to bind to the natural folded protein. This makes it possible, in some cases, to use synthetic peptides in vaccines that aim at raising antibodies against a pathogen protein.

4-9 Antigen–antibody interactions involve a variety of forces.

The interaction between an antibody and its antigen can be disrupted by high salt concentrations, by extremes of pH, by detergents, and sometimes

Petitioner Merck, Ex. 1170, p. 51
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

by competition with high concentrations of the pure epitope itself. The binding is therefore a reversible noncovalent interaction. The forces, or bonds, involved in these noncovalent interactions are outlined in Fig. 4.9.

Electrostatic interactions occur between charged amino acid side chains, as in salt bridges. Most antibody–antigen interactions involve at least one electrostatic interaction. Interactions also occur between electric dipoles, as in hydrogen bonds, or can involve short-range van der Waals forces. High salt concentrations and extremes of pH disrupt antigen–antibody binding by weakening electrostatic interactions and/or hydrogen bonds. This principle is employed in the purification of antigens by using affinity columns of immobilized antibodies, and vice versa for antibody purification (see Appendix I, Section A-5). Hydrophobic interactions occur when two hydrophobic surfaces come together to exclude water. The strength of a hydrophobic interaction is proportional to the surface area that is hidden from water, and for some antigens, hydrophobic interactions probably account for most of the binding energy. In some cases, water molecules are trapped in pockets in the interface between antigen and antibody. These trapped water molecules, especially those between polar amino acid residues, may also contribute to binding and hence to the specificity of the antibody.

The contribution of each of these forces to the overall interaction depends on the particular antibody and antigen involved. A striking difference between antibody interactions with protein antigens and most other natural protein–protein interactions is that antibodies often have many aromatic amino acids in their antigen-binding sites. These amino acids participate mainly in van der Waals and hydrophobic interactions, and sometimes in hydrogen bonds. Tyrosine, for example, can take part in both hydrogen bonding and hydrophobic interactions; it is therefore particularly suitable for providing diversity in antigen recognition and is over-represented in antigen-binding sites. In general, the hydrophobic and van der Waals forces operate over very short ranges and serve to pull together two surfaces that are complementary in shape: hills on one surface must fit into valleys on the other for good binding to occur. In contrast, electrostatic interactions between charged side chains, and hydrogen bonds bridging oxygen and/or nitrogen atoms, accommodate specific features or reactive groups while strengthening the interaction overall.

An example of a reaction involving a specific amino acid in the antigen can be seen in the complex of hen egg-white lysozyme with the antibody D1.3

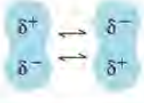
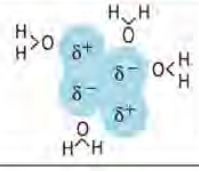
Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$-\overset{\oplus}{N}H_3 \quad \overset{\ominus}{O}OC-$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N, O)	$>N^{\delta-}-H^{\delta+}\cdots O^{\delta-}=C<$
Van der Waals forces	Fluctuations in electron clouds around molecules polarize neighboring atoms oppositely	
Hydrophobic forces	Hydrophobic groups interact unfavorably with water and tend to pack together to exclude water molecules. The attraction also involves van der Waals forces	

Fig. 4.9 The noncovalent forces that hold together the antigen:antibody complex. Partial charges found in electric dipoles are shown as δ^+ or δ^-. Electrostatic forces diminish as the inverse square of the distance separating the charges, whereas van der Waals forces, which are more numerous in most antigen–antibody contacts, fall off as the sixth power of the separation and therefore operate only over very short ranges. Covalent bonds never occur between antigens and naturally produced antibodies.

Petitioner Merck, Ex. 1170, p. 52
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

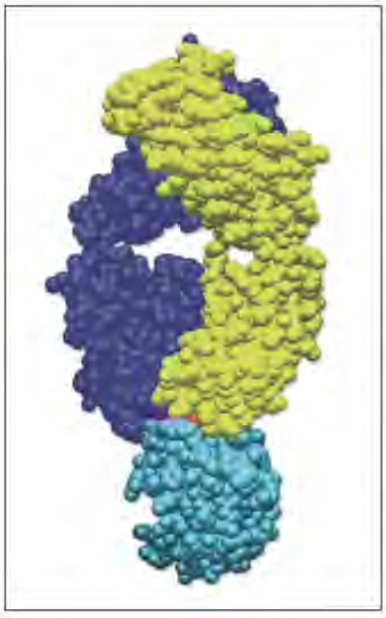


Fig. 4.10 The complex of lysozyme with the antibody D1.3. The interaction of the Fab fragment of D1.3 with hen egg-white lysozyme is shown, with the lysozyme in blue, the heavy chain in purple and the light chain in green. A glutamine residue of lysozyme, shown in red, protrudes between the two V domains of the antigen-binding site and makes hydrogen bonds that are important to the antigen–antibody binding. Courtesy of R.J. Poljak.

(Fig. 4.10), where strong hydrogen bonds are formed between the antibody and a particular glutamine in the lysozyme molecule that protrudes between the V_H and V_L domains. Lysozymes from partridge and turkey have another amino acid in place of the glutamine and do not bind to this antibody. In the high-affinity complex of hen egg-white lysozyme with another antibody, HyHel5 (see Fig. 4.8c), two salt bridges between two basic arginines on the surface of the lysozyme interact with two glutamic acids, one each from the V_H CDR1 and CDR2 loops. Lysozymes that lack one of the two arginine residues show a 1000-fold decrease in affinity for HyHel5. Overall surface complementarity must have an important role in antigen–antibody interactions, but in most antibodies that have been studied at this level of detail only a few residues make a major contribution to the binding energy and hence to the final specificity of the antibody. Although many antibodies naturally bind their ligands with high affinity (in the nanomolar range), genetic engineering by site-directed mutagenesis can tailor an antibody to bind even more strongly to its epitope.

Summary.

X-ray crystallographic analyses of antigen:antibody complexes have shown that the hypervariable loops (complementarity-determining regions, CDRs) of immunoglobulin V regions determine the binding specificity of an antibody. Contact between an antibody molecule and a protein antigen usually occurs over a broad area of the antibody surface that is complementary to the surface recognized on the antigen. Electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interactions can all contribute to binding. Depending on the size of the antigen, amino acid side chains in most or all of the CDRs make contact with antigen and determine both the specificity and the affinity of the interaction. Other parts of the V region normally play little part in the direct contact with the antigen, but they provide a stable structural framework for the CDRs and help to determine their position and conformation. Antibodies raised against intact proteins usually bind to the surface of the protein and make contact with residues that are discontinuous in the primary structure of the molecule; they may, however, occasionally bind peptide fragments of the protein, and antibodies raised against peptides derived from a protein can sometimes be used to detect the native protein molecule. Peptides binding to antibodies usually bind in a cleft or pocket between the V regions of the heavy and light chains, where they make specific contact with some, but not necessarily all, of the CDRs. This is also the usual mode of binding for carbohydrate antigens and small molecules such as haptens.

Antigen recognition by T cells.

In contrast to the immunoglobulins, which interact with pathogens and their toxic products in the extracellular spaces of the body, T cells recognize foreign antigens only when they are displayed on the surfaces of the body's own cells. These antigens can derive from pathogens such as viruses or intracellular bacteria, which replicate within cells, or from pathogens or their products that have been internalized by endocytosis from the extracellular fluid.

T cells detect the presence of an intracellular pathogen because the infected cells display on their surface peptide fragments of the pathogen's proteins. These foreign peptides are delivered to the cell surface by specialized host-cell glycoproteins—the MHC molecules. These are encoded in a large cluster

Petitioner Merck, Ex. 1170, p. 53
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

of genes that were first identified by their powerful effects on the immune response to transplanted tissues. For that reason, the gene complex was called the major histocompatibility complex (MHC) and the peptide-binding glycoproteins are known as MHC molecules. The recognition of antigen as a small peptide fragment bound to an MHC molecule and displayed at the cell surface is one of the most distinctive features of T cells, and will be the focus of this part of the chapter. How the peptide fragments of antigen are generated and become associated with MHC molecules will be described in Chapter 6.

We describe here the structure and properties of the T-cell receptor (TCR). As might be expected from their function as highly variable antigen-recognition structures, the genes for T-cell receptors are closely related to those for immunoglobulins. There are, however, important differences between T-cell receptors and immunoglobulins that reflect the special features of antigen recognition by T cells.

4-10 The T-cell receptor is very similar to a Fab fragment of immunoglobulin.

T-cell receptors were first identified by using monoclonal antibodies that bound to a single cloned T-cell line: such antibodies either specifically inhibit antigen recognition by the clone or specifically activate it by mimicking the antigen (see Appendix I, Section A-19). These **clonotypic** antibodies were then used to show that each T cell bears about 30,000 identical antigen receptors on its surface, each receptor consisting of two different polypeptide chains, termed the **T-cell receptor α** (**TCRα**) and **β** (**TCRβ**) chains, linked by a disulfide bond. The **α:β heterodimers** are very similar in structure to the Fab fragment of an immunoglobulin molecule (Fig. 4.11), and they account for antigen recognition by most T cells. A minority of T cells bear an alternative, but structurally similar, receptor made up of a different pair of polypeptide chains designated **γ** and **δ**. The **γ:δ T-cell receptors** seem to have different antigen-recognition properties from the **α:β T-cell receptors**, and the function of γ:δ T cells in immune responses is not yet entirely clear (see Section 3-24). In the rest of this chapter and elsewhere in the book we use the term T-cell receptor to mean the α:β receptor, except where specified otherwise. Both types of T-cell receptors differ from the membrane-bound immunoglobulin that serves as the B-cell receptor in two main ways. A T-cell receptor has only one antigen-binding site, whereas a B-cell receptor has two, and T-cell receptors are never secreted, whereas immunoglobulin can be secreted as antibody.

Further insights into the structure and function of the α:β T-cell receptor came from studies of cloned cDNA encoding the receptor chains. The amino acid sequences predicted from the cDNA showed that both chains of the T-cell receptor have an amino-terminal variable (V) region with homology to an immunoglobulin V domain, a constant (C) region with homology to an immunoglobulin C domain, and a short stalk segment containing a cysteine residue that forms the interchain disulfide bond (Fig. 4.12). Each chain spans the lipid bilayer by a hydrophobic transmembrane domain, and ends in a short cytoplasmic tail. These close similarities of T-cell receptor chains to

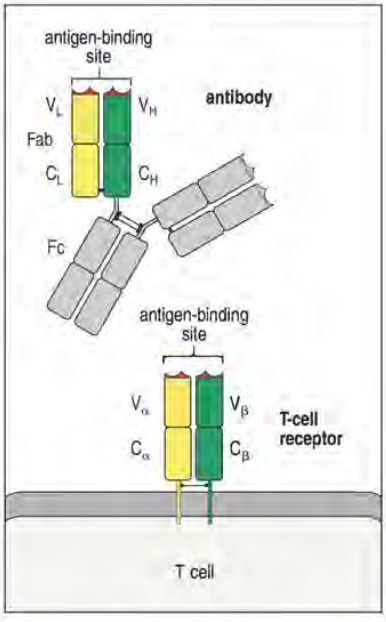


Fig. 4.11 The T-cell receptor resembles a membrane-bound Fab fragment. The Fab fragment of an antibody molecule is a disulfide-linked heterodimer, each chain of which contains one immunoglobulin C domain and one V domain; the juxtaposition of the V domains forms the antigen-binding site (see Section 4-6). The T-cell receptor is also a disulfide-linked heterodimer, with each chain containing an immunoglobulin C-like domain and an immunoglobulin V-like domain. As in the Fab fragment, the juxtaposition of the V domains forms the site for antigen recognition.

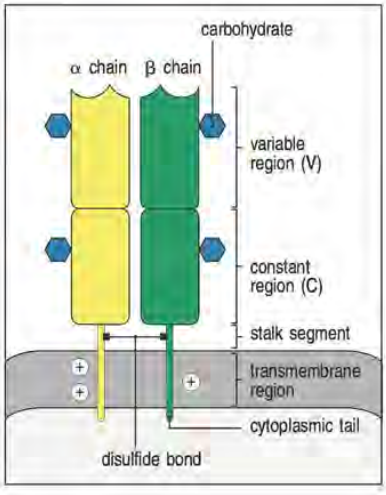


Fig. 4.12 Structure of the T-cell receptor. The T-cell receptor heterodimer is composed of two transmembrane glycoprotein chains, α and β. The extracellular portion of each chain consists of two domains, resembling immunoglobulin V and C domains, respectively. Both chains have carbohydrate side chains attached to each domain. A short stalk segment, analogous to an immunoglobulin hinge region, connects the Ig-like domains to the membrane and contains the cysteine residue that forms the interchain disulfide bond. The transmembrane helices of both chains are unusual in containing positively charged (basic) residues within the hydrophobic transmembrane segment. The α chain carries two such residues; the β chain has one.

Petitioner Merck, Ex. 1170, p. 54
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

the heavy and light immunoglobulin chains first enabled prediction of the structural resemblance of the T-cell receptor heterodimer to a Fab fragment of immunoglobulin.

The three-dimensional structure of the T-cell receptor has since been determined by X-ray crystallography, and the two structures are indeed similar: the T-cell receptor chains fold in much the same way as those of a Fab fragment (Fig. 4.13a), although the final structure appears a little shorter and wider. There are, however, some distinct structural differences between T-cell receptors and Fab fragments. The most striking is in the C_α domain, where the fold is unlike that of any other Ig-like domain. The half of the domain that is juxtaposed with the C_β domain forms a β sheet similar to that found in other Ig-like domains, but the other half of the domain is formed of loosely packed strands and a short segment of α helix (Fig. 4.13b). In a C_α domain the intramolecular disulfide bond, which in Ig-like domains normally joins two β strands, joins a β strand to this segment of α helix.

There are also differences in the way in which the domains interact. The interface between the V and C domains of both T-cell receptor chains is more extensive than in most antibodies. The interaction between the C_α and C_β domains is distinctive as it might be assisted by carbohydrate, with a sugar group from the C_α domain making a number of hydrogen bonds to the C_β domain (see Fig. 4.13b). Finally, a comparison of the variable binding sites shows that, although the CDR loops align fairly closely with those of antibody molecules, there is some relative displacement (see Fig. 4.13c). This is particularly marked in the V_α CDR2 loop, which is oriented at roughly right angles to the equivalent loop in antibody V domains, as a result of a shift in the β strand that anchors one end of the loop from one face of the domain to the other. A strand displacement also causes a change in the orientation of the V_β CDR2 loop in some V_β domains whose structures are known. As relatively few crystallographic structures have been solved to this level of resolution, it remains to be seen to what degree all T-cell receptors share these features, and whether there are more differences to be discovered.

4-11 A T-cell receptor recognizes antigen in the form of a complex of a foreign peptide bound to an MHC molecule.

Antigen recognition by T-cell receptors clearly differs from recognition by B-cell receptors and antibodies. The immunoglobulin on B cells binds directly to the intact antigen and, as discussed in Section 4-8, antibodies typically bind to the surface of protein antigens, contacting amino acids that are discontinuous in the primary structure but are brought together in the folded protein. T cells, in contrast, respond to short continuous amino acid

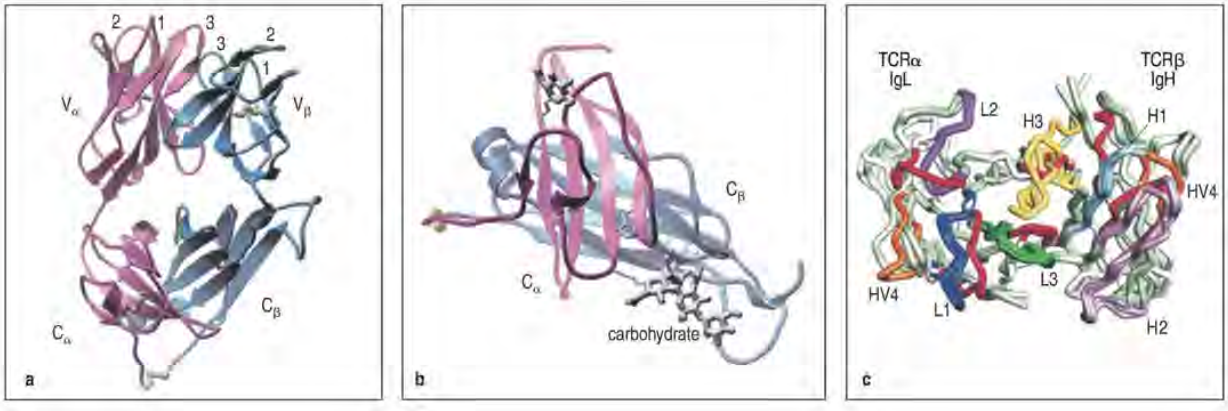


Fig. 4.13 The crystal structure of an α:β T-cell receptor resolved at 2.5 Å. In panels a and b the α chain is shown in pink and the β chain in blue. Disulfide bonds are shown in green. In panel a, the T-cell receptor is viewed from the side as it would sit on a cell surface, with the CDR loops that form the antigen-binding site (labeled 1, 2, and 3) arrayed across its relatively flat top. In panel b, the C_α and C_β domains are shown. The C_α domain does not fold into a typical Ig-like domain; the face of the domain away from the C_β domain is mainly composed of irregular strands of polypeptide rather than β sheet. The intramolecular disulfide bond joins a β strand to this segment of α helix. The interaction between the C_α and C_β domains is assisted by carbohydrate (colored gray and labeled on the figure), with a sugar group from the C_α domain making hydrogen bonds to the C_β domain. In panel c, the T-cell receptor is shown aligned with the antigen-binding sites from three different antibodies. This view is looking down into the binding site. The V_α domain of the T-cell receptor is aligned with the V_L domains of the antigen-binding sites of the antibodies, and the V_β domain is aligned with the V_H domains. The CDRs of the T-cell receptor and immunoglobulin molecules are colored, with CDRs 1, 2, and 3 of the TCR shown in red and the HV4 loop in orange. For the immunoglobulin V domains, the CDR1 loops of the heavy chain (H1) and light chain (L1) are shown in light and dark blue, respectively, and the CDR2 loops (H2, L2) in light and dark purple, respectively. The heavy-chain CDR3 loops (H3) are in yellow; the light-chain CDR3s (L3) are in bright green. The HV4 loops of the TCR (orange) have no hypervariable counterparts in immunoglobulins. Model structures courtesy of I.A. Wilson.

Petitioner Merck, Ex. 1170, p. 55
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

sequences. These sequences are often buried within the native structure of the protein and thus cannot be recognized directly by T-cell receptors unless the protein is unfolded and processed into peptide fragments (Fig. 4.14). We shall see in Chapter 6 how this occurs.

The nature of the antigen recognized by T cells became clear with the realization that the peptides that stimulate T cells are recognized only when bound to an MHC molecule. The ligand recognized by the T cell is thus a complex of peptide and MHC molecule. The evidence for involvement of the MHC in T-cell recognition of antigen was at first indirect, but it has been proved conclusively by stimulating T cells with purified peptide:MHC complexes. The T-cell receptor interacts with this ligand by making contacts with both the MHC molecule and the antigen peptide.

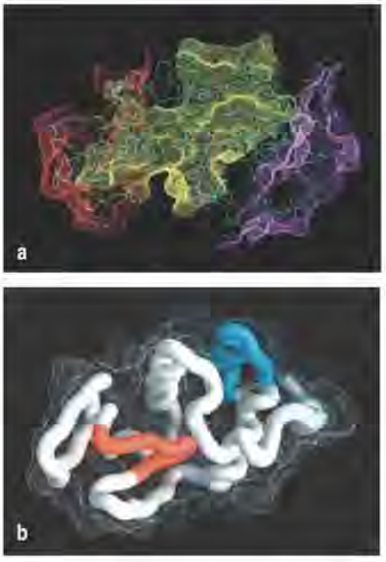


Fig. 4.14 Differences in the recognition of hen egg-white lysozyme by immunoglobulins and T-cell receptors. Antibodies can be shown by X-ray crystallography to bind epitopes on the surface of proteins, as shown in panel a, where the epitopes for three antibodies are shown in different colors on the surface of hen egg-white lysozyme (see also Fig. 4.10). In contrast, the epitopes recognized by T-cell receptors need not lie on the surface of the molecule, because the T-cell receptor recognizes not the antigenic protein itself but a peptide fragment of the protein. The peptides corresponding to two T-cell epitopes of lysozyme are shown in panel b. One epitope, shown in blue, lies on the surface of the protein but a second, shown in red, lies mostly within the core and is inaccessible in the folded protein. For this residue to be accessible to the T-cell receptor, the protein must be unfolded and processed. Panel a courtesy of S. Sheriff.

4-12 There are two classes of MHC molecules with distinct subunit compositions but similar three-dimensional structures.

There are two classes of MHC molecules—**MHC class I** and **MHC class II**—which differ in both their structure and in their expression pattern in the tissues of the body. As shown in Figs 4.15 and 4.16, MHC class I and MHC class II molecules are closely related in overall structure but differ in their subunit compositions. In both classes, the two paired protein domains nearest to the membrane resemble immunoglobulin domains, whereas the two domains furthest away from the membrane fold together to create a long cleft, or groove, which is the site at which a peptide binds. Purified peptide:MHC class I and peptide:MHC class II complexes have been characterized structurally, allowing us to describe in detail both the MHC molecules themselves and the way in which they bind peptides.

MHC class I molecules (see Fig. 4.15) consist of two polypeptide chains. One chain, the α chain, is encoded in the MHC (on chromosome 6 in humans) and is noncovalently associated with a smaller chain, **β_2-microglobulin**, which is not polymorphic and is encoded on a different chromosome—chromosome 15 in humans. Only the class I α chain spans the membrane. The complete molecule has four domains, three formed from the MHC-encoded α chain, and one contributed by β_2-microglobulin. The α_3 domain and β_2-microglobulin closely resemble Ig-like domains in their folded structure. The folded α_1 and α_2 domains form the walls of a cleft on the surface of the molecule; this is where the peptide binds and is known as the **peptide-binding cleft** or **peptide-binding groove**. The MHC molecules are highly polymorphic and the major differences between the different forms are located in the peptide-binding cleft, influencing which peptides will bind and thus the specificity of the dual antigen presented to T cells.

An MHC class II molecule consists of a noncovalent complex of two chains, α and β, both of which span the membrane (see Fig. 4.16). The MHC class II α chain is a different protein from the class I α chain. The MHC class II α and β chains are both encoded within the MHC. The crystallographic structure of the MHC class II molecule shows that it is folded very much like the MHC class I molecule, but the peptide-binding cleft is formed by two domains from different chains, the α_1 and β_1 domains. The major differences lie at the ends of the peptide-binding cleft, which are more open in MHC class II molecules than in MHC class I molecules. Consequently, the ends of a peptide bound to an MHC class I molecule are substantially buried within the molecule, whereas the ends of peptides bound to MHC class II molecules are not. In both MHC class I and class II molecules, bound peptides are sandwiched between the two α-helical segments of the MHC molecule (Fig. 4.17). The T-cell receptor interacts with this compound ligand, making contacts with both the MHC molecule and the peptide antigen. The sites of major polymorphism in MHC class II molecules are again located in the peptide-binding cleft.

Petitioner Merck, Ex. 1170, p. 56
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

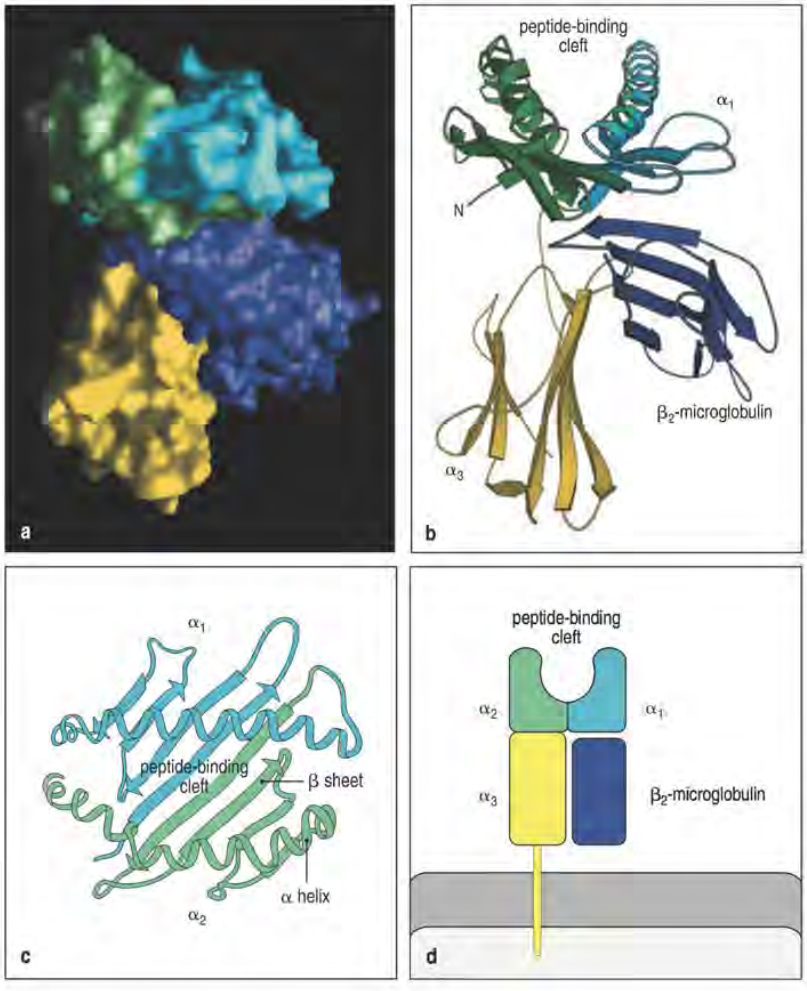


Fig. 4.15 The structure of an MHC class I molecule determined by X-ray crystallography. Panel a shows a computer graphic representation of a human MHC class I molecule, HLA-A2, which has been cleaved from the cell surface by the enzyme papain. The surface of the molecule is shown, colored according to the domains shown in panels b–d and described below. Panels b and c show a ribbon diagram of that structure. Shown schematically in panel d, the MHC class I molecule is a heterodimer of a membrane-spanning α chain (molecular weight 43 kDa) bound noncovalently to β_2-microglobulin (12 kDa), which does not span the membrane. The α chain folds into three domains: α_1, α_2, and α_3. The α_3 domain and β_2-microglobulin show similarities in amino acid sequence to immunoglobulin C domains and have similar folded structures, whereas the α_1 and α_2 domains fold together into a single structure consisting of two separated α helices lying on a sheet of eight antiparallel β strands. The folding of the α_1 and α_2 domains creates a long cleft or groove, which is the site at which peptide antigens bind to the MHC molecules. The transmembrane region and the short stretch of peptide that connects the external domains to the cell surface are not seen in panels a and b because they have been removed by the digestion with papain. As can be seen in panel c, looking down on the molecule from above, the sides of the cleft are formed from the inner faces of the two α helices; the β-pleated sheet formed by the pairing of the α_1 and α_2 domains creates the floor of the cleft.

4-13 Peptides are stably bound to MHC molecules, and also serve to stabilize the MHC molecule on the cell surface.

An individual can be infected by a wide variety of pathogens, whose proteins will not generally have peptide sequences in common. If T cells are to be alerted to all possible infections, the MHC molecules on each cell (both class I and class II) must be able to bind stably to many different peptides. This behavior is quite distinct from that of other peptide-binding receptors, such as those for peptide hormones, which usually bind only a single type of peptide. The crystal structures of peptide:MHC complexes have helped to show how a single binding site can bind peptides with high affinity while retaining the ability to bind a wide variety of different peptides.

An important feature of the binding of peptides to MHC molecules is that the peptide is bound as an integral part of the MHC molecule's structure, and MHC molecules are unstable when peptides are not bound. Stable peptide binding is important, because otherwise peptide exchanges occurring at the cell surface would prevent peptide:MHC complexes from being reliable indicators of infection or of uptake of a specific antigen. When MHC molecules are purified from cells, their stably bound peptides co-purify with them, and this has enabled the peptides bound by particular MHC molecules to be

Petitioner Merck, Ex. 1170, p. 57
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

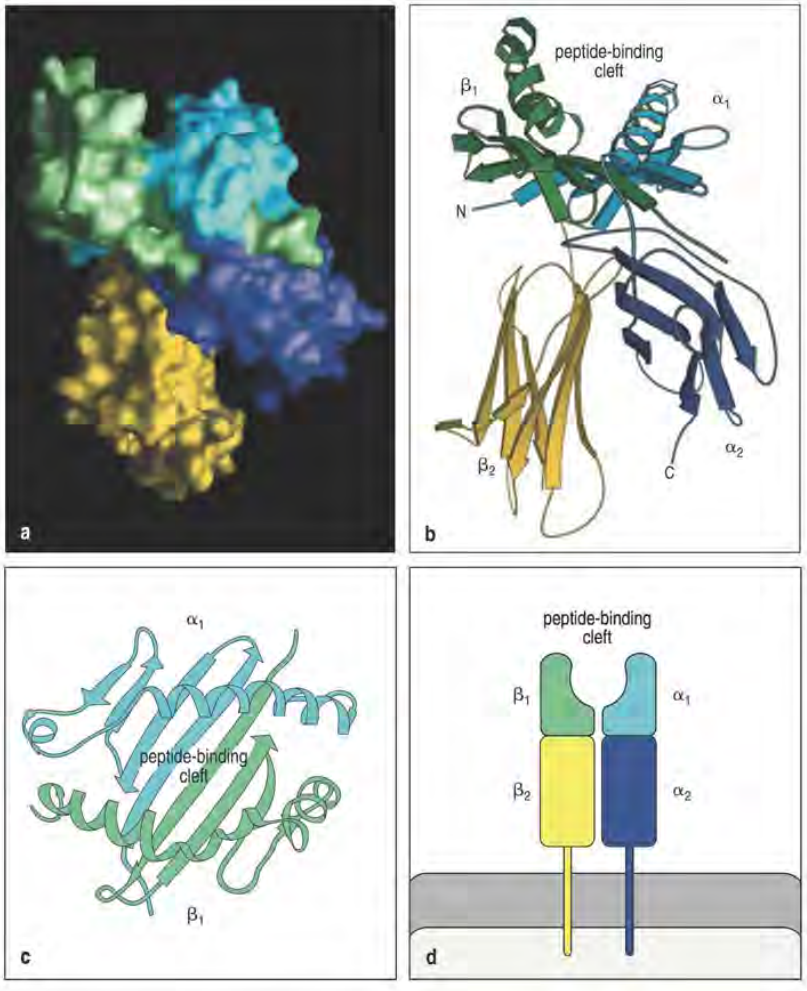


Fig. 4.16 MHC class II molecules resemble MHC class I molecules in overall structure. The MHC class II molecule is composed of two transmembrane glycoprotein chains, α (34 kDa) and β (29 kDa), as shown schematically in panel d. Each chain has two domains, and the two chains together form a compact four-domain structure similar to that of the MHC class I molecule (compare with panel d of Fig. 4.15). Panel a shows a computer graphic representation of the surface of the MHC class II molecule, in this case the human protein HLA-DR1, and panel b shows the equivalent ribbon diagram. N, amino terminus; C, carboxy terminus. The α_2 and β_2 domains, like the α_3 and β_2-microglobulin domains of the MHC class I molecule, have amino acid sequence and structural similarities to immunoglobulin C domains; in the MHC class II molecule the two domains forming the peptide-binding cleft are contributed by different chains and are therefore not joined by a covalent bond (see panels c and d). Another important difference, not apparent in this diagram, is that the peptide-binding groove of the MHC class II molecule is open at both ends.

analyzed. Peptides are released from the MHC molecules by denaturing the complex in acid, and they are then purified and sequenced. Pure synthetic peptides can also be incorporated into empty MHC molecules and the structure of the complex determined, revealing details of the contacts between the MHC molecule and the peptide. From such studies a detailed picture of the binding interactions has been built up. We first discuss the peptide-binding properties of MHC class I molecules.

4-14 MHC class I molecules bind short peptides of 8–10 amino acids by both ends.

Binding of a peptide to an MHC class I molecule is stabilized at both ends of the peptide-binding cleft by contacts between atoms in the free amino and carboxy termini of the peptide and invariant sites that are found at each end of the cleft in all MHC class I molecules (Fig. 4.18). These are thought to be the main stabilizing contacts for peptide:MHC class I complexes, because synthetic peptide analogs lacking terminal amino and carboxyl groups fail to bind stably to MHC class I molecules. Other residues in the peptide serve as additional anchors. Peptides that bind to MHC class I molecules are usually 8–10 amino acids long. Longer peptides are thought to bind, however, particularly

Petitioner Merck, Ex. 1170, p. 58
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

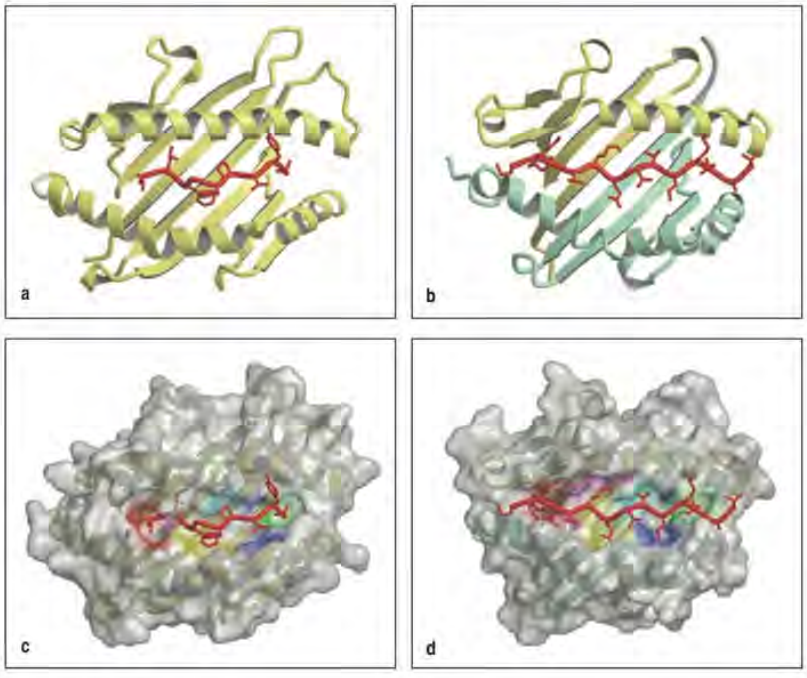


Fig. 4.17 MHC molecules bind peptides tightly within the cleft. When MHC molecules are crystallized with a single synthetic peptide antigen, the details of peptide binding are revealed. In MHC class I molecules (panels a and c) the peptide is bound in an elongated conformation with both ends tightly bound at either end of the cleft. In MHC class II molecules (panels b and d) the peptide is also bound in an elongated conformation but the ends of the peptide are not tightly bound and the peptide extends beyond the cleft. The upper surface of the peptide:MHC complex is recognized by T cells, and is composed of residues of the MHC molecule and the peptide. In representations c and d, the electrostatic potential of the MHC molecule surface is shown, with blue areas indicating a positive potential and red a negative potential. Structures courtesy of R.L. Stanfield and I.A. Wilson.

if they can bind at their carboxy terminus, but they are subsequently cleaved by exopeptidases present in the endoplasmic reticulum, which is where MHC class I molecules bind peptides. The peptide lies in an elongated conformation along the cleft; variations in peptide length seem to be accommodated, in most cases, by a kinking in the peptide backbone. However, two examples of MHC class I molecules in which the peptide is able to extend out of the cleft at the carboxy terminus suggest that some length variation can also be accommodated in this way.

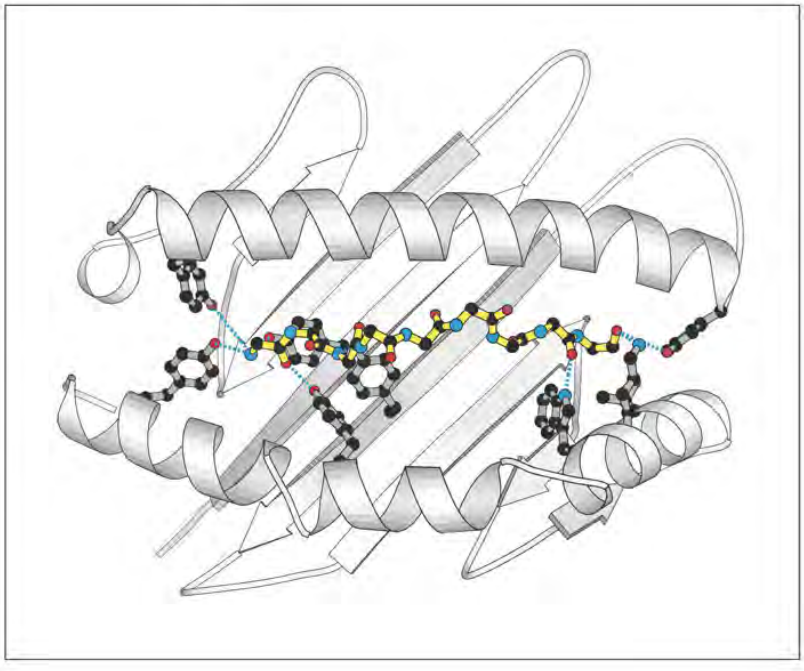


Fig. 4.18 Peptides are bound to MHC class I molecules by their ends. MHC class I molecules interact with the backbone of a bound peptide (shown in yellow) through a series of hydrogen bonds and ionic interactions (shown as dotted blue lines) at each end of the peptide. The amino terminus of the peptide is to the left, the carboxy terminus to the right. Black circles are carbon atoms; red are oxygen; blue are nitrogen. The amino acid residues in the MHC molecule that form these bonds are common to all MHC class I molecules, and their side chains are shown in full (in gray) on a ribbon diagram of the MHC class I groove. A cluster of tyrosine residues common to all MHC class I molecules forms hydrogen bonds to the amino terminus of the bound peptide, while a second cluster of residues forms hydrogen bonds and ionic interactions with the peptide backbone at the carboxy terminus and with the carboxy terminus itself.

Petitioner Merck, Ex. 1170, p. 59
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

These interactions give MHC class I molecules a broad peptide-binding specificity. In addition, MHC molecules are highly polymorphic. There are hundreds of different versions, or **alleles**, of the MHC class I genes in the human population, and each individual carries only a small selection. The main differences between allelic MHC variants are found at certain sites in the peptide-binding cleft, resulting in different amino acids in key peptide-interaction sites. In consequence, the different MHC variants preferentially bind different peptides. The peptides that can bind to a given MHC variant have the same or very similar amino acid residues at two or three particular positions along the peptide sequence. The amino acid side chains at these positions insert into pockets in the MHC molecule that are lined by the polymorphic amino acids. Because this binding anchors the peptide to the MHC molecule, the peptide residues involved are called the **anchor residues**. Both the position and identity of these anchor residues can vary, depending on the particular MHC class I variant that is binding the peptide. However, most peptides that bind to MHC class I molecules have a hydrophobic (or sometimes basic) anchor residue at the carboxy terminus (Fig. 4.19). Whereas changing an anchor residue will in most cases prevent the peptide from binding, not every synthetic peptide of suitable length that contains these anchor residues will bind the appropriate MHC class I molecule, and so the overall binding must also depend on the nature of the amino acids at other positions in the peptide. In some cases, particular amino acids are preferred in certain positions, whereas in others the presence of particular amino acids prevents binding. These additional amino acid positions are called 'secondary anchors.' These features of peptide binding enable an individual MHC class I molecule to bind a wide variety of different peptides, yet allow different MHC class I allelic variants to bind different sets of peptides.

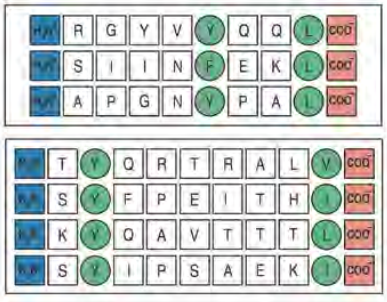


Fig. 4.19 Peptides bind to MHC molecules through structurally related anchor residues. Peptides eluted from two different MHC class I molecules are shown in the upper and lower panels, respectively. The anchor residues (green) differ for peptides that bind different alleles of MHC class I molecules but are similar for all peptides that bind to the same MHC molecule. The anchor residues that bind a particular MHC molecule need not be identical, but are always related (for example, phenylalanine (F) and tyrosine (Y) are both aromatic amino acids, whereas valine (V), leucine (L), and isoleucine (I) are all large hydrophobic amino acids). Peptides also bind to MHC class I molecules through their amino (blue) and carboxy (red) termini.

4-15 The length of the peptides bound by MHC class II molecules is not constrained.

Peptide binding to MHC class II molecules has also been analyzed by elution of bound peptides and by X-ray crystallography, and differs in several ways from peptide binding to MHC class I molecules. Natural peptides that bind to MHC class II molecules are at least 13 amino acids long and can be much longer. The clusters of conserved residues that bind the two ends of a peptide in MHC class I molecules are not found in MHC class II molecules, and the ends of the peptide are not bound. Instead, the peptide lies in an extended conformation along the peptide-binding cleft. It is held there both by peptide side chains that protrude into shallow and deep pockets lined by polymorphic residues and by interactions between the peptide backbone and side chains of conserved amino acids that line the peptide-binding cleft in all MHC class II molecules (Fig. 4.20). Structural data show that amino acid side chains at residues 1, 4, 6, and 9 of an MHC class II-bound peptide can be held in these binding pockets.

The binding pockets of MHC class II molecules accommodate a greater variety of side chains than those of MHC class I molecules, making it more difficult to define anchor residues and to predict which peptides will be able to bind a particular MHC class II variant (Fig. 4.21). Nevertheless, by comparing the sequences of known binding peptides it is usually possible to detect patterns of amino acids that permit binding to different MHC class II variants, and to model how the amino acids of this peptide sequence motif will interact with the amino acids of the peptide-binding cleft. Because the peptide is bound by its backbone and allowed to emerge from both ends of the binding groove, there is, in principle, no upper limit to the length of peptides that could bind to MHC class II molecules. However, it seems that longer peptides bound to MHC class II molecules are trimmed by peptidases to a length of around 13–17 amino acids in most cases. Like MHC class I molecules, MHC class II molecules that lack bound peptide are unstable.

Petitioner Merck, Ex. 1170, p. 60
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

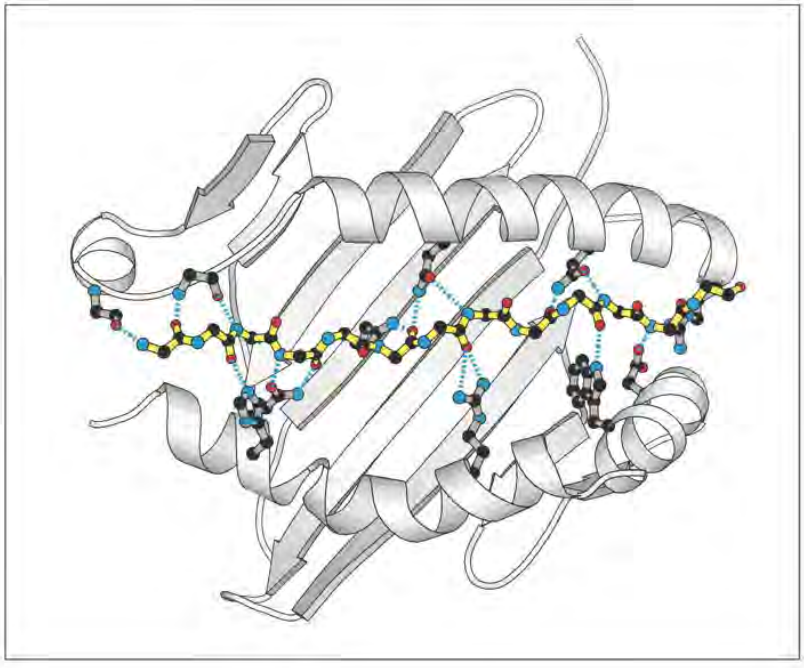


Fig. 4.20 Peptides bind to MHC class II molecules by interactions along the length of the binding groove. A peptide (yellow; shown as the peptide backbone only, with the amino terminus to the left and the carboxy terminus to the right) is bound by an MHC class II molecule through a series of hydrogen bonds (dotted blue lines) that are distributed along the length of the peptide. The hydrogen bonds toward the amino terminus of the peptide are made with the backbone of the MHC class II polypeptide chain, whereas throughout the peptide's length bonds are made with residues that are highly conserved in MHC class II molecules. The side chains of these residues are shown in gray on the ribbon diagram of the MHC class II groove.

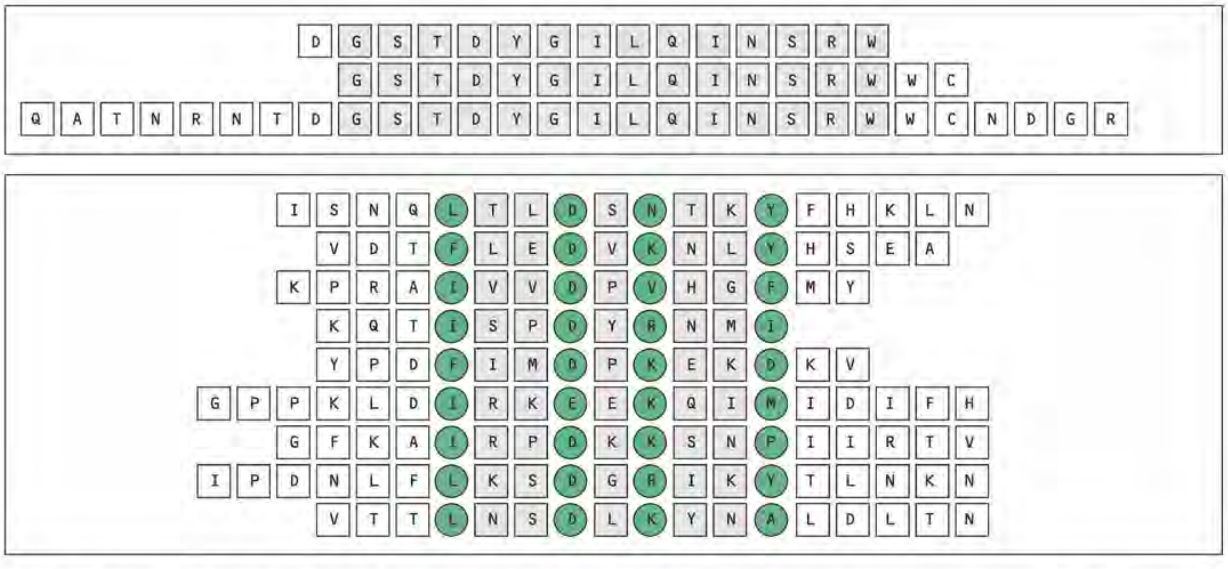


Fig. 4.21 Peptides that bind MHC class II molecules are variable in length and their anchor residues lie at various distances from the ends of the peptide. The sequences of a set of peptides that bind to the mouse MHC class II A^k allele are shown in the upper panel. All contain the same core sequence (shaded) but differ in length. In the lower panel, different peptides binding to the human MHC class II allele HLA-DR3 are shown. Anchor residues are shown as green circles. The lengths of these peptides can vary, and so by convention the first anchor residue is denoted as residue 1. Note that all of the peptides share a hydrophobic residue in position 1, a negatively charged residue (aspartic acid (D) or glutamic acid (E)) in position 4, and a tendency to have a basic residue (lysine (K), arginine (R), histidine (H), glutamine (Q), or asparagine (N)) in position 6 and a hydrophobic residue (for example, tyrosine (Y), leucine (L), phenylalanine (F)) in position 9.

Petitioner Merck, Ex. 1170, p. 61
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

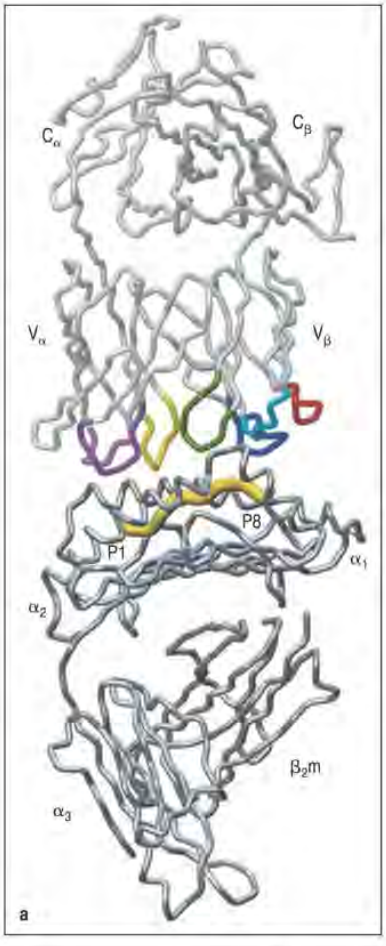
4-16 The crystal structures of several peptide:MHC:T-cell receptor complexes show a similar orientation of the T-cell receptor over the peptide:MHC complex.

At the time that the first X-ray crystallographic structure of a T-cell receptor was published, a structure of the same T-cell receptor bound to a peptide:MHC class I ligand was also produced. This structure (Fig. 4.22), which had been predicted by site-directed mutagenesis of the MHC class I molecule, showed the T-cell receptor aligned diagonally over the peptide and the peptide-binding cleft, with the TCRα chain lying over the α_2 domain and the amino-terminal end of the bound peptide, the TCRβ chain lying over the α_1 domain and the carboxy-terminal end of the peptide, and the CDR3 loops of both TCRα and TCRβ chains meeting over the central amino acids of the peptide. The T-cell receptor is threaded through a valley between the two high points on the two surrounding α helices that form the walls of the peptide-binding cleft.

Analysis of other peptide:MHC class I:T-cell receptor complexes and of peptide:MHC class II:T-cell receptor complexes (Fig. 4.23) shows that all have a very similar orientation, particularly for the V_α domain, although some variability does occur in the location and orientation of the V_β domain. In this orientation, the V_α domain makes contact primarily with the amino-terminal half of the bound peptide, whereas the V_β domain contacts primarily the carboxy-terminal half. Both chains also interact with the α helices of the MHC class I molecule (see Fig. 4.22). The T-cell receptor contacts are not symmetrically distributed over the MHC molecule: whereas the V_α CDR1 and CDR2 loops are in close contact with the helices of the peptide:MHC complex around the amino terminus of the bound peptide, the β-chain CDR1 and CDR2 loops, which interact with the complex at the carboxy terminus of the bound peptide, have variable contributions to the binding.

Comparison of the three-dimensional structure of an unliganded T-cell receptor and the same T-cell receptor complexed to its peptide:MHC ligand shows that the binding results in some degree of conformational change, or 'induced fit,' particularly within the V_α CDR3 loop. It has also been shown that subtly different peptides can have strikingly different effects on the recognition of an otherwise identical peptide:MHC ligand by the same T cell. The flexibility in the CDR3 loop demonstrated by these two structures helps to explain how the T-cell receptor can adopt conformations that recognize related, but different, ligands.

From an examination of the available structures it is hard to predict whether the main binding energy is contributed by T-cell receptor contacts with the bound peptide or by T-cell receptor contacts with the MHC molecule.



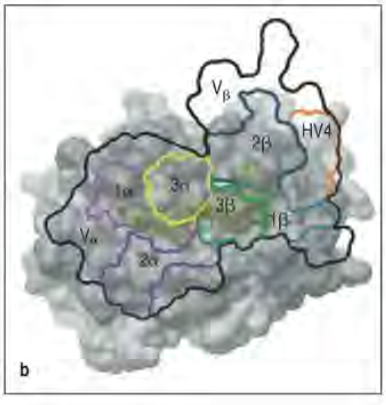


Fig. 4.22 The T-cell receptor binds to the peptide:MHC complex. Panel a: the T-cell receptor binds to the top of the peptide:MHC complex, straddling, in the case of the MHC class I molecule shown here, both the α_1 and α_2 domain helices. The CDRs of the T-cell receptor are indicated in color: the CDR1 and CDR2 loops of the β chain in light and dark blue, respectively; and the CDR1 and CDR2 loops of the α chain in light and dark purple, respectively. The α-chain CDR3 loop is in yellow, and the β-chain CDR3 loop is in green. The β-chain HV4 loop is in red. The thick yellow line P1–P8 is the bound peptide. Panel b: the outline of the T-cell receptor's antigen-binding site (thick black line) is superimposed on the top surface of the peptide:MHC complex (the peptide is shaded dull yellow). The T-cell receptor lies diagonally across the peptide:MHC complex, with the α and β CDR3 loops of the T-cell receptor (3α, 3β, yellow and green, respectively) contacting the center of the peptide. The α-chain CDR1 and CDR2 loops (1α, 2α, light and dark purple, respectively) contact the MHC helices at the amino terminus of the bound peptide, whereas the β-chain CDR1 and CDR2 loops (1β, 2β, light and dark blue, respectively) make contact with the helices at the carboxy terminus of the bound peptide. Courtesy of I.A. Wilson.

Petitioner Merck, Ex. 1170, p. 62
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

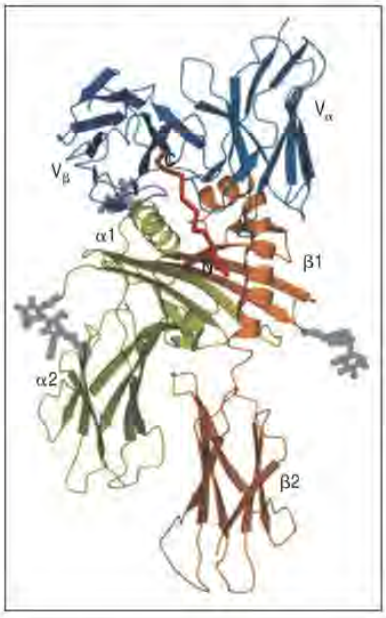


Fig. 4.23 The T-cell receptor interacts with MHC class I and MHC class II molecules in a similar fashion. The structure of a T-cell receptor binding to an MHC class II molecule has been determined, and shows the T-cell receptor binding to an equivalent site, and in an equivalent orientation, to the way in which T-cell receptors bind to MHC class I molecules (see Fig. 4.22). Only the V_α and V_β domains of the T-cell receptor are shown, colored in blue. The peptide is colored red, and carbohydrate residues are indicated in gray. The T-cell receptor sits in a shallow saddle formed between the α-helical regions of the MHC class II α (yellow-green) and β chain (orange), at roughly 90° to the long axis of the MHC class II molecule and the bound peptide. Courtesy of E.L. Reinherz and J-H. Wang.

Measurements of the kinetics of T-cell receptor binding to peptide:MHC ligands suggest that the interactions between the T-cell receptor and the MHC molecule might predominate at the start of the contact, guiding the receptor into the correct position where a second, more detailed, interaction with the peptide as well as the MHC molecule dictates the final outcome of the interaction—binding or dissociation. As with antibody–antigen interactions, only a few amino acids at the interface might provide the essential contacts that determine the specificity and strength of binding. Simply changing a leucine to isoleucine in the peptide, for example, is sufficient to alter a T-cell response from strong killing to no response at all. Mutations of single residues in the presenting MHC molecules can have the same effect. Thus, the specificity of T-cell recognition involves both the peptide and its presenting MHC molecule. This dual specificity underlies the MHC restriction of T-cell responses, a phenomenon that was observed long before the peptide-binding properties of MHC molecules were known. We will recount the story of how MHC restriction was discovered when we return to the issue of how MHC polymorphism affects antigen recognition by T cells in Chapter 6. Another consequence of this dual specificity is a need for T-cell receptors to be able to interact appropriately with the antigen-presenting surface of MHC molecules. It seems that some inherent specificity for MHC molecules is encoded in the T-cell receptor genes, and there is selection during T-cell development for a repertoire of receptors able to interact appropriately with the particular MHC molecules present in that individual (discussed in Chapter 8).

4-17 The CD4 and CD8 cell-surface proteins of T cells are required to make an effective response to antigen.

As well as engaging a peptide:MHC ligand via its antigen receptor, a T cell makes additional interactions with the MHC molecule that stabilize the interaction and are required for the cell to respond effectively to antigen. T cells fall into two major classes, which have different effector functions and are distinguished by the expression of the cell-surface proteins **CD4** and **CD8**. CD8 is carried by cytotoxic T cells, while CD4 is carried by T cells whose function is to activate other cells (see Section 1-19). CD4 and CD8 were known as markers for these functional sets for some time before it became clear that the distinction was based on the ability of T cells to recognize different classes of MHC molecules: CD8 recognizes MHC class I molecules and CD4 recognizes MHC class II. During antigen recognition, CD4 or CD8 (depending on the type of T cell) associates on the T-cell surface with the T-cell receptor and binds to invariant sites on the MHC portion of the composite peptide:MHC ligand, away from the peptide-binding site. This binding is required for the T cell to make an effective response, and so CD4 and CD8 are called **co-receptors**.

CD4 is a single-chain protein composed of four Ig-like domains (Fig. 4.24). The first two domains (D1 and D2) are packed tightly together to form a rigid rod about 60 Å long, which is joined by a flexible hinge to a similar rod formed by the third and fourth domains (D3 and D4). The MHC-binding region on CD4 is located mainly on a lateral face of the D1 domain, and CD4 binds to a hydrophobic crevice formed at the junction of the α_2 and β_2 domains of the MHC class II molecule. This site is well away from the site where the T-cell receptor binds (Fig. 4.25a), and so the CD4 molecule and the T-cell receptor can bind simultaneously to the same peptide:MHC class II complex. The intracellular portion of CD4 interacts strongly with a cytoplasmic tyrosine kinase called Lck, and brings Lck close to the intracellular signaling components associated with the T-cell receptor. This results in enhancement of the signal that is generated when the T-cell receptor binds its ligand, as we discuss in Chapter 7. When CD4 and the T-cell receptor bind simultaneously to the same MHC class II:peptide complex, the T cell is about a hundredfold more sensitive to the antigen than if CD4 were absent.

Petitioner Merck, Ex. 1170, p. 63
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

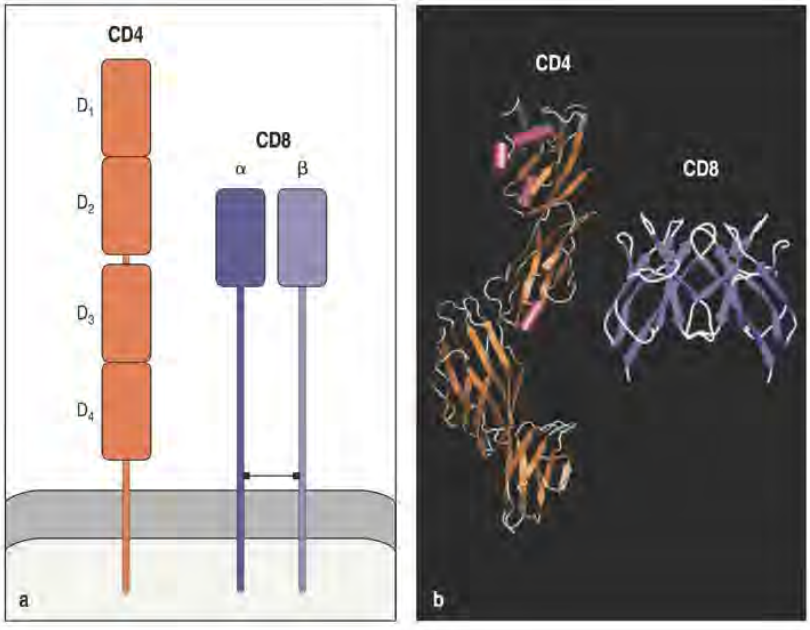


Fig. 4.24 The structures of the CD4 and CD8 co-receptor molecules. The CD4 molecule contains four Ig-like domains, shown in schematic form in panel a and as a ribbon diagram of the crystal structure in panel b. The amino-terminal domain, D_1, is similar in structure to an immunoglobulin V domain. The second domain, D_2, although clearly related to an immunoglobulin domain, is different from both V and C domains and has been termed a C2 domain. The first two domains of CD4 form a rigid rod-like structure that is linked to the two carboxy-terminal domains by a flexible link. The binding site for MHC class II molecules is thought to involve mainly the D_1 domain. The CD8 molecule is a heterodimer of an α and a β chain covalently linked by a disulfide bond; an alternative form of CD8 exists as a homodimer of α chains. The heterodimer is depicted in panel a, whereas the ribbon diagram in panel b is of the homodimer. CD8α and CD8β chains have very similar structures, each having a single domain resembling an immunoglobulin V domain and a stretch of polypeptide chain, believed to be in a relatively extended conformation, that anchors the V-like domain to the cell membrane.

The structure of CD8 is quite different. It is a disulfide-linked dimer of two different chains, called α and β, each containing a single Ig-like domain linked to the membrane by a segment of extended polypeptide (see Fig. 4.24). This segment is extensively glycosylated, which is thought to maintain it in an extended conformation and protect it from cleavage by proteases. CD8α chains can form homodimers, although these are not found when the CD8β chains are present. The CD8α homodimer may have a specific function in recognizing a specialized subset of nonclassical MHC class I molecules that we describe in Chapter 6.

CD8 binds weakly to an invariant site in the α_3 domain of an MHC class I molecule (see Fig. 4.25b). Although only the interaction of the CD8α homodimer with MHC class I is known in detail, it shows that the binding site on the CD8 α:β heterodimer is formed by the interaction of the CD8α and β chains. In addition, CD8 interacts (most probably through its α chain) with residues in the base of the α_2 domain of the MHC class I molecule. The strength of binding of CD8 to the MHC class I molecule is influenced by the glycosylation state of the CD8 molecule; increased numbers of sialic acid residues added to the CD8 carbohydrate structures decrease the strength of the interaction. The pattern of sialylation of CD8 changes during the maturation of T cells and also on activation, and this is likely to have a role in modulating antigen recognition.

By binding to the membrane-proximal domains of the MHC class I and class II molecules, the co-receptors leave the upper surface of the MHC molecule exposed and free to interact with a T-cell receptor, as shown for CD8 in Fig. 4.26. Both CD4 and CD8 bind Lck—in the case of the CD8α:β heterodimer through the cytoplasmic tail of the α chain—and bring it into close proximity to the T-cell receptor. As with CD4, the presence of CD8 increases the sensitivity of T cells to antigen presented by MHC class I molecules about a hundredfold. Thus, CD4 and CD8 have similar functions and bind to the same approximate location in MHC class I and MHC class II molecules, even though the structures of the two co-receptor proteins are only distantly related.

Petitioner Merck, Ex. 1170, p. 64
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

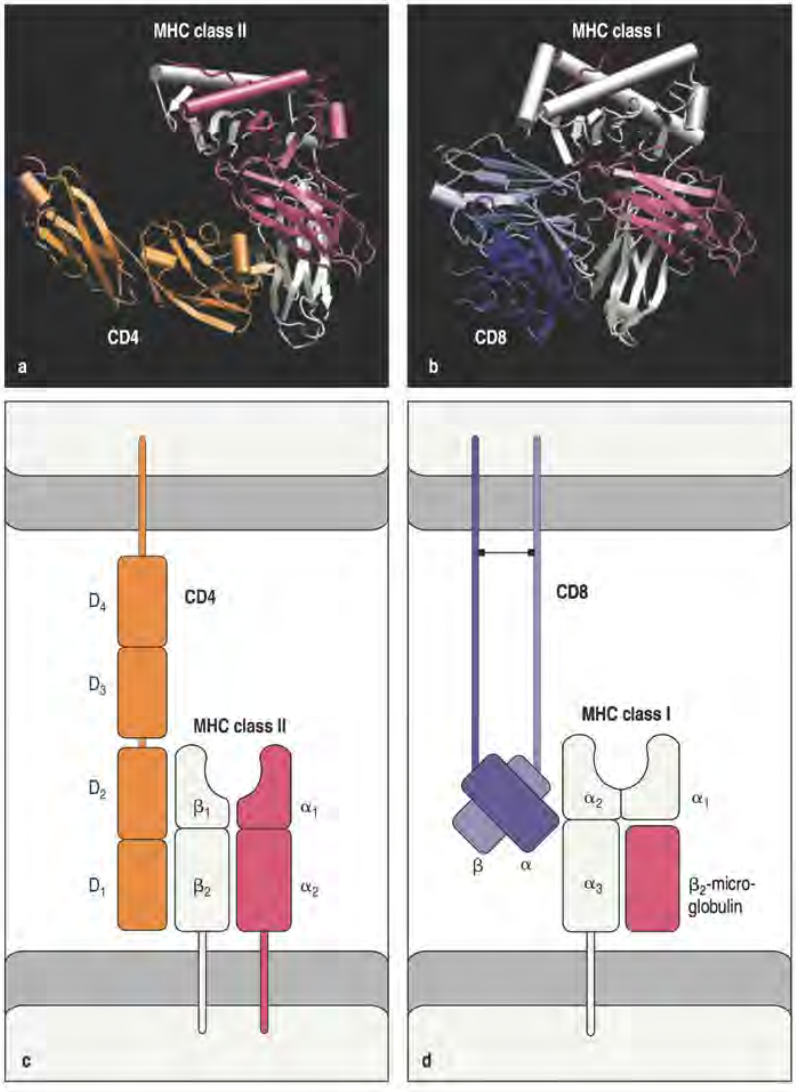


Fig. 4.25 The binding sites for CD4 and CD8 on MHC class II and class I molecules lie in the Ig-like domains. The binding sites for CD4 and CD8 on the MHC class II and class I molecules, respectively, lie in the Ig-like domains nearest to the membrane and distant from the peptide-binding cleft. The binding of CD4 to an MHC class II molecule is shown as a structure graphic in panel a and schematically in panel c. The α chain of the MHC class II molecule is shown in pink, and the β chain in white, while CD4 is in gold. Only the D_1 and D_2 domains of the CD4 molecule are shown in panel a. The binding site for CD4 lies at the base of the β_2 domain of an MHC class II molecule, in the hydrophobic crevice between the β_2 and α_2 domains. The binding of CD8 to an MHC class I molecule is shown in panel b and schematically in panel d. The class I heavy chain and β_2-microglobulin are shown in white and pink, respectively, and the two chains of the CD8 dimer are shown in light and dark purple. The structure is actually of the binding of the CD8α homodimer, but the CD8α:β heterodimer is believed to bind in a similar way. The binding site for CD8 on the MHC class I molecule lies in a similar position to that of CD4 in the MHC class II molecule, but CD8 binding also involves the base of the α_1 and α_2 domains, and thus the binding of CD8 to MHC class I is not completely equivalent to the binding of CD4 to MHC class II.



4-18 The two classes of MHC molecules are expressed differentially on cells.

MHC class I and MHC class II molecules have distinct distributions among cells, and these reflect the different effector functions of the T cells that recognize them (Fig. 4.27). MHC class I molecules present peptides from pathogens, commonly viruses, to CD8 cytotoxic T cells, which are specialized

Fig. 4.26 CD8 binds to a site on MHC class I molecules distant from that to which the T-cell receptor binds. The relative positions of the T-cell receptor and CD8 molecules bound to the same MHC class I molecule can be seen in this hypothetical reconstruction of the interaction of an MHC class I molecule (the α chain is shown in green; β_2-microglobulin (dull yellow) can be seen faintly in the background) with a T-cell receptor and CD8. The α and β chains of the T-cell receptor are shown in pink and purple, respectively. The CD8 structure is that of a CD8α homodimer, but is colored to represent the likely orientation of the subunits in the heterodimer, with the CD8β subunit in red and the CD8α subunit in blue. Courtesy of G. Gao.

Petitioner Merck, Ex. 1170, p. 65
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

to kill any cell that they specifically recognize. Because viruses can infect any nucleated cell, almost all such cells express MHC class I molecules, although the level of constitutive expression varies from one cell type to the next. For example, cells of the immune system express abundant MHC class I on their surface, whereas liver cells (hepatocytes) express relatively low levels (see Fig. 4.27). Nonnucleated cells, such as mammalian red blood cells, express little or no MHC class I, and thus the interior of red blood cells is a site in which an infection can go undetected by cytotoxic T cells. Because red blood cells cannot support viral replication, this is of no great consequence for viral infection, but it might be the absence of MHC class I that allows the *Plasmodium* parasites that cause malaria to live in this privileged site.

In contrast, the main function of the CD4 T cells that recognize MHC class II molecules is to activate other effector cells of the immune system. Thus, MHC class II molecules are normally found on B lymphocytes, dendritic cells, and macrophages—cells that participate in immune responses—but not on other tissue cells (see Fig. 4.27). When CD4 T cells recognize peptides bound to MHC class II molecules on B cells, they stimulate the B cells to produce antibody. Similarly, CD4 T cells recognizing peptides bound to MHC class II molecules on macrophages activate these cells to destroy the pathogens in their vesicles. We shall see in Chapter 9 that MHC class II molecules are also expressed on specialized antigen-presenting cells, the dendritic cells, in lymphoid tissues where naive T cells encounter antigen and are first activated.

The expression of both MHC class I and MHC class II molecules is regulated by cytokines, in particular interferons, released in the course of immune responses. Interferon-α (IFN-α) and IFN-β increase the expression of MHC class I molecules on all types of cells, whereas IFN-γ increases the expression of both MHC class I and MHC class II molecules, and can induce the expression of MHC class II molecules on certain cell types that do not normally express them. Interferons also enhance the antigen-presenting function of MHC class I molecules by inducing the expression of key components of the intracellular machinery that enables peptides to be loaded onto the MHC molecules.

Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+++	+*
B cells	+++	+++
Macrophages	+++	++
Dendritic cells	+++	+++
Epithelial cells of the thymus	+	+++
Other nucleated cells		
Neutrophils	+++	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	–†
Nonnucleated cells		
Red blood cells	–	–

Fig. 4.27 The expression of MHC molecules differs between tissues. MHC class I molecules are expressed on all nucleated cells, although they are most highly expressed in hematopoietic cells. MHC class II molecules are normally expressed only by a subset of hematopoietic cells and by thymic stromal cells, although they may be expressed by other cell types on exposure to the inflammatory cytokine IFN-γ.
*In humans, activated T cells express MHC class II molecules, whereas in mice all T cells are MHC class II-negative.
†In the brain, most cell types are MHC class II-negative, but microglia, which are related to macrophages, are MHC class II-positive.

4-19 A distinct subset of T cells bears an alternative receptor made up of γ and δ chains.

During the search for the gene for the TCRα chain, another T-cell receptor-like gene was unexpectedly discovered. This gene was named TCRγ, and its discovery led to a search for further T-cell receptor genes. Another receptor chain was identified by using antibody against the predicted sequence of the γ chain and was called the δ chain. It was soon discovered that a minority population of T cells bore a distinct type of T-cell receptor made up of γ:δ heterodimers rather than α:β heterodimers. The development of these cells is described in Sections 8-11 and 8-12.

The crystallographic structure of a γ:δ T-cell receptor reveals that, as expected, it is similar in shape to α:β T-cell receptors (Fig. 4.28). γ:δ T-cell receptors may be specialized to bind certain kinds of ligands, including heat-shock proteins and nonpeptide ligands such as phosphorylated ligands or mycobacterial lipid antigens. It seems likely that γ:δ T-cell receptors are not restricted by the 'classical' MHC class I and class II molecules. They may bind the free antigen, much as immunoglobulins do, and/or they may bind to peptides or other antigens presented by nonclassical MHC-like molecules. These are proteins that resemble MHC class I molecules but are relatively nonpolymorphic and are described in Chapter 6. We still know little about how γ:δ T-cell receptors bind antigen and thus how these cells function, and what their role is in immune responses. The structure and rearrangement of the genes for γ:δ T-cell receptors are covered in Sections 5-11 and 8-12.

Petitioner Merck, Ex. 1170, p. 66
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

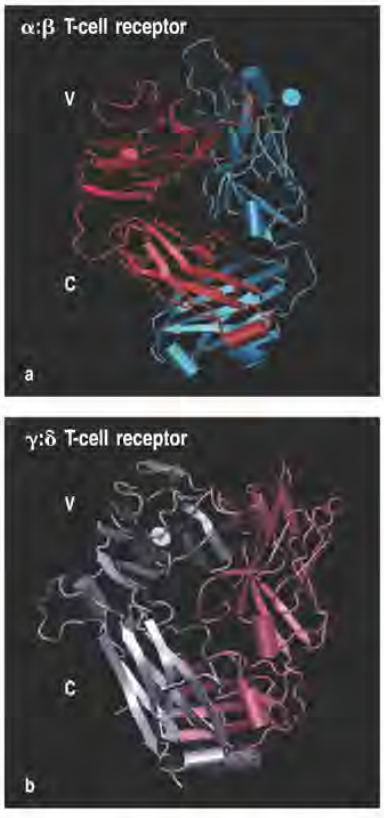


Fig. 4.28 Structures of α:β and γ:δ T-cell receptors. The structures of the α:β and the γ:δ T-cell receptors have both been determined by X-ray crystallography. The α:β T-cell receptor is shown in panel a, with the α chain colored red and the β chain blue. Panel b shows the γ:δ receptor, with the γ chain colored purple and the δ chain pink. The receptors have very similar structures, somewhat resembling that of a Fab fragment of an immunoglobulin molecule. The C_δ domain is more like an immunoglobulin domain than is the corresponding C_α domain of the α:β T-cell receptor.

Summary.

The receptor for antigen on most T cells, the α:β T-cell receptor, is composed of two protein chains, TCRα and TCRβ, and resembles in many respects a single Fab fragment of immunoglobulin. T-cell receptors are always membrane-bound. α:β T-cell receptors do not recognize antigen in its native state, in contrast with the immunoglobulin receptors of B cells, but recognize a composite ligand of a peptide antigen bound to an MHC molecule. MHC molecules are highly polymorphic glycoproteins encoded by genes in the major histocompatibility complex (MHC). Each MHC molecule binds a wide variety of different peptides, but the different variants each preferentially recognize sets of peptides with particular sequence and physical features. The peptide antigen is generated intracellularly, and is bound stably in a peptide-binding cleft on the surface of the MHC molecule. There are two classes of MHC molecules, and these are bound in their nonpolymorphic domains by CD8 and CD4 molecules that distinguish two different functional classes of α:β T cells. CD8 binds MHC class I molecules and can bind simultaneously to the same peptide:MHC class I complex being recognized by a T-cell receptor, thus acting as a co-receptor and enhancing the T-cell response; CD4 binds MHC class II molecules and acts as a co-receptor for T-cell receptors that recognize peptide:MHC class II ligands. A T-cell receptor interacts directly both with the antigenic peptide and with polymorphic features of the MHC molecule that displays it, and this dual specificity underlies the MHC restriction of T-cell responses. A second type of T-cell receptor, composed of a γ and a δ chain, is structurally similar to the α:β T-cell receptor, but it seems to bind different ligands, including nonpeptide ligands. It is thought not to be MHC restricted and is found on a minority population of T cells, the γ:δ T cells.

Summary to Chapter 4.

B cells and T cells use different, but structurally similar, molecules to recognize antigen. The antigen-recognition molecules of B cells are immunoglobulins, and are made both as a membrane-bound receptor for antigen, the B-cell receptor, and as secreted antibodies that bind antigens and elicit humoral effector functions. The antigen-recognition molecules of T cells, in contrast, are made only as cell-surface receptors. Immunoglobulins and T-cell receptors are highly variable molecules, with the variability concentrated in that part of the molecule, the variable (V) region, that binds to antigen. Immunoglobulins bind a wide variety of chemically different antigens, whereas the major α:β type of T-cell receptor predominantly recognizes peptide fragments of foreign proteins bound to the MHC molecules that are ubiquitous on cell surfaces.

Binding of antigen by immunoglobulins has chiefly been studied with antibodies. The binding of antibody to its corresponding antigen is highly specific, and this specificity is determined by the shape and physicochemical properties of the antigen-binding site. The part of the antibody that elicits effector functions, once the variable part has bound an antigen, is located at the other end of the molecule from the antigen-binding sites, and is termed the constant region. There are five main functional classes of antibodies, each encoded by a different type of constant region. As we will see in Chapter 10, these interact with different components of the immune system to incite an inflammatory response and eliminate the antigen.

T-cell receptors differ in several respects from the B-cell immunoglobulins. One is the absence of a secreted form of the receptor. This reflects the functional differences between T cells and B cells. B cells deal with pathogens

Petitioner Merck, Ex. 1170, p. 67
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

and their protein products circulating within the body; secretion of a soluble antigen-recognition molecule by the activated B cell after antigen has been encountered enables them to mop up antigen effectively throughout the extracellular spaces of the body. T cells, in contrast, are specialized for cell–cell interactions. They either kill cells that are infected with intracellular pathogens and that bear foreign antigenic peptides on their surface, or interact with cells of the immune system that have taken up foreign antigen and are displaying it on the cell surface. T-cell recognition does not require a soluble, secreted receptor.

The second distinctive feature of the T-cell receptor is that it recognizes a composite ligand made up of the foreign peptide bound to a self MHC molecule. This means that T cells can interact only with a body cell displaying the antigen, not with the intact pathogen or protein. Each T-cell receptor is specific for a particular combination of peptide and a self MHC molecule.

MHC molecules are encoded by a family of highly polymorphic genes; although each individual expresses several of these genes, this represents only a small selection of all possible variants. During T-cell development, the T-cell receptor repertoire is selected so that the T cells of each individual recognize antigen only in conjunction with their own MHC molecules. Expression of multiple variant MHC molecules, each with a different peptide-binding repertoire, helps to ensure that T cells from an individual will be able to recognize at least some peptides generated from nearly every pathogen.

Questions.

4.1 The immunoglobulin superfamily is one of the most abundant families of protein domain structures. (a) What are the characteristics of an immunoglobulin domain, and how do the various subtypes of these domains differ? (b) What regions of the V-type immunoglobulin domain contribute to its complementarity-determining regions (CDRs), and how do the V-type and C-type immunoglobulin domains differ in those regions?

4.2 How do antibodies, which all have the same basic shape, recognize antigens of a wide variety of different shapes?

4.3 Although the antigen receptors on B cells and T cells are structurally related, there are important differences between them. (a) Describe the similarities and differences in the antigen-recognition properties of B-cell and T-cell antigen receptors. (b) How do these differences influence which antigens are recognized by B cells and T cells? (c) Given these differences, what would you say is the essential difference in the function of B cells and T cells?

4.4 There are two kinds of MHC molecules: class I and class II. (a) What role do MHC molecules have in the activation of antigen-specific T cells? (b) Explain how the peptide-binding region of MHC class I and class II molecules can be so similar, even though one is encoded by a single gene and the other is encoded by two different genes. (c) Besides interactions with the T-cell receptors, what additional interactions made by MHC molecules with T cells help to functionally distinguish between antigens presented by MHC class I and MHC class II molecules?

Petitioner Merck, Ex. 1170, p. 68
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

General references.

Ager, A., Callard, R., Ezine, S., Gerard, C., and Lopez-Botet, M.: **Immune receptor Supplement.** *Immunol. Today* 1996, 17.

Davies, D.R., and Chacko, S.: **Antibody structure.** *Acc. Chem. Res.* 1993, **26**:421–427.

Frazer, K., and Capra, J.D.: **Immunoglobulins: structure and function**, in Paul W.E. (ed): *Fundamental Immunology*, 4th ed. New York, Raven Press, 1998.

Germain, R.N.: **MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation.** *Cell* 1994, **76**:287–299.

Honjo, T., and Alt, F.W. (eds): *Immunoglobulin Genes*, 2nd ed. London, Academic Press, 1996.

Moller, G. (ed): **Origin of major histocompatibility complex diversity.** *Immunol. Rev.* 1995, **143**:5–292.

Poljak, R.J.: **Structure of antibodies and their complexes with antigens.** *Mol. Immunol.* 1991, **28**:1341–1345.

Rudolph, M.G., Stanfield, R.L., and Wilson, I.A: **How TCRs bind MHCs, peptides, and coreceptors.** *Annu. Rev. Immunol.* 2006, **24**:419–466.

Section references.

4-1 IgG antibodies consist of four polypeptide chains.

Edelman, G.M.: **Antibody structure and molecular immunology.** *Scand. J. Immunol.* 1991, **34**:4–22.

Faber, C., Shan, L., Fan, Z., Guddat, L.W., Furebring, C., Ohlin, M., Borrebaeck, C.A.K., and Edmundson, A.B.: **Three-dimensional structure of a human Fab with high affinity for tetanus toxoid.** *Immunotechnology* 1998, **3**:253–270.

Harris, L.J., Larson, S.B., Hasel, K.W., Day, J., Greenwood, A., and McPherson, A.: **The three-dimensional structure of an intact monoclonal antibody for canine lymphoma.** *Nature* 1992, **360**:369–372.

4-2 Immunoglobulin heavy and light chains are composed of constant and variable regions.

&

4-3 The antibody molecule can readily be cleaved into functionally distinct fragments.

Porter, R.R.: **Structural studies of immunoglobulins.** *Scand. J. Immunol.* 1991, **34**:382–389.

Yamaguchi, Y., Kim, H., Kato, K., Masuda, K., Shimada, I., and Arata, Y.: **Proteolytic fragmentation with high specificity of mouse IgG—mapping of proteolytic cleavage sites in the hinge region.** *J. Immunol. Meth.* 1995, **181**:259–267.

4-4 The immunoglobulin molecule is flexible, especially at the hinge region.

Gerstein, M., Lesk, A.M., and Chothia, C.: **Structural mechanisms for domain movements in proteins.** *Biochemistry* 1994, **33**:6739–6749.

Jimenez, R., Salazar, G., Baldridge, K.K., and Romesberg, F.E.: **Flexibility and molecular recognition in the immune system.** *Proc. Natl Acad. Sci. USA* 2003, **100**:92–97.

Saphire, E.O., Stanfield, R.L., Crispin, M.D., Parren, P.W., Rudd, P.M., Dwek, R.A., Burton, D.R., and Wilson, I.A.: **Contrasting IgG structures reveal extreme asymmetry and flexibility.** *J. Mol. Biol.* 2002, **319**:9–18.

4-5 The domains of an immunoglobulin molecule have similar structures.

Barclay, A.N., Brown, M.H., Law, S.K., McKnight, A.J., Tomlinson, M.G., and van der Merwe, P.A. (eds): *The Leukocyte Antigen Factsbook*, 2nd ed. London, Academic Press, 1997.

Brummendorf, T., and Lemmon, V.: **Immunoglobulin superfamily receptors: *cis*-interactions, intracellular adapters and alternative splicing regulate adhesion.** *Curr. Opin. Cell Biol.* 2001, **13**:611–618.

Marchalonis, J.J., Jensen, I., and Schluter, S.F.: **Structural, antigenic and evolutionary analyses of immunoglobulins and T cell receptors.** *J. Mol. Recog.* 2002, **15**:260–271.

Ramsland, P.A., and Farrugia, W.: **Crystal structures of human antibodies: a detailed and unfinished tapestry of immunoglobulin gene products.** *J. Mol. Recog.* 2002, **15**:248–259.

4-6 Localized regions of hypervariable sequence form the antigen-binding site.

Chitarra, V., Alzari, P.M., Bentley, G.A., Bhat, T.N., Eiselé, J.-L., Houdusse, A., Lescar, J., Souchon, H., and Poljak, R.J.: **Three-dimensional structure of a heteroclitic antigen-antibody cross-reaction complex.** *Proc. Natl Acad. Sci. USA* 1993, **90**:7711–7715.

Decanniere, K., Muyldermans, S., and Wyns, L.: **Canonical antigen-binding loop structures in immunoglobulins: more structures, more canonical classes?** *J. Mol. Biol.* 2000, **300**:83–91.

Gilliland, L.K., Norris, N.A., Marquardt, H., Tsu, T.T., Hayden, M.S., Neubauer, M.G., Yelton, D.E., Mittler, R.S., and Ledbetter, J.A.: **Rapid and reliable cloning of antibody variable regions and generation of recombinant single-chain antibody fragments.** *Tissue Antigens* 1996, **47**:1–20.

Johnson, G., and Wu, T.T.: **Kabat Database and its applications: 30 years after the first variability plot.** *Nucleic Acids Res.* 2000, **28**:214–218.

Wu, T.T., and Kabat, E.A.: **An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity.** *J. Exp. Med.* 1970, **132**:211–250.

Xu, J., Deng, Q., Chen, J., Houk, K.N., Bartek, J., Hilvert, D., and Wilson, I.A.: **Evolution of shape complementarity and catalytic efficiency from a primordial antibody template.** *Science* 1999, **286**:2345–2348.

4-7 Antibodies bind antigens via contacts with amino acids in CDRs, but the details of binding depend upon the size and shape of the antigen.

&

4-8 Antibodies bind to conformational shapes on the surfaces of antigens.

Ban, N., Day, J., Wang, X., Ferrone, S., and McPherson, A.: **Crystal structure of an anti-anti-idiotype shows it to be self-complementary.** *J. Mol. Biol.* 1996, **255**:617–627.

Davies, D.R., and Cohen, G.H.: **Interactions of protein antigens with antibodies.** *Proc. Natl Acad. Sci. USA* 1996, **93**:7–12.

Decanniere, K., Desmyter, A., Lauwereys, M., Ghahroudi, M.A., Muyldermans, S., and Wyns, L.: **A single-domain antibody fragment in complex with RNase A: non-canonical loop structures and nanomolar affinity using two CDR loops.** *Structure Fold. Des.* 1999, **7**:361–370.

Padlan, E.A.: **Anatomy of the antibody molecule.** *Mol. Immunol.* 1994, **31**:169–217.

Saphire, E.O., Parren, P.W., Pantophlet, R., Zwick, M.B., Morris, G.M., Rudd, P.M., Dwek, R.A., Stanfield, R.L., Burton, D.R., and Wilson, I.A.: **Crystal structure of a neutralizing human IGG against HIV-1: a template for vaccine design.** *Science* 2001, **293**:1155–1159.

Stanfield, R.L., and Wilson, I.A.: **Protein–peptide interactions.** *Curr. Opin. Struct. Biol.* 1995, **5**:103–113.

Tanner, J.J., Komissarov, A.A., and Deutscher, S.L.: **Crystal structure of an antigen-binding fragment bound to single-stranded DNA.** *J. Mol. Biol.* 2001, **314**:807–822.

Wilson, I.A., and Stanfield, R.L.: **Antibody–antigen interactions: new structures and new conformational changes.** *Curr. Opin. Struct. Biol.* 1994, **4**:857–867.

4-9 Antigen–antibody interactions involve a variety of forces.

Braden, B.C., and Poljak, R.J.: **Structural features of the reactions between antibodies and protein antigens.** *FASEB J.* 1995, **9**:9–16.

Braden, B.C., Goldman, E.R., Mariuzza, R.A., and Poljak, R.J.: **Anatomy of an antibody molecule: structure, kinetics, thermodynamics and mutational studies of the antilysozyme antibody D1.3.** *Immunol. Rev.* 1998, **163**:45–57.

Petitioner Merck, Ex. 1170, p. 69
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Ros, R., Schwesinger, F., Anselmetti, D., Kubon, M., Schäfer, R., Plückthun, A., and Tiefenauer, L.: **Antigen binding forces of individually addressed single-chain Fv antibody molecules.** *Proc. Natl Acad. Sci. USA* 1998, **95**:7402–7405.

4-10 The T-cell receptor is very similar to a Fab fragment of immunoglobulin.

Al-Lazikani, B., Lesk, A.M., and Chothia, C.: **Canonical structures for the hypervariable regions of T cell αβ receptors.** *J. Mol. Biol.* 2000, **295**:979–995.

Kjer-Nielsen, L., Clements, C.S., Brooks, A.G., Purcell, A.W., McCluskey, J., and Rossjohn, J.: **The 1.5 Å crystal structure of a highly selected antiviral T cell receptor provides evidence for a structural basis of immunodominance.** *Structure (Camb.)* 2002, **10**:1521–1532.

Machius, M., Cianga, P., Deisenhofer, J., and Ward, E.S.: **Crystal structure of a T cell receptor Vα11 (AV11S5) domain: new canonical forms for the first and second complementarity determining regions.** *J. Mol. Biol.* 2001, **310**:689–698.

4-11 A T-cell receptor recognizes antigen in the form of a complex of a foreign peptide bound to an MHC molecule.

Garcia, K.C., and Adams, E.J.: **How the T cell receptor sees antigen—a structural view.** *Cell* 2005, **122**:333–336.

Hennecke, J., and Wiley, D.C.: **Structure of a complex of the human αβ T cell receptor (TCR) HA1.7, influenza hemagglutinin peptide, and major histocompatibility complex class II molecule, HLA-DR4 (DRA*0101 and DRB1*0401): insight into TCR cross-restriction and alloreactivity.** *J. Exp. Med.* 2002, **195**:571–581.

Luz, J.G., Huang, M., Garcia, K.C., Rudolph, M.G., Apostolopoulos, V., Teyton, L., and Wilson, I.A.: **Structural comparison of allogeneic and syngeneic T cell receptor–peptide–major histocompatibility complex complexes: a buried alloreactive mutation subtly alters peptide presentation substantially increasing V_β interactions.** *J. Exp. Med.* 2002, **195**:1175–1186.

Reinherz, E.L., Tan, K., Tang, L., Kern, P., Liu, J., Xiong, Y., Hussey, R.E., Smolyar, A., Hare, B., Zhang, R., *et al.* **The crystal structure of a T cell receptor in complex with peptide and MHC class II.** *Science* 1999, **286**:1913–1921.

Rudolph, M.G., Stanfield, R.L., and Wilson, I.A.: **How TCRs bind MHCs, peptides, and coreceptors.** *Annu. Rev. Immunol.* 2006, **24**:419–466.

4-12 There are two classes of MHC molecules with distinct subunit compositions but similar three-dimensional structures.
&
4-13 Peptides are stably bound to MHC molecules, and also serve to stabilize the MHC molecule on the cell surface.

Bouvier, M.: **Accessory proteins and the assembly of human class I MHC molecules: a molecular and structural perspective.** *Mol. Immunol.* 2003, **39**:697–706.

Dessen, A., Lawrence, C.M., Cupo, S., Zaller, D.M., and Wiley, D.C.: **X-ray crystal structure of HLA-DR4 (DRA*0101, DRB1*0401) complexed with a peptide from human collagen II.** *Immunity* 1997, **7**:473–481.

Fremont, D.H., Hendrickson, W.A., Marrack, P., and Kappler, J.: **Structures of an MHC class II molecule with covalently bound single peptides.** *Science* 1996, **272**:1001–1004.

Fremont, D.H., Matsumura, M., Stura, E.A., Peterson, P.A. and Wilson, I.A.: **Crystal structures of two viral peptides in complex with murine MHC class 1 H-2K^b.** *Science* 1992, **257**:919–927.

Fremont, D.H., Monnaie, D., Nelson, C.A., Hendrickson, W.A., and Unanue, E.R.: **Crystal structure of I-Ak in complex with a dominant epitope of lysozyme.** *Immunity* 1998, **8**:305–317.

Macdonald, W.A., Purcell, A.W., Mifsud, N.A., Ely, L.K., Williams, D.S., Chang, L., Gorman, J.J., Clements, C.S., Kjer-Nielsen, L., Koelle, D.M., *et al.*: **A naturally selected dimorphism within the HLA-B44 supertype alters class I structure, peptide repertoire, and T cell recognition.** *J. Exp. Med.* 2003, **198**:679–691.

Zhu, Y., Rudensky, A.Y., Corper, A.L., Teyton, L., and Wilson, I.A.: **Crystal structure of MHC class II I-Ab in complex with a human CLIP peptide: prediction of an I-Ab peptide-binding motif.** *J. Mol. Biol.* 2003, **326**:1157–1174.

4-14 MHC class I molecules bind short peptides of 8–10 amino acids by both ends.

Bouvier, M., and Wiley, D.C.: **Importance of peptide amino and carboxyl termini to the stability of MHC class I molecules.** *Science* 1994, **265**:398–402.

Govindarajan, K.R., Kangueane, P., Tan, T.W., and Ranganathan, S.: **MPID: MHC-Peptide Interaction Database for sequence–structure–function information on peptides binding to MHC molecules.** *Bioinformatics* 2003, **19**:309–310.

Saveanu, L., Fruci, D., and van Endert, P.: **Beyond the proteasome: trimming, degradation and generation of MHC class I ligands by auxiliary proteases.** *Mol. Immunol.* 2002, **39**:203–215.

Weiss, G.A., Collins, E.J., Garboczi, D.N., Wiley, D.C., and Schreiber, S.L.: **A tricyclic ring system replaces the variable regions of peptides presented by three alleles of human MHC class I molecules.** *Chem. Biol.* 1995, **2**:401–407.

4-15 The length of the peptides bound by MHC class II molecules is not constrained.

Conant, S.B., and Swanborg, R.H.: **MHC class II peptide flanking residues of exogenous antigens influence recognition by autoreactive T cells.** *Autoimmun. Rev.* 2003, **2**:8–12.

Guan, P., Doytchinova, I.A., Zygouri, C., and Flower, D.R.: **MHCPred: a server for quantitative prediction of peptide–MHC binding.** *Nucleic Acids Res.* 2003, **31**:3621–3624.

Lippolis, J.D., White, F.M., Marto, J.A., Luckey, C.J., Bullock, T.N., Shabanowitz, J., Hunt, D.F., and Engelhard, V.H.: **Analysis of MHC class II antigen processing by quantitation of peptides that constitute nested sets.** *J. Immunol.* 2002, **169**:5089–5097.

Park, J.H., Lee, Y.J., Kim, K.L., and Cho, E.W.: **Selective isolation and identification of HLA-DR-associated naturally processed and presented epitope peptides.** *Immunol. Invest.* 2003, **32**:155–169.

Rammensee, H.G.: **Chemistry of peptides associated with MHC class I and class II molecules.** *Curr. Opin. Immunol.* 1995, **7**:85–96.

Rudensky, A.Y., Preston-Hurlburt, P., Hong, S.C., Barlow, A., and Janeway, C.A., Jr. **Sequence analysis of peptides bound to MHC class II molecules.** *Nature* 1991, **353**:622–627.

Sercarz, E.E., and Maverakis, E.: **MHC-guided processing: binding of large antigen fragments.** *Nat. Rev. Immunol.* 2003, **3**:621–629.

Sinnathamby, G., and Eisenlohr, L.C.: **Presentation by recycling MHC class II molecules of an influenza hemagglutinin-derived epitope that is revealed in the early endosome by acidification.** *J. Immunol.* 2003, **170**:3504–3513.

4-16 The crystal structures of several peptide:MHC:T-cell receptor complexes show a similar orientation of the T-cell receptor over the peptide:MHC complex.

Buslepp, J., Wang, H., Biddison, W.E., Appella, E., and Collins, E.J.: **A correlation between TCR Vα docking on MHC and CD8 dependence: implications for T cell selection.** *Immunity* 2003, **19**:595–606.

Ding, Y.H., Smith, K.J., Garboczi, D.N., Utz, U., Biddison, W.E., and Wiley, D.C.: **Two human T cell receptors bind in a similar diagonal mode to the HLA-A2/Tax peptide complex using different TCR amino acids.** *Immunity* 1998, **8**:403–411.

Kjer-Nielsen, L., Clements, C.S., Purcell, A.W., Brooks, A.G., Whisstock, J.C., Burrows, S.R., McCluskey, J., and Rossjohn, J.: **A structural basis for the selection of dominant αβ T cell receptors in antiviral immunity.** *Immunity* 2003, **18**:53–64.

Garcia, K.C., Degano, M., Pease, L.R., Huang, M., Peterson, P.A., Leyton, L., and Wilson, I.A.: **Structural basis of plasticity in T cell receptor recognition of a self peptide-MHC antigen.** *Science* 1998, **279**:1166–1172.

Reiser, J.B., Darnault, C., Gregoire, C., Mosser, T., Mazza, G., Kearney, A., van der Merwe, P.A., Fontecilla-Camps, J.C., Housset, D., and Malissen, B.: **CDR3 loop flexibility contributes to the degeneracy of TCR recognition.** *Nat. Immunol.* 2003, **4**:241–247.

Sant'Angelo, D.B., Waterbury, G., Preston-Hurlburt, P., Yoon, S.T., Medzhitov, R., Hong, S.C., and Janeway, C.A., Jr. **The specificity and orientation of a TCR to its peptide-MHC class II ligands.** *Immunity* 1996, **4**:367–376.

Petitioner Merck, Ex. 1170, p. 70
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Teng, M.K., Smolyar, A., Tse, A.G.D., Liu, J.H., Liu, J., Hussey, R.E., Nathenson, S.G., Chang, H.C., Reinherz, E.L., and Wang, J.H.: **Identification of a common docking topology with substantial variation among different TCR–MHC–peptide complexes.** *Curr. Biol.* 1998, **8**:409–412.

4-17 The CD4 and CD8 cell-surface proteins of T cells are required to make an effective response to antigen.

Chang, H.C., Tan, K., Ouyang, J., Parisini, E., Liu, J.H., Le, Y., Wang, X., Reinherz, E. L., and Wang, J. H. **Structural and mutational analyses of CD8αβ heterodimer and comparison with the CD8αα homodimer.** *Immunity* 2005, **6**: 661–671.

Gao, G.F., Tormo, J., Gerth, U.C., Wyer, J.R., McMichael, A.J., Stuart, D.I., Bell, J.I., Jones, E.Y., and Jakobsen, B.Y.: **Crystal structure of the complex between human CD8αα and HLA-A2.** *Nature* 1997, **387**:630–634.

Gaspar, R., Jr, Bagossi, P., Bene, L., Matko, J., Szollosi, J., Tozser, J., Fesus, L., Waldmann, T.A., and Damjanovich, S.: **Clustering of class I HLA oligomers with CD8 and TCR: three-dimensional models based on fluorescence resonance energy transfer and crystallographic data.** *J. Immunol.* 2001, **166**:5078–5086.

Kim, P.W., Sun, Z.Y., Blacklow, S.C., Wagner, G., and Eck, M.J.: **A zinc clasp structure tethers Lck to T cell coreceptors CD4 and CD8.** *Science* 2003, **301**:1725–1728.

Moody, A.M., North, S.J., Reinhold, B., Van Dyken, S.J., Rogers, M.E., Panico, M., Dell, A., Morris, H.R., Marth, J.D., and Reinherz, E.L.: **Sialic acid capping of CD8β core 1-*O*-glycans controls thymocyte-major histocompatibility complex class I interaction.** *J. Biol. Chem.* 2003, **278**:7240–7260.

Wang, J.H., and Reinherz, E.L.: **Structural basis of T cell recognition of peptides bound to MHC molecules.** *Mol. Immunol.* 2002, **38**:1039–1049.

Wu, H., Kwong, P.D., and Hendrickson, W.A.: **Dimeric association and segmental variability in the structure of human CD4.** *Nature* 1997, **387**:527–530.

Zamoyska, R.: **CD4 and CD8: modulators of T cell receptor recognition of antigen and of immune responses?** *Curr. Opin. Immunol.* 1998, **10**:82–86.

4-18 The two classes of MHC molecules are expressed differentially on cells.

Steimle, V., Siegrist, C.A., Mottet, A., Lisowska-Grospierre, B., and Mach, B.: **Regulation of MHC class II expression by interferon-γ mediated by the transactivator gene CIITA.** *Science* 1994, **265**:106–109.

4-19 A distinct subset of T cells bears an alternative receptor made up of γ and δ chains.

Allison, T.J., and Garboczi, D.N.: **Structure of γδ T cell receptors and their recognition of non-peptide antigens.** *Mol. Immunol.* 2002, **38**:1051–1061.

Allison, T.J., Winter, C.C., Fournie, J.J., Bonneville, M., and Garboczi, D.N.: **Structure of a human γδ T-cell antigen receptor.** *Nature* 2001, **411**:820–824.

Carding, S.R., and Egan, P.J.: **γδ T cells: functional plasticity and heterogeneity.** *Nat. Rev. Immunol.* 2002, **2**:336–345.

Das, H., Wang, L., Kamath, A., and Bukowski, J.F.: **$V_\gamma 2V_\delta 2$ T-cell receptor-mediated recognition of aminobisphosphonates.** *Blood* 2001, **98**:1616–1618.

Wilson, I.A., and Stanfield, R.L.: **Unraveling the mysteries of γδ T cell recognition.** *Nat. Immunol.* 2001, **2**:579–581.

Wu, J., Groh, V., and Spies, T.: **T cell antigen receptor engagement and specificity in the recognition of stress-inducible MHC class I-related chains by human epithelial γ δ T cells.** *J. Immunol.* 2002, **169**:1236–1240.

Petitioner Merck, Ex. 1170, p. 71
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

8 The Development and Survival of Lymphocytes

The antigen receptors carried by B lymphocytes and T lymphocytes are immensely variable in their antigen specificity, enabling an individual to make immune responses against the wide range of pathogens encountered during a lifetime. This diverse repertoire of B-cell receptors and T-cell receptors is generated during the development of B cells and T cells from their uncommitted precursors. The production of new lymphocytes, or **lymphopoiesis**, takes place in specialized lymphoid tissues—the **central lymphoid tissues**—which are the bone marrow for most B cells and the thymus for most T cells. Lymphocyte precursors originate in the bone marrow, but whereas B cells complete most of their development there, the precursors of most T cells migrate to the thymus, where they develop into mature T cells. B cells also originate and develop in the fetal liver and the neonatal spleen. Some T cells that form specialized populations within the gut epithelium may migrate as immature precursors from the bone marrow to develop in sites called 'cryptopatches' just under the intestinal epithelial crypts. We will mainly focus here on the development of bone marrow derived B cells and thymus-derived T cells.

In the fetus and the juvenile, the central lymphoid tissues are the sources of large numbers of new lymphocytes, which migrate to populate the **peripheral lymphoid tissues** such as lymph nodes, spleen, and mucosal lymphoid tissue (these tissues are also known as secondary lymphoid tissues). In mature individuals, the development of new T cells in the thymus slows down, and T-cell numbers are maintained through long-lived individual T cells together with the division of mature T cells outside the central lymphoid organs. New B cells, in contrast, are continually produced from the bone marrow, even in adults.

The structure of the antigen receptor genes expressed by B cells and T cells, and the mechanisms by which a complete antigen receptor is assembled, were described in Chapters 4 and 5. This chapter builds on that foundation to explain how B and T lymphocytes themselves develop from a common progenitor through a series of stages, and how each of these stages tests for the proper assembly of antigen receptors.

Once an antigen receptor has been formed, rigorous testing is required to select lymphocytes that carry useful antigen receptors—that is, antigen receptors that can recognize pathogens and yet will not react against an individual's own cells. Given the incredible diversity of receptors that the rearrangement process can generate, it is important that those lymphocytes that mature are likely to be useful in recognizing and responding to foreign antigens, especially as an individual can express only a small fraction of the total possible receptor repertoire in his or her lifetime. We describe how the specificity and affinity of the receptor for self ligands are tested to determine whether the immature lymphocyte will survive into the mature repertoire, or die. In general, it seems that developing lymphocytes whose receptors interact weakly

Petitioner Merck, Ex. 1170, p. 72
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

with self antigens, or that bind self antigens in a particular way, receive a signal that enables them to survive; this type of selection is known as **positive selection**. Positive selection is particularly critical in the development of α:β T cells, which recognize composite antigens consisting of peptides bound to MHC molecules, because it ensures that an individual's T cells will be able to respond to peptides bound to his or her MHC molecules.

In contrast, lymphocytes with strongly self-reactive receptors must be eliminated to prevent autoimmune reactions; this process of **negative selection** is one of the ways in which the immune system is made self-tolerant. The default fate of developing lymphocytes, in the absence of any signal being received from the receptor, is death, and as we will see, the vast majority of developing lymphocytes die before emerging from the central lymphoid organs or before completing maturation in the peripheral lymphoid organs.

In this chapter we describe the different stages of the development of B cells and T cells in mice and humans from the uncommitted stem cell up to the mature, functionally specialized lymphocyte with its unique antigen receptor ready to respond to a foreign antigen. The final stages in the life history of a mature lymphocyte, in which an encounter with its antigen activates it to become an effector or memory lymphocyte, are discussed in Chapters 9–11. The chapter is divided into four parts. The first two describe B-cell and T-cell development, respectively. We then look at the positive and negative selection of T cells in the thymus. Next, we describe the fate of newly generated lymphocytes as they leave the central lymphoid organs and migrate to the peripheral lymphoid tissues, where further maturation occurs. Mature lymphocytes continually recirculate between the blood and peripheral lymphoid tissues (see Chapter 1) and, in the absence of infection, their numbers remain relatively constant, despite the continual production of new lymphocytes. Finally, we look at the factors that govern the survival of naive lymphocytes in the peripheral lymphoid organs, and the maintenance of lymphocyte **homeostasis**—the regulation of their numbers and turnover.

Development of B lymphocytes.

The main phases of a B lymphocyte's life history are shown in Fig. 8.1. The stages in both B-cell and T-cell development are defined mainly by the successive steps in the assembly and expression of functional antigen-receptor genes. At each step of lymphocyte development, the progress of gene rearrangement is monitored, and the major recurring theme is that successful gene rearrangement leads to the production of a protein chain that serves as a signal for the cell to progress to the next stage. We will see that a developing B cell is presented with opportunities for multiple rearrangements that increase the likelihood of expressing a functional antigen receptor, but there are also checkpoints that reinforce the requirement that each B cell expresses just one receptor specificity. We will start by looking at how the earliest recognizable cells of the B-cell lineage develop from the multipotent hematopoietic stem cells in the bone marrow, and at what point the B-cell and T-cell lineages diverge.

8-1 Lymphocytes derive from hematopoietic stem cells in the bone marrow.

The cells of the lymphoid lineage—B cells, T cells, and NK cells—are all derived from common lymphoid progenitor cells, which themselves derive from the multipotent **hematopoietic stem cells** (**HSCs**) that give rise to all blood cells

Petitioner Merck, Ex. 1170, p. 73
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

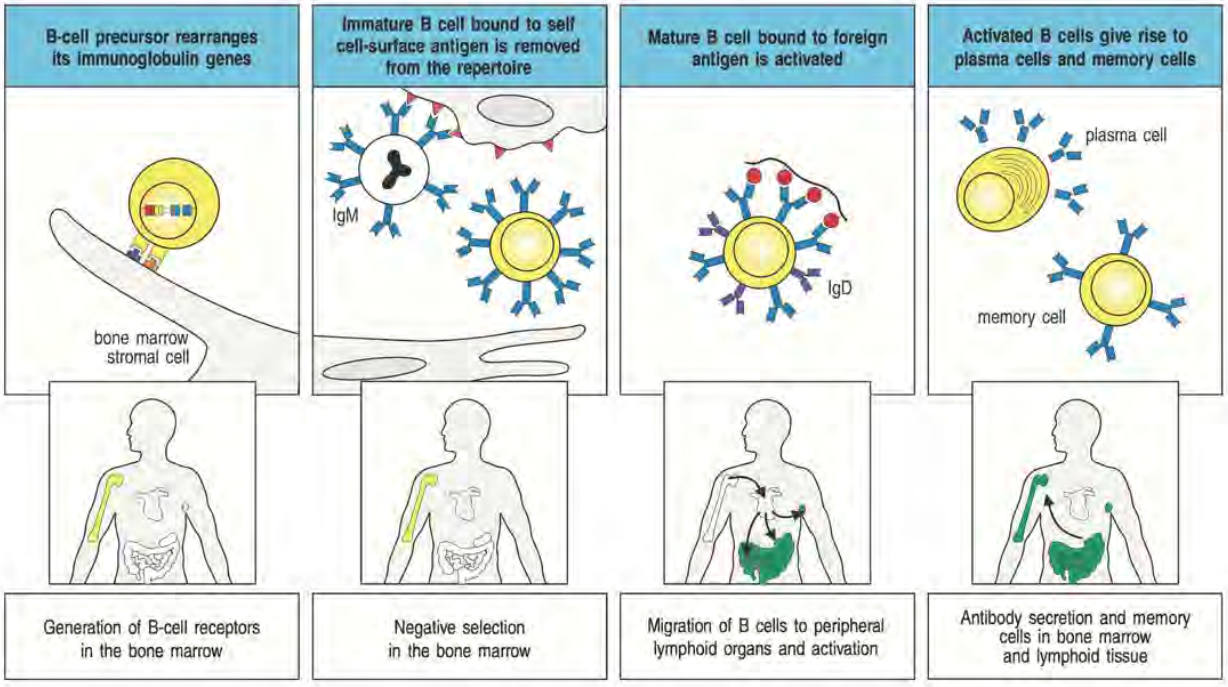


Fig. 8.1 B cells develop in the bone marrow and migrate to peripheral lymphoid organs, where they can be activated by antigens. In the first phase of development, progenitor B cells in the bone marrow rearrange their immunoglobulin genes. This phase is independent of antigen but is dependent on interactions with bone marrow stromal cells (first panels). It ends in an immature B cell that carries an antigen receptor in the form of cell-surface IgM and can now interact with antigens in its environment. Immature B cells that are strongly stimulated by antigen at this stage either die or are inactivated in a process of negative selection, thus removing many self-reactive B cells from the repertoire (second panels). In the third phase of development, the surviving immature B cells emerge into the periphery and mature to express IgD as well as IgM. They can now be activated by encounter with their specific foreign antigen in a peripheral lymphoid organ (third panels). Activated B cells proliferate, and differentiate into antibody-secreting plasma cells and long-lived memory cells (fourth panels).

(see Fig. 1.3). Development from the precursor stem cell into cells that are committed to becoming B cells or T cells follows the basic principles of cell differentiation. Properties that are essential for the function of the mature cell are gradually acquired, along with the loss of properties that are more characteristic of the immature cell. In the case of lymphocyte development, cells become committed first to the lymphoid, as opposed to the myeloid, lineage, and then to either the B-cell or the T-cell lineages (Fig. 8.2).

The specialized microenvironment of the bone marrow provides signals both for the development of lymphocyte progenitors from hematopoietic stem cells and for the subsequent differentiation of B cells. Such signals act on the developing lymphocytes to switch on key genes that direct the developmental program and are produced by the network of specialized non-lymphoid connective-tissue **stromal cells** that are in intimate contact with the developing lymphocytes (Fig. 8.3). The contribution of the stromal cells is twofold. First, they form specific adhesive contacts with the developing lymphocytes by interactions between cell-adhesion molecules and their ligands. Second, they provide soluble and membrane-bound cytokines and chemokines that control lymphocyte differentiation and proliferation.

The hematopoietic stem cell first differentiates into **multipotent progenitor cells** (**MPPs**), which can produce both lymphoid and myeloid cells but are no longer self-renewing stem cells. Multipotent progenitors express a cell-surface receptor tyrosine kinase known as FLT3 (originally called stem-cell kinase 1 (STK1) in humans and Flt3/Flk2 in mice) that binds the membrane-bound FLT3 ligand on stromal cells. Signaling through FLT3 is needed for differentiation to the next stage, the **common lymphoid progenitor** (**CLP**). This cell gives rise to the earliest committed B-lineage cell, the **pro-B cell** (see Fig. 8.3). The common lymphoid progenitor is so called because it was originally thought to give rise to both the B-cell and T-cell lineages. But although it can do so in culture, it is not yet clear whether it does so *in vivo*.

The production of lymphocyte progenitors from the multipotent progenitor cell is accompanied by expression of the receptor for interleukin-7 (IL-7),

Petitioner Merck, Ex. 1170, p. 74
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

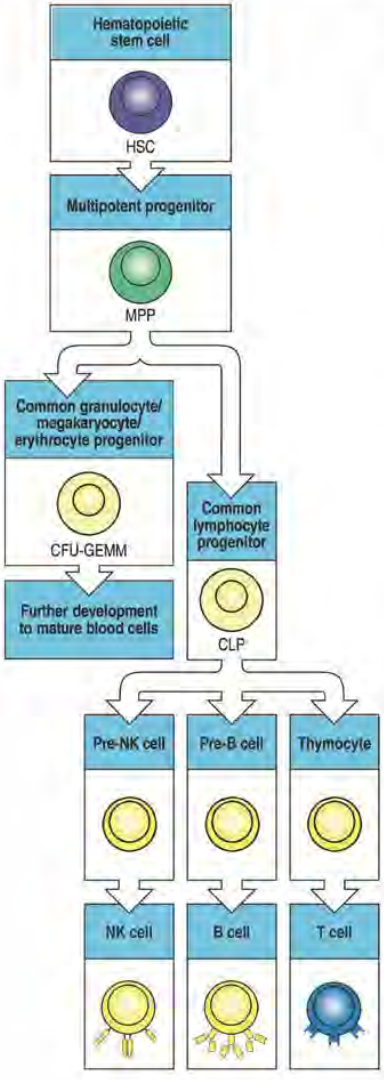


Fig. 8.2 A multipotent hematopoietic stem cell generates all the cells of the immune system. In the bone marrow or other hematopoietic sites, the multipotent stem cell gives rise to cells with progressively more limited potential. A simplified progression is shown here. The multipotent progenitor (MPP), for example, has lost its stem-cell properties. The first branch leads to cells with myeloid and erythroid potential on the one hand (CFU-GEMMs) and on the other to the common lymphoid progenitors (CLPs), with lymphoid potential. The former give rise to all nonlymphoid cellular blood elements, including circulating monocytes and granulocytes and the macrophages and dendritic cells that reside in tissues and peripheral lymphoid organs (not shown). The CLP can give rise to NK cells, T cells, or B cells through successive stages of differentiation in either the bone marrow or thymus. There may be considerable plasticity in these pathways, in that in certain circumstances progenitor cells may switch their commitment. For example, a progenitor cell may give rise to either B cells or macrophages; however, for simplicity these alternative pathways are not shown. Some dendritic cells are also thought to be derived from the lymphoid progenitor.

which is induced by FLT3 signaling together with the activity of the transcription factor PU.1. The cytokine IL-7, secreted by bone marrow stromal cells, is essential for the growth and survival of developing B cells in mice (but possibly not in humans). Another essential factor is stem-cell factor (SCF), a membrane-bound cytokine present on stromal cells that stimulates the growth of hematopoietic stem cells and the earliest B-lineage progenitors. SCF interacts with the receptor tyrosine kinase Kit on the precursor cells (see Fig. 8.3). The chemokine CXCL12 (stromal cell-derived factor 1, SDF-1) is also essential for the early stages of B-cell development. It is produced constitutively by bone marrow stromal cells, and one of its roles may be to retain developing B-cell precursors in the marrow microenvironment. Thymic stroma-derived lymphopoietin (**TSLP**) resembles IL-7 and binds a receptor sharing the common γ chain of the IL-7 receptor. Despite its name, TSLP may promote B-cell development in the embryonic liver and, in the perinatal period at least, in the mouse bone marrow.

A definitive B-cell fate, the pro-B cell, is specified by induction of the B-lineage-specific transcription factor E2A, which is present as two alternatively spliced forms called E12 and E47, and the early B-cell factor (EBF). It is not clear what initiates the expression of E2A in some progenitors, but it is known that the transcription factors PU.1 and Ikaros are required for E2A expression. E2A then induces the expression of EBF. IL-7 signaling promotes the survival of these committed progenitors, while E2A and EBF act together to drive the expression of proteins that determine the pro-B cell state.

As B-lineage cells mature, they migrate within the marrow, remaining in contact with the stromal cells. The earliest stem cells lie in a region called the **endosteum**, which lines the inner cavity of the long bones. Developing B-lineage cells make contact with reticular stromal cells in the trabecular spaces, and as they mature they move toward the central sinus of the marrow cavity. The final stages of development of **immature B cells** into **mature B cells** occur in peripheral lymphoid organs such as the spleen, which we describe in the fourth part of this chapter.

8-2 B-cell development begins by rearrangement of the heavy-chain locus.

The stages of B-cell development are, in the order that they occur: **early pro-B cell**, **late pro-B cell**, **large pre-B cell**, **small pre-B cell**, and **mature B cell** (Fig. 8.4). Only one gene locus is rearranged at a time, in a fixed sequence. The first rearrangement to take place involves the locus that contains D gene segments—the immunoglobulin heavy-chain (IgH) locus in the case of B cells—and the joining of a D gene segment to a J segment. D to J_H rearrangement takes place mostly in the early pro-B-cell stage, but can be seen as early as

Petitioner Merck, Ex. 1170, p. 75
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

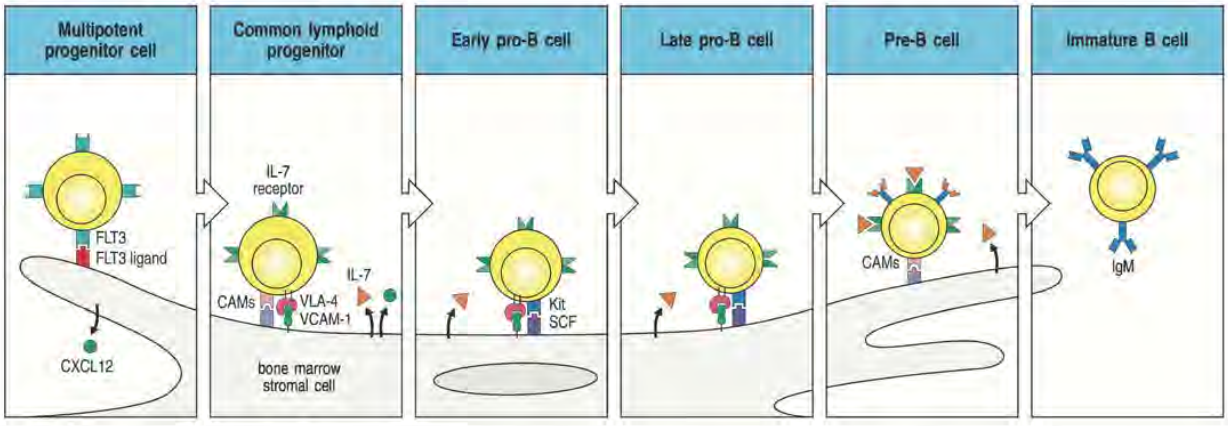


Fig. 8.3 The early stages of B-cell development are dependent on bone marrow stromal cells. Interaction of B-cell progenitors with bone marrow stromal cells is required for development to the immature B-cell stage. The designations pro-B cell and pre-B cell refer to defined phases of B-cell development as described in Fig. 8.4. Multipotent progenitor cells express the receptor tyrosine kinase FLT3, which binds to its ligand on stromal cells. Signaling through FLT3 is required for differentiation to the next stage, the common lymphoid progenitor. The chemokine CXCL12 (SDF-1) acts to retain stem cells and lymphoid progenitors to appropriate stromal cells in the bone marrow. The receptor for interleukin-7 (IL-7) is present from this stage, and IL-7 produced by stromal cells is required for the development of B-lineage cells. Progenitor cells bind to the adhesion molecule VCAM-1 on stromal cells through the integrin VLA-4 and also interact through other cell-adhesion molecules (CAMs). The adhesive interactions promote the binding of the receptor tyrosine kinase Kit (CD117) on the surface of the pro-B cell to stem-cell factor (SCF) on the stromal cell, which activates the kinase and induces the proliferation of B-cell progenitors.

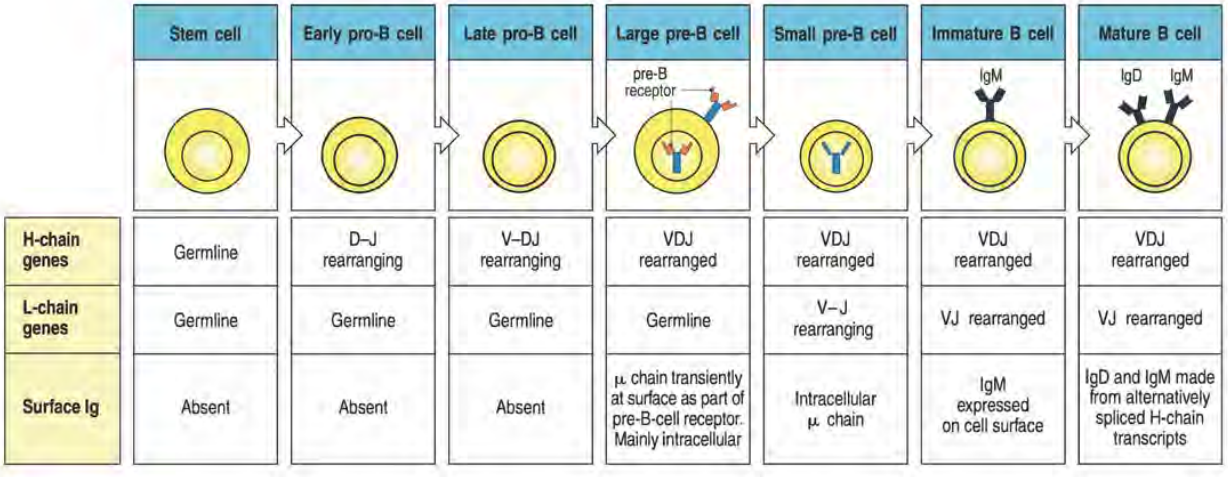


Fig. 8.4 The development of a B-lineage cell proceeds through several stages marked by the rearrangement and expression of the immunoglobulin genes. The stem cell has not yet begun to rearrange its immunoglobulin (Ig) gene segments; they are in the germline configuration as found in all nonlymphoid cells. The heavy-chain (H-chain) locus rearranges first. Rearrangement of a D gene segment to a J_H gene segment starts in the common lymphoid progenitor and occurs mostly in early pro-B cells, generating late pro-B cells in which V_H to DJ_H rearrangement occurs. A successful VDJ_H rearrangement leads to the expression of a complete immunoglobulin heavy chain as part of the pre-B-cell receptor, which is found mainly in the cytoplasm and to some degree on the surface of the cell. Once this occurs, the cell is stimulated to become a large pre-B cell, which proliferates. Large pre-B cells then cease dividing and become small resting pre-B cells, at which point they cease expression of the surrogate light chains and express the μ heavy chain alone in the cytoplasm. Small pre-B cells reexpress the RAG proteins and start to rearrange the light-chain (L-chain) genes. Upon successfully assembling a light-chain gene, a cell becomes an immature B cell that expresses a complete IgM molecule at the cell surface. Mature B cells produce a δ heavy chain as well as a μ heavy chain, by a mechanism of alternative mRNA splicing (see Fig. 5.17), and are marked by the additional appearance of IgD on the cell surface.

Petitioner Merck, Ex. 1170, p. 76
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

the common lymphoid progenitor. As shown in Fig. 8.4, expression of a functional heavy chain allows the formation of the **pre-B-cell receptor**, which is the signal to the cell to proceed to the next stage of development, the rearrangement of a light-chain gene. The transcription factors E2A and EBF in the early pro-B cell induce the expression of several key proteins that enable gene rearrangement to occur, including the RAG-1 and RAG-2 components of the V(D)J recombinase (see Chapter 5). Thus, E2A and EBF allow the initiation of V(D)J recombination at the heavy-chain locus and the expression of a heavy chain. In the absence of E2A or EBF, even the earliest identifiable stage in B-cell development, D to J_H joining, fails to occur.

Another key protein induced by E2A and EBF is the transcription factor Pax5, one isoform of which is known as the B-cell activator protein (BSAP). Among the targets of Pax5 are the gene for the B-cell co-receptor component CD19 and the gene for Igα, a signaling component of both the pre-B-cell receptor and the B-cell receptor (see Section 7-7). In the absence of Pax5, pro-B cells fail to develop further down the B-cell pathway but can be induced to give rise to T cells and myeloid cell types, indicating that Pax5 is required for commitment of the pro-B cell to the B-cell lineage. Pax5 also induces the expression of the B-cell linker protein (BLNK), an SH2-containing scaffold protein that is required for further development of the pro-B cell and for signaling from the mature B-cell antigen receptor (see Section 7-16). The temporal expression of some of the surface proteins, receptors, and transcription factors required for B-cell development are listed in Fig. 8.5.

Although the V(D)J recombinase system operates in both B- and T-lineage cells and uses the same core enzymes, rearrangements of T-cell receptor

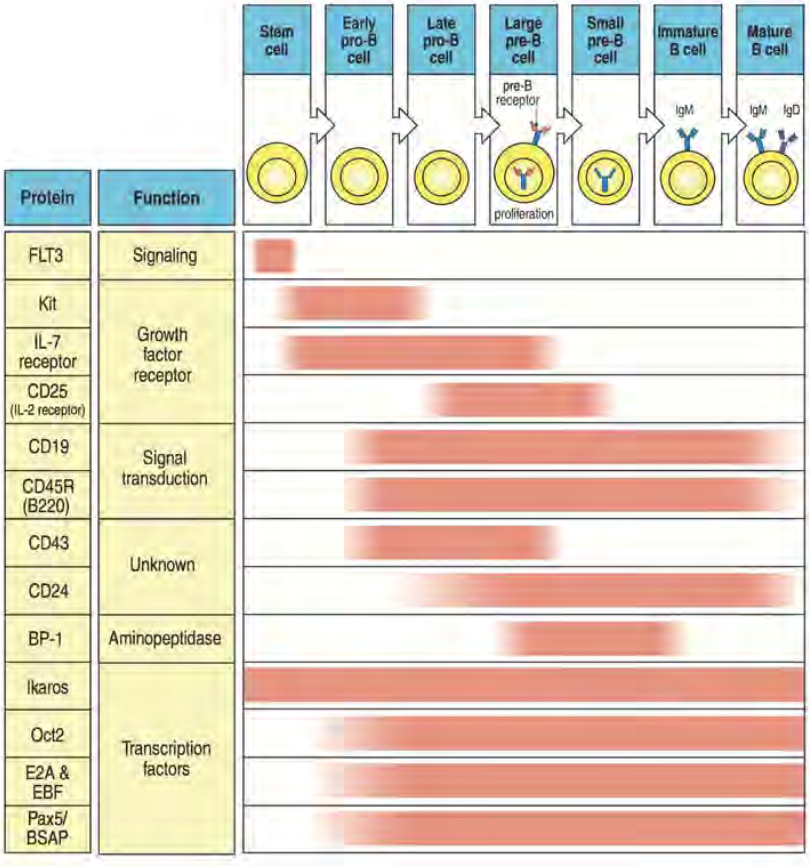


Fig. 8.5 Expression of surface proteins, receptors, and transcription factors in B-cell development. The stages of B-cell development corresponding to those shown in Fig. 8.4 are shown at the top of the figure. The receptor FLT3 is expressed on hematopoietic stem cells and the common lymphoid progenitor. The earliest B-lineage surface markers are CD19 and CD45R (B220 in the mouse), which are expressed throughout B-cell development. A pro-B cell is also distinguished by the expression of CD43 (a marker of unknown function), Kit (CD117), and the IL-7 receptor. A late pro-B cell starts to express CD24 (a marker of unknown function) and the IL-2 receptor α chain CD25. A pre-B cell is phenotypically distinguished by the expression of the enzyme BP-1, whereas Kit and the IL-7 receptor are no longer expressed. The actions of the listed transcription factors in B-cell development are discussed in the text, with the exception of the octamer transcription factor, Oct2, which binds the octamer ATGCAAAT found in the heavy-chain promoter and elsewhere.

Petitioner Merck, Ex. 1170, p. 77
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

genes do not occur in B-lineage cells, nor do complete rearrangements of immunoglobulin genes occur in T cells. The ordered rearrangement events that do occur are associated with lineage-specific low-level transcription of the gene segments about to be joined.

The initial D to J_H rearrangements in the immunoglobulin heavy-chain locus (Fig. 8.6) typically occur on both alleles, at which point the cell becomes a late pro-B cell. Most D to J_H joins in humans are potentially useful, because most human D gene segments can be translated in all three reading frames without encountering a stop codon. Thus, there is no need of a special mechanism for distinguishing successful D to J_H joins, and at this early stage there is also no need to ensure that only one allele undergoes rearrangement. Indeed, given the likely rate of failure at later stages, starting off with two successfully rearranged D–J_H sequences is an advantage.

To produce a complete immunoglobulin heavy chain, the late pro-B cell now proceeds with a rearrangement of a V_H gene segment to a DJ_H sequence. In contrast to D to J_H rearrangement, V_H to DJ_H rearrangement occurs first on only one chromosome. A successful rearrangement leads to the production of

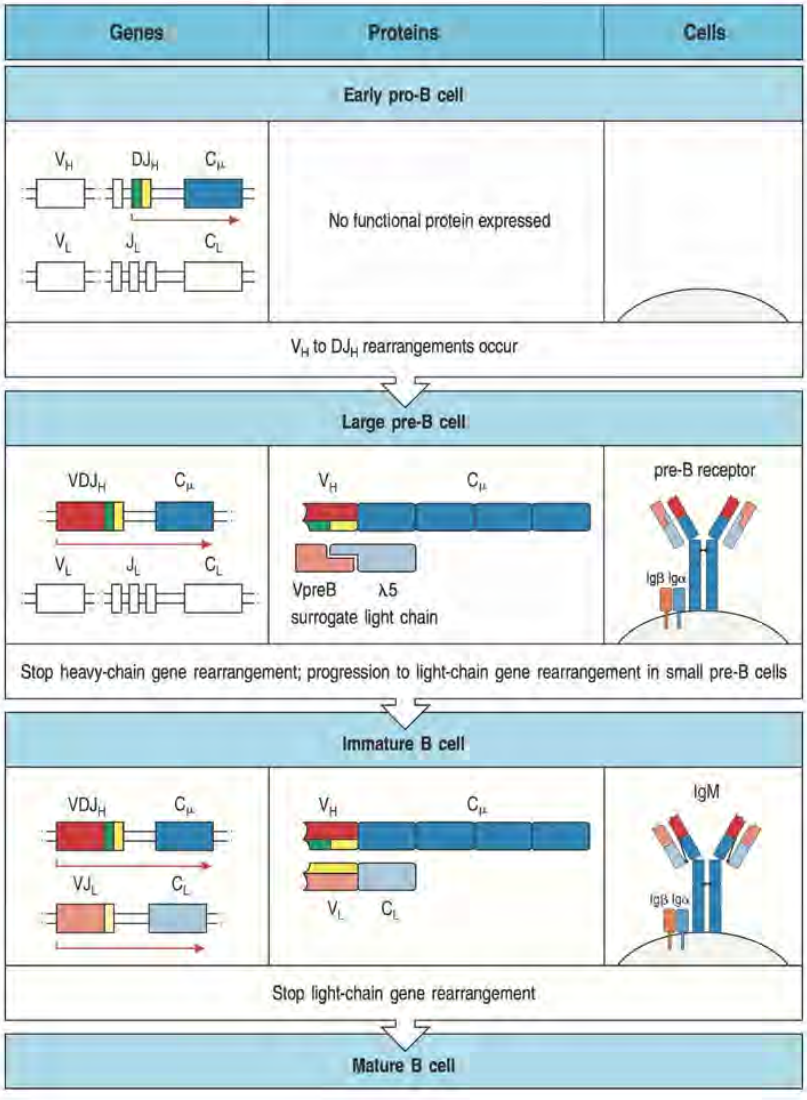


Fig. 8.6 A productively rearranged immunoglobulin gene is immediately expressed as a protein by the developing B cell. In early pro-B cells, heavy-chain gene rearrangement is not yet complete and no functional μ protein is expressed, although transcription occurs (red arrow), as shown in the top panel. As soon as a productive heavy-chain gene rearrangement takes place, μ chains are expressed by the cell in a complex with two other chains, λ5 and VpreB, which together make up a surrogate light chain. The whole immunoglobulin-like complex is known as the pre-B-cell receptor (center panel). It is associated with two other protein chains, Igα and Igβ, which signal the B cell to halt heavy-chain gene rearrangement, which drives the transition to the large pre-B cell stage by inducing proliferation. The progeny of large pre-B cells stop dividing and become small pre-B cells, in which light-chain gene rearrangements commence. Successful light-chain gene rearrangement results in the production of a light chain that binds the μ chain to form a complete IgM molecule, which is expressed together with Igα and Igβ at the cell surface, as shown in the bottom panel. Signaling via this surface receptor complex is thought to trigger the cessation of light-chain gene rearrangement.

Petitioner Merck, Ex. 1170, p. 78
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

intact μ heavy chains, after which V_H to DJ_H rearrangement ceases and the cell becomes a pre-B cell. Pro-B cells that do not produce a μ chain are eliminated, and at least 45% of pro-B cells are lost at this stage. In at least two out of three cases the first V_H to DJ_H rearrangement is nonproductive, and rearrangement then occurs on the other chromosome, again with a theoretical two in three chance of failure. A rough estimate of the chance of generating a pre-B cell is thus 55% $(1/3 + (2/3 \times 1/3) = 0.55)$. The actual frequency is somewhat lower, because the V gene segment repertoire contains pseudogenes that can rearrange yet have major defects that prevent the expression of a functional protein. An initial nonproductive rearrangement need not signal the immediate failure of development of the pro-B cell, because it is possible for most loci to undergo successive rearrangements on the same chromosome, and where that fails, the locus on the other chromosome will rearrange.

The diversity of the B-cell antigen-receptor repertoire is enhanced at this stage by the enzyme terminal deoxynucleotidyl transferase (TdT). TdT is expressed by the pro-B cell and adds nontemplated nucleotides (N-nucleotides) at the joints between rearranged gene segments (see Section 5-8). In adult humans it is expressed in pro-B cells during heavy-chain gene rearrangement, but its expression declines at the pre-B-cell stage during light-chain gene rearrangement. This explains why N-nucleotides are found in the V–D and D–J joints of nearly all heavy-chain genes but only in about a quarter of human light-chain joints. N-nucleotides are rarely found in mouse light-chain V–J joints, showing that TdT is switched off slightly earlier in the development of mouse B cells. In fetal development, when the peripheral immune system is first being supplied with T and B lymphocytes, TdT is expressed at low levels, if at all.

8-3 The pre-B-cell receptor tests for successful production of a complete heavy chain and signals for the transition from the pro-B cell to pre-B cell stage.

The imprecise nature of V(D)J recombination is a double-edged sword. Although it produces increased diversity in the antibody repertoire, it also results in many unsuccessful rearrangements. Pro-B cells therefore need a way of testing that a potentially functional heavy chain has been produced. They do this by incorporating a functional heavy chain into a receptor that can signal its successful production. This test takes place in the absence of light chains, whose loci have not yet rearranged. Instead, pro-B cells make two invariant 'surrogate' proteins that together have a structural resemblance to the light chain and can pair with the μ chain to form the pre-B-cell receptor (pre-BCR) (see Fig. 8.6). The assembly of a pre-B-cell receptor signals to the B cell that a productive rearrangement has been made, and the cell is then considered a pre-B cell.

The surrogate chains are encoded by nonrearranging genes separate from the antigen-receptor loci, and their expression is induced by E2A and EBF. One is called **λ5** because of its close resemblance to the C domain of the λ light chain; the other, called **VpreB**, resembles a light-chain V domain but has an extra region at the amino-terminal end. Other proteins expressed by pro-B cells and pre-B cells are also required for the formation of a functional receptor complex and are essential for B-cell development. The invariant proteins Igα and Igβ are components of both the pre-B-cell receptor and the B-cell receptor complexes on the cell surface. Igα and Igβ transduce signals from these receptors by interacting with intracellular tyrosine kinases through their cytoplasmic tails (see Section 7-7). Igα and Igβ are expressed from the pro-B-cell stage until the death of the cell or until its terminal differentiation into an antibody-secreting plasma cell.

Formation of the pre-B-cell receptor is an important checkpoint that mediates the transition between the pro-B cell and the pre-B cell. In mice that

Petitioner Merck, Ex. 1170, p. 79
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

either lack λ5 or have mutant heavy-chain genes that cannot produce the transmembrane domain, the pre-B-cell receptor cannot be formed and B-cell development is blocked after heavy-chain gene rearrangement. In normal B-cell development, the pre-B-cell receptor complex is expressed transiently, perhaps because the production of λ5 mRNA stops as soon as pre-B-cell receptors begin to be formed. Although present at only low levels on the cell surface, the pre-B-cell receptor generates signals required for the transition from pro-B cell to pre-B cell. No antigen or other external ligand seems to be involved in signaling by the receptor. Instead, pre-B-cell receptors are thought to interact with each other, forming dimers or oligomers that generate signals as described in Section 7-16. Dimerization involves 'unique' regions in the amino termini of λ5 and VpreB proteins that are not present in other immunoglobulin-like domains and which mediate the cross-linking of adjacent pre-B-cell receptors on the cell surface (Fig. 8.7). The signaling generated by receptor clustering halts further rearrangement of the heavy-chain locus and allows the pro-B cell to become sensitive to IL-7. This induces cell proliferation, initiating the transition to the large pre-B cell. Pre-B-cell receptor signaling requires the scaffold protein BLNK and Bruton's tyrosine kinase (Btk), an intracellular Tec-family tyrosine kinase (see Section 7-16). In humans and mice, deficiency of BLNK leads to a block in B-cell development at the pro-B-cell stage. In humans, mutations in the *BTK* gene cause a profound B-lineage-specific immune deficiency, Bruton's X-linked agammaglobulinemia (XLA), in which no mature B cells are produced. The block in B-cell development caused by mutations in *BTK* is almost total, interrupting the transition from pre-B cell to immature B cell. A similar, but less severe, defect called **X-linked immunodeficiency** or xid arises from mutations in the *Btk* gene in mice.



X-linked Agammaglobulinemia

8-4 Pre-B-cell receptor signaling inhibits further heavy-chain locus rearrangement and enforces allelic exclusion.

Successful rearrangements at both heavy-chain alleles could result in a B cell producing two receptors of different antigen specificities. To prevent this, signaling by the pre-B-cell receptor enforces **allelic exclusion**, the state in which only one of the two alleles of a gene is expressed in a diploid cell. Allelic exclusion, which occurs at both the heavy-chain locus and the light-chain loci, was

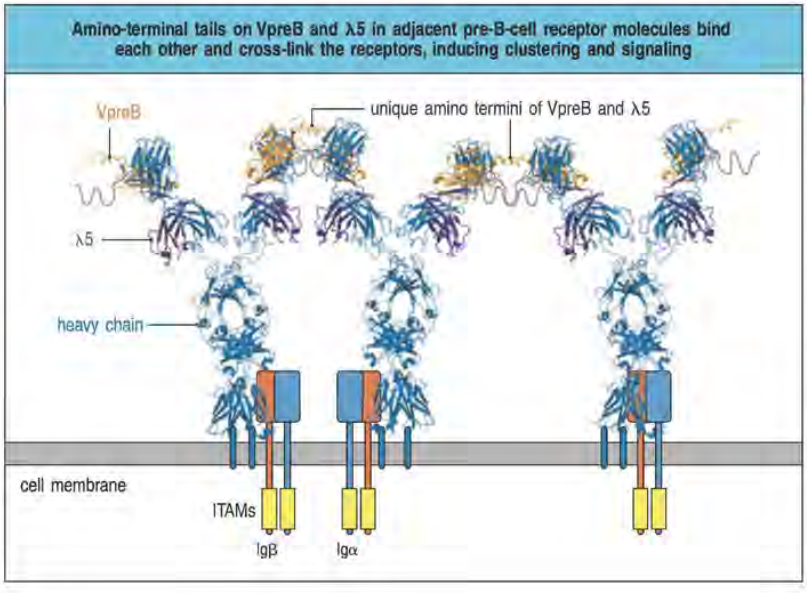


Fig. 8.7 The pre-B-cell receptor initiates signaling through spontaneous dimerization induced by the unique regions of VpreB and λ5. Two surrogate protein chains, VpreB (orange) and λ5 (purple), substitute for a light chain and bind to a heavy chain, thus allowing its surface expression. VpreB substitutes for the light-chain V region in this surrogate interaction, while λ5 takes the part of the light-chain constant region. Both VpreB and λ 5 contain 'unique' amino-terminal regions that are not present in other immunoglobulin-like domains, shown here as unstructured tails extending out from the globular domains. These amino-terminal regions associated with one pre-B-cell receptor can interact with the corresponding regions on the adjacent pre-B cell receptor, promoting the spontaneous formation of pre-B-cell receptor dimers on the cell surface. Dimerization generates signaling from the pre-B-cell receptor that is dependent on the presence of the ITAM-containing signaling chains Igα and Igβ. The signals cause the inhibition of RAG-1 and RAG-2 expression and the proliferation of the large pre-B cell. Courtesy of Chris Garcia.

Petitioner Merck, Ex. 1170, p. 80
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

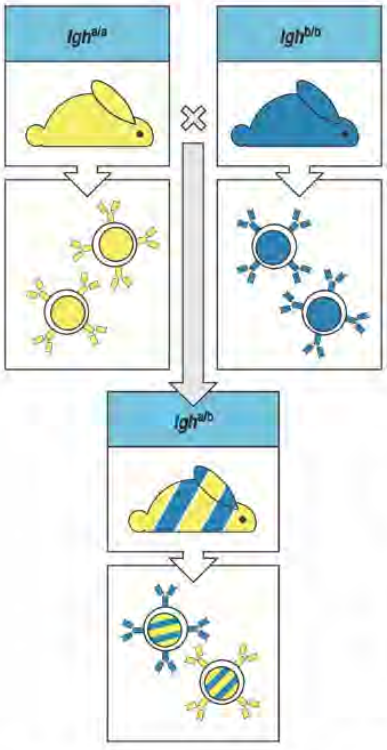


Fig. 8.8 Allelic exclusion in individual B cells. Most species have genetic polymorphisms of the constant regions of their immunoglobulin heavy-chain and light-chain genes; these are known as allotypes (see Appendix I, Section A-10). In rabbits, for example, all of the B cells in an individual homozygous for the *a* allele of the immunoglobulin heavy-chain locus ($Igh^{a/a}$) will express immunoglobulin of allotype a, whereas in an individual homozygous for the *b* allele ($Igh^{b/b}$) all the B cells make immunoglobulin of allotype b. In a heterozygous animal ($Igh^{a/b}$), which carries the *a* allele at one of the Igh loci and the *b* allele on the other, individual B cells can be shown to express surface immunoglobulin of either the a-allotype or the b-allotype, but not both. This allelic exclusion reflects the productive rearrangement of only one of the two *Igh* alleles in the B cell, because the production of a successfully rearranged immunoglobulin heavy chain forms a pre-B-cell receptor, which signals the cessation of further heavy-chain gene rearrangement.

discovered more than 30 years ago and provided one of the original pieces of experimental support for the theory that one lymphocyte expresses one type of antigen receptor (Fig. 8.8).

Signaling from the pre-B-cell receptor promotes heavy-chain allelic exclusion in three ways. First, it reduces V(D)J recombinase activity by directly reducing the expression of the *RAG-1* and *RAG-2* genes. Second, it further reduces levels of RAG-2 by indirectly causing this protein to be targeted for degradation, which occurs when RAG-2 is phosphorylated in response to the entry of the pro-B cell into S phase (the DNA synthesis phase) of the cell cycle. Finally, pre-B-cell receptor signaling reduces access of the heavy-chain locus to the recombinase machinery, although the precise details are not clear. At a later stage of B-cell development, RAG proteins will again be expressed in order to carry out light-chain locus rearrangement, but at that point the heavy-chain locus does not undergo further rearrangement. In the absence of pre-B-cell receptor signaling, allelic exclusion of the heavy-chain locus does not occur. For example, in λ5 knockout mice, in which the pre-B-cell receptor is not formed and the signal for V_H to DJ_H rearrangement to stop is not given, rearrangements of the heavy-chain genes are found on both chromosomes in all B-cell precursors, so that about 10% of the cells have two productive VDJ_H rearrangements.

8-5 Pre-B cells rearrange the light-chain locus and express cell-surface immunoglobulin.

The transition from the pro-B cell to the large pre-B-cell stage is accompanied by several rounds of cell division, expanding the population of cells with successful in-frame joins by about 30–60-fold before they become resting small pre-B cells. A large pre-B cell with a particular rearranged heavy-chain gene therefore gives rise to numerous small pre-B cells. RAG proteins are produced again in the small pre-B cells, and rearrangement of the light-chain locus begins. Each of these cells can make a different rearranged light-chain gene and so cells with many different antigen specificities are generated from a single pre-B cell, which makes an important contribution to overall B-cell receptor diversity.

Light-chain rearrangement also exhibits allelic exclusion. Rearrangements at the light-chain locus generally take place at only one allele at a time. The light-chain loci lack D segments and rearrangement occurs by V to J joining, and if a particular VJ rearrangement fails to produce a functional light chain, repeated rearrangements of unused V and J gene segments at the same allele can occur (Fig. 8.9). Several attempts at productive rearrangement of a light-chain gene can therefore be made on one chromosome before initiating any rearrangements on the second chromosome. This greatly increases the chances of eventually generating an intact light chain, especially as there are two different light-chain loci. As a result, many cells that reach the pre-B-cell stage succeed in generating progeny that bear intact IgM molecules and can be classified as **immature B cells**. Figure 8.10 lists some of the proteins involved in V(D)J recombination and shows how their expression is regulated throughout B-cell development. Figure 8.11 summarizes the stages of B-cell development up to the point of assembly of a complete surface immunoglobulin, indicating the points at which developing B cells can be lost as a result of failure to produce a productive join.

As well as allelic exclusion, light chains also display **isotypic exclusion**; that is, the expression of only one type of light chain—κ or λ—by an individual B cell. In mice and humans, the κ light-chain locus tends to rearrange before the λ locus. This was first deduced from the observation that myeloma cells secreting λ light chains generally have both their κ and λ light-chain genes rearranged, whereas in myelomas secreting κ light chains, generally only the

Petitioner Merck, Ex. 1170, p. 81
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

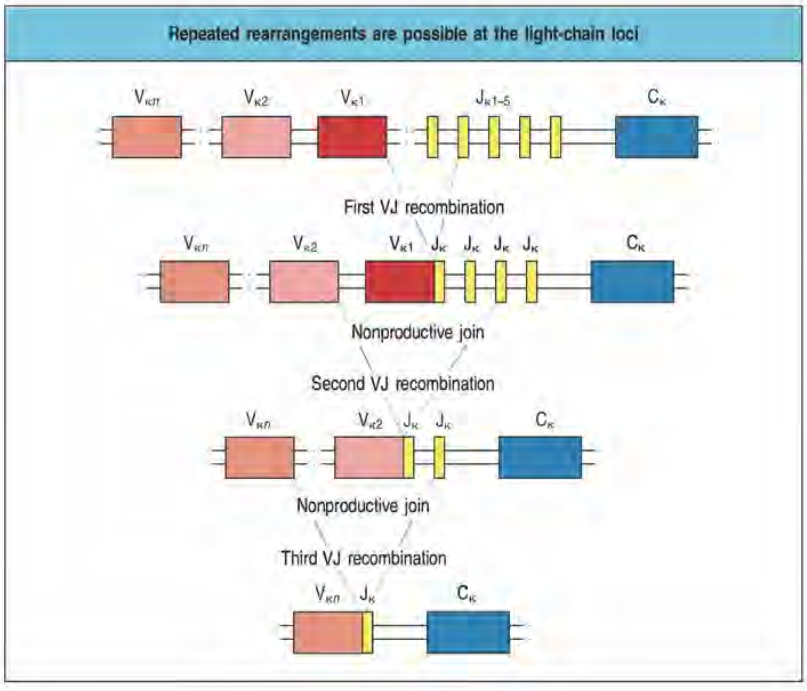


Fig. 8.9 Nonproductive light-chain gene rearrangements can be rescued by further rearrangement. The organization of the light-chain loci in mice and humans offers many opportunities for the rescue of pre-B cells that initially make an out-of-frame rearrangement. Light-chain rescue is illustrated here at the human κ locus. If the first rearrangement is nonproductive, a 5′ V_κ gene segment can recombine with a 3′ J_κ gene segment to remove the out-of-frame join located between them and to replace it with a new rearrangement. In principle, this can happen up to five times on each chromosome, because there are five functional J_κ gene segments in humans. If all rearrangements of κ-chain genes fail to yield a productive light-chain join, λ-chain gene rearrangement may succeed (not shown; see Fig. 8.11).

κ genes are rearranged. This order is occasionally reversed, however, and λ gene rearrangement does not absolutely require the previous rearrangement of the κ genes. The ratios of κ-expressing versus λ-expressing mature B cells vary from one extreme to the other in different species. In mice and rats it is 95% κ to 5% λ, in humans it is typically 65%:35%, and in cats it is 5%:95%, the opposite of that in mice. These ratios correlate most strongly with the number of functional V_κ and V_λ gene segments in the genome of the species. They also reflect the kinetics and efficiency of gene segment rearrangements. The κ:λ ratio in the mature lymphocyte population is useful in clinical diagnostics, because an aberrant κ:λ ratio indicates the dominance of one clone and the presence of a lymphoproliferative disorder, which may be malignant.

8-6 Immature B cells are tested for autoreactivity before they leave the bone marrow.

Once a rearranged light chain has paired with a μ chain, IgM can be expressed on the cell surface (sIgM) and the pre-B cell becomes an **immature B cell**. At this stage, the antigen receptor is first tested for reactivity to self antigens, or autoreactivity. The elimination or inactivation of autoreactive B cells ensures that the B-cell population as a whole will be tolerant of self antigens. The **tolerance** produced at this stage of B-cell development is known as **central tolerance** because it arises in a central lymphoid organ, the bone marrow. As we shall see later in the chapter and in Chapter 15, self-reactive B cells that escape this test and go on to mature may still be removed from the repertoire after they have left the bone marrow, a process that produces **peripheral tolerance**.

sIgM associates with Igα and Igβ to form a functional B-cell receptor complex, and the fate of an immature B cell in the bone marrow depends on signals delivered from this receptor complex on its interaction with ligands in the environment. Igα signaling is particularly important in dictating the

Petitioner Merck, Ex. 1170, p. 82
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

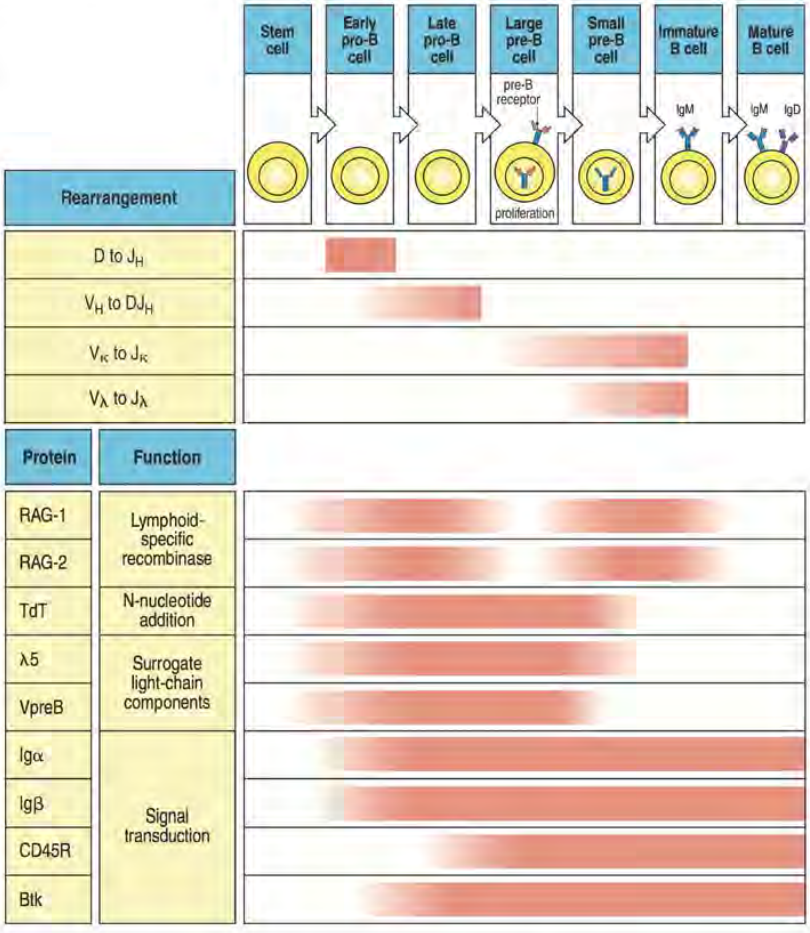


Fig 8.10 Expression of proteins involved in gene rearrangement and the production of pre-B-cell and B-cell receptors. The proteins listed here have been included because of their proven importance in the developmental sequence, largely on the basis of studies in mice. Their individual contributions to B-cell development are discussed in the text. Also shown is the temporal sequence of gene rearrangement. Signaling proteins and transcription factors involved in early B-lineage development are listed in Fig. 8.5.

emigration of B cells from the bone marrow and/or their survival in the periphery: mice that express Igα with a truncated cytoplasmic domain that cannot signal show a fourfold reduction in the number of immature B cells in the marrow, and a hundredfold reduction in the number of peripheral B cells.

Immature B cells that have no strong reactivity to self antigens are allowed to mature (Fig. 8.12, first panel). They leave the marrow via sinusoids that enter the central sinus and are carried by the venous blood supply to the spleen. If, however, the newly expressed receptor encounters a strongly cross-linking antigen in the bone marrow—that is, if the B cell is strongly self-reactive—development is arrested and the cell will not mature. This was first demonstrated by experiments in which antigen receptors on immature B cells were experimentally stimulated *in vivo* using anti-μ chain antibodies (see Appendix I, Section A-10); the outcome was elimination of the immature B cells.

More recent experiments using mice expressing transgenes that enforce the expression of self-reactive B-cell receptors confirmed these earlier findings, but also showed that immediate elimination is not the only possible outcome of binding to a self antigen. There are four possible fates for self-reactive immature B cells, depending on the nature of the ligand they recognize (Fig. 8.12, last three panels). These fates are: cell death by apoptosis or clonal deletion; the production of a new receptor by a process known as receptor editing; the induction of a permanent state of unresponsiveness, or anergy, to antigen; and a state of immunological ignorance.

Petitioner Merck, Ex. 1170, p. 83
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

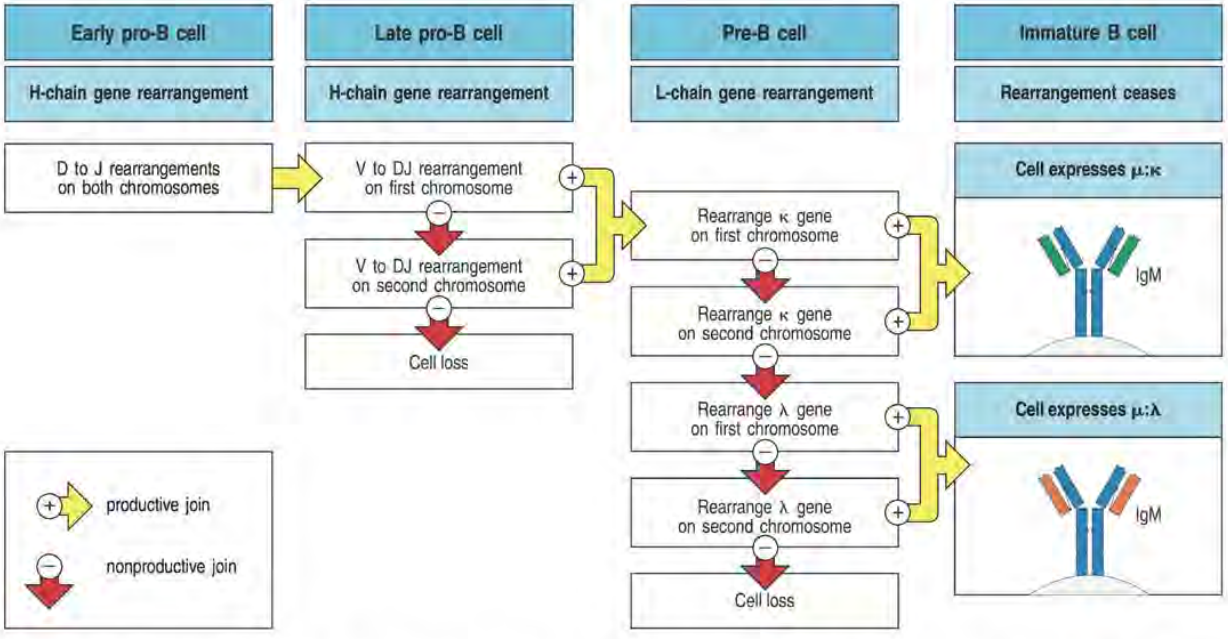


Fig. 8.11 The steps in immunoglobulin gene rearrangement at which developing B cells can be lost. The developmental program rearranges the heavy-chain (H-chain) locus first and then the light-chain (L-chain) loci. Cells are allowed to progress to the next stage when a productive rearrangement has been achieved. Each rearrangement has about a one in three chance of being successful, but if the first attempt is nonproductive, development is suspended and there is a chance for one or more further attempts, so by simple mathematics four in nine rearrangements give rise to a heavy chain. The scope for repeated rearrangements is greater at the light-chain loci (see Fig. 8.9), so that fewer cells are lost between the pre-B and immature B-cell stages than in the pro-B to pre-B transition.

Clonal deletion, or the removal of cells of a particular antigen specificity from the repertoire, seems to predominate when the interacting self antigen is multivalent. The effect of an encounter with a multivalent antigen was tested in mice transgenic for both chains of an immunoglobulin specific for H-2K^b MHC class I molecules. In such mice, nearly all the B cells that develop bear the anti-MHC immunoglobulin as sIgM. If the transgenic mouse does not express H-2K^b, normal numbers of B cells develop, all bearing transgene-encoded anti-H-2K^b receptors. However, in mice expressing both H-2K^b and the immunoglobulin transgenes, B-cell development is blocked. Normal numbers of pre-B cells and immature B cells are found, but B cells expressing the anti-H-2K^b immunoglobulin as sIgM never mature to populate the spleen and lymph nodes; instead, most of these immature B cells die in the bone marrow by apoptosis.

Not all lymphocytes with strongly autoreactive receptors undergo clonal deletion. There is an interval before cell death during which the autoreactive B cell can be rescued by further gene rearrangements that replace the autoreactive receptor with a new receptor that is not self-reactive. This mechanism is termed **receptor editing** (Fig. 8.13). When an immature B cell first produces sIgM, RAG protein is still being made. If the receptor is not self-reactive, the absence of sIgM cross-linking allows gene rearrangement to cease and B-cell development continues, with RAG proteins eventually disappearing. For an autoreactive receptor, however, an encounter with the self antigen results in strong cross-linking of sIgM, RAG expression continues, and light-chain gene rearrangement can continue, as described in Fig. 8.9. These secondary rearrangements can rescue immature self-reactive B cells by deleting the self-reactive light-chain gene and replacing it with another sequence. If the

Petitioner Merck, Ex. 1170, p. 84
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

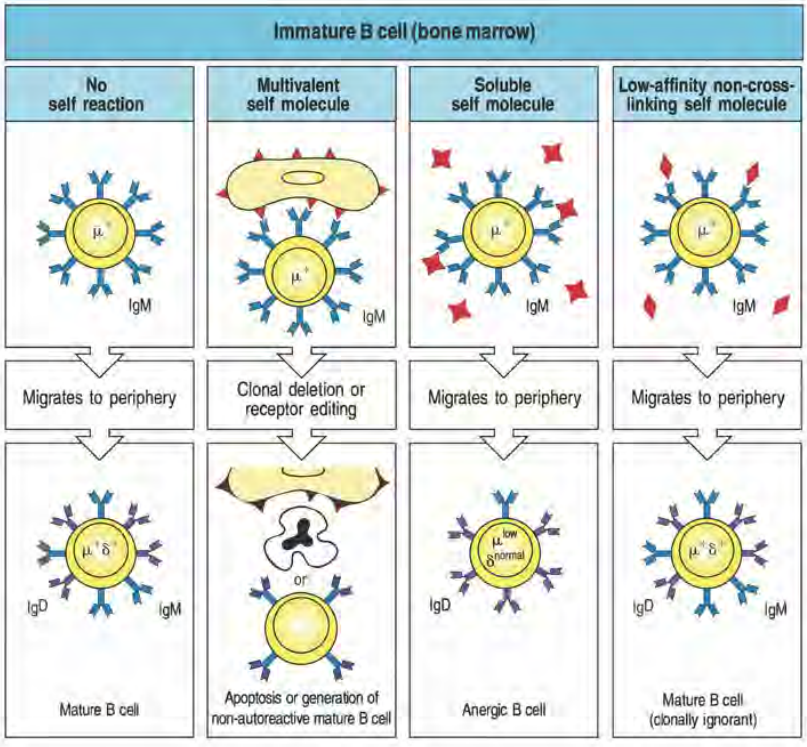


Fig. 8.12 Binding to self molecules in the bone marrow can lead to the death or inactivation of immature B cells. First panels: immature B cells that do not encounter antigen mature normally; they migrate from the bone marrow to the peripheral lymphoid tissues, where they may become mature recirculating B cells bearing both IgM and IgD on their surface. Second panels: when developing B cells express receptors that recognize multivalent ligands, for example ubiquitous self cell-surface molecules such as those of the MHC, these receptors are deleted from the repertoire. The B cells either undergo receptor editing (see Fig. 8.13), so that the self-reactive receptor specificity is deleted, or the cells themselves undergo programmed cell death or apoptosis (clonal deletion). Third panels: immature B cells that bind soluble self antigens able to cross-link the B-cell receptor are rendered unresponsive to the antigen (anergic) and bear little surface IgM. They migrate to the periphery, where they express IgD but remain anergic; if in competition with other B cells in the periphery, they are rapidly lost. Fourth panels: immature B cells whose antigen is inaccessible to them, or which bind monovalent or soluble self antigens with low affinity, do not receive any signal and mature normally. Such cells are potentially self-reactive, however, and are said to be clonally ignorant because their ligand is present but is unable to activate them.

new light chain is not autoreactive, the B cell continues normal development. If the receptor remains autoreactive, rearrangement continues until a non-autoreactive receptor is produced or light-chain V and J gene segments are exhausted. Cells that remain autoreactive undergo apoptosis.

Receptor editing has been shown definitively in mice bearing transgenes for autoantibody heavy and light chains that have been placed within the immunoglobulin loci by homologous recombination (see Appendix I, Section A-46, for details of this method). The transgene imitates a primary gene rearrangement and is surrounded by unused endogenous gene segments. In mice that express the antigen recognized by the transgene-encoded receptor, the mature B cells that emerge into the periphery have used these surrounding gene segments for rearrangements that replace the autoreactive light-chain transgene with a non-autoreactive gene.

It is not clear whether receptor editing occurs at the heavy-chain locus. There are no available D segments at a rearranged heavy-chain locus, so a new rearrangement cannot simply occur by the normal mechanism and remove the preexisting one. Instead, a process of V_H replacement may use embedded recombination signal sequences in a recombination event that displaces the V gene segment from the self-reactive rearrangement and replaces it with a new V gene segment. This has been observed in some B-cell tumors, but whether it occurs during normal B-cell development in response to signals from autoreactive B-cell receptors is not certain.

It was originally thought that the successful production of a heavy chain and a light chain caused the almost instantaneous shutdown of light-chain locus rearrangement and that this ensured both allelic and isotypic exclusion. The unexpected ability of self-reactive B cells to continue to rearrange their light-chain genes, even after having made a productive rearrangement, has raised questions about this supposed mechanism of allelic exclusion. This

Petitioner Merck, Ex. 1170, p. 85
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

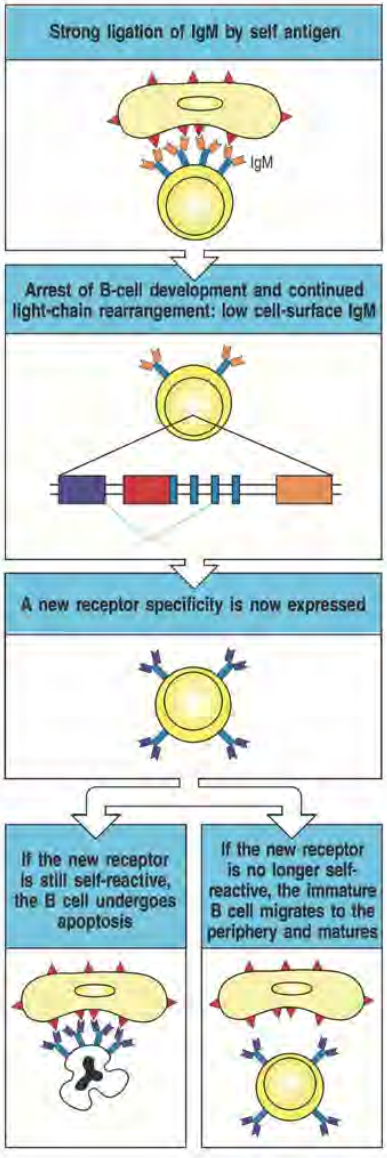


Fig. 8.13 Replacement of light chains by receptor editing can rescue some self-reactive B cells by changing their antigen specificity. When a developing B cell expresses antigen receptors that are strongly cross-linked by multivalent self antigens such as MHC molecules on cell surfaces (top panel), its development is arrested. The cell decreases surface expression of IgM and does not turn off the *RAG* genes (second panel). Continued synthesis of RAG proteins allows the cell to continue light-chain gene rearrangement. This usually leads to a new productive rearrangement and the expression of a new light chain, which combines with the previous heavy chain to form a new receptor (receptor editing; third panel). If this new receptor is not self-reactive, the cell is 'rescued' and continues normal development much like a cell that had never reacted with self (bottom right panel). If the cell remains self-reactive it may be rescued by another cycle of rearrangement, but if it continues to react strongly with self it will undergo programmed cell death or apoptosis and be deleted from the repertoire (clonal deletion; bottom left panel).

suggests that the fall in the level of RAG protein that follows a successful non-autoreactive rearrangement could be the principal, but not necessarily the sole, mechanism behind allelic exclusion at the light-chain locus. It seems that allelic exclusion is not absolute as there are rare B cells that express two different light chains.

We have so far discussed the fate of newly formed B cells that undergo multivalent cross-linking of their sIgM. Immature B cells that encounter more weakly cross-linking self antigens of low valence, such as small soluble proteins, respond differently. In this situation, self-reactive B cells tend to be inactivated and enter a state of permanent unresponsiveness, or **anergy**, but do not immediately die (see Fig. 8.12). Anergic B cells cannot be activated by their specific antigen even with help from antigen-specific T cells. Again, this phenomenon was elucidated using transgenic mice. Hen egg-white lysozyme (HEL) was expressed in soluble form from a transgene in mice that were also transgenic for high-affinity anti-HEL immunoglobulin. The HEL-specific B cells matured but could not respond to antigen. Anergic cells retain their IgM within the cell and transport little to the surface. In addition, they develop a partial block in signal transduction so that, despite normal levels of HEL-binding sIgD, the cells cannot be stimulated by cross-linking this receptor. The signaling defect may involve the action of two ubiquitin ligases (see Section 7-5), Cbl and Cbl-b. These proteins seem to target Igα and Syk for degradation, reducing sIgM expression and BLNK phosphorylation (see Section 7-16). In mice lacking these proteins, B cells that would normally become anergic go on to develop into mature B cells with normal surface expression of IgM.

The migration of anergic B cells within peripheral lymphoid organs is impaired, and their life-span and their ability to compete with immuno-competent B cells are compromised. In normal circumstances, in which B cells binding a soluble self antigen are rare, self-reactive anergic B cells are detained in the T-cell areas of peripheral lymphoid tissues and are excluded from lymphoid follicles. Anergic B cells cannot be activated by T cells, because all the T cells will be tolerant to soluble antigens. Instead they die relatively quickly, presumably because they fail to get survival signals from T cells, which ensures that the long-lived pool of peripheral B cells is purged of potentially self-reactive cells.

The fourth potential fate of self-reactive immature B cells is that nothing happens to them; they remain in a state of **immunological ignorance** of their self antigen (see Fig. 8.12). Immunologically ignorant cells have affinity for a self antigen but for various reasons do not sense and respond to it. The antigen may not be accessible to developing B cells in the bone marrow or spleen, or may be in low concentration, or may bind so weakly to the B-cell receptor that it does not generate an activating signal. Because some ignorant cells can be (and in fact are) activated under certain conditions such as inflammation or

Petitioner Merck, Ex. 1170, p. 86
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

when the self antigen becomes available or reaches an unusually high concentration, they should not be considered inert, and they are fundamentally different from cells with non-autoreactive receptors that could never be activated by self antigens.

The fact that central tolerance is not perfect and some self-reactive B cells are allowed to mature reflects the balance that the immune system strikes between purging all self-reactivity and maintaining the ability to respond to pathogens. If the elimination of self-reactive cells were too efficient, the receptor repertoire might become too limited and thus unable to recognize a wide variety of pathogens. Some autoimmune disease is the price of this balance: we shall see in Chapter 15 that ignorant self-reactive lymphocytes can be activated and cause disease under certain circumstances. Normally, however, ignorant B cells are held in check by a lack of T-cell help, the continued inaccessibility of the self antigen, or the tolerance that can be induced in mature B cells, which is described later in this chapter.

Summary.

So far we have followed B-cell development from the earliest progenitors in the bone marrow to the immature B cell that is ready to emerge into the peripheral lymphoid tissue. The heavy-chain locus is rearranged first and, if this is successful, a μ heavy chain is produced that combines with surrogate light chains to form the pre-B-cell receptor; this is the first checkpoint in B-cell development. Production of the pre-B-cell receptor signals successful heavy-chain gene rearrangement, and causes cessation of this rearrangement, thus enforcing allelic exclusion. It also initiates pre-B-cell proliferation, generating numerous progeny in which subsequent light-chain rearrangement can be attempted. If the initial light-chain gene rearrangement is productive, a complete immunoglobulin B-cell receptor is formed, gene rearrangement again ceases, and the B cell continues its development. If the first light-chain gene rearrangement is unsuccessful, rearrangement continues until either a productive rearrangement is made or all available J regions are used up. If no productive rearrangement is made, the developing B cell dies. In the next part of the chapter, we turn to T-cell development.

The development of T lymphocytes in the thymus.

Like B cells, T lymphocytes derive from the multipotent hematopoietic stem cells in the bone marrow. However, their progenitor cells migrate from the bone marrow via the blood to the thymus, where they mature (Fig. 8.14); this is the reason for the name thymus-dependent (T) lymphocytes or T cells. T-cell development parallels that of B cells in many ways, including the orderly and stepwise rearrangement of antigen-receptor genes, the sequential testing for successful gene rearrangement, and the eventual assembly of a heterodimeric antigen receptor. Nevertheless, T-cell development in the thymus has some features not seen for B cells, such as the generation of two distinct lineages of T cells, the γ:δ lineage and the α:β lineage, which express distinct antigen-receptor genes. Developing T cells, which are known generally as **thymocytes**, also undergo rigorous selection that depends on interactions with thymic cells and that shapes the mature repertoire of T cells to ensure self-MHC restriction as well as self-tolerance. We begin with a general overview of the stages of thymocyte development and its relationship to thymic anatomy before considering gene rearrangement and the mechanisms of selection.

Petitioner Merck, Ex. 1170, p. 87
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

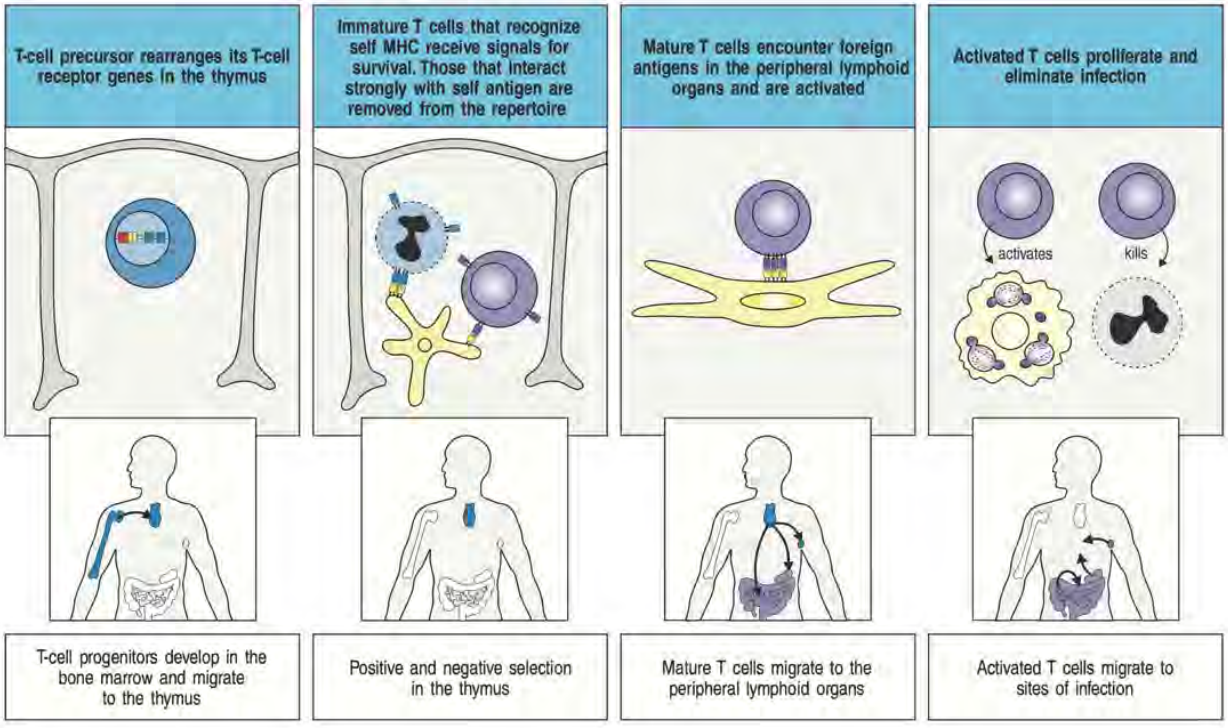


Fig. 8.14 T cells undergo development in the thymus and migrate to the peripheral lymphoid organs, where they are activated by foreign antigens. T-cell precursors migrate from the bone marrow to the thymus, where the T-cell receptor genes are rearranged (first panels); α:β T-cell receptors that are compatible with self-MHC molecules transmit a survival signal on interacting with thymic epithelium, leading to positive selection of the cells that bear them. Self-reactive receptors transmit a signal that leads to cell death, and cells bearing them are removed from the repertoire in a process of negative selection (second panels). T cells that survive selection mature and leave the thymus to circulate in the periphery; they repeatedly leave the blood to migrate through the peripheral lymphoid organs, where they may encounter their specific foreign antigen and become activated (third panels). Activation leads to clonal expansion and differentiation into effector T cells. These are attracted to sites of infection, where they can kill infected cells or activate macrophages (fourth panels); others are attracted into B-cell areas, where they help to activate an antibody response (not shown).

8-7 T-cell progenitors originate in the bone marrow, but all the important events in their development occur in the thymus.

The thymus is situated in the upper anterior thorax, just above the heart. It consists of numerous lobules, each clearly differentiated into an outer cortical region—the **thymic cortex**—and an inner **medulla** (Fig. 8.15). In young individuals, the thymus contains large numbers of developing T-cell precursors embedded in a network of epithelia known as the **thymic stroma**. This provides a unique microenvironment for T-cell development analogous to that provided for B cells by the stromal cells of the bone marrow.

T lymphocytes develop from a lymphoid progenitor in the bone marrow that also gives rise to B lymphocytes. Some of these progenitors leave the bone marrow and migrate to the thymus. In the thymus, the progenitor cell receives a signal, most probably from stromal cells, that is transduced through a receptor called Notch1 to switch on specific genes. Notch signaling is widely used in animal development to specify tissue differentiation; in lymphocyte development, the Notch signal instructs the precursor to commit to the T-cell lineage rather than the B-cell lineage. Notch signaling is required throughout T-cell development and is also thought to help regulate other T-cell lineage choices, including the α:β versus γ:δ choice and the CD4 versus CD8 decision.

The thymic epithelium arises early in embryonic development from endoderm-derived structures known as the third pharyngeal pouches. These epithelial tissues form a rudimentary thymus, or **thymic anlage**. This is colonized by cells of hematopoietic origin that give rise to large numbers of thymocytes, which are committed to the T-cell lineage, and to **intrathymic dendritic cells**. Thymocytes are not simply passengers within the thymus: they influence the arrangement of the thymic epithelial cells on which they depend for survival, inducing the formation of a reticular epithelial structure that surrounds the developing thymocytes (Fig. 8.16). The thymus is independently colonized by numerous macrophages, also of bone marrow origin.

Petitioner Merck, Ex. 1170, p. 88
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

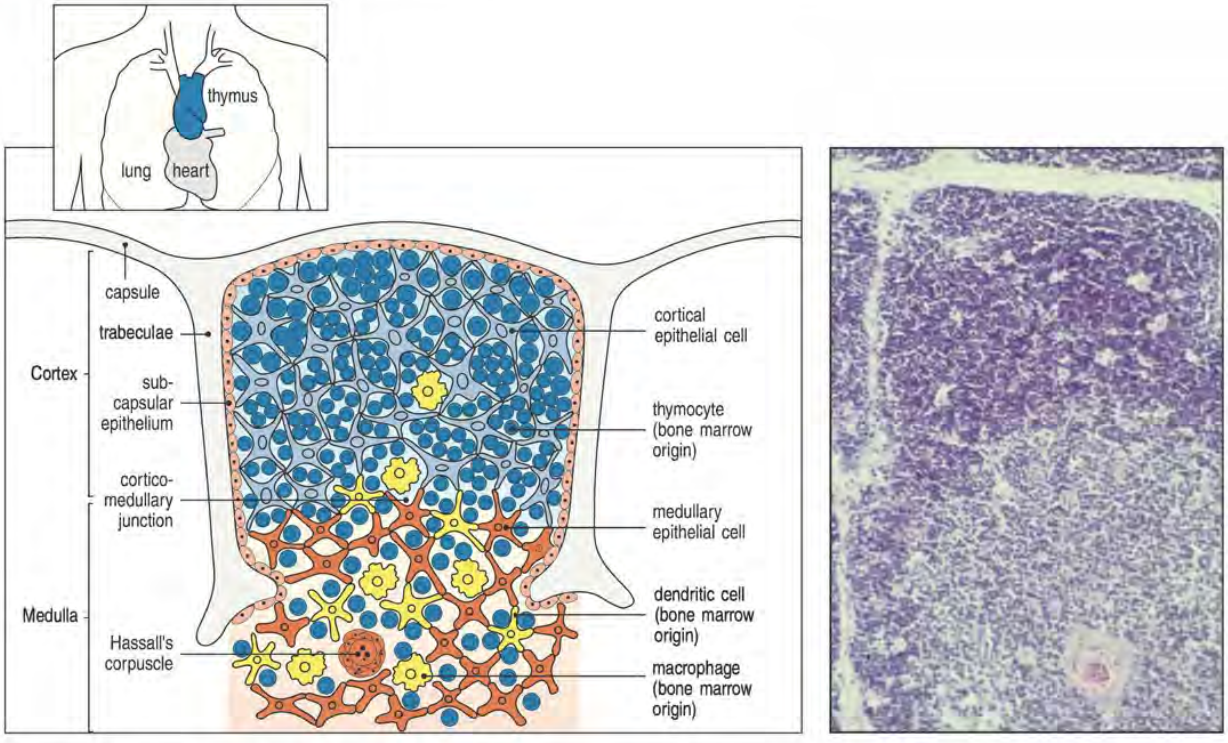


Fig. 8.15 The cellular organization of the human thymus. The thymus, which lies in the midline of the body, above the heart, is made up of several lobules, each of which contains discrete cortical (outer) and medullary (central) regions. As shown in the diagram on the left, the cortex consists of immature thymocytes (dark blue), branched cortical epithelial cells (pale blue), with which the immature cortical thymocytes are closely associated, and scattered macrophages (yellow), which are involved in clearing apoptotic thymocytes. The medulla consists of mature thymocytes (dark blue) and medullary epithelial cells (orange), along with macrophages (yellow) and dendritic cells (yellow) of bone marrow origin. Hassall's corpuscles are probably also sites of cell destruction. The thymocytes in the outer cortical cell layer are proliferating immature cells, whereas the deeper cortical thymocytes are mainly immature T cells undergoing thymic selection. The photograph shows the equivalent section of a human thymus, stained with hematoxylin and eosin. The cortex is darkly staining, whereas the medulla is lightly stained. The large body in the medulla is a Hassall's corpuscle. Photograph courtesy of C.J. Howe.

The cellular architecture of the human thymus is illustrated in Fig. 8.15. Bone marrow derived cells are differentially distributed between the thymic cortex and medulla. The cortex contains only immature thymocytes and scattered macrophages, whereas more mature thymocytes, along with dendritic cells and macrophages, are found in the medulla. This reflects the different developmental events that occur in these two compartments.

The importance of the thymus in immunity was first discovered through experiments on mice; indeed, most of our knowledge of T-cell development in the thymus comes from the mouse. It was found that surgical removal of the thymus (**thymectomy**) at birth resulted in immunodeficient mice, focusing interest on this organ at a time when the difference between T and B lymphocytes in mammals had not been defined. Much evidence, including observations in immunodeficient children, has since confirmed the importance of the thymus in T-cell development. In **DiGeorge syndrome** in humans and in mice with the ***nude*** mutation, the thymus does not form

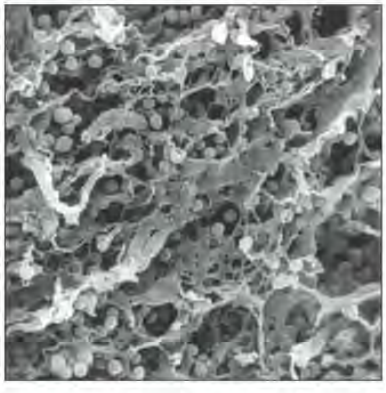


Fig. 8.16 The epithelial cells of the thymus form a network surrounding developing thymocytes. In this scanning electron micrograph of the thymus, the developing thymocytes (the spherical cells) occupy the interstices of an extensive network of epithelial cells. Photograph courtesy of W. van Ewijk.

Petitioner Merck, Ex. 1170, p. 89
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

and the affected individual produces B lymphocytes but few T lymphocytes. DiGeorge syndrome is a complex combination of cardiac, facial, endocrine, and immune defects associated with deletions of chromosome 22q11. The *nude* mutation in mice is due to a defect in the gene for Foxn1, a transcription factor required for terminal epithelial cell differentiation; the name *nude* was given to this mutation because it also causes hairlessness. Rare cases of a defect in the human *FOXN1* gene (which is on chromosome 17) have been associated with T-cell immunodeficiency, absence of a thymus, congenital alopecia, and nail dystrophy.

DiGeorge Syndrome

The crucial role of the thymic stroma in inducing the differentiation of the bone marrow derived T-cell progenitors can be demonstrated by the results of tissue grafts between two mutant mice, each lacking mature T cells for a different reason. In *nude* mice the thymic epithelium fails to differentiate, whereas in *scid* mice B and T lymphocytes fail to develop because of a defect in antigen-receptor gene rearrangement (see Section 5-5). Reciprocal grafts of thymus and bone marrow between these immunodeficient strains show that *nude* bone marrow precursors develop normally in a *scid* thymus (Fig. 8.17).

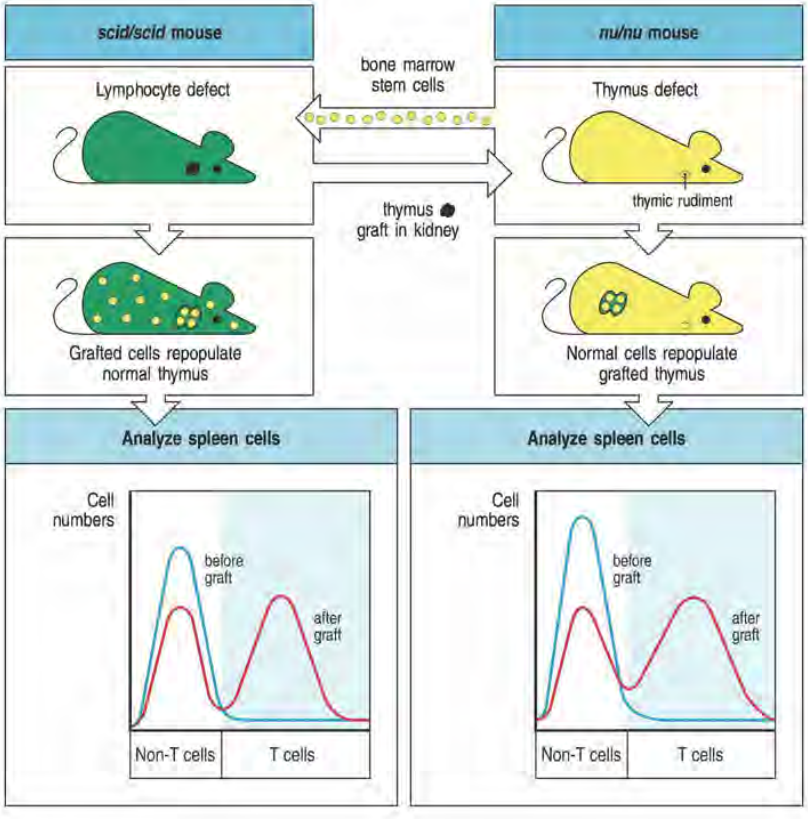


Fig. 8.17 The thymus is critical for the maturation of bone marrow derived cells into T cells. Mice with the *scid* mutation (upper left photograph) have a defect that prevents lymphocyte maturation, whereas mice with the *nude* mutation (upper right photograph) have a defect that affects the development of the cortical epithelium of the thymus. T cells do not develop in either strain of mouse: this can be demonstrated by staining spleen cells with antibodies specific for mature T cells and analyzing them in a flow cytometer (see Appendix I, Section A-22), as represented by the blue line in the graphs in the bottom panels. Bone marrow cells from *nude* mice can restore T cells to *scid* mice (red line in graph on left), showing that, in the correct environment, the *nude* bone marrow cells are intrinsically normal and capable of producing T cells. Thymic epithelial cells provided by a thymus graft from *scid* mice into the kidney of *nude* mice can induce the maturation of T cells in *nude* mice (red line in graph on right), demonstrating that the thymus provides the essential microenvironment for T-cell development.

Petitioner Merck, Ex. 1170, p. 90
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Thus, the defect in *nude* mice is in the thymic stromal cells. Transplanting a *scid* thymus into a *nude* mouse leads to T-cell development. However, *scid* bone marrow cannot develop T cells, even in a wild-type recipient.

In mice, the thymus continues to develop for 3–4 weeks after birth, whereas in humans it is fully developed at birth. The rate of T-cell production by the thymus is greatest before puberty. After puberty, the thymus begins to shrink and the production of new T cells in adults is lower, although it does continue throughout life. In both mice and humans, removal of the thymus after puberty is not accompanied by any notable loss of T-cell function or numbers. Thus, it seems that once the T-cell repertoire is established, immunity can be sustained without the production of significant numbers of new T cells; the pool of peripheral T cells is instead maintained by long-lived T cells and also by some division of mature T cells.

8-8 T-cell precursors proliferate extensively in the thymus, but most die there.

T-cell precursors arriving in the thymus from the bone marrow spend up to a week differentiating there before they enter a phase of intense proliferation. In a young adult mouse the thymus contains about 10^8 to 2×10^8 thymocytes. About 5×10^7 new cells are generated each day; however, only about 10^6 to 2×10^6 (roughly 2–4%) of these leave the thymus each day as mature T cells. Despite the disparity between the number of T cells generated in the thymus and the number leaving, the thymus does not continue to grow in size or cell numbers. This is because about 98% of the thymocytes that develop in the thymus also die in the thymus by apoptosis (see Section 1-14). Changes in the plasma membrane of cells undergoing apoptosis lead to their rapid phagocytosis, and apoptotic bodies, which are the residual condensed chromatin of apoptotic cells, are seen inside macrophages throughout the thymic cortex (Fig. 8.18). This apparently profligate waste of thymocytes is a crucial part of T-cell development because it reflects the intensive screening that each thymocyte undergoes for the ability to recognize self-peptide:self-MHC complexes and for self-tolerance.

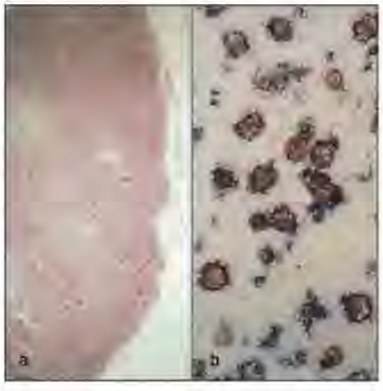


Fig. 8.18 Developing T cells that undergo apoptosis are ingested by macrophages in the thymic cortex. Panel a shows a section through the thymic cortex and part of the medulla in which cells have been stained for apoptosis with a red dye. Thymic cortex is to the right of the photograph. Apoptotic cells are scattered throughout the cortex but are rare in the medulla. Panel b shows a section of thymic cortex at higher magnification that has been stained red for apoptotic cells and blue for macrophages. The apoptotic cells can be seen within macrophages. Magnifications: panel a, ×45; panel b, ×164. Photographs courtesy of J. Sprent and C. Surh.

8-9 Successive stages in the development of thymocytes are marked by changes in cell-surface molecules.

Like developing B cells, developing thymocytes pass through a series of distinct stages. These are marked by changes in the status of the T-cell receptor genes and in the expression of the T-cell receptor, and by changes in the expression of cell-surface proteins such as the CD3 complex (see Section 7-7) and the co-receptor proteins CD4 and CD8 (see Section 4-17). These surface changes reflect the state of functional maturation of the cell, and particular combinations of cell-surface proteins are used as markers for T cells at different stages of differentiation. The principal stages are summarized in Fig. 8.19. Two distinct lineages of T cells—α:β and γ:δ, which have different types of T-cell receptor chains—are produced early in T-cell development. Later, α:β T cells develop into two distinct functional subsets—CD4 T cells and CD8 T cells.

When progenitor cells first enter the thymus from the bone marrow, they lack most of the surface molecules characteristic of mature T cells, and their receptor genes are not rearranged. These cells give rise to the major population of α:β T cells and the minor population of γ:δ T cells. If injected into the peripheral circulation, these lymphoid progenitors can even give rise to B cells and NK cells. Interactions with the thymic stroma trigger an initial phase of differentiation along the T-cell lineage pathway followed by cell proliferation and the expression of the first cell-surface molecules specific for T cells,

Petitioner Merck, Ex. 1170, p. 91
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

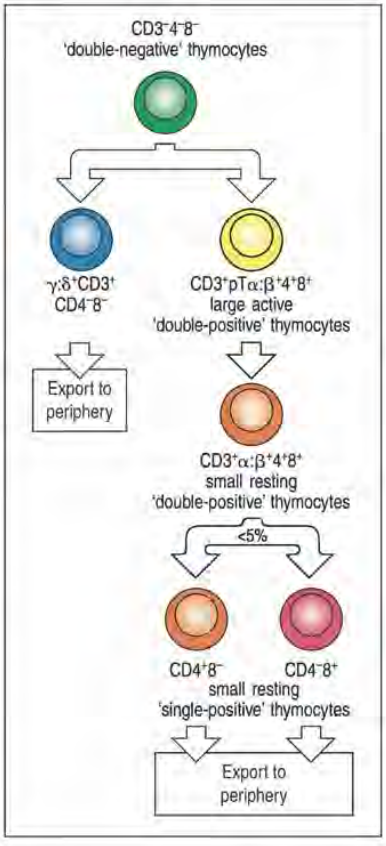


Fig. 8.19 Two distinct lineages of thymocytes are produced in the thymus. CD4, CD8, and T-cell receptor complex molecules (CD3, and the T-cell receptor α and β chains) are important cell-surface molecules for identifying thymocyte subpopulations. The earliest cell population in the thymus does not express any of these proteins, and because they do not express CD4 or CD8 they are called 'double-negative' thymocytes. These cells include precursors that give rise to two T-cell lineages: the minority population of γ:δ T cells (which lack CD4 or CD8 even when mature), and the majority α:β T-cell lineage. The development of prospective α:β T cells proceeds through stages in which both CD4 and CD8 are expressed by the same cell; these are known as 'double-positive' thymocytes. These cells enlarge and divide. Later, they become small resting double-positive cells that express low levels of the T-cell receptor. Most thymocytes die within the thymus after becoming small double-positive cells, but those cells whose receptors can interact with self-peptide:self-MHC molecular complexes lose expression of either CD4 or CD8 and increase the level of expression of the T-cell receptor. The outcome of this process is the 'single-positive' thymocytes, which, after maturation, are exported from the thymus as mature single-positive CD4 or CD8 T cells.

for example CD2 and (in mice) Thy-1. At the end of this phase, which can last about a week, the thymocytes bear distinctive markers of the T-cell lineage but do not express any of the three cell-surface markers that define mature T cells. These are the CD3:T-cell receptor complex and the co-receptors CD4 or CD8. Because of the absence of CD4 and CD8, such cells are called **double-negative thymocytes** (see Fig. 8.19).

In the fully developed thymus, immature double-negative T cells constitute about 60% of the thymocytes that lack both CD4 and CD8. This pool (about 5% of all thymocytes) also includes two populations of more mature T cells that belong to minority lineages. One of these, representing about 20% of the double-negative cells, comprises cells that have rearranged and are expressing the genes encoding the γ:δ T-cell receptor; we will return to these cells in Section 8-12. The second, representing another 20% of all double-negative thymocytes, includes cells bearing α:β T-cell receptors of very limited diversity. These cells also express the NK1.1 receptor commonly found on NK cells; they are therefore known as **invariant NKT cells (iNKT cells)**. iNKT cells are activated as part of the early response to many infections; they differ from the major lineage of α:β T cells in recognizing CD1 molecules rather than MHC class I or MHC class II molecules (see Section 6-19) and they are not shown in Fig. 8.19. In this and subsequent discussions, we reserve the term 'double-negative thymocytes' for the immature thymocytes that do not yet express a complete T-cell receptor molecule. These cells give rise to both γ:δ and α:β T cells (see Fig. 8.19), although most of them develop along the α:β pathway.

The α:β pathway is shown in more detail in Fig. 8.20. The double-negative stage can be further subdivided into four stages on the basis of expression of the adhesion molecule CD44, CD25 (the α chain of the IL-2 receptor), and Kit, the receptor for SCF (see Section 8-1). At first, double-negative thymocytes express Kit and CD44 but not CD25 and are called **DN1** cells; in these cells, the genes encoding both chains of the T-cell receptor are in the germline configuration. As thymocytes mature, they begin to express CD25 on their surface and are called **DN2** cells; later, expression of CD44 and Kit is reduced, and they are called **DN3** cells.

Rearrangement of the T-cell receptor β-chain locus begins in DN2 cells with some D_β to J_β rearrangements and continues in DN3 cells with V_β to DJ_β rearrangement. Cells that fail to make a successful rearrangement of the β-chain locus remain at the DN3 ($CD44^{low}CD25^+$) stage and soon die, whereas cells that make productive β-chain gene rearrangements and express the β chain lose expression of CD25 once again and progress to the **DN4** stage, in which they proliferate. The functional significance of the transient expression of CD25 is unclear: T cells develop normally in mice in which the IL-2 gene has been deleted by gene knockout (see Appendix I, Section A-46). By contrast,

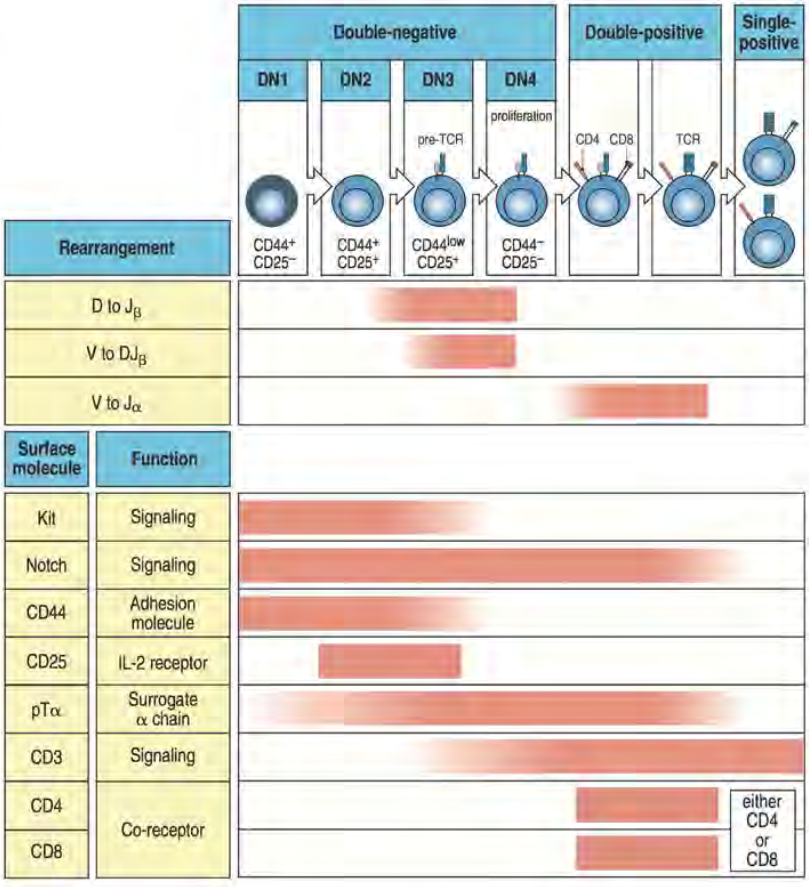


Fig. 8.20 The correlation of stages of α:β T-cell development in the mouse thymus with the program of gene rearrangement and the expression of cell-surface proteins. Lymphoid precursors are triggered to proliferate and become thymocytes committed to the T-cell lineage through interactions with the thymic stroma. These double-negative (DN1) cells express CD44 and Kit, and at a later stage (DN2) express CD25, the α chain of the IL-2 receptor. After this, the DN2 ($CD44^+CD25^+$) cells begin to rearrange the β-chain locus, becoming $CD44^{low}$ and Kit^{low} as this occurs, and become DN3 cells. The DN3 cells are arrested in the $CD44^{low}CD25^+$ stage until they productively rearrange the β-chain locus; the in-frame β chain then pairs with a surrogate chain called pTα to form the pre-T-cell receptor (pre-TCR) and is expressed on the cell surface, which triggers entry into the cell cycle. Expression of small amounts of pTα:β on the cell surface in association with CD3 signals the cessation of β-chain gene rearrangement and triggers rapid cell proliferation, which causes the loss of CD25. The cells are then known as DN4 cells. Eventually, the DN4 cells cease to proliferate and CD4 and CD8 are expressed. The small $CD4^+CD8^+$ double-positive cells begin efficient rearrangement at the α-chain locus. The cells then express low levels of an α:β T-cell receptor and the associated CD3 complex and are ready for selection. Most cells die by failing to be positively selected or as a consequence of negative selection, but some are selected to mature into CD4 or CD8 single-positive cells and eventually to leave the thymus. Expression of some other cell-surface proteins is depicted with respect to the stages of thymocyte development. Their individual contributions to T-cell development are discussed in the text.

Kit is quite important for the development of the earliest double-negative thymocytes, in that mice lacking Kit have a much smaller number of double-negative T cells. In addition, IL-7 produced by the thymic stroma is essential for early T-cell development, because there is a severe block to development when the IL-7 receptor is defective. Finally, continuous Notch signaling is important for progression through each stage of T-cell development.

In DN3 thymocytes, the expressed β chains pair with a surrogate pre-T-cell receptor α chain called **pTα** (pre-T-cell α), which allows the assembly of a complete **pre-T-cell receptor** (pre-TCR) that is analogous in structure and function to the pre-B-cell receptor. The pre-TCR is expressed on the cell surface in a complex with the CD3 molecules that provide the signaling components of T-cell receptors (see Section 7-7). As with the pre-B-cell receptor, the assembly of the CD3:pre-T-cell receptor complex causes constitutive signaling that does not require interaction with a ligand. Recent structural evidence shows that the pre-TCR forms dimers in a manner similar to pre-BCR dimerization. The pTα Ig domain makes two important contacts. It associates with the constant-region Ig domain of the Vβ subunit to form the pre-TCR itself. A distinct surface of the pre-Tα then binds to a Vβ domain from another pre-TCR molecule, forming a bridge between two different pre-TCRs. Importantly, the region of contact with the Vβ involves residues that are highly conserved across many Vβ families. In this way, expression of the pre-TCR induces ligand-independent dimerization, which leads to cell proliferation, the arrest of further β-chain gene rearrangement, and the expression of both CD8 and CD4. These **double-positive thymocytes** make up the vast majority

Petitioner Merck, Ex. 1170, p. 93
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

of thymocytes. Once the large double-positive thymocytes have ceased to proliferate and have become small double-positive cells, the α-chain locus begins to rearrange. As we will see later in this chapter, the structure of the α locus (see Section 5-9) allows multiple successive attempts at rearrangement, so that it is successfully rearranged in most developing thymocytes. Thus, most double-positive cells produce an α:β T-cell receptor during their relatively short life-span.

Small double-positive thymocytes initially express low levels of the T-cell receptor. Most of these receptors cannot recognize self-peptide:self-MHC molecular complexes; they will fail positive selection and the cells will die. In contrast, those double-positive cells that recognize self-peptide:self-MHC complexes, and can therefore be positively selected, go on to mature, and express high levels of the T-cell receptor. At the same time they cease to express one or other of the two co-receptor molecules, becoming either CD4 or CD8 **single-positive thymocytes**. Thymocytes also undergo negative selection during and after the double-positive stage, which eliminates those cells capable of responding to self antigens. About 2% of the double-positive thymocytes survive this dual screening and mature as single-positive T cells that are gradually exported from the thymus to form the peripheral T-cell repertoire. The time between the entry of a T-cell progenitor into the thymus and the export of its mature progeny is estimated to be about 3 weeks in the mouse.

8-10 Thymocytes at different developmental stages are found in distinct parts of the thymus.



The thymus is divided into two main regions, a peripheral cortex and a central medulla (see Fig. 8.15). Most T-cell development takes place in the cortex; only mature single-positive thymocytes are seen in the medulla. Initially, progenitors from the bone marrow enter at the cortico-medullary junction and migrate to the outer cortex (Fig. 8.21). At the outer edge of the cortex, in the subcapsular region of the thymus, large immature double-negative thymocytes proliferate vigorously; these cells are thought to represent committed thymocyte progenitors and their immediate progeny and will give rise to all subsequent thymocyte populations. Deeper in the cortex, most of the thymocytes are small double-positive cells. The cortical stroma is composed of epithelial cells with long branching processes that express both MHC class II and MHC class I molecules on their surface. The thymic cortex is

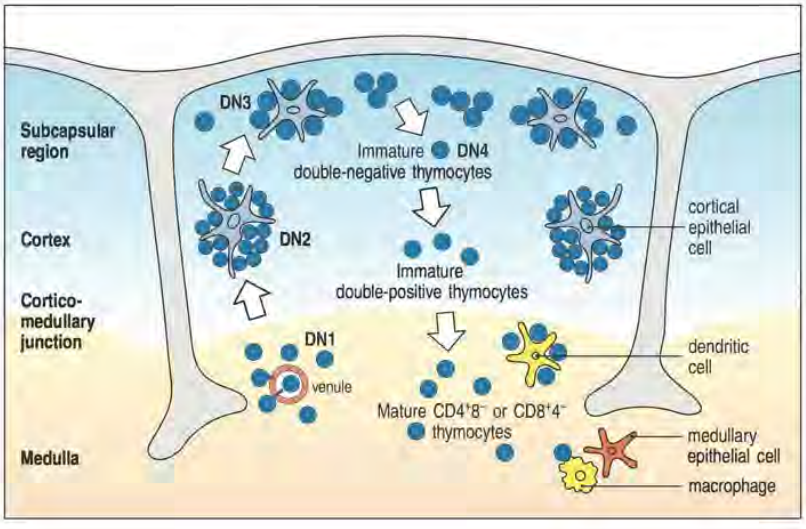


Fig. 8.21 Thymocytes at different developmental stages are found in distinct parts of the thymus. The earliest precursor thymocytes enter the thymus from the bloodstream via venules near the cortico-medullary junction. Ligands that interact with the receptor Notch1 are expressed in the thymus and act on the immigrant cells to commit them to the T-cell lineage. As these cells differentiate through the early $CD4^-CD8^-$ double-negative (DN) stages described in the text, they migrate through the cortico-medullary junction and to the outer cortex. DN3 cells reside near the subcapsular region. As the progenitor matures further to the $CD4^+CD8^+$ double-positive stage, it migrates back through the cortex. Finally, the medulla contains only mature single-positive T cells, which eventually leave the thymus.

Petitioner Merck, Ex. 1170, p. 94
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

densely packed with thymocytes, and the branching processes of the thymic cortical epithelial cells make contact with almost all cortical thymocytes (see Fig. 8.16). Contact between the MHC molecules on thymic cortical epithelial cells and the receptors of developing T cells has a crucial role in positive selection, as we will see later in this chapter.

After positive selection, developing T cells migrate from the cortex to the medulla. The medulla contains fewer lymphocytes, and those present are predominantly the newly matured single-positive T cells that will eventually leave the thymus. The medulla plays a role in negative selection. The antigen-presenting cells in this environment include dendritic cells that express co-stimulatory molecules, which are generally absent from the cortex. In addition, specialized medullary epithelial cells present peripheral antigens for the negative selection of T cells reactive for these self antigens.

8-11 T cells with α:β or γ:δ receptors arise from a common progenitor.

T cells bearing γ:δ receptors differ from α:β T cells in that they are found primarily in epithelial and mucosal sites and lack expression of the CD4 and CD8 co-receptors; in comparison with α:β T cells, relatively little is known about the ligands they recognize and the γ:δ receptor is thought not to be MHC restricted (see Sections 3-24 and 4-19). Recall from Section 5-11 that different genetic loci are used to make these two types of T-cell receptors. The β, γ, and δ loci begin to undergo rearrangement almost simultaneously in developing thymocytes, and the two cell lineages diverge from a common precursor only after certain gene rearrangements have already occurred (Fig. 8.22). This can be deduced from the pattern of gene rearrangements found in thymocytes and in mature γ:δ and α:β T cells. Mature γ:δ T cells can contain rearranged β-chain genes, although 80% of these are nonproductive, and mature α:β T cells often contain rearranged, but mostly out-of-frame, γ-chain genes.

The decision of a precursor to commit to the γ:δ or the α:β lineage is thought to depend on which type of receptor—a functional γ:δ receptor or the pre-T-cell receptor (β:pTα)—is expressed first at the DN stage of thymocyte development. Formation of a pre-T-cell receptor is more likely to occur first, as formation of a γ:δ receptor requires functional rearrangements at both the γ and the δ loci, whereas formation of a pre-T-cell receptor requires only a functional β locus rearrangement, to produce a β chain that pairs with pTα (see Section 8-9). If a γ:δ receptor is expressed first, the double-negative precursor commits to the γ:δ lineage, fails to express CD4 and CD8 co-receptors and migrates from the thymus to the periphery without undergoing positive or negative selection. If the pre-T-cell receptor is expressed first, the cell commits to the α:β lineage and goes on to express CD4 and CD8.

This difference in fate between the two lineages is thought to result from differences in the quality of signaling from the two types of receptors. The γ:δ T-cell receptor seems to deliver a stronger signal than the pre-T-cell receptor as a result of greater phosphorylation of the mitogen-activated kinase Erk (see Section 7-13). It is not yet clear whether ligand interactions are involved in signaling by the γ:δ T-cell receptor at this step. Strong Erk activation in the double-negative T-cell precursor induces expression of the transcription factor Id3, which promotes the γ:δ lineage and opposes the α:β lineage. Some mature γ:δ cells actually carry productive rearrangements at the β-chain locus, and this can be explained if the γ:δ receptor is able to activate Erk and induce Id3 before the cell is able to assemble a functional pre-T-cell receptor.

In most thymocytes a β-chain gene rearranges successfully before productive rearrangements of both the γ and δ genes have occurred. The production of a pre-T-cell receptor then arrests further gene rearrangement and signals the thymocyte to proliferate, to express its co-receptor genes, and eventually to start rearranging the α-chain genes. Once the α-chain locus starts rearranging,

Petitioner Merck, Ex. 1170, p. 95
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

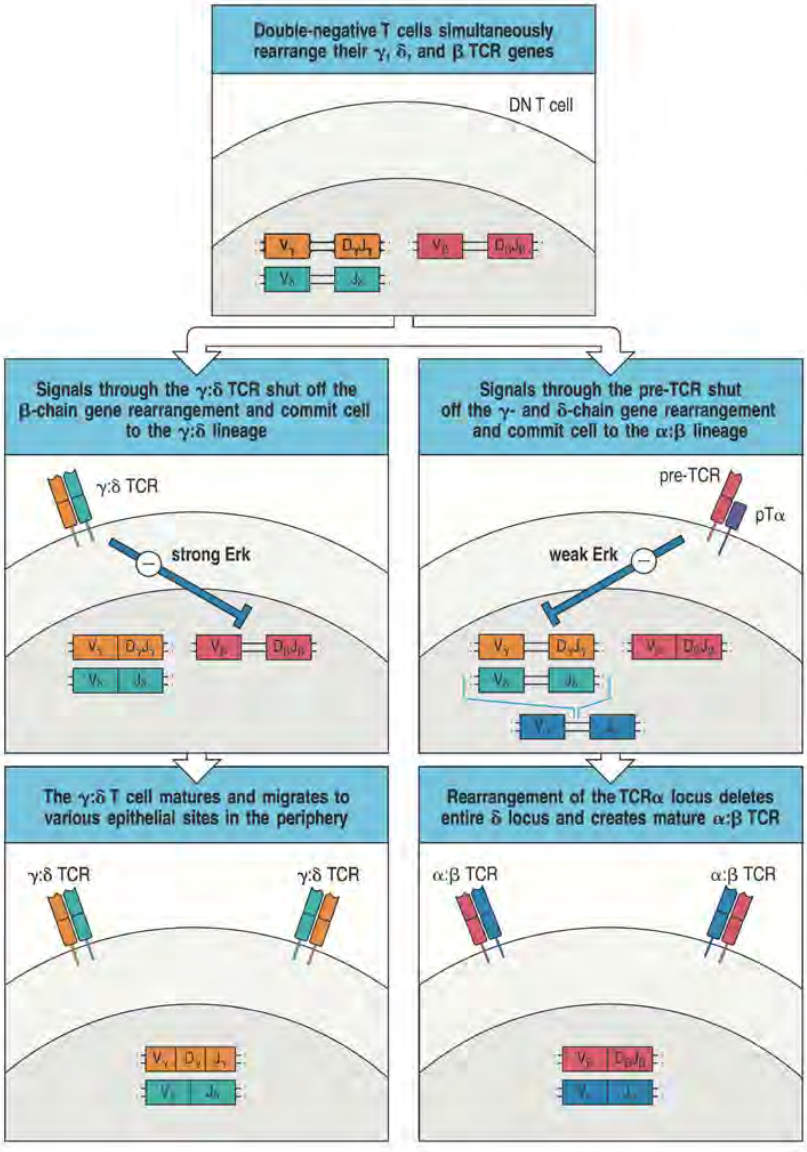


Fig. 8.22 Signals through the γ:δ T-cell receptor and the pre-T-cell receptor compete to determine the fate of thymocytes. During the development of T cells in the thymus, double-negative (DN) thymocytes begin to rearrange the γ, δ, and β T-cell receptor loci simultaneously (top panel). If a complete γ:δ T-cell receptor is formed before a successful β-chain gene rearrangement has led to the production of the pre-T-cell receptor (left panels), the thymocyte receives signals through the γ:δ receptor, which shuts off further rearrangement of the β-chain gene. Signaling by the γ:δ receptor induces strong activation of Erk (see Section 7-13) that commits the cell to the γ:δ lineage. This cell then matures into a γ:δ T cell and migrates out of the thymus into the peripheral circulation (bottom left panel). If a functional β chain is formed before a complete γ:δ receptor, it pairs with the pTα to generate a pre-T-cell receptor (right panels). In this case, the developing thymocyte receives a signal through the pre-T-cell receptor that shuts off rearrangements of the γ and δ loci. Weaker Erk activation via the pre-T-cell receptor in comparison with the γ:δ receptor leads to commitment to the α:β lineage. The thymocyte passes from the DN3 stage through the proliferating DN4 stage into the double-positive stage, at which the TCRα-chain locus rearranges and a mature α:β T-cell receptor is produced (bottom right panel). α-chain locus rearrangement deletes the δ genes, thus precluding the production of a γ:δ receptor on the same cell.

the δ-chain gene segments located within the α-chain locus are deleted as an extrachromosomal circle. This ensures that cells committed to the α:β lineage cannot make a complete γ:δ receptor.

8-12 T cells expressing particular γ- and δ-chain V regions arise in an ordered sequence early in life.

During embryonic development, the generation of the various types of T cells—even the particular V region assembled in γ:δ cells—is developmentally controlled. The first T cells to appear carry γ:δ T-cell receptors (Fig 8.23). In the mouse, γ:δ T cells first appear in discrete waves or bursts in the fetus, with the T cells in each wave populating distinct sites in the adult animal.

The first wave of γ:δ T cells populates the epidermis; the T cells become wedged among the keratinocytes and adopt a dendritic-like form that has given them

Petitioner Merck, Ex. 1170, p. 96
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

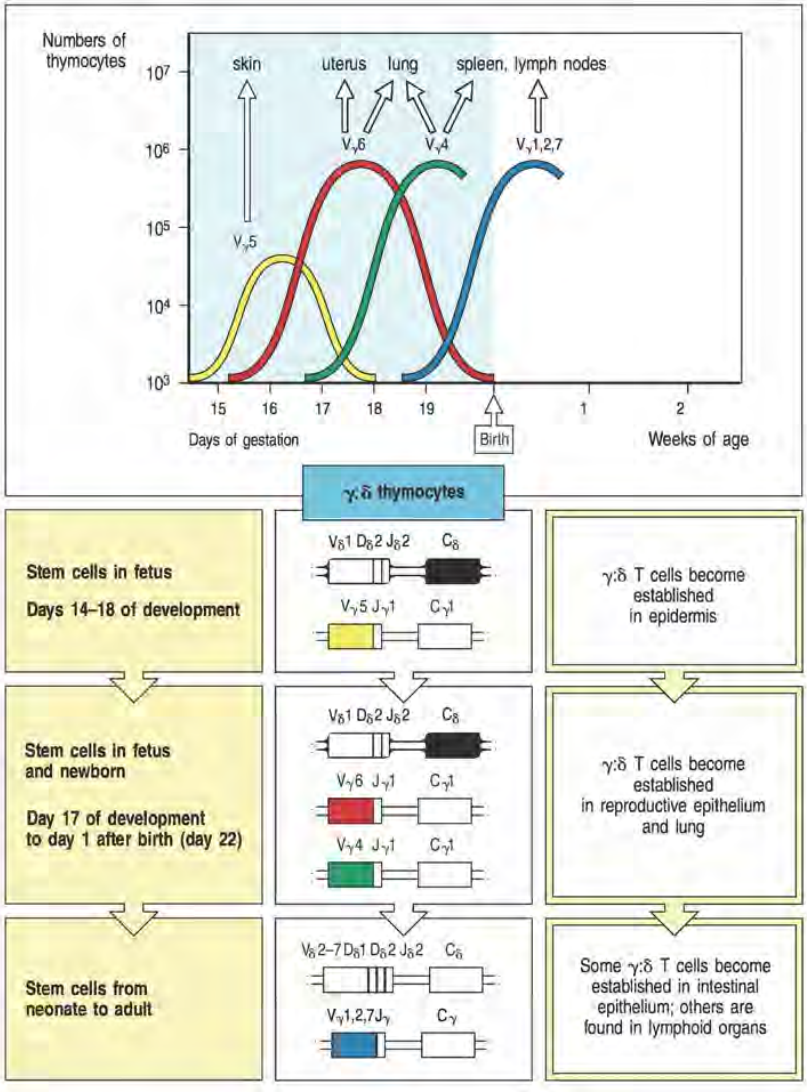


Fig. 8.23 The rearrangement of T-cell receptor γ and δ genes in the mouse proceeds in waves of cells expressing different V_γ and V_δ gene segments. At about 2 weeks of gestation, the $C\gamma_1$ locus is expressed with its closest V gene ($V\gamma_5$). After a few days, $V\gamma_5$-bearing cells decline (upper panel) and are replaced by cells expressing the next most proximal gene, $V\gamma_6$. Both these rearranged γ chains are expressed with the same rearranged δ-chain gene, as shown in the lower panels, and there is little junctional diversity in either the Vγ or the Vδ chain. As a consequence, most of the γ:δ T cells produced in each of these early waves have the same specificity, although the antigen recognized in each case is not known. The $V\gamma_5$-bearing cells become established selectively in the epidermis, whereas the $V\gamma_6$-bearing cells become established in the epithelium of the reproductive tract. After birth, the α:β T-cell lineage becomes dominant and, although γ:δ T cells are still produced, they are a much more heterogeneous population, bearing receptors with a great deal of junctional diversity. Note that Vγ gene segments are described using the system proposed by Tonegawa.

the name of **dendritic epidermal T cells** (**dETCs**) (Fig. 8.24). The second wave homes to the epithelia of the reproductive tract. Remarkably, given the large number of theoretically possible rearrangements, the receptors expressed by these early waves of γ:δ T cells are essentially invariant. All the cells in each wave assemble the same V_γ and V_δ regions, but each wave uses a different set of V, D, and J gene segments. Why certain V, D, and J gene segments are selected for rearrangement at particular times during embryonic development is still poorly understood. There are no N-nucleotides contributing additional diversity at the junctions between V, D, and J gene segments, reflecting the absence of the enzyme TdT in these fetal γ:δ T cells.

After the initial waves, T cells are produced continuously and α:β T cells predominate, making up more than 95% of thymocytes. The γ:δ T cells produced at this stage are different from those produced earlier. They have much more diverse receptors, for which several different V gene segments have been used, and the receptor sequences have abundant N-nucleotide additions. Most of these γ:δ T cells, like α:β T cells, migrate to peripheral lymphoid tissues rather than to epithelia.

Petitioner Merck, Ex. 1170, p. 97
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The developmental changes in V gene segment usage and N-nucleotide addition in murine γ:δ T cells parallel changes in B-cell populations during fetal development, which are discussed later (see Section 8-28). Their functional significance is unclear, however, and not all of these changes in the pattern of receptors expressed by γ:δ T cells occur in humans. The dETCs, for example, do not seem to have exact human counterparts, although there are γ:δ T cells in the epithelial linings of the human reproductive and gastrointestinal tracts. The mouse dETCs may serve as sentinel cells that are activated upon local tissue damage or as cells that regulate inflammatory processes.

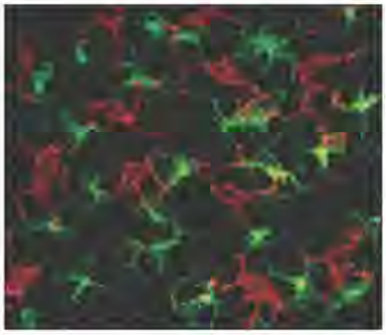


Fig. 8.24 Dendritic epidermal T cells reside within the epithelial layer, forming an interdigitating network with Langerhans cells. This face-on view of a murine epidermal sheet shows Langerhans cells (green) and dendritic epidermal T cells (dETCs) (red) forming an interdigitating network within the layers of the epidermis. The epidermal epithelial cells are not visible in this fluorescence image. The branching dendritic-like form of these γ:δ T cells is the source of their name. Although the ligands for all γ:δ T-cell receptors are not known, some γ:δ T cells recognize nonclassical MHC molecules (see Section 6-17), which can be induced in epithelia by stresses such as UV damage or pathogens. Thus, dETCs may serve as sentinels of such damage, producing cytokines that activate the innate immune response, and in turn, adaptive immunity. Courtesy of Adrian Hayday.

8-13 Successful synthesis of a rearranged β chain allows the production of a pre-T-cell receptor that triggers cell proliferation and blocks further β-chain gene rearrangement.

We now return to the development of α:β T cells. The rearrangement of the β- and α-chain loci closely parallels the rearrangement of immunoglobulin heavy-chain and light-chain loci during B-cell development (see Sections 8-2 and 8-5). As shown in Fig. 8.25, the β-chain gene segments rearrange first, with the D_β gene segments rearranging to J_β gene segments, and this is followed by V_β to DJ_β rearrangement. If no functional β chain can be synthesized from this rearrangement, the cell will not be able to produce a pre-T-cell receptor and will die unless it makes successful rearrangements at both the γ and the δ loci (see Fig. 8.22). However, unlike B cells with nonproductive heavy-chain gene rearrangements, thymocytes with nonproductive β-chain VDJ rearrangements can be rescued by further rearrangement, which is possible because of the two clusters of D_β and J_β gene segments upstream of two C_β genes (see Fig. 5.9). The likelihood of a productive VDJ join at the β locus is therefore somewhat higher than the 55% chance of a productive immunoglobulin heavy-chain gene arrangement.

Once a productive β-chain gene rearrangement has occurred, the β chain is expressed together with the invariant pTα and the CD3 molecules (see Fig. 8.25) and is transported in this complex to the cell surface. The β:pTα complex is a functional pre-T-cell receptor analogous to the μ:VpreB:λ5 pre-B-cell receptor complex in B-cell development (see Section 8-3). Expression of the pre-T-cell receptor at the DN3 stage of thymocyte development induces signals that cause the phosphorylation and degradation of RAG-2, thus halting β-chain gene rearrangement and ensuring allelic exclusion at the β locus. These signals also induce the DN4 stage in which rapid cell proliferation occurs, and eventually the co-receptor proteins CD4 and CD8 are expressed. The pre-T-cell receptor signals constitutively via the cytoplasmic protein kinase Lck, a Src-family tyrosine kinase (see Fig. 7.12), but seems not to require a ligand on the thymic epithelium. Lck subsequently associates with the co-receptor proteins. In mice genetically deficient in Lck, T-cell development is arrested before the CD4CD8 double-positive stage, and no α-chain gene rearrangements can be made.

The role of the expressed β chain in suppressing further β-locus rearrangement can be demonstrated in mice containing a rearranged TCRβ transgene: these mice express the transgenic β chain on virtually 100% of their T cells, and rearrangement of their endogenous β-chain genes is strongly suppressed. The importance of pTα has been shown in mice deficient in pTα, in which there is a hundredfold decrease in α:β T cells and an absence of allelic exclusion at the β locus.

During the proliferation of DN4 cells triggered by expression of the pre-T-cell receptor, the *RAG-1* and *RAG-2* genes are repressed. Hence, no rearrangement of the α-chain locus occurs until the proliferative phase ends, when *RAG-1* and *RAG-2* are transcribed again, and the functional RAG-1:RAG-2 complex

Petitioner Merck, Ex. 1170, p. 98
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

accumulates. This ensures that each cell in which a β-chain gene has been successfully rearranged gives rise to many CD4CD8 thymocytes. Once the cells stop dividing, each of them can independently rearrange its α-chain genes, so that a single functional β chain can be associated with many different α chains in the progeny cells. During the period of α-chain gene rearrangement, α:β T-cell receptors are first expressed and selection by self-peptide:self-MHC complexes on the thymus cells can begin.

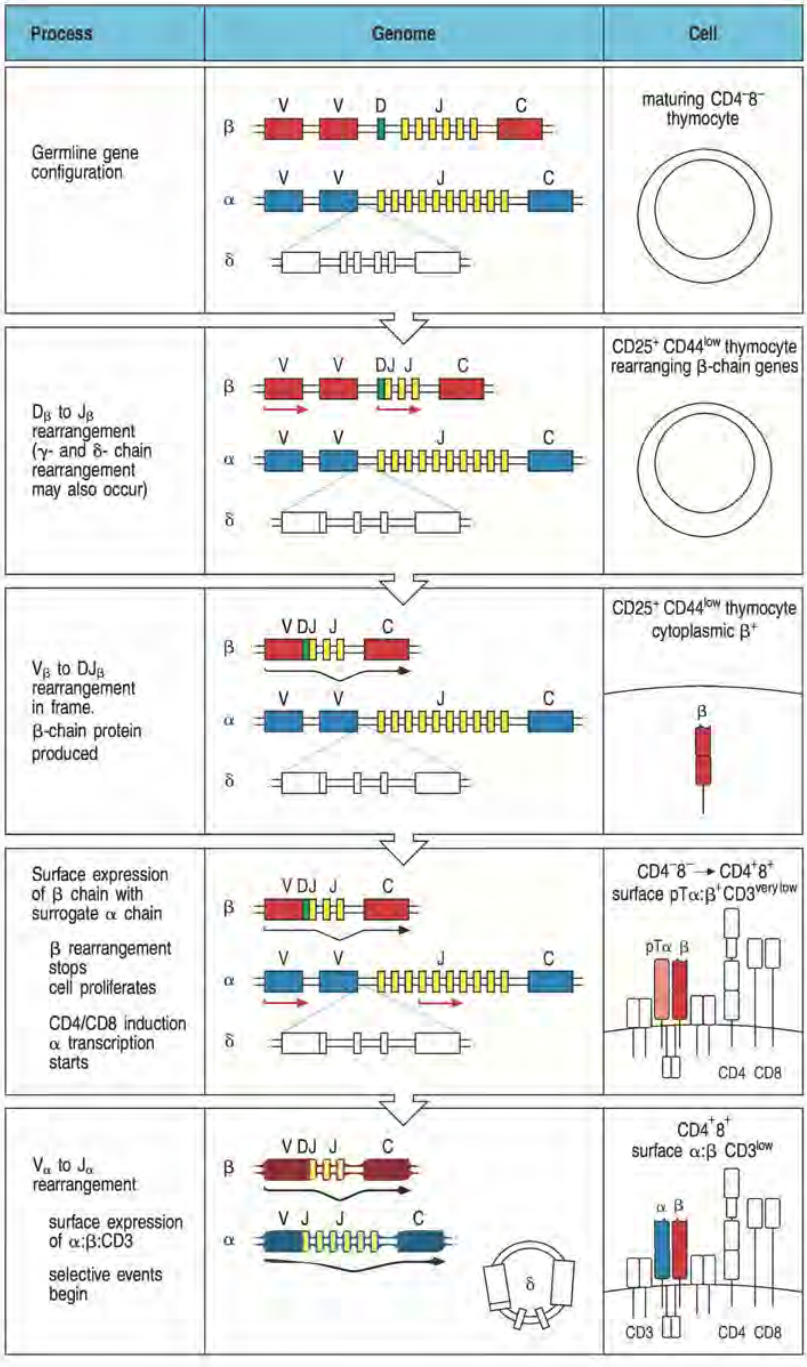


Fig. 8.25 The stages of gene rearrangement in α:β T cells. The sequence of gene rearrangements is shown, together with an indication of the stage at which the events take place and the nature of the cell-surface receptor molecules expressed at each stage. The β-chain locus rearranges first, in $CD4^-CD8^-$ double-negative thymocytes expressing CD25 and low levels of CD44. As with immunoglobulin heavy-chain genes, D to J gene segments rearrange before V gene segments rearrange to DJ (second and third panels). It is possible to make up to four attempts to generate a productive rearrangement at the β-chain locus, because there are four D gene segments with two sets of J gene segments associated with each TCR β chain locus (not shown). The productively rearranged gene is expressed initially within the cell and then at low levels on the cell surface. It associates with pTα, a surrogate 33 kDa α chain that is equivalent to λ5 in B-cell development, and this pTα:β heterodimer forms a complex with the CD3 chains (fourth panel). The expression of the pre-T-cell receptor signals the developing thymocytes to halt β-chain gene rearrangement and to undergo multiple cycles of division. At the end of this proliferative burst, the CD4 and CD8 molecules are expressed, the cell ceases cycling, and the α chain is now able to undergo rearrangement. The first α-chain gene rearrangement deletes all δ D, J, and C gene segments on that chromosome, although these are retained as a circular DNA, proving that these are nondividing cells (bottom panel). This permanently inactivates the δ-chain gene. Rearrangements at the α-chain locus can proceed through several cycles, because of the large number of V_α and J_α gene segments, so that productive rearrangements almost always occur. When a functional α chain is produced that pairs efficiently with the β chain, the $CD3^{low}CD4^+CD8^+$ thymocyte is ready to undergo selection for its ability to recognize self peptides in association with self-MHC molecules.

The progression of thymocytes from the double-negative to the double-positive, and finally to the single-positive, stage is accompanied by a distinct pattern of expression of proteins involved in DNA rearrangement, signaling, and T-cell-specific gene expression (Fig. 8.26). TdT, the enzyme responsible for the insertion of N-nucleotides, is expressed throughout T-cell receptor gene rearrangement; N-nucleotides are found at the junctions of all rearranged α and β genes. Lck and another tyrosine kinase, ZAP-70, are both expressed from an early stage in thymocyte development. As well as its key role in signaling from the pre-T-cell receptor, Lck is also important for γ:δ T-cell development. In contrast, gene knockout studies (see Appendix I, Section A-46) show that ZAP-70, although expressed from the double-negative stage onward, has an essential role later: it promotes the development of single-positive thymocytes from double-positive thymocytes. Fyn, a Src-family kinase similar to Lck, is expressed at increasing levels from the double-positive stage onward. It is not essential for the development of α:β thymocytes as long as Lck is present, but is required for the development of iNKT cells.

Finally, several transcription factors have been identified that guide the development of thymocytes from one stage to the next. Ikaros and GATA3 are expressed in early T-cell progenitors; in the absence of either, T-cell development is generally disrupted. Moreover, these factors also have roles in the normal functioning of mature T cells. In contrast, Ets1, though also expressed in early progenitors, is not essential for T-cell development, although mice

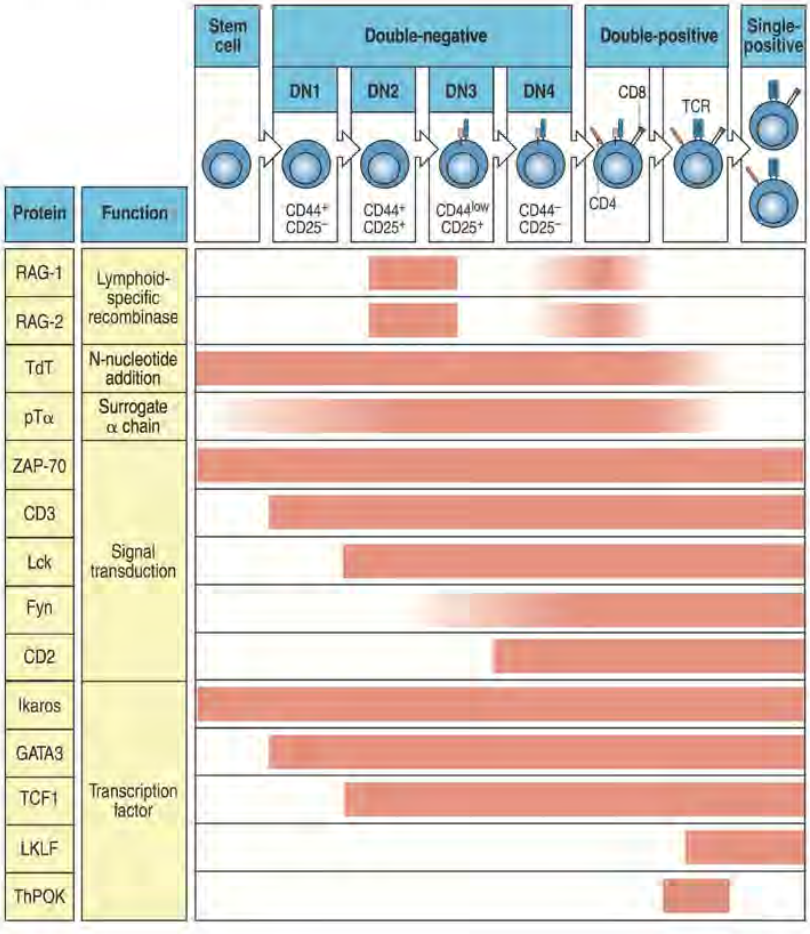


Fig. 8.26 The temporal pattern of expression of some cellular proteins important in early T-cell development. Expression is depicted with regard to the stages of thymocyte development as determined by cell-surface marker expression. The proteins listed are a selection of those known to be associated with early T-lineage development and have been included because of their proven importance in the developmental sequence, largely on the basis of studies in mice. Some of these proteins are involved in gene rearrangement and signaling through receptors, and their individual contributions are discussed in the text. Several transcription factors have been identified that guide the development of thymocytes from one stage to the next by regulating gene expression. Ikaros and GATA3 are expressed in early T-cell progenitors; in the absence of either, T-cell development is generally disrupted. These proteins also have roles in mature T cells. In the absence of TCF1 (T-cell factor-1), double-negative T cells that make productive β-chain gene rearrangements do not proliferate in response to the pre-T-cell receptor signal, thus preventing the efficient production of double-positive thymocytes. LKLF (lung Kruppel-like factor) is first expressed at the single-positive stage; if it is absent, thymocytes exhibit a defect in emigration to populate peripheral lymphoid tissues, due in part to their failure to express receptors involved in trafficking, such as the sphingosine-1-phosphate (S1P) receptor, $S1P_1$ (see Chapter 9). The transcription factor Ets1 (not shown on this figure) is not essential for T-cell development, but mice lacking this factor do not make NK cells.

Petitioner Merck, Ex. 1170, p. 100
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

lacking this factor do not make NK cells. TCF1 (T-cell factor-1) is first expressed during the double-negative stage. In its absence, double-negative T cells that make productive β-chain gene rearrangements do not proliferate, preventing the efficient production of double-positive thymocytes. Thus, transcription factors expressed at various developmental stages control normal thymocyte development by controlling the expression of appropriate genes.

8-14 T-cell α-chain genes undergo successive rearrangements until positive selection or cell death intervenes.

The T-cell receptor α-chain genes are comparable to the immunoglobulin κ and λ light-chain genes in that they do not have D gene segments and are rearranged only after their partner receptor chain has been expressed. As with the light-chain genes, repeated attempts at α-chain gene rearrangement are possible, as illustrated in Fig. 8.27. The presence of multiple V_α gene segments, and about 60 J_α gene segments spread over some 80 kilobases of DNA, allows many successive V_α to J_α rearrangements to take place at both α-chain alleles. This means that T cells with an initial nonproductive α-gene rearrangement are much more likely to be rescued by a subsequent rearrangement than are B cells with a nonproductive light-chain gene rearrangement.

One key difference between B and T cells is that the final assembly of an immunoglobulin leads to the cessation of gene rearrangement and initiates the further differentiation of the B cell, whereas rearrangement of the V_α gene segments continues in T cells unless there is signaling by a self-peptide:self-MHC complex that positively selects the receptor. This means that many T cells have in-frame rearrangements on both chromosomes and so can produce two types of α chains. This is possible because expression of the T-cell receptor is not in itself sufficient to shut off gene rearrangement. Continued rearrangements on both chromosomes can allow several different α chains to be produced successively as well as simultaneously in each developing T cell and to be tested for self-peptide:self-MHC recognition in partnership with

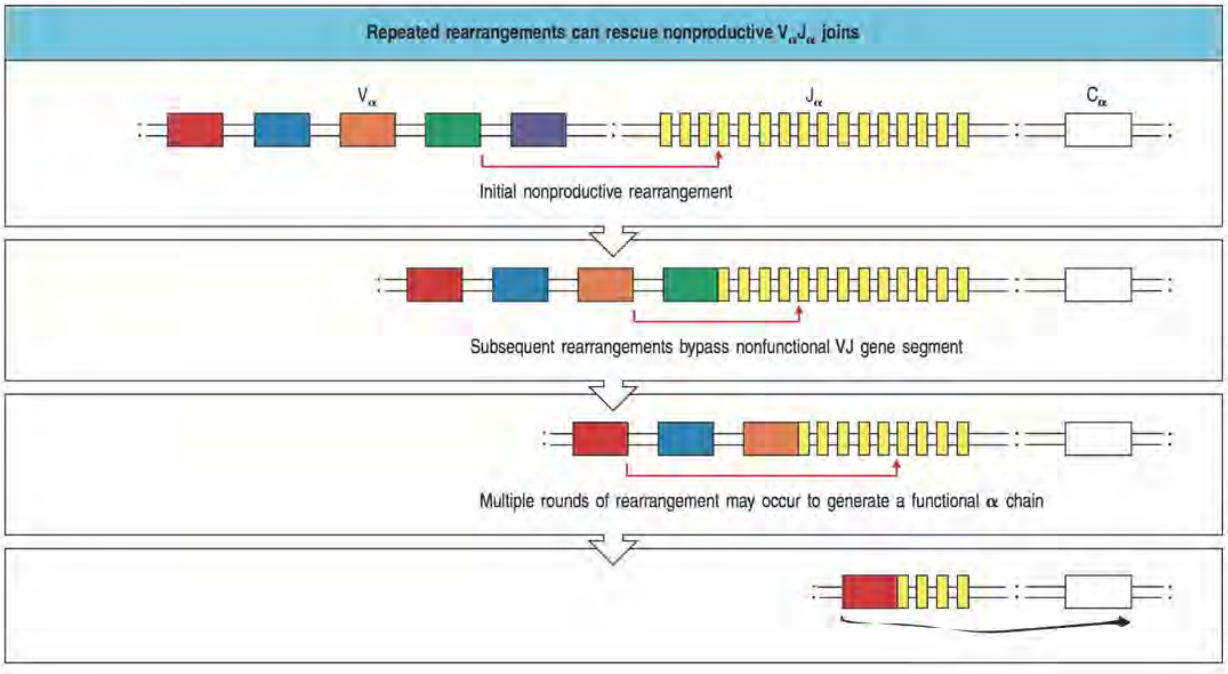


Fig. 8.27 Multiple successive rearrangement events can rescue nonproductive T-cell receptor α-chain gene rearrangements. The multiplicity of V and J gene segments at the α-chain locus allows successive rearrangement events to 'leapfrog' over previously rearranged VJ segments, deleting any intervening gene segments. The α-chain rescue pathway resembles that of the immunoglobulin κ light-chain genes (see Section 8-5), but the number of possible successive rearrangements is greater. α-chain gene rearrangement continues until either a productive rearrangement leads to positive selection or the cell dies.

Petitioner Merck, Ex. 1170, p. 101
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

the same β chain. This phase of gene rearrangement lasts for 3 or 4 days in the mouse and ceases only when positive selection occurs as a consequence of receptor engagement, or when the cell dies. One can predict that if the frequency of positive selection is sufficiently low, roughly one in three mature T cells will express two productively rearranged α chains at the cell surface. This has been confirmed for both human and mouse T cells. Thus, in the strict sense, T-cell receptor α-chain genes are not subject to allelic exclusion.

T cells with dual specificity might be expected to give rise to inappropriate immune responses if the cell is activated through one receptor yet can act upon target cells recognized by the second receptor. However, only one of the two receptors is likely to be able to recognize peptide presented by a self-MHC molecule, and so the T cell will have only a single functional specificity. This is because once a thymocyte has been positively selected by self-peptide:self-MHC recognition, α-chain gene rearrangement ceases. Thus, the existence of cells with two α-chain genes productively rearranged and two α chains expressed at the cell surface does not truly challenge the idea that a single functional specificity is expressed by each cell.

Summary.

The thymus provides a specialized and architecturally organized microenvironment for the development of mature T cells. Precursors of T cells migrate from the bone marrow to the thymus, where they interact with environmental cues, such as ligands for the Notch receptor, that drive commitment to the T lineage. Developing thymocytes develop along one of three T-cell lineages: γ:δ T cells, α:β T cells, and iNKT cells (which are T cells with α:β receptors of very limited diversity).

T-cell progenitors make a choice between the γ:δ and the α:β T-cell lineages. Early in ontogeny, the production of γ:δ T cells predominates over α:β T cells, and these cells populate several peripheral tissues, including the skin, reproductive epithelium, and intestine. Later, more than 90% of thymocytes express α:β T-cell receptors. In developing thymocytes, the γ, δ, and β genes rearrange virtually simultaneously; signaling by a functional γ:δ receptor commits the precursor toward the γ:δ lineage; these cells halt further gene rearrangement and do not express CD4 and CD8 co-receptors. Production of a functionally rearranged β-chain gene and signaling by the pre-T-cell receptor commits the precursor to the α:β lineage.

T cells of the conventional α:β lineage pass through a series of stages distinguished by rearrangement of the α-chain gene and the production of an α:β T-cell receptor, the differential expression of CD44 and CD25, CD3:T-cell receptor complex proteins, and the co-receptors CD4 and CD8. Most steps in T-cell development take place in the thymic cortex, whereas the medulla contains mainly mature T cells.

Positive and negative selection of T cells.

Up to the stage at which an α:β receptor is produced, T-cell development has been independent of antigen. From this point onward, developmental decisions in the α:β T-cell lineage depend on the interaction of the receptor with peptide:MHC ligands it encounters in the thymus, and we now consider this phase of T-cell development.

T-cell precursors committed to the α:β lineage at the DN3 stage undergo vigorous proliferation in the subcapsular region—the DN4 stage. These cells

Petitioner Merck, Ex. 1170, p. 102
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

then rapidly transit through an immature CD8-single-positive stage and then into double-positive cells that express low levels of the T-cell receptor and both the CD4 and CD8 co-receptors and move deeper into the thymic cortex. These double-positive cells have a life-span of only about 3–4 days unless they are rescued by engagement of their T-cell receptor. The rescue of double-positive cells from programmed cell death and their maturation into CD4 or CD8 single-positive cells is the process known as positive selection. Only about 10–30% of the T-cell receptors generated by gene rearrangement will be able to recognize self-peptide:self-MHC complexes and thus function in self-MHC-restricted responses to foreign antigens (see Chapter 4); those that have this capability are selected for survival in the thymus. Double-positive cells also undergo negative selection: T cells whose receptors recognize self-peptide:self-MHC complexes too strongly undergo apoptosis, thus eliminating potentially self-reactive cells. In this section we examine the interactions between developing double-positive thymocytes and different thymic components and examine the mechanisms by which these interactions shape the mature T-cell repertoire.

8-15 The MHC type of the thymic stroma selects a repertoire of mature T cells that can recognize foreign antigens presented by the same MHC type.

Positive selection was first demonstrated in classic experiments using mice whose bone marrow had been completely replaced by bone marrow from a mouse that had a different MHC genotype but was otherwise genetically identical. These mice are known as **bone marrow chimeras** (see Appendix I, Section A-42). The recipient mouse is first irradiated to destroy all its own lymphocytes and bone marrow progenitor cells; after bone marrow transplantation, all bone marrow derived cells will be of the donor genotype. These will include all lymphocytes, as well as the antigen-presenting cells they interact with. The rest of the animal's tissues, including the nonlymphoid stromal cells of the thymus, will be of the recipient MHC genotype.

In the experiments that demonstrated positive selection (Fig. 8.28), the donor mice were F_1 hybrids derived from MHC^a and MHC^b parents and thus were of the $MHC^{a\times b}$ genotype. The irradiated recipients were one of the parental strains, either MHC^a or MHC^b. Because of MHC restriction, individual T cells recognize either MHC^a or MHC^b, but not both. Normally, roughly equal numbers of the $MHC^{a\times b}$ T cells from $MHC^{a\times b}$ F_1 hybrid mice will recognize antigen presented by MHC^a or MHC^b. However, in bone marrow chimeras in which T cells of $MHC^{a\times b}$ genotype develop in an MHC^a thymus, T cells immunized to a particular antigen turn out to recognize that antigen mainly, if not exclusively, when it is presented by MHC^a molecules, even though the antigen-presenting cells display antigen bound to both MHC^a and MHC^b. These experiments demonstrated that the MHC molecules present in the environment in which T cells develop determine the MHC restriction of the mature T-cell receptor repertoire.

A similar kind of experiment, using grafts of thymic tissue, showed that the radioresistant cells of the thymic stroma are responsible for positive selection. In these experiments, the recipient animals were athymic *nude* or thymectomized mice of $MHC^{a\times b}$ genotype that were given thymic stromal grafts of MHC^a genotype. Thus, all their cells except the thymic stroma carried both MHC^a and MHC^b. The $MHC^{a\times b}$ bone marrow cells of these mice matured into T cells that recognized antigens presented by MHC^a but not by MHC^b. This result showed that it is the MHC molecules expressed by the thymic stromal cells that determine what mature T cells consider to be self MHC. These results also argued that the MHC restriction seen in the immunized bone marrow chimeras could originate in the thymus, presumably by the selection of T cells as they developed.

Petitioner Merck, Ex. 1170, p. 103
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

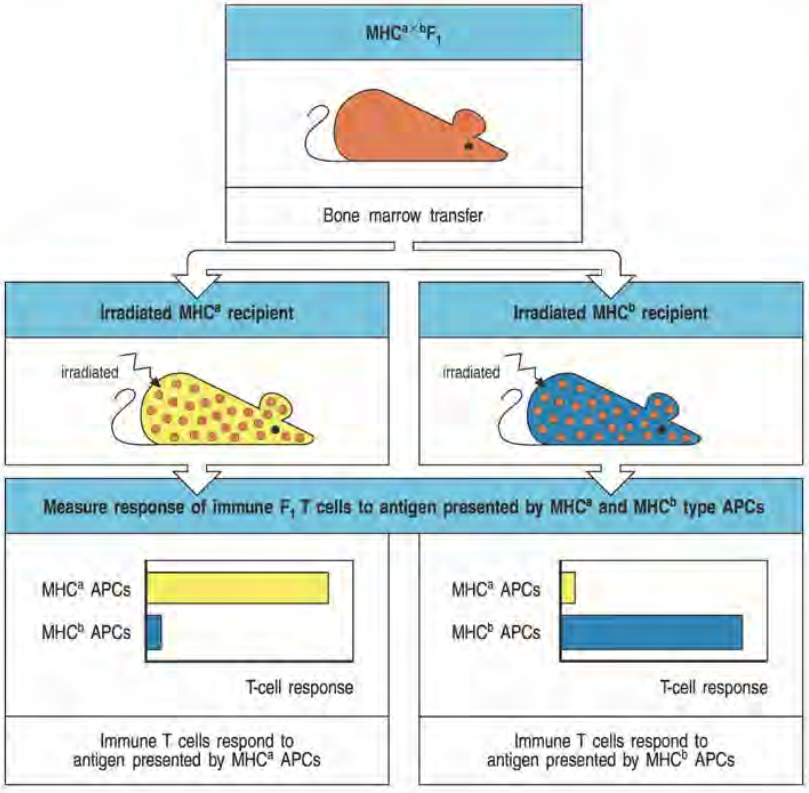


Fig. 8.28 Positive selection is revealed by bone marrow chimeric mice. As shown in the top two sets of panels, bone marrow from an MHC$^{a\times b}$ F$_1$ hybrid mouse is transferred to a lethally irradiated recipient mouse of either parental MHC type (MHC^a or MHC^b). When these chimeric mice are immunized with antigen, the antigen can be presented by the bone marrow derived MHC$^{a\times b}$ antigen-presenting cells (APCs) in association with both MHC^a and MHC^b molecules. The T cells from an MHC$^{a\times b}$ F$_1$ mouse include cells that respond to antigen presented by APCs from MHC^a mice and cells that respond to APCs from MHC^b mice (not shown). But when immunized T cells from the chimeric animals are tested *in vitro* with APCs bearing MHC^a or MHC^b only, they respond far better to antigen presented by the MHC molecules of the recipient MHC type, as shown in the bottom panels. This shows that the T cells have undergone positive selection for MHC restriction in the recipient thymus.

8-16 Only thymocytes whose receptors interact with self-peptide: self-MHC complexes can survive and mature.

Bone marrow chimeras and thymic grafting provided evidence that MHC molecules in the thymus influence the MHC-restricted T-cell repertoire. However, mice transgenic for rearranged T-cell receptor genes provided the first conclusive evidence that the interaction of the T cell with self-peptide:self-MHC complexes is necessary for the survival of immature T cells and their maturation into naive CD4 or CD8 T cells. For these experiments, the rearranged α- and β-chain genes were cloned from a T-cell clone (see Appendix I, Section A-24) whose origin, antigen specificity, and MHC restriction were known. When such genes are introduced into the mouse genome, they are expressed early during thymocyte development and the rearrangement of endogenous T-cell receptor genes is inhibited; endogenous β-chain gene rearrangement is inhibited completely but that of α-chain genes is inhibited only incompletely. The result is that most of the developing thymocytes express the T-cell receptor encoded by the transgenes.

By introducing T-cell receptor transgenes specific for a known MHC genotype, the effect of MHC molecules on the maturation of thymocytes with receptors of known specificity can be studied directly without the need for immunization and analysis of effector function. Such studies showed that thymocytes bearing a particular T-cell receptor could develop to the double-positive stage in thymuses that expressed different MHC molecules from those in which the cell bearing the T-cell receptor originally developed. However, these transgenic thymocytes only developed beyond the double-positive stage and became mature T cells if the thymus expressed the same self-MHC molecule as that on which the original T-cell clone was selected (Fig. 8.29). Such experiments also discovered the fate of T cells that fail

Petitioner Merck, Ex. 1170, p. 104
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

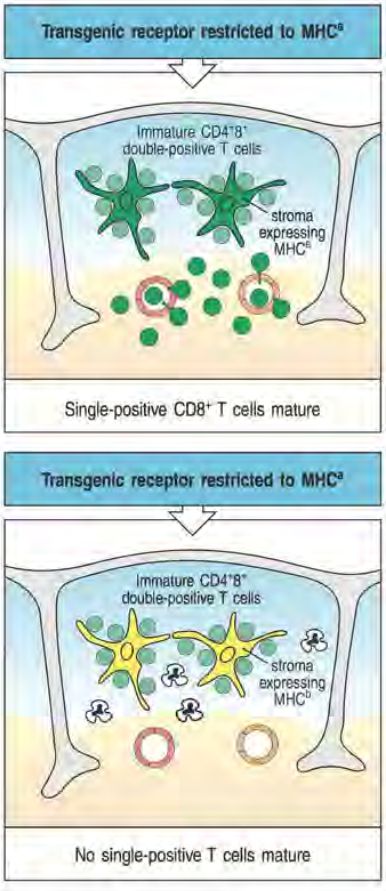


Fig. 8.29 Positive selection is demonstrated by the development of T cells expressing rearranged T-cell receptor transgenes. In mice transgenic for rearranged α:β T-cell receptor genes, the maturation of T cells depends on the MHC haplotype expressed in the thymus. If the transgenic mice express the same MHC haplotype in their thymic stromal cells as the mouse from which the rearranged TCRα-chain and TCRβ-chain genes originally developed (both MHC^a, top panels), then the T cells expressing the transgenic T-cell receptor will develop from the double-positive stage (pale green) into mature T cells (dark green), in this case mature CD8$^+$ single-positive cells. If the MHC^a-restricted TCR transgenes are genetically crossed into a different MHC background (MHC^b, yellow) (bottom panel), then developing T cells expressing the transgenic receptor will progress to the double-positive stage but will fail to mature further. This failure is due to the absence of an interaction between the transgenic T-cell receptor with MHC molecules on the thymic cortex, and thus no signal for positive selection is delivered, leading to apoptotic death by neglect.

positive selection. Rearranged receptor genes from a mature T cell specific for a peptide presented by a particular MHC molecule were introduced into a recipient mouse lacking that MHC molecule, and the fate of thymocytes was investigated by staining with antibodies specific for the transgenic receptor. Antibodies against other molecules such as CD4 and CD8 were used at the same time to mark the stages of T-cell development. It was found that cells that fail to recognize the MHC molecules present on the thymic epithelium never progress further than the double-positive stage and die in the thymus within 3 or 4 days of their last division.

8-17 Positive selection acts on a repertoire of T-cell receptors with inherent specificity for MHC molecules.

Positive selection acts on a repertoire of T-cell receptors whose specificity is determined by randomly generated combinations of V, D, and J gene segments (see Section 5-7). Despite this, T-cell receptors exhibit a bias toward recognition of MHC molecules even before positive selection. If the specificity of the unselected repertoire were completely random, only a very small proportion of thymocytes would be expected to recognize any MHC molecule. However, an inherent specificity of T-cell receptors for MHC molecules has been detected by examining mature T cells that represent the unselected receptor repertoire. Such T cells can be produced *in vitro* from fetal thymuses that lack expression of MHC class I and MHC class II molecules by triggering generalized 'positive selection' using antibodies that bind to the V_β chain of T-cell receptors and to the CD4 co-receptor. When such antibody-selected CD4 T cells are examined, roughly 5% can respond to any one MHC class II genotype. Because these cells developed without selection by MHC molecules, this reactivity must reflect an inherent MHC-specificity encoded in the germline V gene segments. This specificity should significantly increase the proportion of receptors that can be positively selected by any individual's MHC molecules.

The germline-encoded reactivity seems to be due to specific amino acids in the CDR1 and CDR2 regions of T-cell receptor V_β and V_α regions. The CDR1 and CDR2 regions are encoded in the germline V gene segments and are highly variable (see Section 5-8). But among this variability, certain amino acids are conserved and common to many V segments. Analysis of numerous crystal structures has revealed that when the T-cell receptor binds a peptide:MHC complex, specific amino acids of the V_β region interact with a particular part of the MHC protein. For example, in many human and mouse V_β regions, the CRD2 has a tyrosine at position 48, and this interacts with a region in the middle of the α1 helix of MHC class I and class II proteins (see Fig. 6.22). Two other amino acids commonly found in other V_β regions (tyrosine at 46 and glutamic acid at 54) interact with the same region of MHC. T cells expressing V_β genes with mutations at any of these positions showed reduced positive selection, demonstrating that the interaction of such V regions with MHC molecules contributes to T-cell development.

8-18 Positive selection coordinates the expression of CD4 or CD8 with the specificity of the T-cell receptor and the potential effector functions of the T cell.

At the time of positive selection, the thymocyte expresses both CD4 and CD8 co-receptor molecules. By the end of thymic selection, mature α:β T cells ready for export to the periphery have stopped expressing one of these co-receptors and belong to one of three categories: conventional CD4 or CD8 T cells, or a subset of regulatory T cells expressing CD4 and high levels of CD25. Moreover, almost all mature T cells that express CD4 have receptors

Petitioner Merck, Ex. 1170, p. 105
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

that recognize peptides bound to self-MHC class II molecules and are programmed to become cytokine-secreting helper T cells. In contrast, most of the cells that express CD8 have receptors that recognize peptides bound to self-MHC class I molecules and are programmed to become cytotoxic effector cells. Thus, positive selection also determines the cell-surface phenotype and functional potential of the mature T cell, selecting the appropriate co-receptor for efficient antigen recognition and the appropriate program for the T cell's eventual functional differentiation in an immune response.

Experiments with mice transgenic for rearranged T-cell receptor genes show clearly that the specificity of the T-cell receptor for self-peptide:self-MHC molecule complexes determines which co-receptor a mature T cell will express. If the transgenes encode a T-cell receptor specific for antigen presented by self-MHC class I molecules, mature T cells that express the transgenic receptor are CD8 T cells. Similarly, in mice made transgenic for a receptor that recognizes antigen with self-MHC class II molecules, mature T cells that express the transgenic receptor are CD4 T cells (Fig. 8.30).

The importance of MHC molecules in this selection is illustrated by the class of human immunodeficiency diseases known as **bare lymphocyte syndromes**, which are caused by mutations that lead to an absence of MHC molecules on lymphocytes and thymic epithelial cells. People who lack MHC class II molecules have CD8 T cells but only a few, highly abnormal, CD4 T cells; a similar result has been obtained in mice in which MHC class II expression has been eliminated by targeted gene disruption (see Appendix I, Section A-46). Likewise, mice and humans that lack MHC class I molecules lack CD8 T cells. Thus, MHC class II molecules are absolutely required for CD4 T-cell development, whereas MHC class I molecules are similarly required for CD8 T-cell development.

In mature T cells, the co-receptor functions of CD8 and CD4 depend on their respective abilities to bind invariant sites on MHC class I and MHC class II molecules (see Section 4-17). Co-receptor binding to an MHC molecule is also required for normal positive selection, as shown for CD4 in the experiment discussed in the next section. Thus, positive selection depends on engagement of both the antigen receptor and co-receptor by an MHC molecule, and determines the survival of single-positive cells that express only the appropriate co-receptor. Commitment to either the CD4 or CD8 lineage is coordinated with receptor specificity and it seems that the developing thymocyte integrates signals from both the antigen receptor and the co-receptor. Co-receptor-associated Lck signals are most effectively delivered when CD4 rather than CD8 is engaged as a co-receptor, and these Lck signals play a large part in the decision to become a mature CD4 cell. When a T cell receives a signal inducing positive selection, it first downregulates both CD4 and CD8, but then reexpresses CD4, making a $CD4^+CD8^{low}$ cell regardless of whether the T-cell receptor has been engaged by MHC class I or MHC class II molecules (Fig. 8.31). It appears that the strength of signaling in this $CD4^+CD8^{low}$ cell is what determines the lineage choice. If the cell is being selected by MHC class II, the reexpression of CD4 provides a stronger or more sustained signal, mediated in part by Lck, and this is responsible for further differentiation along the CD4 pathway, with the complete loss of CD8. If the cell is being

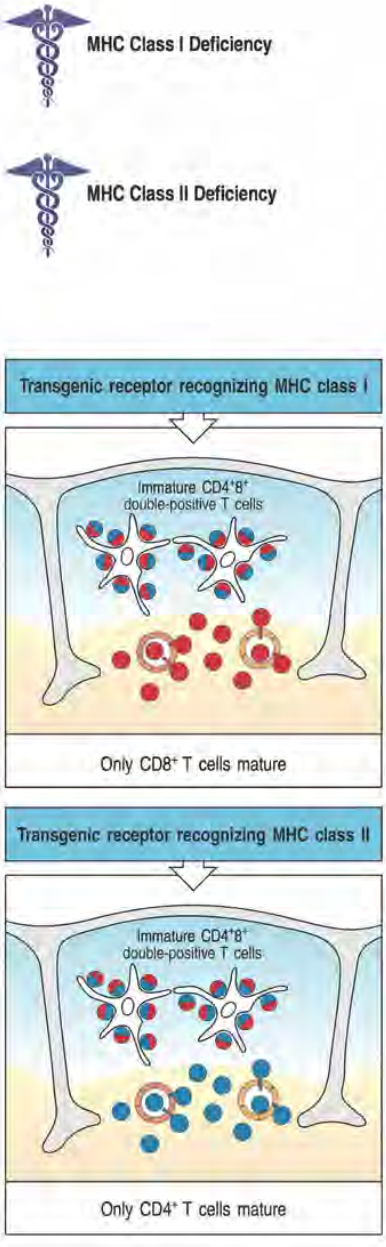


Fig. 8.30 The MHC molecules that induce positive selection determine co-receptor specificity. In mice transgenic for T-cell receptors restricted by an MHC class I molecule (top panel), the mature T cells that develop all have the CD8 (red) phenotype. In mice transgenic for receptors restricted by an MHC class II molecule (bottom panel), all mature T cells have the CD4 (blue) phenotype. In both cases, normal numbers of immature, double-positive thymocytes (half blue, half red) are found. The specificity of the T-cell receptor determines the outcome of the developmental pathway, ensuring that the only T cells that mature are those equipped with a co-receptor that is able to bind the same self-MHC molecule as the T-cell receptor.

Petitioner Merck, Ex. 1170, p. 106
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

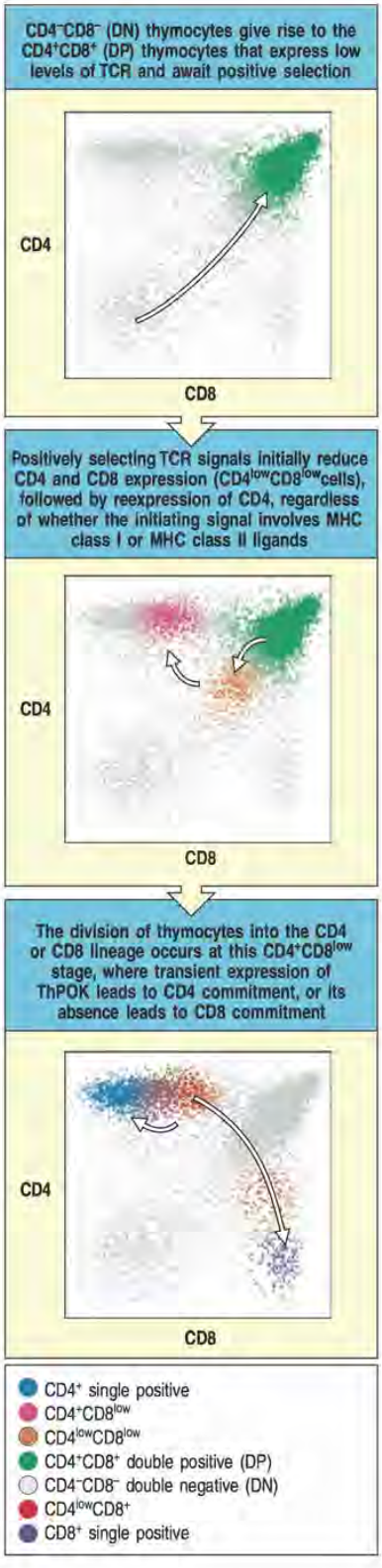


Fig. 8.31 Stages in the positive selection of α:β T cells as identified by FACS analysis. The diagram represents FACS analysis (see Appendix I, Fig. A.25) of various stages in thymocyte development with reference to the co-receptor molecules CD4 and CD8. Each dot represents an individual cell, and its position on the axes represents the quantity of CD4 and CD8 expression that was determined at the time of analysis. Double-negative (DN) cells are located in the bottom left corner of the plot. DN cells that have successfully rearranged a β chain receive a signal through pre-TCR and undergo proliferation, and then induce expression of the CD8 and CD4 co-receptors to become double-positive (DP) cells (green). Rearrangement of the α-chain locus occurs in these DP cells, with expression of a T-cell receptor on the cell surface first at low and then at intermediate levels. In these cells, signaling is co-receptor dependent. If the expressed T-cell receptor interacts successfully with MHC molecules on thymic stroma to induce positive selection, the cell initially reduces CD8 and CD4 expression (orange), followed by a subsequent increased expression of CD4 to generate the $CD4^+CD8^{low}$ population (red). If selection was provided by an MHC class II molecule, signaling in the $CD4^+CD8^{low}$ T cell is of a longer duration and commitment to CD4 occurs, with maintenance of CD4 and loss of CD8 expression (blue). If the selection was provided by an MHC class I molecule, signaling in the $CD4^+CD8^{low}$ T cell will be of shorter duration, and this leads to commitment to the CD8 lineage, with reexpression of CD8 and loss of CD4 (purple).

selected by MHC class I, reexpression of CD4 will not lead to further signaling via Lck; this weaker signal in turn determines CD8 commitment, with a subsequent loss of CD4 expression and the later reexpression of CD8.

T-cell receptor signaling regulates this choice of the CD4 versus the CD8 lineage by controlling the expression of two transcription factors, ThPOK and Runx3 (see Fig. 8.31). ThPOK was identified through a naturally occurring loss-of-function mutation in mice that lacked CD4 T-cell development. In mice lacking ThPOK, MHC class II-restricted thymocytes are redirected toward the CD8 lineage. ThPOK is not expressed in double-positive thymocytes, but strong T-cell receptor signaling at the $CD4^+CD8^{low}$ stage of development induces its expression. ThPOK in turn represses expression of Runx3; together, the expression of ThPOK and absence of Runx3 lead to CD4 commitment and the ability to express cytokine genes typical of CD4 cells. If T-cell signaling is of insufficient strength or duration, however, ThPOK is not induced, and Runx3 is allowed to be expressed. This leads to silencing of CD4 expression, reexpression of CD8 and the ability to express genes typical of CD8 T cells that encode proteins involved in target-cell killing.

The majority of double-positive thymocytes that undergo positive selection develop into either CD4 or CD8 single-positive T cells. The thymus also generates a smaller population of single-positive CD4 T cells that represent a distinct lineage known as **natural regulatory T cells (T_{reg} cells)**. Like CD4 and CD8 cells, T_{reg} cells arise at the $CD4^+CD8^{low}$ stage of development. Commitment to the T_{reg} lineage is determined by expression of a Forkhead-family transcription factor called **FoxP3** and seems to be caused by T-cell receptor signaling that is stronger than that inducing conventional CD4 commitment but is not strong enough to induce deletion (discussed in Section 8-20). T_{reg} cells express high levels of the surface proteins CD25 and CTLA-4 (see Section 7-18). It is thought that the T-cell receptors expressed by T_{reg} cells strongly recognize self antigens expressed in the thymus, which triggers stable expression of FoxP3.

8-19 Thymic cortical epithelial cells mediate positive selection of developing thymocytes.

The thymus transplantation studies described in Section 8-15 suggested that stromal cells were important for positive selection. These cells form a web of cell processes that make close contacts with the double-positive T cells undergoing positive selection (see Fig. 8.16), and T-cell receptors can be seen clustering with MHC molecules at the sites of contact. Direct evidence that thymic cortical epithelial cells mediate positive selection comes from

Petitioner Merck, Ex. 1170, p. 107
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

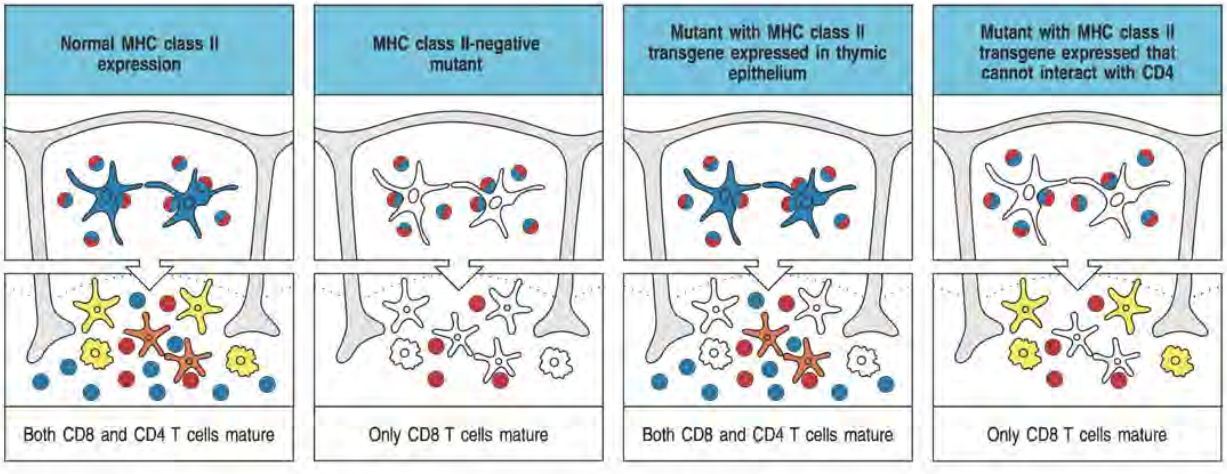
an ingenious manipulation of mice whose MHC class II genes have been eliminated by targeted gene disruption (Fig. 8.32). Mutant mice that lack MHC class II molecules do not normally produce CD4 T cells. To test the role of the thymic epithelium in positive selection, an MHC class II gene was placed under the control of a promoter that restricted its expression to thymic cortical epithelial cells. This was then introduced as a transgene into the CD4-mutant mice, and CD4 T-cell development was restored. A variant of this experiment showed that, to promote the development of CD4 T cells, the MHC class II molecule on the thymic cortical epithelium must be able to interact effectively with CD4. Thus, when the MHC class II transgene expressed in the thymus contains a mutation that prevents its binding to CD4, very few CD4 T cells develop. Equivalent studies of CD8 interaction with MHC class I molecules showed that co-receptor binding is also necessary for the positive selection of CD8 cells.

The critical role of the thymic cortical epithelium in positive selection raises the question of whether there is anything distinctive about the antigen-presenting properties of these cells. The thymic stromal cells may simply be in closest proximity to the developing thymocytes, as there are very few macrophages and dendritic cells in the cortex. In addition, however, thymic epithelium differs from other tissues in the expression of key proteases that are involved in MHC class I and II antigen processing (see Section 6-8). Cortical epithelial cells express cathepsin L as opposed to the more widely expressed cathepsin S, and mice deficient in cathepsin L have severely impaired CD4 T-cell development. Thymic epithelial cells from mice lacking cathepsin L exhibit a relatively high density of MHC class II molecules on their surface that retain the invariant chain-associated peptide (CLIP) (see Fig. 6.10). Cortical epithelial cells also express a unique proteasome subunit, β5T, whereas other cells express β5 or β5i. Mice deficient in β5T have severely impaired CD8 T-cell development. Because mice that lack either cathepsin L or β5 still have normal levels of MHC on the surface of their thymic cortical cells, it would seem that it is the peptide repertoire displayed by the MHC molecules on cortical epithelial cells that is responsible for altering CD8 T-cell development, although the mechanism is still unclear.

Fig. 8.32 Thymic cortical epithelial cells mediate positive selection. In the thymus of normal mice (first panels), which express MHC class II molecules on epithelial cells in the thymic cortex (blue) as well as on medullary epithelial cells (orange) and bone marrow derived cells (yellow), both CD4 (blue) and CD8 (red) T cells mature. Double-positive thymocytes are shown as half red and half blue. The second panels represent mutant mice in which MHC class II expression has been eliminated by targeted gene disruption; in these mice, few CD4 T cells develop, although CD8 T cells develop normally. In MHC class II-negative mice containing an MHC class II transgene engineered so that it is expressed only on the epithelial cells of the thymic cortex (third panels), normal numbers of CD4 T cells mature. In contrast, if a mutant MHC class II molecule with a defective CD4-binding site is expressed (fourth panel), positive selection of CD4 T cells does not take place. This indicates that the cortical epithelial cells are the critical cell type mediating positive selection and that the MHC class II molecule needs to be able to interact with the CD4 protein.

8-20 T cells that react strongly with ubiquitous self antigens are deleted in the thymus.

When the T-cell receptor of a mature naive T cell is strongly ligated by a peptide:MHC complex displayed on a specialized antigen-presenting cell in a



Petitioner Merck, Ex. 1170, p. 108
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

peripheral lymphoid organ, the T cell is activated to proliferate and produce effector T cells. In contrast, when the T-cell receptor of a developing thymocyte is similarly ligated by a self-peptide:MHC complex in the thymus, it dies by apoptosis. The response of immature T cells to stimulation by antigen is the basis of negative selection. Elimination of immature T cells in the thymus prevents their potentially harmful activation later, should they encounter the same self peptides when they are mature T cells.

Negative selection has been demonstrated by the use of both artificial and naturally occurring self peptides. Negative selection of thymocytes reactive to an artificial self peptide was demonstrated by using TCR-transgenic mice in which the majority of thymocytes expressed a T-cell receptor specific for a peptide of ovalbumin bound to an MHC class II molecule. When the ovalbumin peptide was injected into these mice, most of the CD4CD8 double-positive thymocytes in the thymic cortex died by apoptosis (Fig. 8.33). Negative selection by a natural self peptide was observed by using TCR-transgenic mice expressing T-cell receptors specific for self peptides normally expressed only in male mice. Thymocytes bearing these receptors disappear from the developing T-cell population in male mice at the double-positive stage of development, and no single-positive cells bearing the transgenic receptors mature. By contrast, in female mice, which lack the male-specific peptide, T cells bearing the transgenic receptors mature normally. Negative selection to male-specific peptides has also been demonstrated in normal mice and also occurs by deletion of T cells.

TCR transgenic mice were very useful for the classic experiments above, but they express a functional T-cell receptor earlier during development than normal mice and have a very high frequency of cells reactive to any particular peptide. A more realistic system for evaluating negative selection involves the transgenic expression of only the β chain of a T-cell receptor reactive to a given peptide antigen. In such mice, the β chain pairs with endogenous α chains, yet the frequency of peptide-reactive T cells is sufficient for detection using peptide:MHC tetramers (see Appendix I, Section A-28). These and other

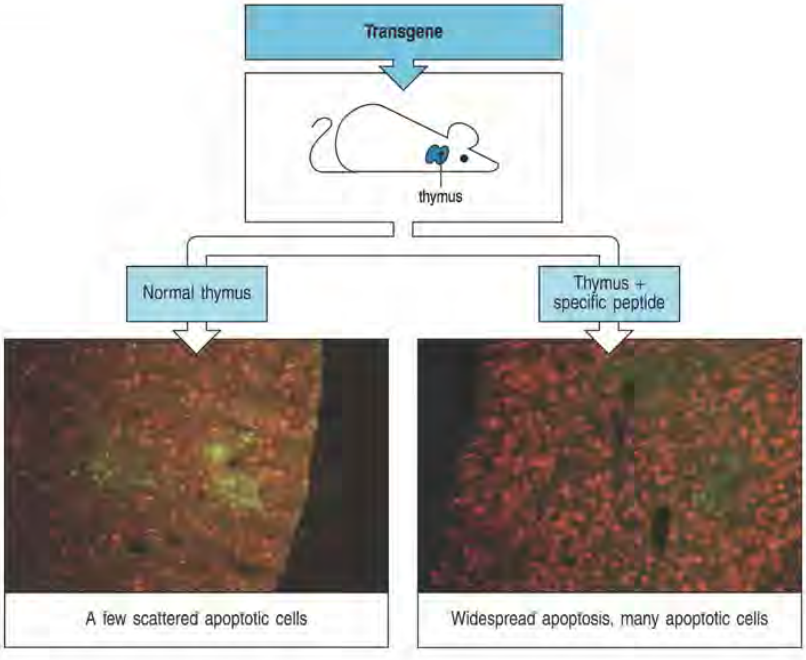


Fig. 8.33 T cells specific for self antigens are deleted in the thymus. In mice transgenic for a T-cell receptor that recognizes a known peptide antigen complexed with self MHC, all the T cells have the same specificity. In the absence of the peptide, most thymocytes mature and emigrate to the periphery. This can be seen in the bottom left panel, where a normal thymus is stained with antibody to identify the medulla (in green), and by the TUNEL technique (see Appendix I, Section A-32) to identify apoptotic cells (in red). If the mice are injected with the peptide that is recognized by the transgenic T-cell receptor, massive cell death occurs in the thymus, as shown by the increased numbers of apoptotic cells in the right-hand bottom panel. Photographs courtesy of A. Wack and D. Kioussis.

Petitioner Merck, Ex. 1170, p. 109
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

more physiologic approaches showed that clonal deletion can occur at either the double-positive or single-positive stage, presumably depending on where the T cell encounters the antigen that causes deletion.

These experiments illustrate the principle that self-peptide:self-MHC complexes encountered in the thymus purge the mature T-cell repertoire of cells bearing self-reactive receptors. One obvious problem with this scheme is that many tissue-specific proteins, such as pancreatic insulin, would not be expected to be expressed in the thymus. However, it is now clear that many such 'tissue-specific' proteins are expressed by certain stromal cells in the thymic medulla; thus, intrathymic negative selection could apply even to proteins that are otherwise restricted to tissues outside the thymus. The expression of some, but not all, tissue-specific proteins in the thymic medulla is controlled by a gene called ***AIRE*** (**autoimmune regulator**). *AIRE* is expressed in medullary stromal cells (Fig. 8.34), interacts with many proteins involved in transcription, and seems to lengthen transcripts from promoters that would otherwise terminate. Mutations in *AIRE* give rise to the human autoimmune disease known as **autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy** (**APECED**) or **autoimmune polyglandular syndrome type I**, highlighting the important role of intrathymic expression of tissue-specific proteins in maintaining tolerance to self. Negative selection of developing T cells involves interactions with ubiquitous self antigens and tissue-restricted self antigens, and can take place in both the thymic cortex and the thymic medulla.

It is unlikely that all possible self proteins are expressed in the thymus. Thus, negative selection in the thymus may not remove all T cells reactive to self antigens that appear exclusively in other tissues or are expressed at different stages in development. There are, however, several mechanisms operating in the periphery that can prevent mature T cells from responding to tissue-specific antigens; these are discussed in Chapter 15, when we consider the problem of autoimmune responses and their avoidance.

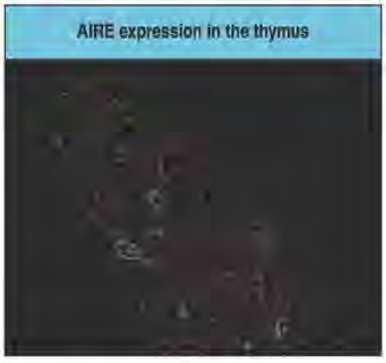


Fig. 8.34 AIRE is expressed in the medulla of the thymus and promotes the expression of proteins normally expressed in peripheral tissues. Expression of AIRE by thymic medullary cells is limited to the medullary region of the thymus, where it is expressed in a subset of epithelial-like cells. The expression of the thymic medullary epithelial marker MTS10 is shown in red. AIRE expression is shown in green by immunofluorescence, and is present in only a fraction of the medullary epithelial cells. Photograph courtesy of R.K. Chin and Y.-X. Fu.



Autoimmune Polyendocrinopathy–Candidiasis–Ectodermal Dystrophy (APECED)

8-21 Negative selection is driven most efficiently by bone marrow derived antigen-presenting cells.

As discussed above, negative selection occurs throughout thymocyte development, both in the thymic cortex and in the medulla, and so is likely to be mediated by antigen presentation by several different cell types. There does seem to be a hierarchy in the effectiveness of cells in mediating negative selection. At the top are bone marrow derived dendritic cells and macrophages. These are antigen-presenting cells that also activate mature T cells in peripheral lymphoid tissues, as we shall see in Chapter 9. The self antigens presented by these cells are therefore the most important source of potential autoimmune responses, and T cells responding to such self peptides must be eliminated in the thymus.

Experiments using bone marrow chimeric mice have clearly shown the role of thymic macrophages and dendritic cells in negative selection (Fig. 8.35). In these experiments, $MHC^{a\times b}$ F_1 bone marrow is grafted into one of the parental strains (MHC^a in Fig. 8.35). The $MHC^{a\times b}$ T cells developing in the grafted animals are thus exposed to MHC^a thymic epithelium. Bone marrow derived dendritic cells and macrophages will, however, express both MHC^a and MHC^b. The bone marrow chimeras will tolerate skin grafts from either MHC^a or MHC^b animals, and from the acceptance of both grafts we can infer that the developing T cells are not self-reactive for either of the two MHC antigens. The only cells that could present self-peptide:MHC^b complexes to thymocytes, and thus induce tolerance to MHC^b, are the bone marrow derived cells. The dendritic cells and macrophages are therefore assumed to have a crucial role in negative selection.

Petitioner Merck, Ex. 1170, p. 110
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

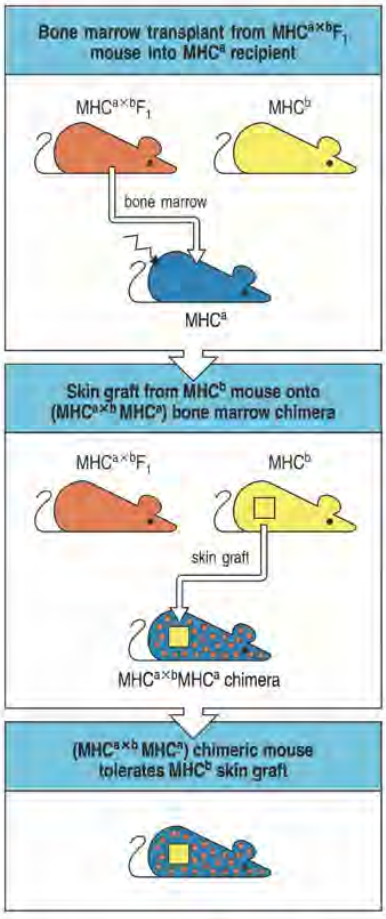


Fig. 8.35 Bone marrow derived cells mediate negative selection in the thymus. When MHCa$^{a\times b}$ F_1 bone marrow is injected into an irradiated MHC^a mouse, the T cells mature on thymic epithelium expressing only MHC^a molecules. Nevertheless, the chimeric mice are tolerant to skin grafts expressing MHC^b molecules (provided that these grafts do not present skin-specific peptides that differ between strains a and b). This implies that the T cells whose receptors recognize self antigens presented by MHC^b have been eliminated in the thymus. Because the transplanted MHC$^{a\times b}$ F_1 bone marrow cells are the only source of MHC^b molecules in the thymus, bone marrow derived cells must be able to induce negative selection.

In addition, both the thymocytes themselves and the thymic epithelial cells can cause the deletion of self-reactive cells. Such reactions may normally be of secondary significance compared with the dominant role of bone marrow derived cells. In patients undergoing bone marrow transplantation from an unrelated donor, however, where all the thymic macrophages and dendritic cells are of donor type, negative selection mediated by thymic epithelial cells can assume a special importance in maintaining tolerance to the recipient's own antigens.

8-22 The specificity and/or the strength of signals for negative and positive selection must differ.

T cells undergo both positive selection for self-MHC restriction and negative selection for self-tolerance by interacting with self-peptide:self-MHC complexes expressed on stromal cells in the thymus. An unresolved issue is how the interaction of the T-cell receptor with self-peptide:self-MHC complexes distinguishes between these opposite outcomes. First, more receptor specificities must be positively selected than are negatively selected. Otherwise, all the cells that were positively selected in the thymic cortex would be eliminated by negative selection, and no T cells would ever be produced (Fig. 8.36, left panels). Second, the consequences of the interactions that lead to positive and negative selection must differ: cells that recognize self-peptide:self-MHC complexes on cortical epithelial cells are induced to mature, whereas those whose receptors might confer strong and potentially damaging autoreactivity are induced to die.

Currently, the choice between positive and negative selection is thought to hinge on the strength of self-peptide:MHC binding by the T-cell receptor, an idea known as the **affinity hypothesis** (Fig. 8.36, right panels). Low-affinity interactions rescue the cell from death by neglect, leading to positive selection; high-affinity interactions induce apoptosis and thus negative selection. Because more complexes are likely to bind weakly than to bind strongly, this model explains the positive selection of a larger repertoire of cells than are negatively selected. Using T-cell receptor transgenic thymocytes, it was shown that variants of the antigenic peptide could induce positive selection in thymic organ cultures or *in vivo*. Peptide variants that induced positive selection had a lower affinity for the T-cell receptor than did antigenic peptide. How this quantitative difference in receptor affinity translates into a qualitatively distinct cell fate is still an area of active investigation. Many of the biochemical signals induced by low-affinity interactions are weaker or of shorter duration than those from high-affinity interactions. However, low-affinity interactions lead to increased activation of the protein kinase Erk, whereas high-affinity interactions lead to only transient activation of Erk, suggesting that differential activation of this or other MAPKs might determine the outcome of thymic selection.

Finally, other populations besides α:β T cells emerge from the thymus; they are numerically minor but functionally important. These include the T_{reg} cells discussed earlier (Section 8-18), the iNKT cells described in Section 8-9 and in Section 3-24, and CD8α:α intraepithelial lymphocytes (IELs) (see Section 12-10); each has unique developmental requirements. T_{reg} cells require signaling downstream of the IL-2 receptor, whereas other T cells do not. iNKT cells require a T-cell receptor interaction with nonclassical MHC molecules expressed on thymocytes and signal through the adaptor protein SAP, unlike other T cells. They have a memory/activated phenotype and have been suggested to develop in response to 'agonist' signaling—in other words, interactions of a T-cell receptor with a self peptide that would normally activate a T cell. Such interactions are also known to cause clonal deletion, and at this point it is not clear which activating interactions lead to clonal deletion in the thymus and which lead to selection of the nonconventional iNKT cells.

Petitioner Merck, Ex. 1170, p. 111
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

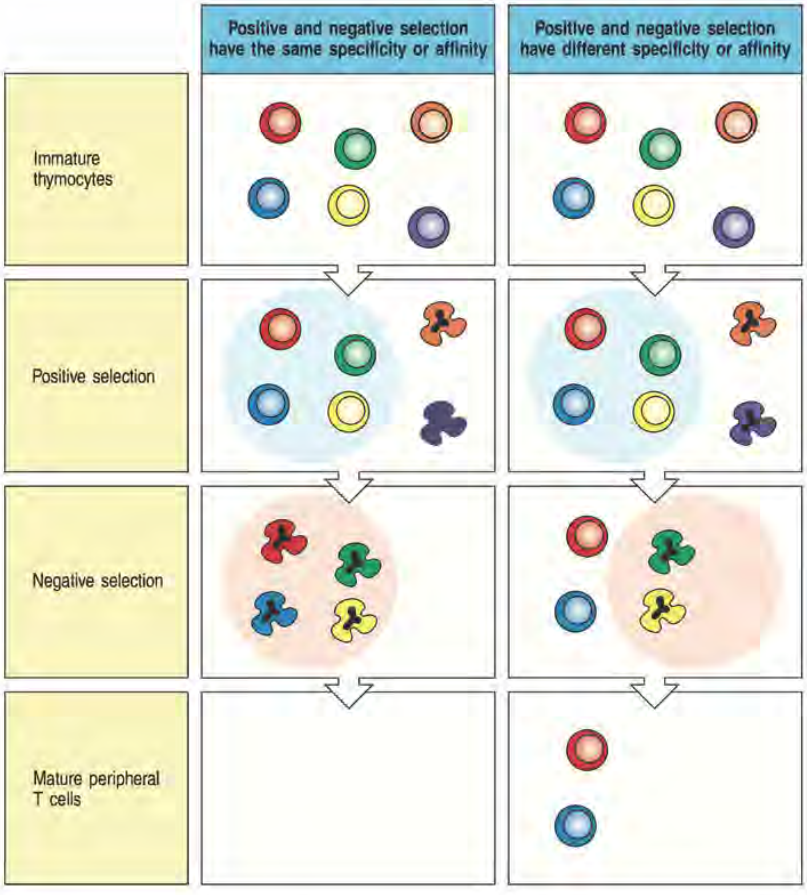


Fig. 8.36 The specificity or affinity of positive selection must differ from that of negative selection. Immature T cells are positively selected in such a way that only those thymocytes whose receptors can engage the peptide:MHC complexes on thymic epithelium mature, giving rise to a population of thymocytes restricted for self MHC. Negative selection removes those thymocytes whose receptors can be activated by self peptides complexed with self-MHC molecules, giving a self-tolerant population of thymocytes. If the specificity and affinity of positive and negative selection were the same (left panels), all the T cells that survive positive selection would be deleted during negative selection. Only if the specificity and affinity of negative selection are different from those of positive selection (right panels) can thymocytes mature into T cells.

After surviving positive and negative selection, thymocytes complete their maturation in the thymic medulla and then emigrate to peripheral lymphoid organs. Their final maturation results in changes to the T-cell receptor signaling machinery. Whereas an immature double-positive or single-positive thymocyte stimulated through the T-cell receptor will undergo apoptosis, a mature single-positive thymocyte responds by proliferating. The final maturation stage takes less than 4 days, and functionally competent T cells then emigrate from the thymus into the bloodstream. Emigration requires recognition of the lipid molecule sphingosine 1-phosphate (S1P) by the G-protein-coupled receptor $S1P_1$, which is expressed by thymocytes during their final maturation. S1P is present in high concentration in blood and lymph, and mature thymocytes seem to be drawn toward it. Mature thymocytes also express CD62L (L-selectin), a lymph-node homing receptor that facilitates the localization of mature naive T cells to peripheral lymphoid organs after their emigration from the thymus.

Summary.

The stages of thymocyte development up to the expression of the pre-T-cell receptor—including the decision between commitment to either the α:β or the γ:δ lineage—are all independent of interactions with peptide:MHC antigens. With the successful rearrangement of α-chain genes and expression of the T-cell receptor, α:β thymocytes undergo further development that is determined by the interactions of their T-cell receptors with self peptides

Petitioner Merck, Ex. 1170, p. 112
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

presented by the MHC molecules on the thymic stroma. CD4CD8 double-positive thymocytes whose receptors interact with self-peptide:self-MHC complexes on thymic cortical epithelial cells are positively selected, and will eventually mature into CD4 or CD8 single-positive cells. T cells that react too strongly with self antigens are deleted in the thymus, a process driven most efficiently by bone marrow derived antigen-presenting cells in the medullary region of the thymus. Certain tissue-restricted proteins can be expressed in medullary epithelial cells as a result of the actions of the AIRE protein, and so can mediate negative selection in the thymus. The outcome of positive and negative selection is the generation of a mature T-cell repertoire that is both MHC-restricted and self-tolerant. Precisely how the recognition of self-peptide:self-MHC ligands by the T-cell receptor leads either to positive or negative selection remains an unsolved problem.

Survival and maturation of lymphocytes in peripheral lymphoid tissues.

Once B and T lymphocytes have completed their development in the central lymphoid tissues, they are carried in the blood to the peripheral lymphoid tissues. These tissues have a highly organized architecture, with distinct areas of B cells and T cells, and organization that is determined by interactions between lymphocytes and the other cell types that make up the lymphoid tissues. Before considering the factors governing the survival and maturation of newly formed lymphocytes in the periphery, we will briefly look at the development and organization of peripheral lymphoid tissues and the signals that guide lymphocytes to their correct locations. Normally, a lymphocyte will leave peripheral lymphoid tissue and recirculate via lymph and blood (see Section 1-15), continually reentering lymphoid tissues until antigen is encountered or the lymphocyte dies. When it meets its antigen, the lymphocyte stops recirculating, proliferates, and differentiates, as described in Chapters 9–11. When a lymphocyte dies, its place is taken by a newly formed lymphocyte; this enables a turnover of the receptor repertoire and ensures that lymphocyte numbers remain constant.

8-23 Different lymphocyte subsets are found in particular locations in peripheral lymphoid tissues.

The various peripheral lymphoid organs are organized roughly along the same lines, as discussed in Chapter 1, with distinct areas of B cells and T cells, and they also contain macrophages, dendritic cells, and non-leukocyte stromal cells. The lymphoid tissue of the spleen is the white pulp, whose overall architecture is illustrated in Fig. 1.19. Each area of white pulp is demarcated by a **marginal sinus**, a vascular network that branches from the central arteriole. The **marginal zone** of the white pulp, the outer border of which is the edge of the marginal sinus, is a highly organized region of cells whose function is poorly understood. It has few T cells but is rich in macrophages and contains a unique population of B cells, the **marginal zone B cells**, which do not recirculate. Pathogens reaching the bloodstream are efficiently trapped in the marginal zone by macrophages, and it could be that marginal zone B cells are adapted to provide the first responses to such pathogens.

The white pulp contains clearly separated areas of T cells and B cells. T cells are clustered around the central arteriole, and the globular B-cell areas or follicles are located farther out. Some follicles may contain **germinal centers**, in which B cells involved in an adaptive immune response are proliferating

Petitioner Merck, Ex. 1170, p. 113
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

and undergoing somatic hypermutation (see Section 5-18). In follicles with germinal centers, the resting B cells that are not part of the immune response are pushed outward to make up the **mantle zone** around the proliferating lymphocytes. The antigen-driven production of germinal centers will be described in detail when we consider B-cell responses in Chapter 10.

Other types of cells are found within the B-cell and T-cell areas. The B-cell zone contains a network of **follicular dendritic cells** (**FDCs**), which are concentrated mainly in the area of the follicle most distant from the central arteriole. FDCs have long processes, from which they get their name, and these are in contact with B cells. FDCs are a distinct type of cell from the dendritic cells we have encountered previously (see Section 1-3), in that they are not leukocytes and are not derived from bone marrow precursors; in addition, they are not phagocytic and do not express MHC class II proteins. FDCs seem to be specialized to capture antigen in the form of immune complexes—complexes of antigen, antibody, and complement. The immune complexes are not internalized but remain intact on the surface of the FDC, where the antigen can be recognized by B cells. FDCs are also important in the development of B-cell follicles.

T-cell zones contain a network of bone marrow derived dendritic cells, sometimes known as **interdigitating dendritic cells** from the way in which their processes interweave among the T cells. There are two subtypes of these dendritic cells, distinguished by characteristic cell-surface proteins; one expresses the α chain of CD8, whereas the other is CD8 negative but expresses CD11b:CD18, an integrin that is also expressed by macrophages.

As in the spleen, the T cells and B cells in lymph nodes are organized into discrete T-cell and B-cell areas (see Fig. 1.18). B-cell follicles have a similar structure and composition to those in the spleen and are located just under the outer capsule of the lymph node. T-cell zones surround the follicles in the paracortical areas. Unlike the spleen, lymph nodes have connections to both the blood system and the lymphatic system. Lymph enters into the subcapsular space, which is also known as the marginal sinus, and brings in antigen and antigen-bearing dendritic cells from the tissues.

The **mucosa-associated lymphoid tissues** (**MALT**) are associated with the body's epithelial surfaces that provide physical barriers against infection. Peyer's patches are part of the MALT and are lymph node-like structures interspersed at intervals just beneath the gut epithelium. They have B-cell follicles and T-cell zones (see Fig. 1.20), and the epithelium overlying them contains specialized M cells that are adapted to channel antigens and pathogens from the gut lumen to the Peyer's patch (see Section 1-15 and Chapter 12). Peyer's patches and similar tissue present in the tonsils provide specialized sites where B cells can become committed to synthesizing IgA. The stromal cells of the MALT secrete the cytokine TGF-β, which has been shown to induce IgA secretion by B cells in culture. In addition, as discussed in Section 8-12, during fetal development waves of γ:δ T cells with specific γ- and δ-gene rearrangements leave the thymus and migrate to these epithelial barriers. The mucosal immune system is discussed in more detail in Chapter 12.

8-24 The development of peripheral lymphoid tissues is controlled by lymphoid tissue inducer cells and proteins of the tumor necrosis factor family.

Before discussing how lymphocytes find their way to their respective zones in the peripheral lymphoid organs, we shall briefly look at how these organs and the zones within them develop in the first place. Lymphatic vessels are formed during embryonic development from endothelial cells that originate in blood vessels. Some endothelial cells in the early venous system begin to

Petitioner Merck, Ex. 1170, p. 114
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

express the homeobox transcription factor Prox1. These cells bud from the vein, migrate away, and reassociate to form a parallel network of lymphatic vessels. Mice lacking Prox1 have normal arteries and veins, but fail to form a lymphatic system, showing this factor to be critical in establishing the identity of lymphatic endothelium. As the lymphatic vessels form, blood-lineage cells called **lymphoid tissue inducer (LTi) cells** arise in the fetal liver and are carried in the bloodstream to sites of prospective lymph nodes and Peyer's patches. LTi cells initiate the formation of lymph nodes and Peyer's patches by interacting with stromal cells and inducing the production of cytokines and chemokines, which recruit other lymphoid cells to these sites. Members of the tumor necrosis factor (TNF)/TNF receptor (TNFR) family of cytokines turn out to be critically involved in the interactions between LTi cells and stromal cells.

The role of this family of cytokines in the formation of peripheral lymphoid organs has been demonstrated in a series of knockout mice in which either the TNF family ligand or its receptor was inactivated (Fig. 8.37). These knockouts have complicated phenotypes, which is partly due to the fact that individual TNF-family proteins can bind to multiple receptors and, conversely, many receptors can bind more than one protein. In addition, it seems clear that there is some overlapping function or cooperation between TNF-family proteins. Nonetheless, some general conclusions can be drawn.

Lymph-node development depends on the expression of TNF-family proteins known as the **lymphotoxins (LTs)**, and different types of lymph nodes depend on signals from different LTs. **LT-α_3**, a soluble homotrimer of the LT-α chain, supports the development of cervical and mesenteric lymph nodes, and possibly lumbar and sacral lymph nodes. All these lymph nodes drain mucosal sites. LT-α_3 probably exerts its effects by binding to TNFR-I. The membrane-bound heterotrimer comprising LT-α and the distinct protein LT-β (**LT-α_2:β_1**), often known as **LT-β**, binds only to the LT-β receptor and supports the development of all the other lymph nodes. Peyer's patches also do not form in the absence of LT-β. The effects of the LT knockouts are not reversible in adult animals and there are certain critical developmental periods during which the absence or inhibition of these LT-family proteins will permanently prevent the development of lymph nodes and Peyer's patches.

LTi cells express LT-β, which engages the LT-β receptors on stromal cells in the prospective lymphoid site, activating the NFκB pathway (see Section 7-22). This induces the stromal cells to express adhesion molecules and chemokines such as CXCL13 (B-lymphocyte chemokine, BLC), which in turn recruits more LTi cells, which have receptors for these molecules, eventually generating large clusters of cells that will become lymph nodes or Peyer's patches. The chemokines also attract cells such as lymphocytes and other hematopoietic-lineage cells with appropriate receptors to populate the forming lymphoid organ. The principles, and even some of the molecules, underlying the development of peripheral lymphoid organs in the fetus are

Fig. 8.37 Normal architecture of the peripheral lymphoid organs requires TNF family members and their receptors. The role of TNF family members in the development of peripheral lymphoid organs has been deduced mainly from the study of knockout mice deficient in one or more TNF-family ligands or receptors. Some receptors bind more than one ligand, and some ligands bind more than one receptor, complicating the effects of their deletion. (Note that receptors are named for the first ligand known to bind them.) The defects are organized here with respect to the two main receptors, TNFR-I and the LT-β receptor and their ligands, TNF-α and the lymphotoxins (LTs). In some cases, the loss of ligands that bind the same receptor leads to different phenotypes. This is due to the ability of the ligand to bind another receptor, as indicated in the figure. The LT-α protein chain contributes to two distinct ligands, the trimer LT-α_3 and the heterodimer LT-α_2:β_1, each of which acts through a distinct receptor. In general, signaling through the LT-β receptor is required for lymph node and FDC development and normal splenic architecture, whereas signaling through TNFR-I is also required for FDCs and normal splenic architecture but not for lymph-node development.

		Effects seen in knockout (KO) mice				
Receptor	Ligands	Spleen	Peripheral lymph node	Mesenteric lymph node	Peyer's patch	Follicular dendritic cells
TNFR-I	TNF-α LT-α_3	Distorted architecture	Present in TNF-α KO Absent in LT-α KO owing to lack of LT-β signals	Present	Reduced	Absent
LT-β receptor	TNF-α LT-α_2/β_1	Distorted No marginal zones	Absent	Present in LT-β KO Absent in LT-β receptor KO	Absent	Absent

Petitioner Merck, Ex. 1170, p. 115
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

very similar to those that maintain the organization of lymphoid organs in the adult, as we shall see in the next section.

Although the spleen develops in mice deficient in any of the known TNF or TNFR family members, its architecture is abnormal in many of these mutants (see Fig. 8.37). LT (most probably the membrane-bound LT-β) is required for the normal segregation of T-cell and B-cell zones in the spleen. TNF-α, binding to TNFR-I, also contributes to the organization of the white pulp: when TNF-α signals are disrupted, B cells surround T-cell zones in a ring rather than forming discrete follicles. In addition, the marginal zones are not well defined when TNF-α or its receptor is absent.

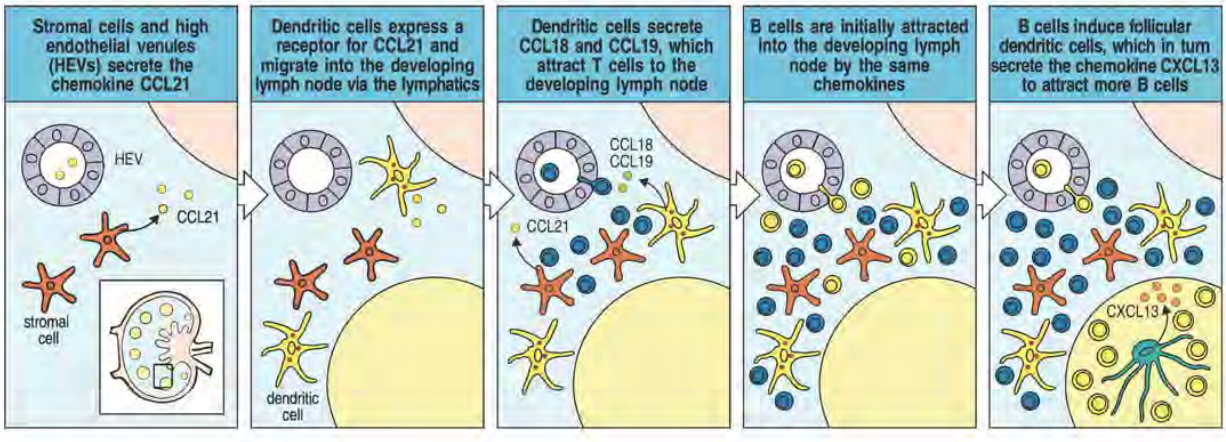
Perhaps the most important role of TNF-α and TNFR-I in lymphoid organ development is in the development of FDCs, as these cells are lacking in mice with knockouts of either TNF-α or TNFR-I (see Fig. 8.37). The knockout mice do have lymph nodes and Peyer's patches, because they express LTs, but these structures lack FDCs. LT-β is also required for FDC development: mice that cannot form LT-β or signal through it lack normal FDCs in the spleen and any residual lymph nodes. Unlike the disruption of lymph-node development, the disorganized lymphoid architecture in the spleen is reversible if the missing TNF-family member is restored. B cells are the likely source of the LT-β, because normal B cells can restore FDCs and follicles when transferred to RAG-deficient recipients (which lack lymphocytes).

8-25 The homing of lymphocytes to specific regions of peripheral lymphoid tissues is mediated by chemokines.

Newly formed lymphocytes enter the spleen via the blood, exiting first in the marginal sinus, from which they migrate to the appropriate areas of the white pulp. Lymphocytes that survive their passage through the spleen most probably leave via venous sinuses in the red pulp. In lymph nodes, lymphocytes enter from the blood through the walls of specialized blood vessels, the high endothelial venules (HEVs), which are located within the T-cell zones. Naive B cells migrate through the HEVs in the T-cell area and come to rest in the follicle where, unless they encounter their specific antigen and become activated, they remain for about a day. B cells and T cells leave in the lymph via the efferent lymphatic, which returns them eventually to the blood. The precise location of B cells, T cells, macrophages, and dendritic cells in peripheral lymphoid tissue is controlled by chemokines, which are produced by both stromal cells and bone marrow derived cells (Fig. 8.38).

B cells constitutively express the chemokine receptor CXCR5 and are attracted to the follicles by the ligand for this receptor, the chemokine CXCL13. The

Fig. 8.38 The organization of a lymphoid organ is orchestrated by chemokines. The cellular organization of lymphoid organs is initiated by stromal cells and vascular endothelial cells, which secrete the chemokine CCL21 (first panel). Dendritic cells with a receptor for CCL21, CCR7, are attracted to the site of the developing lymph node by CCL21 (second panel); it is not known whether at the earliest stages of lymph node development immature dendritic cells enter from the bloodstream or via the lymphatics, as they do later in life. Once in the lymph node, the dendritic cells express the chemokines CCL18 (also called DC-CK1) and CCL19, for which T cells express receptors. Together, the chemokines secreted by stromal cells and dendritic cells attract T cells to the developing lymph node (third panel). The same combination of chemokines also attracts B cells into the developing lymph node (fourth panel). The B cells are able to either induce the differentiation of the non-leukocyte FDCs (which are a distinct lineage from the bone marrow derived dendritic cells) or direct their recruitment into the lymph node. Once present, the FDCs secrete a chemokine, CXCL13, which is a chemoattractant for B cells. The production of CXCL13 drives the organization of B cells into discrete B-cell areas (follicles) around the FDCs and contributes to the further recruitment of B cells from the circulation into the lymph node (fifth panel).



Petitioner Merck, Ex. 1170, p. 116
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

most likely source of CXCL13 is the FDC, possibly along with other follicular stromal cells. This is reminiscent of the expression of CXCL13 by stromal cells during the formation of the lymph node. B cells are, in turn, the source of the LT that is required for the development of FDCs, which is reminiscent of LTi cells expressing the LT required to activate stromal cells. The reciprocal dependence of B cells and FDCs, and LTis and stromal cells, illustrates the complex web of interactions that organizes peripheral lymphoid tissues. T cells can also express CXCR5, although at a lower level, and this may explain how T cells are able to enter B-cell follicles, which they do when activated, to participate in the formation of the germinal center.

Movie 8.2 

T-cell localization to the T zones involves two chemokines, CCL19 (MIP-3β) and CCL21 (secondary lymphoid chemokine, SLC). Both of these bind the receptor CCR7, which is expressed by T cells; mice that lack CCR7 do not form normal T-cell zones and have impaired primary immune responses. CCL21 is produced by stromal cells of the T-cell zone in spleen, and by the endothelial cells of HEVs in lymph nodes and Peyer's patches. Another source of CCL19 and CCL21 is the interdigitating dendritic cells, which are also prominent in the T zones. Indeed, dendritic cells themselves express CCR7 and will localize to T zones even in RAG-deficient mice. Thus, in lymph-node development, the T zone might be organized first through the attraction of dendritic cells and T cells by CCL21 produced by stromal cells. This organization would then be reinforced by CCL21 and CCL19 secreted by resident mature dendritic cells, which in turn attract more T cells and immature dendritic cells.

8-26 Lymphocytes that encounter sufficient quantities of self antigens for the first time in the periphery are eliminated or inactivated.

Large numbers of autoreactive lymphocytes have been purged from the population of new lymphocytes in the central lymphoid organs (discussed in Section 8-20); however, this only involves lymphocytes specific for autoantigens that are expressed in or can reach these organs. Not all potential self antigens are expressed in the central lymphoid organs. Some, like the thyroid product thyroglobulin, are highly tissue specific, or are compartmentalized so that little if any is available in the circulation. Therefore, newly emigrated self-reactive lymphocytes that encounter their specific autoantigen for the first time in the periphery must be eliminated or inactivated. This tolerance mechanism is known as peripheral tolerance. Like self-reactive lymphocytes in the central lymphoid organs, lymphocytes that encounter self antigens *de novo* in the periphery can have several fates: deletion, anergy, or survival.

In the absence of an infection, newly mature B cells that encounter a strongly cross-linking antigen in the periphery will undergo clonal deletion. This was elegantly shown in studies of B cells expressing B-cell receptors specific for H-2K^b MHC class I molecules. These cells are deleted even when, in transgenic animals, the expression of the H-2K^b molecule is restricted to the liver by the use of a liver-specific gene promoter. There is no receptor editing: B cells that encounter strongly cross-linking antigens in the periphery undergo apoptosis directly, unlike their counterparts in the bone marrow, which attempt further receptor rearrangements. The different outcomes may be due to the fact that the B cells in the periphery are more mature and can no longer rearrange their light-chain loci.

As with immature B cells, mature B cells that encounter and bind an abundant soluble antigen become anergized. This was demonstrated in mice by placing the *HEL* transgene under the control of an inducible promoter that can be regulated by changes in the diet. It is thus possible to induce the production of lysozyme at any time and thereby study its effects on HEL-specific B cells at different stages of maturation. These experiments have

Petitioner Merck, Ex. 1170, p. 117
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

shown that both mature and immature B cells are inactivated when they are chronically exposed to soluble antigen.

The situation is similar for T cells. Again, our understanding of the fates of autoreactive T cells in the periphery comes mainly from the study of mice transgenic for self-reactive T-cell receptors. In some cases, T cells reacting to self antigens in the periphery are eliminated. This usually follows a brief period of activation and cell division, and so is known as **activation-induced cell death**. In other cases, the self-reactive cells may be rendered anergic. When studied *in vitro*, these anergic T cells prove refractory to signals delivered through the T-cell receptor.

The question immediately arises: if the encounter of a mature naive lymphocyte with a self antigen leads to cell death or anergy, why does this not also happen to a mature lymphocyte that recognizes a pathogen-derived antigen? The answer is that infection sets up inflammation, which induces the expression of co-stimulatory molecules on the antigen-presenting dendritic cells and the production of cytokines promoting lymphocyte activation. The outcome of an encounter with antigen in these conditions is the activation, proliferation, and differentiation of the lymphocyte to effector-cell status. In the absence of infection or inflammation, dendritic cells still process and present self antigens, but in the absence of co-stimulatory and other signals, any interaction of a mature lymphocyte with its specific antigen seems to result in a tolerance-inducing (**tolerogenic**) signal from the antigen receptor.

8-27 Immature B cells arriving in the spleen turn over rapidly and require cytokines and positive signals through the B-cell receptor for maturation and survival.

When B cells emerge from bone marrow into the periphery, they are still functionally immature. Immature B cells express high levels of sIgM but little sIgD, whereas mature B cells express low levels of IgM and high levels of IgD. Most immature B cells leaving the bone marrow will not survive to become fully mature B cells. Figure 8.39 shows the possible fates of newly produced B cells

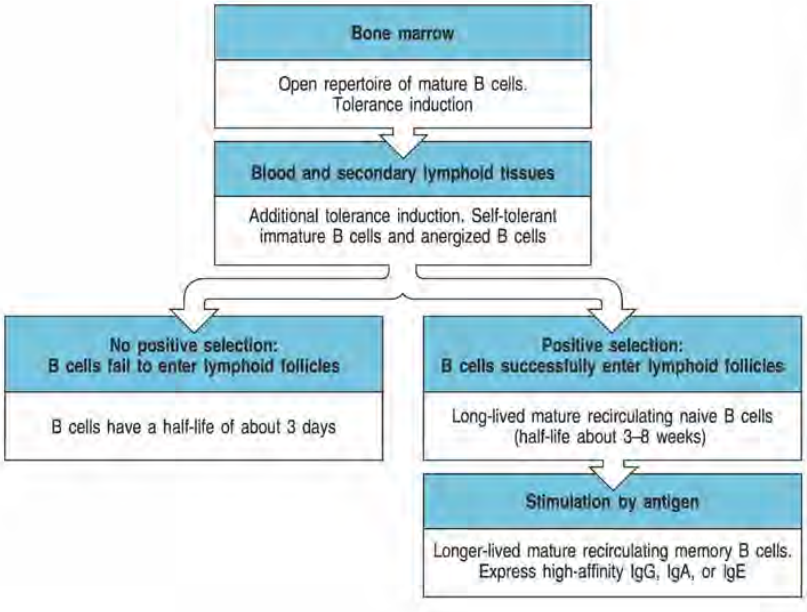


Fig. 8.39 Proposed population dynamics of conventional B cells. B cells are produced as receptor-positive immature B cells in the bone marrow. The most avidly self-reactive B cells are removed at this stage. B cells then migrate to the periphery, where they enter the peripheral lymphoid tissues. It is estimated that $10–20 \times 10^6$ B cells are produced by the bone marrow and exported each day in a mouse, and an equal number are lost from the periphery. There seem to be two classes of peripheral B cells: long-lived B cells and rapidly turning-over B cells. The short-lived B cells are, by definition, recently formed B cells. Most of the turnover of short-lived B cells might result from B cells that fail to enter lymphoid follicles. In some cases this is a consequence of being rendered anergic by binding to soluble self antigen; for the remaining immature B cells, entry into lymphoid follicles is thought to entail some form of positive selection. Thus, the remainder of the short-lived B cells fail to join the long-lived pool because they are not positively selected. About 90% of all peripheral B cells are relatively long-lived mature B cells that seem to have undergone positive selection in the periphery. These mature naive B cells recirculate through peripheral lymphoid tissues and have a half-life of 6–8 weeks in mice. Memory B cells, which have been activated previously by antigen and T cells, are thought to have a longer life.

Petitioner Merck, Ex. 1170, p. 118
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

that enter the periphery. The daily output of new B cells from the bone marrow is roughly 5–10% of the total B-lymphocyte population in the steady-state peripheral pool. The size of this pool seems to remain constant in unimmunized animals, a regulatory process known as homeostasis, and so the stream of new B cells needs to be balanced by the removal of an equal number of peripheral B cells. However, the majority of peripheral mature B cells are long-lived, and only 1–2% of these die each day. Most of the B cells that die are in the rapidly turning-over immature peripheral B-cell population, of which more than 50% die every 3 days. The failure of most newly formed B cells to survive for more than a few days in the periphery seems to be due to competition between peripheral B cells for access to the follicles in peripheral lymphoid tissues. If newly produced immature B cells do not enter a follicle, their passage through the periphery is halted and they eventually die. The limited number of lymphoid follicles cannot accommodate all of the B cells generated each day and so there is continual competition for entry.

The follicle provides signals necessary for B-cell survival. In particular, the TNF-family member **BAFF** (for B-cell activating factor belonging to the TNF family) is made by several cell types, but is produced abundantly by the FDCs. B cells express three different receptors for BAFF, namely BAFF-R, BCMA, and TACI. The BAFF-R is the most important for follicular B-cell survival, because mutants lacking BAFF-R have mainly immature B cells and few long-lived peripheral B cells. BCMA and TACI also bind a related TNF family cytokine APRIL, which is not required for the survival of immature B cells but is important for IgA antibody production, as we shall see in Chapter 10. Immature B cells proceed through two defined **transitional stages** in the spleen, called T1 and T2, defined by the absence or presence of the B-cell co-receptor component CD21 (complement receptor 2) (see Section 2-13). In mice lacking BAFF, immature B cells progress to the T1 stage in the spleen but fail to express CD21, and the mice lack mature B cells.

Peripheral B cells also include memory B cells, which are produced in addition to antibody-producing plasma cells from mature B cells after their first encounter with antigen; we will return to B-cell memory in Chapter 11. Competition for follicular entry favors mature B cells that are already established in the relatively long-lived and stable peripheral B-cell pool. Mature B cells have undergone phenotypic changes that might make their access to the follicles easier; for example, they express CXCR5, the receptor for CXCL13, which is expressed by FDCs (see Fig. 8.38). They also have increased expression of CD21, which enhances the signaling capacity of the B cell.

The B-cell receptor plays a positive role in the maturation and continued recirculation of peripheral B cells. Continuous expression of the B-cell receptor is required for B-cell survival, because conditional gene deletion of the B-cell receptor in mature B cells leads to the loss of these B cells. Mice that lack the tyrosine kinase Syk, which is involved in signaling from the B-cell receptor (see Section 7-16), have immature B cells but fail to develop mature B cells. Thus, a Syk-transduced signal may be required for final B-cell maturation or for the survival of mature B cells. Although each B-cell receptor has a unique specificity, antigen-specific interactions may not induce the signals used for final B-cell maturation; the receptor could, for example, be responsible for 'tonic' signaling, in which a weak but important signal is generated by the assembly of the receptor complex and infrequently triggers some or all of the downstream signaling events.

8-28 B-1 cells and marginal zone B cells are distinct B-cell subtypes with unique antigen receptor specificity.

The receptor specificity is important in shaping the peripheral B-cell pools that derive from immature B cells that reach the spleen. This is shown most

Petitioner Merck, Ex. 1170, p. 119
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

clearly in the role of the B-cell receptor and antigen in the selection of two subsets of B cells that do not reside in B-cell follicles: the so-called **B-1 cells** and the marginal zone B cells of the spleen.

B-1 cells are a unique subset comprising about 5% of all B cells in mice and humans, and are the major population in rabbits. B-1 cells have high levels of sIgM and little sIgD, opposite to the pattern of follicular B cells. They are called B-1 because they are the first to appear during fetal development (Fig. 8.40), before the conventional B cells whose development we have discussed up to now—which are called **B-2 cells**. B-1 cells are found primarily in the peritoneal and pleural cavity fluid. Certain autoantigens and environmental antigens encountered in the periphery are thought to drive the expansion and maintenance of B-1 cells. Some of these antigens, such as phosphocholine, are encountered on the surface of bacteria that colonize the gut.

It is not clear whether B-1 cells arise as a distinct lineage from a unique precursor cell or differentiate to the B-1 phenotype from a precursor cell that could also give rise to B-2 cells. In the mouse, fetal liver produces mainly B-1 cells, whereas adult bone marrow generates predominantly B-2 cells, and this has been interpreted as support for the unique precursor hypothesis. However, the weight of evidence favors the idea that commitment to the B-1 or B-2 subset is due to a selection step, rather than their being distinct lineages like the γ:δ and α:β T cells.

Marginal zone B cells, so called because they reside in the marginal sinus of the white pulp in the spleen, are another unique subset of B cells. They seem to be resting mature B cells, yet they have a different set of surface proteins from those present on the major follicular B-cell population. For example, they express lower levels of CD23 (the low-affinity receptor for IgE), and high

Property	B-1 cells	Conventional B-2 cells	Marginal zone B cells
When first produced	Fetus	After birth	After birth
N-regions in VDJ junctions	Few	Extensive	Yes
V-region repertoire	Restricted	Diverse	Partly restricted
Primary location	Body cavities (peritoneal, pleural)	Secondary lymphoid organs	Spleen
Mode of renewal	Self-renewing	Replaced from bone marrow	Long-lived
Spontaneous production of immunoglobulin	High	Low	Low
Isotypes secreted	IgM >> IgG	IgG > IgM	IgM > IgG
Response to carbohydrate antigen	Yes	Maybe	Yes
Response to protein antigen	Maybe	Yes	Yes
Requirement for T-cell help	No	Yes	Sometimes
Somatic hypermutation	Low to none	High	?
Memory development	Little or none	Yes	?

Fig. 8.40 A comparison of the properties of B-1 cells, conventional B cells (B-2 cells), and marginal zone B cells. B-1 cells can develop in unusual sites in the fetus, such as the omentum, in addition to the liver. B-1 cells predominate in the young animal, although they probably can be produced throughout life. Being produced mainly during fetal and neonatal life, their rearranged variable-region sequences contain few N-nucleotides. In contrast, marginal zone B cells accumulate after birth and do not reach peak levels in the mouse until 8 weeks of age. B-1 cells are best thought of as a partly activated self-renewing pool of lymphocytes that are selected by ubiquitous self and foreign antigens. Because of this selection, and possibly because the cells are produced early in life, the B-1 cells have a restricted repertoire of variable regions and antigen-binding specificities. Marginal zone B cells also have a restricted repertoire that may be selected by a set of antigens similar to those that select B-1 cells. B-1 cells seem to be the major population of B cells in certain body cavities, most probably because of exposure at these sites to antigens that drive B-1 cell proliferation. Marginal zone B cells remain in the marginal zone of the spleen and are not thought to recirculate. Partial activation of B-1 cells leads to the secretion of mainly IgM antibody; B-1 cells contribute much of the IgM that circulates in the blood. The limited diversity of both the B-1 and marginal zone B-cell repertoire and the propensity of these cells to react with common bacterial carbohydrate antigens suggest that they carry out a more primitive, less adaptive, immune response than conventional B cells (B-2 cells). In this regard they are comparable to γ:δ T cells.

Petitioner Merck, Ex. 1170, p. 120
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

levels of both the MHC class I-like molecule CD1 (see Section 6-19) and two receptors for the C3 fragment of complement, CR1 (CD35) and CR2 (CD21). Marginal zone B cells have restricted antigen specificities, biased toward self antigens and common bacterial antigens, and may be adapted to provide a quick response if the latter enter the bloodstream. They may not require T-cell help to become activated. Functionally and phenotypically, marginal zone B cells resemble B-1 cells; recent experiments suggest that they are positively selected for survival by certain self antigens, much as B-1 cells are.

The functions of B-1 cells and marginal zone B cells are being clarified. Their locations suggest a role for B-1 cells in defending the body cavities and a role for marginal zone B cells in defense against bacteria that penetrate the bloodstream. The restricted repertoire of receptors in both cell types seems to equip them for a function in the early, nonadaptive phase of an immune response (see Section 3-24). Indeed, the V gene segments that are used to encode the receptors of B-1 and marginal zone B cells might have evolved by natural selection to recognize common bacterial antigens, thus allowing them to contribute to the very early phases of the adaptive immune response. In practice, it is found that B-1 cells make little contribution to adaptive immune responses to most protein antigens, but contribute strongly to some antibody responses against carbohydrate antigens. Moreover, a large proportion of the IgM that normally circulates in the blood of unimmunized mice derives from B-1 cells. The existence of these so-called **natural antibodies**, which are highly cross-reactive and bind with low affinity to both microbial and self antigens, supports the view that B-1 cells are partly activated because they are selected for self-renewal by ubiquitous self and environmental antigens.

8-29 T-cell homeostasis in the periphery is regulated by cytokines and self-MHC interactions.

When T cells have expressed their receptors and co-receptors, and matured within the thymus for a further week or so, they emigrate to the periphery. Unlike B cells emigrating from bone marrow, only relatively small numbers of T cells are exported from the thymus, roughly $1–2 \times 10^6$ per day in the mouse. As with B cells, the size and composition of the peripheral pool of naive T cells are also regulated by homeostatic mechanisms that maintain it at a roughly constant size and a composition of diverse but potentially functional T-cell receptors. These mechanisms involve both cytokines and signals received through the T-cell receptor in response to its interaction with self-MHC molecules.

A requirement for the cytokine IL-7 and interactions with self-peptide:self-MHC complexes for T-cell survival in the periphery has been shown experimentally. If T cells are transferred from their normal environment to recipients lacking MHC molecules, or lacking the 'correct' MHC molecules that originally selected the T cells, they do not survive long. In contrast, if T cells are transferred into recipients that have the correct MHC molecules, they survive. Contact with the appropriate self-peptide:self-MHC complex as they circulate through peripheral lymphoid organs leads mature naive T cells to undergo infrequent cell division. This slow increase in T-cell numbers must be balanced by a slow loss of T cells, such that the number of T cells remains roughly constant. Most probably, this loss occurs among the daughters of the dividing naive T cells.

Where do the mature naive CD4 and CD8 T cells encounter their positively selecting ligands? Current evidence favors self-MHC molecules on dendritic cells resident in the T-cell zones of peripheral lymphoid tissues. These cells are similar to the dendritic cells that migrate to the lymph nodes from other tissues but lack sufficient co-stimulatory potential to induce full T-cell

Petitioner Merck, Ex. 1170, p. 121
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

activation. The study of peripheral positive selection is in its infancy, however, and a clear picture has yet to emerge. Memory T cells are also part of the peripheral T-cell pool, and we return to their regulation in Chapter 11.

Summary.

The formation and organization of the peripheral lymphoid tissues is controlled by proteins of the TNF family and their receptors (TNFRs). LTi cells expressing LT-β interact with stromal cells expressing the receptor TNFR-I in the developing embryo to induce chemokine production, which in turn initiates formation of the lymph nodes and Peyer's patches. Similar interactions between lymphotoxin-expressing B cells and TNFR-I-expressing follicular dendritic cells (FDCs) establishes the normal architecture of the spleen and lymph nodes. The homing of B and T cells to distinct areas of lymphoid tissue involves attraction by specific chemokines. B and T lymphocytes that survive selection in the bone marrow and thymus are exported to the peripheral lymphoid organs. Most of the newly formed B cells that emigrate from the bone marrow die soon after their arrival in the periphery, thus keeping the number of circulating B cells fairly constant. A small number mature and become longer-lived naive B cells. T cells leave the thymus as fully mature cells and are produced in smaller numbers than B cells. The fate of mature lymphocytes in the periphery is still controlled by their antigen receptors. In the absence of an encounter with their specific foreign antigen, naive lymphocytes require some tonic signaling through their antigen receptors for long-term survival.

T cells are generally long-lived and are thought to be slowly self-renewing in the peripheral lymphoid tissues, being maintained by repeated contacts with self-peptide:self-MHC complexes that can be recognized by the T-cell receptor but do not cause T-cell activation, in combination with signals derived from IL-7. The evidence for receptor-mediated survival signals is clearest for T cells, but they also seem to be needed for B-1 cells and marginal zone B cells, in which case they may promote differentiation, expansion, and survival, and most probably also for B-2 cells, in which case they promote survival without expansion. The lymphoid follicle, through which B cells must circulate to survive, seems to provide signals for their maturation and survival. A few ligands that select B-1 and marginal zone B cells are known, but in general the ligands involved in B-cell selection are unknown. The distinct minority subpopulations of lymphocytes, such as the B-1 cells, marginal zone B cells, γ:δ T cells, and iNKT cells, have different developmental histories and functional properties from those of conventional B-2 cells and α:β T cells and are likely to be regulated independently of these majority B-cell and T-cell populations.

Summary to Chapter 8.

In this chapter we have learned about the formation of the B-cell and T-cell lineages from an uncommitted hematopoietic stem cell. The somatic gene rearrangements that generate the highly diverse repertoire of antigen receptors—immunoglobulin for B cells, and the T-cell receptor for T cells—occur in the early stages of the development of T cells and B cells from a common bone marrow derived lymphoid progenitor. Mammalian B-cell development takes place in fetal liver and, after birth, in the bone marrow; T cells also originate in the bone marrow but undergo most of their development in the thymus. Much of the somatic recombination machinery, including the RAG proteins that are an essential part of the V(D)J recombinase, is common to both B and T cells. In both B and T cells, gene rearrangements begin with the loci that contain D gene segments, and proceed successively at each locus. The first step in B-cell development is the rearrangement of the locus for the immunoglobulin heavy chain, and for T cells the β chain. In each case, the

Petitioner Merck, Ex. 1170, p. 122
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

developing cell is allowed to proceed to the next stage of development only if the rearrangement has produced an in-frame sequence that can be translated into a protein expressed on the cell surface: either the pre-B-cell receptor or the pre-T cell receptor. Cells that do not generate successful rearrangements for both receptor chains die by apoptosis. The course of conventional B-cell development is summarized in Fig. 8.45, and that of α:β T cells in Fig. 8.46.

Once a functional antigen receptor has appeared on the cell surface, the lymphocyte is tested in two ways. Positive selection tests for the potential usefulness of the antigen receptor, whereas negative selection removes self-reactive cells from the lymphocyte repertoire, rendering it tolerant to the antigens of the body. Positive selection is particularly important for T cells, because it ensures that only cells bearing T-cell receptors that can recognize antigen in combination with self-MHC molecules will continue to mature. Positive selection also coordinates the choice of co-receptor expression. CD4 becomes expressed by T cells harboring MHC class II restricted receptors, and CD8 by cells harboring MHC class I restricted receptors. This ensures the optimal use of these receptors in responses to pathogens. For B cells, positive selection seems to occur at the final transition from immature to mature B cells, which occurs in peripheral lymphoid tissues. Tolerance to self antigens is enforced by negative selection at different stages throughout the development of both B and T cells, and positive selection likewise seems to represent a continuous process.

B and T cells surviving development in the central lymphoid organs emigrate to the periphery, where they home to occupy specific sites. The formation of peripheral lymphoid organs begins during embryonic development by the interaction of lymphoid tissue inducer cells expressing TNF family cytokines with stromal cells. The organization of the peripheral lymphoid organs, such as spleen and lymph nodes, also involves interactions between cells expressing TNF and TNFR family proteins. The homing of B and T cells to different parts of these peripheral tissues involves their expression of distinct chemokine receptors and the secretion of specific chemokines by various stromal elements. Maturation and survival of B and T lymphocytes in these peripheral tissues involves other specific factors. Naive B cells receive survival signals in the follicle through interaction with BAFF. Naive T cells require the cytokines IL-7 and IL-15 for survival, along with signals received through the T-cell receptor interacting with self-MHC molecules.

Petitioner Merck, Ex. 1170, p. 123
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

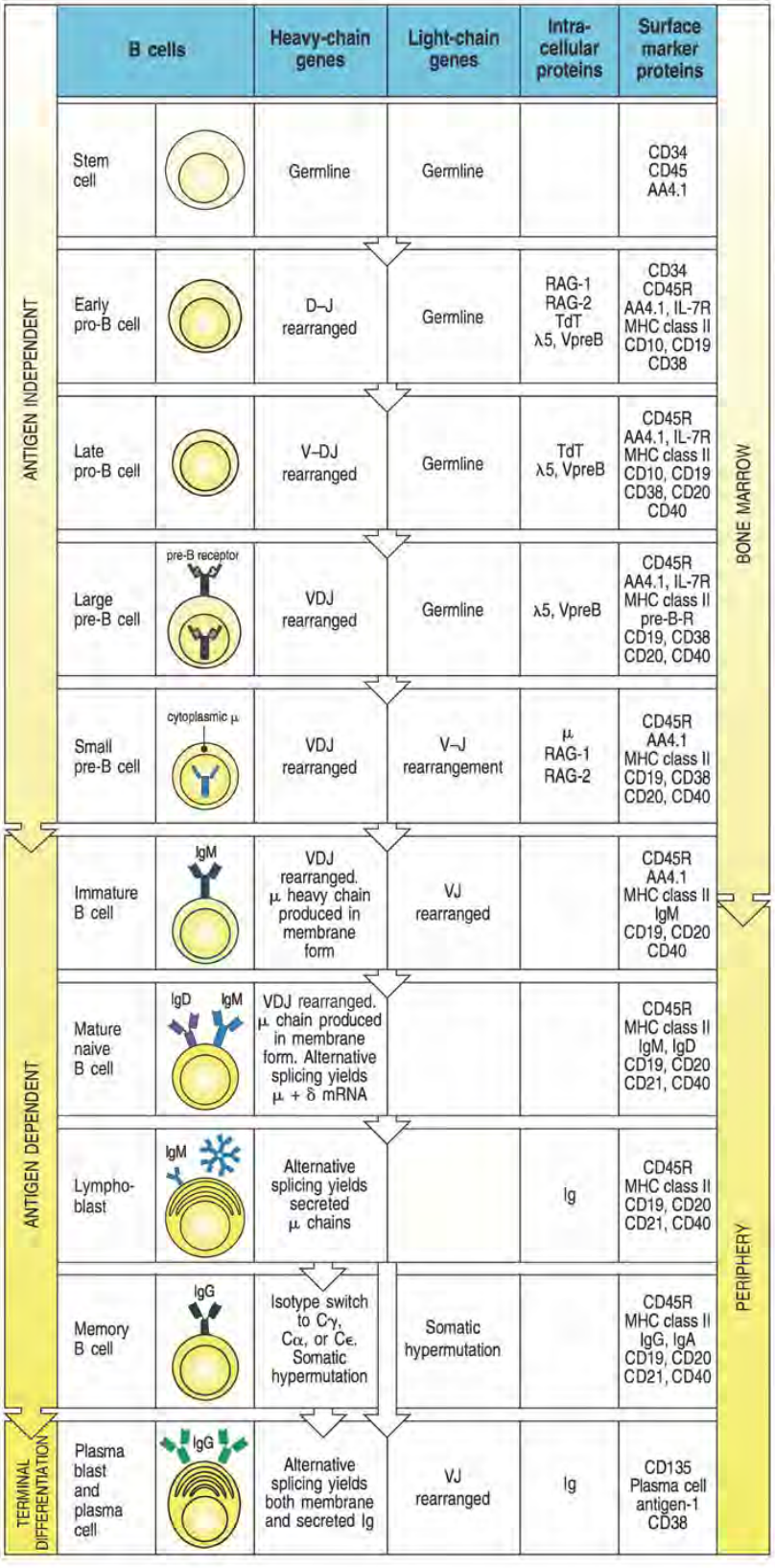
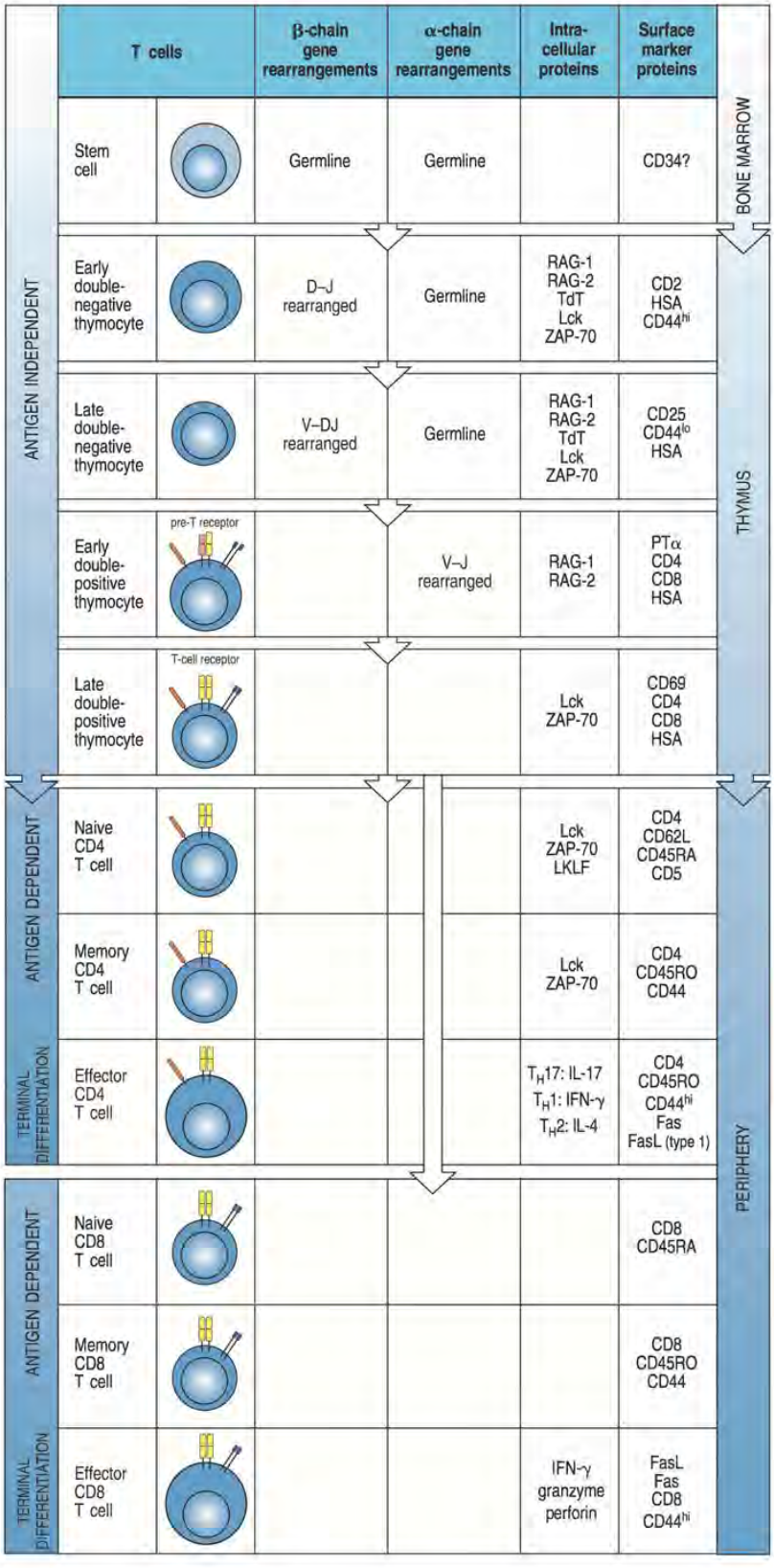


Fig. 8.45 A summary of the development of human conventional B-lineage cells. The state of the immunoglobulin genes, the expression of some essential intracellular proteins, and the expression of some cell-surface molecules are shown for successive stages of B-2-cell development. The immunoglobulin genes undergo further changes during antigen-driven B-cell differentiation, such as class switching and somatic hypermutation (see Chapter 5), which are evident in the immunoglobulins produced by memory cells and plasma cells. These antigen-dependent stages are described in more detail in Chapter 9.

Petitioner Merck, Ex. 1170, p. 124
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Fig. 8.46 A summary of the development of human α:β T cells. The state of the T-cell receptor genes, the expression of some essential intracellular proteins, and the expression of some cell-surface molecules are shown for successive stages of α:β T-cell development. Note that because the T-cell receptor genes do not undergo further changes during antigen-driven development, only the phases during which they are actively undergoing rearrangement in the thymus are indicated. The antigen-dependent phases of CD4 and CD8 cells are depicted separately, and are detailed in Chapter 9.



Questions.

8.1 B-cell development in the bone marrow shares many features with T-cell development in the thymus. (a) What are the two major goals of lymphocyte development? (b) Discuss the ordered steps of receptor rearrangement in B and T cells, drawing the parallels between the two cell types. (c) What is the function of the pre-B-cell receptor and the pre-T-cell receptor? (d) Why do T cells develop in the thymus and B cells develop in the bone marrow?

8.2 Lymphocyte development is notable for huge cell losses at several steps. (a) What are the major reasons that lymphocytes die without progressing beyond the pre-T-cell or pre-B-cell stage? (b) What is the major reason that lymphocytes die after reaching the immature stage of expressing a complete TCR or BCR?

8.3 Discuss the process of positive selection of T cells in the thymus. (a) Where does it take place? (b) What are the ligands? (c) When (at what stage) during T-cell development does positive selection occur? (d) Describe how the choice between expression of the co-receptor—CD4 or CD8—occurs, and identify any known regulators of this process.

8.4 Peripheral lymphoid tissues become organized through communication between several kinds of cells and several kinds of receptor interactions. (a) What families of molecules are critical for the proper organization of peripheral lymphoid tissues? (b) Which are important for organizing the B-cell zones? (c) Which are important for organizing the T-cell zone?

8.5 There are three main subsets of B cells: follicular, marginal zone, and B-1. Compare and contrast their development and functions, covering at least five different categories.

General references.

Loffert, D., Schaal, S., Ehlich, A., Hardy, R.R., Zou, Y.R., Muller, W., and Rajewsky, K.: **Early B-cell development in the mouse—insights from mutations introduced by gene targeting.** *Immunol. Rev.* 1994, **137**:135–153.

Melchers, F., ten Boekel, E., Seidl, T., Kong, X.C., Yamagami, T., Onishi, K., Shimizu, T., Rolink, A.G., and Andersson, J.: **Repertoire selection by pre-B-cell receptors and B-cell receptors, and genetic control of B-cell development from immature to mature B cells.** *Immunol. Rev.* 2000, **175**:33–46.

Starr, T.K., Jameson, S.C., and Hogquist, K.A.: **Positive and negative selection of T cells.** *Annu. Rev. Immunol.* 2003, **21**:139–176.

von Boehmer, H.: **The developmental biology of T lymphocytes.** *Annu. Rev. Immunol.* 1993, **6**:309–326.

Weinberg, R.A.: *The Biology of Cancer*, 1st ed. New York:Garland Science, 2007.

Section references.

8-1 Lymphocytes derive from hematopoietic stem cells in the bone marrow.

Busslinger, M.: **Transcriptional control of early B cell development.** *Annu. Rev. Immunol.* 2004, **22**:55–79.

Chao, M.P., Seita, J., and Weissman, I.L.: **Establishment of a normal hematopoietic and leukemia stem cell hierarchy.** *Cold Spring Harbor Symp. Quant. Biol.* 2008, **73**:439–449.

Funk, P.E., Kincade, P.W., and Witte, P.L.: **Native associations of early hematopoietic stem-cells and stromal cells isolated in bone-marrow cell aggregates.** *Blood* 1994, **83**:361–369.

Jacobsen, K., Kravitz, J., Kincade, P.W., and Osmond, D.G.: **Adhesion receptors on bone-marrow stromal cells—in vivo expression of vascular cell adhesion molecule-1 by reticular cells and sinusoidal endothelium in normal and γ-irradiated mice.** *Blood* 1996, **87**:73–82.

Kiel, M.J., and Morrison, S.J.: **Uncertainty in the niches that maintain haematopoietic stem cells.** *Nat. Rev. Immunol.* 2008, **8**:290–301.

8-2 B-cell development begins by rearrangement of the heavy-chain locus.

Allman, D., Li, J., and Hardy, R.R.: **Commitment to the B lymphoid lineage occurs before DH-JH recombination.** *J. Exp. Med.* 1999, **189**:735–740.

Allman, D., Lindsley, R.C., DeMuth, W., Rudd, K., Shinton, S.A., and Hardy, R.R.: **Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation.** *J. Immunol.* 2001, **167**:6834–6840.

Hardy, R.R., Carmack, C.E., Shinton, S.A., Kemp, J.D., and Hayakawa, K.: **Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow.** *J. Exp. Med.* 1991, **173**:1213–1225.

Petitioner Merck, Ex. 1170, p. 126
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Osmond, D.G., Rolink, A., and Melchers, F.: **Murine B lymphopoiesis: towards a unified model.** *Immunol. Today* 1998, **19**:65–68.

8-3 The pre-B-cell receptor tests for successful production of a complete heavy chain and signals for the transition from the pro-B cell to pre-B cell stage.

Bankovich, A.J., Raunser, S., Juo, Z.S., Walz, T., Davis, M.M., and Garcia, K.C., **Structural insight into pre-B cell receptor function.** *Science* 2007, **316**:291–294.

Grawunder, U., Leu, T.M.J., Schatz, D.G., Werner, A., Rolink, A.G., Melchers, F., and Winkler, T.H.: **Down-regulation of Rag1 and Rag2 gene expression in pre-B cells after functional immunoglobulin heavy-chain rearrangement.** *Immunity* 1995, **3**:601–608.

Monroe, J.G.: **ITAM-mediated tonic signalling through pre-BCR and BCR complexes.** *Nat. Rev. Immunol.* 2006, **6**:283–294.

8-4 Pre-B-cell receptor signaling inhibits further heavy-chain locus rearrangement and enforces allelic exclusion.

Geier, J.K., and Schlissel, M.S.: **Pre-BCR signals and the control of Ig gene rearrangements.** *Semin. Immunol.* 2006, **18**:31–39.

Loffert, D., Ehlich, A., Muller, W., and Rajewsky, K.: **Surrogate light-chain expression is required to establish immunoglobulin heavy-chain allelic exclusion during early B-cell development.** *Immunity* 1996, **4**:133–144.

Melchers, F., ten Boekel, E., Yamagami, T., Andersson, J., and Rolink, A.: **The roles of preB and B cell receptors in the stepwise allelic exclusion of mouse IgH and L chain gene loci.** *Semin. Immunol.* 1999, **11**:307–317.

8-5 Pre-B cells rearrange the light-chain locus and express cell-surface immunoglobulin.

Arakawa, H., Shimizu, T., and Takeda, S.: **Reevaluation of the probabilities for productive rearrangements on the κ-loci and λ-loci.** *Int. Immunol.* 1996, **8**:91–99.

Gorman, J.R., van der Stoep, N., Monroe, R., Cogne, M., Davidson, L., and Alt, F.W.: **The Igk 3' enhancer influences the ratio of Igκ versus Igλ B lymphocytes.** *Immunity* 1996, **5**:241–252.

Hesslein, D.G., and Schatz, D.G.: **Factors and forces controlling V(D)J recombination.** *Adv. Immunol.* 2001, **78**:169–232.

Kee, B.L., and Murre, C.: **Transcription factor regulation of B lineage commitment.** *Curr. Opin. Immunol.* 2001, **13**:180–185.

Sleckman, B.P., Gorman, J.R., and Alt, F.W.: **Accessibility control of antigen receptor variable region gene assembly—role of *cis*-acting elements.** *Annu. Rev. Immunol.* 1996, **14**:459–481.

Takeda, S., Sonoda, E., and Arakawa, H.: **The κ–λ ratio of immature B cells.** *Immunol. Today* 1996, **17**:200–201.

8-6 Immature B cells are tested for autoreactivity before they leave the bone marrow.

Casellas, R., Shih, T.A., Kleinewietfeld, M., Rakonjac, J., Nemazee, D., Rajewsky, K., and Nussenzweig, M.C.: **Contribution of receptor editing to the antibody repertoire.** *Science* 2001, **291**:1541–1544.

Chen, C., Nagy, Z., Radic, M.Z., Hardy, R.R., Huszar, D., Camper, S.A., and Weigert, M.: **The site and stage of anti-DNA B-cell deletion.** *Nature* 1995, **373**:252–255.

Cornall, R.J., Goodnow, C.C., and Cyster, J.G.: **The regulation of self-reactive B cells.** *Curr. Opin. Immunol.* 1995, **7**:804–811.

Huang, F., and Gu, H.: **Negative regulation of lymphocyte development and function by the Cbl family of proteins.** *Immunol. Rev.* 2008, **224**:229–238.

Melamed, D., Benschop, R.J., Cambier, J.C., and Nemazee, D.: **Developmental regulation of B lymphocyte immune tolerance compartmentalizes clonal selection from receptor selection.** *Cell* 1998, **92**:173–182.

Nemazee, D.: **Receptor editing in lymphocyte development and central tolerance.** *Nat. Rev. Immunol.* 2006, **6**:728–740.

Prak, E.L., and Weigert, M.: **Light-chain replacement—a new model for antibody gene rearrangement.** *J. Exp. Med.* 1995, **182**:541–548.

8-7 T-cell progenitors originate in the bone marrow, but all the important events in their development occur in the thymus.

Anderson, G., Moore, N.C., Owen, J.J.T., and Jenkinson, E.J.: **Cellular interactions in thymocyte development.** *Annu. Rev. Immunol.* 1996, **14**:73–99.

Carlyle, J.R., and Zúñiga-Pflücker, J.C.: **Requirement for the thymus in α:β T lymphocyte lineage commitment.** *Immunity* 1998, **9**:187–197.

Ciofani, M., Knowles, G., Wiest, D., von Boehmer, H., and Zúñiga-Pflücker, J.: **Stage-specific and differential Notch dependency at the α:β and γ:δ T lineage bifurcation.** *Immunity* 2006, **25**:105–116.

Gordon, J., Wilson, V.A., Blair, N.F., Sheridan, J., Farley, A., Wilson, L., Manley, N.R., and Blackburn, C.C.: **Functional evidence for a single endodermal origin for the thymic epithelium.** *Nat. Immunol.* 2004, **5**:546–553.

Nehls, M., Kyewski, B., Messerle, M., Waldschütz, R., Schüddekopf, K., Smith, A.J.H., and Boehm, T.: **Two genetically separable steps in the differentiation of thymic epithelium.** *Science* 1996, **272**:886–889.

Rodewald, H.R.: **Thymus organogenesis.** *Annu. Rev. Immunol.* 2008, **26**:355–388.

van Ewijk, W., Hollander, G., Terhorst, C., and Wang, B.: **Stepwise development of thymic microenvironments *in vivo* is regulated by thymocyte subsets.** *Development* 2000, **127**:1583–1591.

8-8 T-cell precursors proliferate extensively in the thymus, but most die there.

Shortman, K., Egerton, M., Spangrude, G.J., and Scollay, R.: **The generation and fate of thymocytes.** *Semin. Immunol.* 1990, **2**:3–12.

Surh, C.D., and Sprent, J.: **T-cell apoptosis detected *in situ* during positive and negative selection in the thymus.** *Nature* 1994, **372**:100–103.

8-9 Successive stages in the development of thymocytes are marked by changes in cell-surface molecules.

Borowski, C., Martin, C., Gounari, F., Haughn, L., Aifantis, I., Grassi, F., and von Boehmer, H.: **On the brink of becoming a T cell.** *Curr. Opin. Immunol.* 2002, **14**:200–206.

Pang, S.S., Berry, R., Chen, Z., Kjer-Nielsenm, L., Perugini, M.A., King, G.F., Wang, C., Chew, S.H., La Gruta, N.L., Williams, N.K., *et al.*: **The structural basis for autonomous dimerization of the pre-T-cell antigen receptor.** *Nature* 2010, **467**:844–848.

Saint-Ruf, C., Ungewiss, K., Groetrup, M., Bruno, L., Fehling, H.J., and von Boehmer, H.: **Analysis and expression of a cloned pre-T-cell receptor gene.** *Science* 1994, **266**:1208–1212.

Shortman, K., and Wu, L.: **Early T lymphocyte progenitors.** *Annu. Rev. Immunol.* 1996, **14**:29–47.

8-10 Thymocytes at different developmental stages are found in distinct parts of the thymus.

Benz, C., Heinzel, K., and Bleul, C.C.: **Homing of immature thymocytes to the subcapsular microenvironment within the thymus is not an absolute requirement for T cell development.** *Eur. J. Immunol.* 2004, **34**:3652–3663.

Bleul, C.C., and Boehm, T.: **Chemokines define distinct microenvironments in the developing thymus.** *Eur. J. Immunol.* 2000, **30**:3371–3379.

Nitta, T., Murata, S., Ueno, T., Tanaka, K., and Takahama, Y.: **Thymic microenvironments for T-cell repertoire formation.** *Adv. Immunol.* 2008, **99**:59–94.

Ueno, T., Saito F., Gray, D.H.D., Kuse, S., Hieshima, K., Nakano, H., Kakiuchi, T., Lipp, M., Boyd, R.L., and Takahama, Y.: **CCR7 signals are essential for cortex–medulla migration of developing thymocytes.** *J. Exp. Med.* 2004, **200**:493–505.

8-11 T cells with α:β or γ:δ receptors arise from a common progenitor.

Fehling, H.J., Gilfillan, S., and Ceredig, R.: **α β/γ δ lineage commitment in the thymus of normal and genetically manipulated mice.** *Adv. Immunol.* 1999, **71**:1–76.

Hayday, A.C., Barber, D.F., Douglas, N., and Hoffman, E.S.: **Signals involved in**

Petitioner Merck, Ex. 1170, p. 127
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

γδT cell versus αβT cell lineage commitment. *Semin. Immunol.* 1999, **11**:239–249.

Hayes, S.M., and Love, P.E.: **Distinct structure and signaling potential of the γδTCR complex.** *Immunity* 2002, **16**:827–838.

Kang, J., and Raulet, D.H.: **Events that regulate differentiation of αβ TCR⁺ and γδTCR⁺ T cells from a common precursor.** *Semin. Immunol.* 1997, **9**:171–179.

Kreslavsky, T., Garbe, A.I., Krueger, A., and von Boehmer, H.: **T cell receptor-instructed αβ versus γδ lineage commitment revealed by single-cell analysis.** *J. Exp. Med.* 2008, **205**:1173–1186.

Lauritsen, J.P., Haks, M.C., Lefebvre, J.M., Kappes, D.J., and Wiest, D.L.: **Recent insights into the signals that control αβ/γδ-lineage fate.** *Immunol. Rev.* 2006, **209**:176–190.

Livak, F., Petrie, H.T., Crispe, I.N., and Schatz, D.G.: **In-frame TCRδ gene rearrangements play a critical role in the αβ/γδ T cell lineage decision.** *Immunity* 1995, **2**:617–627.

Xiong, N., and Raulet, D.H.: **Development and selection of γδT cells.** *Immunol. Rev.* 2007, **215**:15–31.

8-12 T cells expressing particular γ- and δ-chain V regions arise in an ordered sequence early in life.

Carding, S.R., and Egan, P.J.: **γδT cells: functional plasticity and heterogeneity.** *Nat. Rev. Immunol.* 2002, **2**:336–345.

Ciofani, M., Knowles, G.C., Wiest, D.L., von Boehmer, H., and Zúñiga-Pflücker, J.C.: **Stage-specific and differential notch dependency at the α:β and γ:δT lineage bifurcation.** *Immunity* 2006, **25**:105–116.

Dunon, D., Courtois, D., Vainio, O., Six, A., Chen, C.H., Cooper, M.D., Dangy, J.P., and Imhof, B.A.: **Ontogeny of the immune system: γ:δ and α:β T cells migrate from thymus to the periphery in alternating waves.** *J. Exp. Med.* 1997, **186**:977–988.

Haas, W., Pereira, P., Tonegawa, S.: **Gamma/delta cells.** *Annu. Rev. Immunol.* 1993, **11**:637–685.

Lewis, J.M., Girardi, M., Roberts, S.J., Barbee, S.D., Hayday, A.C., and Tigelaar, R.E.: **Selection of the cutaneous intraepithelial γδ⁺ T cell repertoire by a thymic stromal determinant.** *Nat. Immunol.* 2006, **7**:843–850.

Strid, J., Tigelaar, R.E., and Hayday, A.C.: **Skin immune surveillance by T cells—a new order?** *Semin. Immunol.* 2009, **21**:110–120.

8-13 Successful synthesis of a rearranged β chain allows the production of a pre-T-cell receptor that triggers cell proliferation and blocks further β-chain gene rearrangement.

Borowski, C., Li, X., Aifantis, I., Gounari, F., and von Boehmer, H.: **Pre-TCRα and TCRα are not interchangeable partners of TCRβ during T lymphocyte development.** *J. Exp. Med.* 2004, **199**:607–615.

Dudley, E.C., Petrie, H.T., Shah, L.M., Owen, M.J., and Hayday, A.C.: **T-cell receptor β chain gene rearrangement and selection during thymocyte development in adult mice.** *Immunity* 1994, **1**:83–93.

Philpott, K.L., Viney, J.L., Kay, G., Rastan, S., Gardiner, E.M., Chae, S., Hayday, A.C., and Owen, M.J.: **Lymphoid development in mice congenitally lacking T cell receptor αβ-expressing cells.** *Science* 1992, **256**:1448–1453.

von Boehmer, H., Aifantis, I., Azogui, O., Feinberg, J., Saint-Ruf, C., Zober, C., Garcia, C., and Buer, J.: **Crucial function of the pre-T-cell receptor (TCR) in TCRβ selection, TCRβ allelic exclusion and α:β versus γ:δ lineage commitment.** *Immunol. Rev.* 1998, **165**:111–119.

8-14 T-cell α-chain genes undergo successive rearrangements until positive selection or cell death intervenes.

Buch, T., Rieux-Laucat, F., Förster, I., and Rajewsky, K.: **Failure of HY-specific thymocytes to escape negative selection by receptor editing.** *Immunity* 2002, **16**:707–718.

Hardardottir, F., Baron, J.L., and Janeway, C.A., Jr: **T cells with two functional antigen-specific receptors.** *Proc. Natl Acad. Sci. USA* 1995, **92**:354–358.

Huang, C.-Y., Sleckman, B.P., and Kanagawa, O.: **Revision of T cell receptor α chain genes is required for normal T lymphocyte development.** *Proc. Natl Acad. SciU SA* 2005, **102**:14356–14361.

Marrack, P., and Kappler, J.: **Positive selection of thymocytes bearing α:β T cell receptors.** *Curr. Opin. Immunol.* 1997, **9**:250–255.

Padovan, E., Casorati, G., Dellabona, P., Meyer, S., Brockhaus, M., and Lanzavecchia, A.: **Expression of two T-cell receptor α chains: dual receptor T cells.** *Science* 1993, **262**:422–424.

Petrie, H.T., Livak, F., Schatz, D.G., Strasser, A., Crispe, I.N., and Shortman, K.: **Multiple rearrangements in T-cell receptor α-chain genes maximize the production of useful thymocytes.** *J. Exp. Med.* 1993, **178**:615–622.

8-15 The MHC type of the thymic stroma selects a repertoire of mature T cells that can recognize foreign antigens presented by the same MHC type.

Fink, P.J., and Bevan, M.J.: **H-2 antigens of the thymus determine lymphocyte specificity.** *J. Exp. Med.* 1978, **148**:766–775.

Zinkernagel, R.M., Callahan, G.N., Klein, J., and Dennert, G.: **Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus.** *Nature* 1978, **271**:251–253.

8-16 Only thymocytes whose receptors interact with self-peptide:self-MHC complexes can survive and mature.

Hogquist, K.A., Tomlinson, A.J., Kieper, W.C., McGargill, M.A., Hart, M.C., Naylor, S., and Jameson, S.C.: **Identification of a naturally occurring ligand for thymic positive selection.** *Immunity* 1997, **6**:389–399.

Huessman, M., Scott, B., Kisielow, P., and von Boehmer, H.: **Kinetics and efficacy of positive selection in the thymus of normal and T-cell receptor transgenic mice.** *Cell* 1991, **66**:533–562.

Stefanski, H.E., Mayerova, D., Jameson, S.C., and Hogquist, K.A.: **A low affinity TCR ligand restores positive selection of CD8⁺ T cells *in vivo*.** *J. Immunol.* 2001, **166**:6602–6607.

8-17 Positive selection acts on a repertoire of T-cell receptors with inherent specificity for MHC molecules.

Marrack, P., Scott-Browne, J.P., Dai, S., Gapin, L., and Kappler, J.W.: **Evolutionarily conserved amino acids that control TCR-MHC interaction.** *Annu. Rev. Immunol.* 2008, **26**:171–203.

Merkenschlager, M., Graf, D., Lovatt, M., Bommhardt, U., Zamoyska, R., and Fisher, A.G.: **How many thymocytes audition for selection?** *J. Exp. Med.* 1997, **186**:1149–1158.

Scott-Browne, J.P., White, J., Kappler, J.W., Gapin, L., and Marrack, P.: **Germline-encoded amino acids in the αβ T-cell receptor control thymic selection.** *Nature* 2009, **458**:1043–1046.

Zerrahn, J., Held, W., and Raulet, D.H.: **The MHC reactivity of the T cell repertoire prior to positive and negative selection.** *Cell* 1997, **88**:627–636.

8-18 Positive selection coordinates the expression of CD4 or CD8 with the specificity of the T-cell receptor and the potential effector functions of the T cell.

Egawa, T., and Littman, D.R.: **ThPOK acts late in specification of the helper T cell lineage and suppresses Runx-mediated commitment to the cytotoxic T cell lineage.** *Nat. Immunol.* 2008, **9**:1131–1139.

He, X., Park, K., and Kappes, D.J.: **The role of ThPOK in control of CD4/CD8 lineage commitment.** *Annu. Rev. Immunol.* 2010, **28**:295–320.

He, X., Xi, H., Dave, V.P., Zhang, Y., Hua, X., Nicolas, E., Xu, W., Roe, B.A., and Kappes, D.J.: **The zinc finger transcription factor Th-POK regulates CD4 versus CD8 T-cell lineage commitment.** *Nature* 2005, **433**:826–833.

Lundberg, K., Heath, W., Kontgen, F., Carbone, F.R., and Shortman, K.: **Intermediate steps in positive selection: differentiation of CD4⁺8^{int}TCR^{int} thymocytes into CD4⁻8⁺TCR^{hi} thymocytes.** *J. Exp. Med.* 1995, **181**:1643–1651.

Singer, A., Adoro, S., and Park, J.H.: **Lineage fate and intense debate: myths, models and mechanisms of CD4- versus CD8-lineage choice.** *Nat. Rev. Immunol.* 2008, **8**:788–801.

Petitioner Merck, Ex. 1170, p. 128
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

von Boehmer, H., Kisielow, P., Lishi, H., Scott, B., Borgulya, P., and Teh, H.S.: **The expression of CD4 and CD8 accessory molecules on mature T cells is not random but correlates with the specificity of the α:β receptor for antigen.** *Immunol. Rev.* 1989, **109**:143–151.

Zheng, Y., and Rudensky, A.Y.: **Foxp3 in control of the regulatory T cell lineage.** *Nat. Immunol.* 2007, **8**:457–462.

8-19 Thymic cortical epithelial cells mediate positive selection of developing thymocytes.

Cosgrove, D., Chan, S.H., Waltzinger, C., Benoist, C., and Mathis, D.: **The thymic compartment responsible for positive selection of CD4⁺ T cells.** *Int. Immunol.* 1992, **4**:707–710.

Ernst, B.B., Surh, C.D., and Sprent, J.: **Bone marrow-derived cells fail to induce positive selection in thymus reaggregation cultures.** *J. Exp. Med.* 1996, **183**:1235–1240.

Fowlkes, B.J., and Schweighoffer, E.: **Positive selection of T cells.** *Curr. Opin. Immunol.* 1995, **7**:188–195.

Nakagawa, T., Roth, W., Wong, P., Nelson, A., Farr, A., Deussing, J., Villadangos, J.A., Ploegh, H., Peters, C., and Rudensky, A.Y.: **Cathepsin L: critical role in Ii degradation and CD4 T cell selection in the thymus.** *Science* 1998, **280**:450–453.

8-20 T cells that react strongly with ubiquitous self antigens are deleted in the thymus.

Kishimoto, H., and Sprent, J.: **Negative selection in the thymus includes semimature T cells.** *J. Exp. Med.* 1997, **185**:263–271.

Mathis, D., and Benoist, C.: **Aire.** *Annu. Rev. Immunol.* 2009, **27**:287–312.

Zal, T., Volkmann, A., and Stockinger, B.: **Mechanisms of tolerance induction in major histocompatibility complex class II-restricted T cell specific for a blood-borne self antigen.** *J. Exp. Med.* 1994, **180**:2089–2099.

8-21 Negative selection is driven most efficiently by bone marrow derived antigen-presenting cells.

McCaughtry, T.M., Baldwin, T.A., Wilken, M.S., and Hogquist, K.A.: **Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla.** *J. Exp. Med.* 2008, **205**:2575–2584.

Sprent, J., and Webb, S.R.: **Intrathymic and extrathymic clonal deletion of T cells.** *Curr. Opin. Immunol.* 1995, **7**:196–205.

Webb, S.R., and Sprent, J.: **Tolerogenicity of thymic epithelium.** *Eur. J. Immunol.* 1990, **20**:2525–2528.

8-22 The specificity and/or the strength of signals for negative and positive selection must differ.

Alberola-Ila, J., Hogquist, K.A., Swan, K.A., Bevan, M.J., and Perlmutter, R.M.: **Positive and negative selection invoke distinct signaling pathways.** *J. Exp. Med.* 1996, **184**:9–18.

Ashton-Rickardt, P.G., Bandeira, A., Delaney, J.R., Van Kaer, L., Pircher, H.P., Zinkernagel, R.M., and Tonegawa, S.: **Evidence for a differential avidity model of T-cell selection in the thymus.** *Cell* 1994, **76**:651–663.

Bommhardt, U., Basson, M.A., Krummrei, U., and Zamoyska, R.: **Activation of the extracellular signal-related kinase/mitogen-activated protein kinase pathway discriminates CD4 versus CD8 lineage commitment in the thymus.** *J. Immunol.* 1999, **163**:715–722.

Bommhardt, U., Scheuring, Y., Bickel, C., Zamoyska, R., and Hunig, T.: **MEK activity regulates negative selection of immature CD4⁺CD8⁺ thymocytes.** *J. Immunol.* 2000, **164**:2326–2337.

Hogquist, K.A., Jameson, S.C., Heath, W.R., Howard, J.L., Bevan, M.J., and Carbone, F.R.: **T-cell receptor antagonist peptides induce positive selection.** *Cell* 1994, **76**:17–27.

8-23 Different lymphocyte subsets are found in particular locations in peripheral lymphoid tissues.

Liu, Y.J.: **Sites of B lymphocyte selection, activation, and tolerance in spleen.** *J. Exp. Med.* 1997, **186**:625–629.

Loder, F., Mutschler, B., Ray, R.J., Paige, C.J., Sideras, P., Torres, R., Lamers, M.C., and Carsetti, R.: **B cell development in the spleen takes place in discrete steps and is determined by the quality of B cell receptor-derived signals.** *J. Exp. Med.* 1999, **190**:75–89.

Mebius, R.E.: **Organogenesis of lymphoid tissues.** *Nat. Rev. Immunol.* 2003, **3**:292–303.

8-24 The development of peripheral lymphoid tissues is controlled by lymphoid tissue inducer cells and proteins of the tumor necrosis factor family.

Douni, E., Akassoglou, K., Alexopoulou, L., Georgopoulos, S., Haralambous, S., Hill, S., Kassiotis, G., Kontoyiannis, D., Pasparakis, M., Plows, D., *et al.*: **Transgenic and knockout analysis of the role of TNF in immune regulation and disease pathogenesis.** *J. Inflamm.* 1996, **47**:27–38.

Fu, Y.X., and Chaplin, D.D.: **Development and maturation of secondary lymphoid tissues.** *Annu. Rev. Immunol.* 1999, **17**:399–433.

Mariathasan, S., Matsumoto, M., Baranyay, F., Nahm, M.H., Kanagawa, O., and Chaplin, D.D.: **Absence of lymph nodes in lymphotoxin-α (LTα)-deficient mice is due to abnormal organ development, not defective lymphocyte migration.** *J. Inflamm.* 1995, **45**:72–78.

Mebius, R.E., Rennert, P., and Weissman, I.L.: **Developing lymph nodes collect CD4⁺CD3⁻ LTβ⁺ cells that can differentiate to APC, NK cells, and follicular cells but not T or B cells.** *Immunity* 1997, **7**:493–504.

Wigle, J.T., and Oliver, G.: **Prox1 function is required for the development of the murine lymphatic system.** *Cell* 1999, **98**:769–778.

8-25 The homing of lymphocytes to specific regions of peripheral lymphoid tissues is mediated by chemokines.

Ansel, K.M., and Cyster, J.G.: **Chemokines in lymphopoiesis and lymphoid organ development.** *Curr. Opin. Immunol.* 2001, **13**:172–179.

Cyster, J.G.: **Chemokines and cell migration in secondary lymphoid organs.** *Science* 1999, **286**:2098–2102.

Cyster, J.G.: **Leukocyte migration: scent of the T zone.** *Curr. Biol.* 2000, **10**:R30–R33.

Cyster, J.G., Ansel, K.M., Reif, K., Ekland, E.H., Hyman, P.L., Tang, H.L., Luther, S.A., and Ngo, V.N.: **Follicular stromal cells and lymphocyte homing to follicles.** *Immunol. Rev.* 2000, **176**:181–193.

8-26 Lymphocytes that encounter sufficient quantities of self antigens for the first time in the periphery are eliminated or inactivated.

Cyster, J.G., Hartley, S.B., and Goodnow, C.C.: **Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire.** *Nature* 1994, **371**:389–395.

Goodnow, C.C., Crosbie, J., Jorgensen, H., Brink, R.A., and Basten, A.: **Induction of self-tolerance in mature peripheral B lymphocytes.** *Nature* 1989, **342**:385–391.

Lam, K.P., Kuhn, R., and Rajewsky, K.: **In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death.** *Cell* 1997, **90**:1073–1083.

Russell, D.M., Dembic, Z., Morahan, G., Miller, J.F.A.P., Burki, K., and Nemazee, D.: **Peripheral deletion of self-reactive B cells.** *Nature* 1991, **354**:308–311.

Steinman, R.M., and Nussenzweig, M.C.: **Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance.** *Proc. Natl Acad. Sci. USA* 2002, **99**:351–358.

Petitioner Merck, Ex. 1170, p. 129
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

8-27 Immature B cells arriving in the spleen turn over rapidly and require cytokines and positive signals through the B-cell receptor for maturation and survival.

Allman, D.M., Ferguson, S.E., Lentz, V.M., and Cancro, M.P.: **Peripheral B cell maturation. II. Heat-stable antigen^{hi} splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells.** *J. Immunol.* 1993, **151**:4431–4444.

Hardess, S.M., Lentz, V.M., Sah, A.P., Hsu, B.L., Clise-Dwyer, K., Hilbert, D.M., Hayes, C.E., and Cancro, M.P.: **Competition for BLyS-mediated signaling through Bcmd/BR3 regulates peripheral B lymphocyte numbers.** *Curr. Biol.* 2001, **11**:1986–1989.

Levine, M.H., Haberman, A.M., Sant'Angelo, D.B., Hannum, L.G., Cancro, M.P., Janeway, C.A., Jr, and Shlomchik, M.J.: **A B-cell receptor-specific selection step governs immature to mature B cell differentiation.** *Proc. Natl Acad. Sci. USA* 2000, **97**:2743–2748.

Rolink, A.G., Tschopp, J., Schneider, P., and Melchers, F.: **BAFF is a survival and maturation factor for mouse B cells.** *Eur. J. Immunol.* 2002, **32**:2004–2010.

Schiemann, B., Gommerman, J.L., Vora, K., Cachero, T.G., Shulga-Morskaya, S., Dobles, M., Frew, E., and Scott, M.L.: **An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway.** *Science* 2001, **293**:2111–2114.

8-28 B-1 cells and marginal zone B cells are distinct B-cell subtypes with unique antigen receptor specificity.

Clarke, S.H., and Arnold, L.W.: **B-1 cell development: evidence for an uncommitted immunoglobulin (Ig)M$^+$ B cell precursor in B-1 cell differentiation.** *J. Exp. Med.* 1998, **187**:1325–1334.

Hardy, R.R., and Hayakawa, K.: **A developmental switch in B lymphopoiesis.** *Proc. Natl Acad. Sci. USA* 1991, **88**:11550–11554.

Hayakawa, K., Asano, M., Shinton, S.A., Gui, M., Allman, D., Stewart, C.L., Silver, J., and Hardy, R.R.: **Positive selection of natural autoreactive B cells.** *Science* 1999, **285**:113–116.

Martin, F., and Kearney, J.F.: **Marginal-zone B cells.** *Nat. Rev. Immunol.* 2002, **2**:323–335.

8-29 T-cell homeostasis in the periphery is regulated by cytokines and self-MHC interactions.

Judge, A.D., Zhang, X., Fujii, H., Surh, C.D., and Sprent, J.: **Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype CD8$^+$ T cells.** *J. Exp. Med.* 2002, **196**:935–946.

Kassiotis, G., Garcia, S., Simpson, E., and Stockinger, B.: **Impairment of immunological memory in the absence of MHC despite survival of memory T cells.** *Nat. Immunol.* 2002, **3**:244–250.

Ku, C.C., Murakami, M., Sakamoto, A., Kappler, J., and Marrack, P.: **Control of homeostasis of CD8$^+$ memory T cells by opposing cytokines.** *Science* 2000, **288**:675–678.

Murali-Krishna, K., Lau, L.L., Sambhara, S., Lemonnier, F., Altman, J., and Ahmed, R.: **Persistence of memory CD8 T cells in MHC class I-deficient mice.** *Science* 1999, **286**:1377–1381.

Seddon, B., Tomlinson, P., and Zamoyska, R.: **IL-7 and T cell receptor signals regulate homeostasis of CD4 memory cells.** *Nat. Immunol.* 2003, **4**:680–686.

Petitioner Merck, Ex. 1170, p. 130
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

10 The Humoral Immune Response

Many of the bacteria that cause infectious disease in humans multiply in the extracellular spaces of the body, and most intracellular pathogens spread by moving from cell to cell through the extracellular fluids. The extracellular spaces are protected by the **humoral immune response**, in which antibodies produced by B cells cause the destruction of extracellular microorganisms and prevent the spread of intracellular infections. Activation of naive B cells is triggered by antigen and usually requires helper T cells, such as the T follicular helper (T_{FH}) cells introduced in Section 9-17; the activated B cells then differentiate into antibody-secreting **plasma cells** (Fig. 10.1) and memory B cells. In this chapter we use the general term **helper T cell** to mean any effector CD4 T cell that can activate a B cell.

Antibodies contribute to immunity in three main ways (see Fig. 10.1). The first is known as **neutralization**. To enter cells, viruses and intracellular bacteria bind to specific molecules on the target cell surface. Antibodies that bind to the pathogen can prevent this and are said to neutralize the pathogen. Neutralization by antibodies is also important in preventing bacterial toxins from entering cells. Second, antibodies protect against bacteria that multiply outside cells, and do this mainly by facilitating uptake of the pathogen by phagocytes. Coating the surface of a pathogen to enhance phagocytosis is called **opsonization**. Antibodies bound to the pathogen are recognized by phagocytic cells by means of receptors called Fc receptors that bind to the antibody constant region (C region). Third, antibodies coating a pathogen can activate the proteins of the complement system by the classical pathway, as described in Chapter 2. Complement proteins bound to the pathogen surface opsonize the pathogen by binding complement receptors on phagocytes. Other complement components recruit phagocytic cells to the site of infection, and the terminal components of complement can lyse certain microorganisms directly by forming pores in their membranes. Which effector mechanisms are engaged in a particular response is determined by the heavy-chain isotype of the antibodies produced, which determines their class (see Section 5-12).

In the first part of this chapter we describe the interactions of naive B cells with antigen and with helper T cells that lead to the activation of B cells and antibody production. Some important microbial antigens can provoke antibody production without the help of T cells, and we shall also consider these responses here. Most antibody responses undergo a process called affinity maturation, in which antibodies of greater affinity for their target antigen are produced by the somatic hypermutation of antibody variable-region (V-region) genes. The molecular mechanism of somatic hypermutation was described in Chapter 5, and here we look at its immunological consequences. We also revisit class switching (see Section 5-19), which produces antibodies of different functional classes and confers functional diversity on the antibody response. Both affinity maturation and class switching occur only in B cells and require T-cell help. In the rest of the chapter we look in detail at the various effector mechanisms by which antibodies contain and eliminate infections. Like the T-cell response, the humoral immune response produces immunological memory, and this will be discussed in Chapter 11.

Petitioner Merck, Ex. 1170, p. 131
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

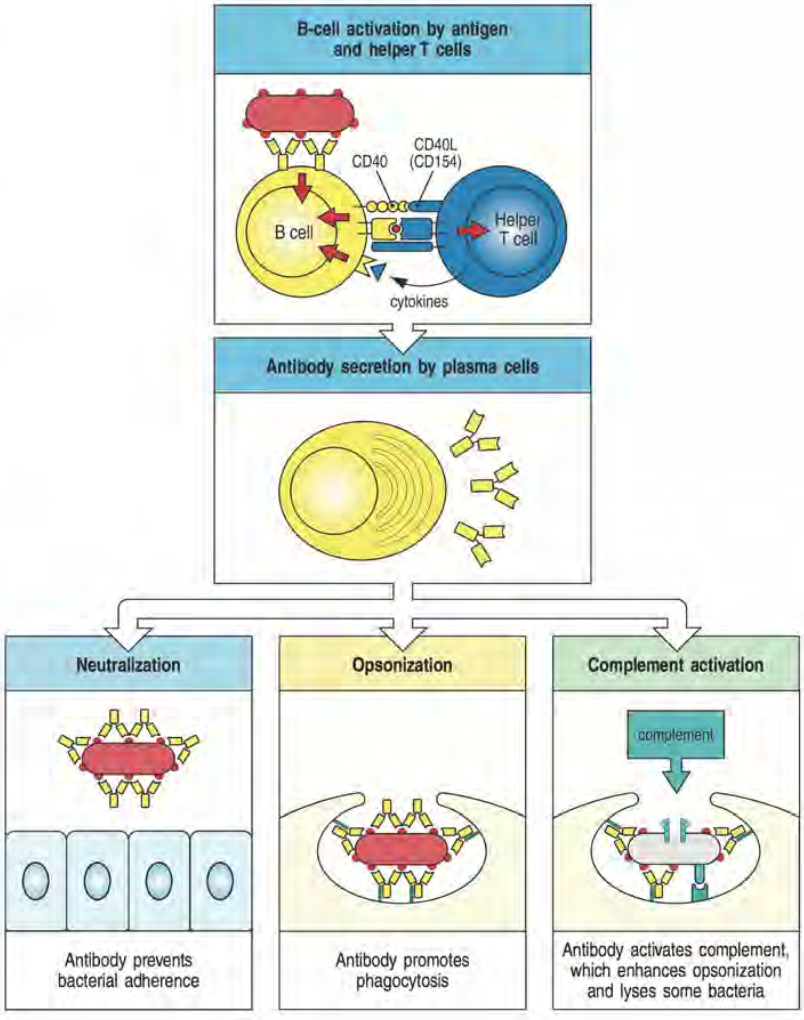


Fig. 10.1 The humoral immune response is mediated by antibodies secreted by plasma cells. Antigen that binds to the B-cell antigen receptor both sends a signal into the B cell and is internalized and processed into peptides that activate effector helper T cells. Signals from the bound antigen and from the helper T cell (in the form of the co-stimulatory CD40 ligand (CD40L) and cytokines) induce the B cell to proliferate and differentiate into plasma cells secreting specific antibody (top two panels). Antibodies protect the host from infection in three main ways. First, they can inhibit the toxic effects or infectivity of pathogens by binding to them, which is termed neutralization (bottom left panel). Second, by coating the pathogens they can enable accessory cells that recognize the Fc portions of arrays of antibodies to ingest and kill the pathogen, a process called opsonization (bottom center panel). Third, antibodies can trigger the activation of the complement system. Complement proteins strongly enhance opsonization, and can directly kill certain bacterial cells (bottom right panel).

B-cell activation by helper T cells.

The surface immunoglobulin that serves as the **B-cell receptor** (**BCR**) for antigen can bind a vast variety of chemical structures. In the context of natural infections it binds native proteins, glycoproteins, and polysaccharides, as well as whole virus particles and bacterial cells, by recognizing epitopes on their surfaces. It has two roles in B-cell activation. First, like the antigen receptor on T cells, it signals to the cell's interior when antigen is bound (see Chapter 7). Second, the B-cell antigen receptor delivers the bound antigen to intracellular sites, where it can be degraded to give peptides that are returned to the B-cell surface bound to MHC class II molecules (see Chapter 6). These peptide:MHC class II complexes are recognized by antigen-specific helper T cells that have already differentiated in response to the same pathogen, as described in Chapter 9. The effector T cells make cytokines that cause the B cell to proliferate and its progeny to differentiate into antibody-secreting cells and into memory B cells. Some microbial antigens can activate B cells directly in the absence of T-cell help, and the ability of B cells to respond directly to these antigens provides a rapid response to many important pathogens.

Petitioner Merck, Ex. 1170, p. 132
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

However, the fine tuning of antibody responses to increase the affinity of the antibody for the antigen and the switching to most immunoglobulin classes other than IgM depend on the interaction of antigen-stimulated B cells with helper T cells and other cells in the peripheral lymphoid organs. Thus, antibodies induced by microbial antigens alone tend to have lower affinity and to be less functionally versatile than those induced with T-cell help.

10-1 The humoral immune response is initiated when B cells that bind antigen are signaled by helper T cells or by certain microbial antigens alone.

It is a general rule in adaptive immunity that naive antigen-specific lymphocytes are difficult to activate by antigen alone. As we saw in Chapter 9, priming of naive T cells requires a co-stimulatory signal from professional antigen-presenting cells; naive B cells also require accessory signals that can come either from a helper T cell or, in some cases, directly from microbial constituents.

Antibody responses to protein antigens require antigen-specific T-cell help. These antigens are unable to induce antibody responses in animals or humans who lack T cells, and they are therefore known as **thymus-dependent** or **TD antigens**. To receive T-cell help, the B cell must be displaying antigen on its surface in a form a T cell can recognize. This occurs when antigen bound by surface immunoglobulin on the B cell is internalized and returned to the cell surface as peptides bound to MHC class II molecules. Helper T cells that recognize the peptide:MHC complex then deliver activating signals to the B cell (Fig. 10.2, top two panels). Thus, protein antigens binding to B cells both provide a specific signal to the B cell by cross-linking its antigen receptors and allow the B cell to attract antigen-specific T-cell help. When an activated helper T cell recognizes and binds to a peptide:MHC class II complex on the B-cell surface it induces the B cell to proliferate and differentiate into antibody-producing plasma cells (Fig. 10.3). The requirement for T-cell help means that before a B cell can be induced to make antibody against the molecules of an infecting pathogen, CD4 T cells specific for peptides from this pathogen must be activated to produce helper T cells. This occurs when naive T cells interact with dendritic cells presenting the appropriate peptides, as described in Chapter 9.

Although peptide-specific helper T cells are required for B-cell responses to protein antigens, some microbial constituents, such as bacterial polysaccharides, can induce antibody production in the absence of helper T cells. These microbial antigens are known as **thymus-independent** or **TI antigens** because they can induce antibody responses in individuals who have no T lymphocytes. The second signal required to activate antibody production against TI antigens is provided either directly by recognition of a common microbial constituent (see Fig. 10.2, bottom panel) or by extensive cross-linking of B-cell receptors, which would occur when a B cell binds repeating epitopes on the bacterial cell. Thymus-independent antibody responses provide some protection against extracellular bacteria, and we will return to them later.

10-2 B-cell responses are enhanced by co-ligation of the B-cell receptor and B-cell co-receptor by antigen and complement fragments on microbial surfaces.

We have already described the **B-cell co-receptor complex** as being composed of three cell-surface proteins: CD19, CD21, and CD81 (see Fig. 7.23). CD21 (also known as complement receptor 2, CR2) is a receptor for the complement fragments C3d and C3dg (see Section 2-13). These complement

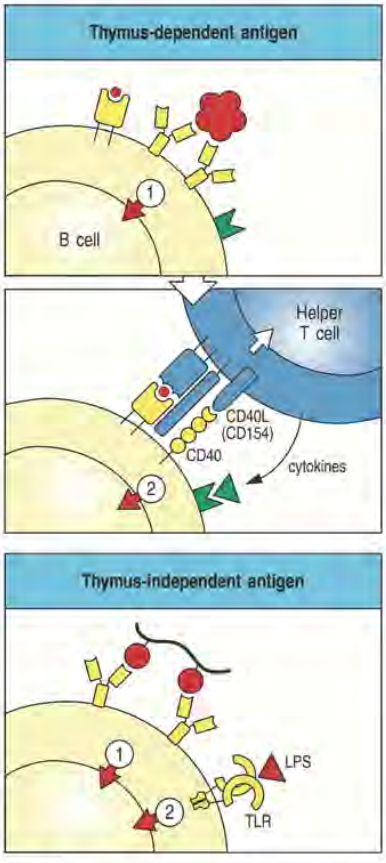


Fig. 10.2 A second signal is required for B-cell activation by either thymus-dependent or thymus-independent antigens. The first signal (indicated as 1 in the figure) required for B-cell activation is delivered through its antigen receptor (top panel). For thymus-dependent antigens, the second signal (indicated as 2) is delivered by a helper T cell that recognizes degraded fragments of the antigen as peptides bound to MHC class II molecules on the B-cell surface (center panel); the interaction between CD40 ligand (CD40L, also called CD154) on the T cell and CD40 on the B cell contributes an essential part of this second signal. For thymus-independent antigens, a second signal can be delivered along with the antigen itself, through Toll-like receptors that recognize antigen-associated TLR ligands, such as bacterial lipopolysaccharide (LPS) or bacterial DNA (bottom panel).

Petitioner Merck, Ex. 1170, p. 133
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

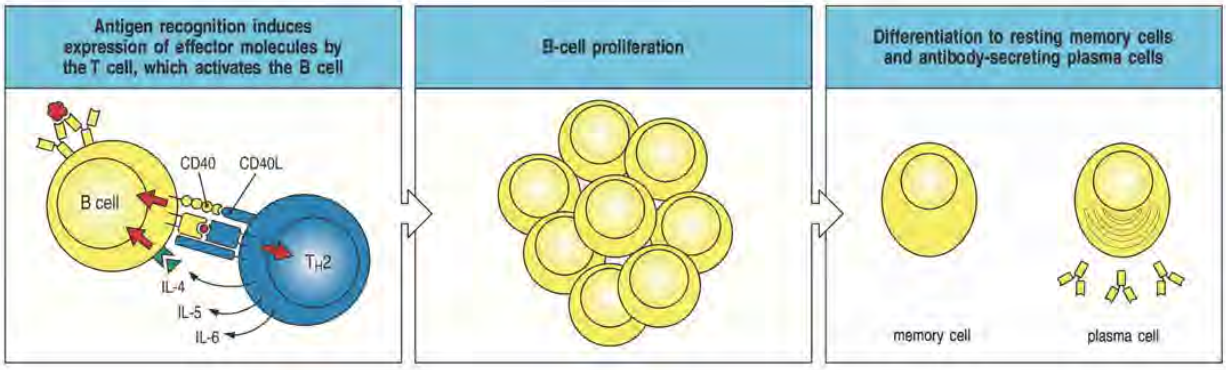


Fig. 10.3 Helper T cells stimulate the proliferation and then the differentiation of antigen-binding B cells. The specific interaction of an antigen-binding B cell with a helper T cell leads to the expression of the B-cell stimulatory molecule CD40 ligand on the helper T-cell surface and to the secretion by the T cell of the B-cell stimulatory cytokines IL-4, IL-5, and IL-6, which drive the proliferation and differentiation of the B cell into antibody-secreting plasma cells. Alternatively, an activated B cell can become a memory cell.

fragments are deposited on the surface of pathogens, such as a bacterial cell, when the complement pathway is activated either by the innate pathways or by antibody bound to the antigen itself. When the B-cell receptor binds to antigen epitopes on surfaces that have also bound C3d or C3dg, CD21 binds to the complement fragments and brings the B-cell receptor together with the co-receptor. This generates signals through CD19 that activate a PI 3-kinase signaling pathway and co-stimulate the B-cell response (see Fig. 7.23) by enhancing proliferation, differentiation, and antibody production.

Simultaneous ligation (co-ligation) of both the antigen receptor and the B-cell co-receptor powerfully amplifies B-cell activation and antibody production. This effect is shown dramatically when mice are immunized with hen egg-white lysozyme coupled to three linked molecules of C3dg. In this case the dose of modified lysozyme needed to induce antibody in the absence of added adjuvant is as little as 1/10,000 of that needed with the unmodified lysozyme.

10-3 Helper T cells activate B cells that recognize the same antigen.

A given B cell can only be activated by helper T cells that respond to the same antigen; this is called **linked recognition**. However, the specific peptide recognized by the helper T cell may be quite distinct from the protein epitope recognized by the B cell's antigen receptor. Indeed, most complex natural antigens, such as viruses and bacteria, are composed of multiple proteins and carry both protein and carbohydrate epitopes. For linked recognition to occur, the peptide recognized by the T cell must be physically associated with the antigen recognized by the B cell, so that the B cell can internalize the antigen through its B-cell receptors and display the appropriate peptide to the T cell. For example, by recognizing an epitope on a viral protein coat, a B cell can bind and internalize a complete virus particle. It then can degrade either coat proteins or internal viral proteins into peptides for display on MHC class II molecules on the B-cell surface. Helper T cells that have been primed earlier in the infection by dendritic cells presenting these peptides can then activate the B cell to make antibodies that recognize the coat protein (Fig. 10.4).

The specific activation of the B cell by its **cognate** T cell—that is, a helper T cell primed by the same antigen—depends on the ability of the antigen-specific B cell to concentrate the appropriate peptide on its surface MHC class II molecules. B cells that bind a particular antigen are up to 10,000 times more efficient at displaying peptide fragments of that antigen on their MHC class II molecules than are B cells that do not bind the antigen. A B cell most efficiently receives help from a T cell that recognizes a peptide that is part of the antigen bound by the B cell.

Petitioner Merck, Ex. 1170, p. 134
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

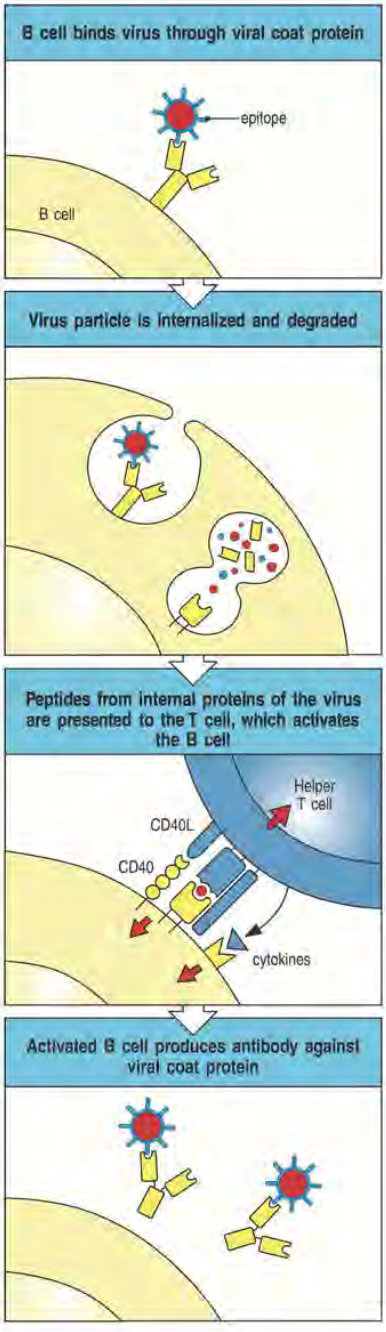


Fig. 10.4 B cells and helper T cells must recognize epitopes of the same molecular complex in order to interact. An epitope on a viral coat protein is recognized by the surface immunoglobulin on a B cell, and the virus is internalized and degraded. Peptides derived from viral proteins, including internal proteins, are returned to the B-cell surface bound to MHC class II molecules (see Chapter 6). Here, these complexes are recognized by helper T cells, which help to activate the B cells to produce antibody against the coat protein. This process is known as linked recognition.

Linked recognition was originally discovered through studies of the production of antibodies against haptens (see Appendix I, Section A-1). Haptens are small chemical groups that cannot elicit antibody responses on their own because they cannot cross-link B-cell receptors and do not recruit T-cell help. When coupled to a carrier protein, however, they become immunogenic, because the protein will carry multiple hapten groups that can now cross-link B-cell receptors. In addition, T-cell dependent responses are possible because T cells can be primed to respond to peptides derived from the protein. Accidental coupling of a hapten to a protein is responsible for the allergic responses shown by many people to the antibiotic penicillin, which reacts with host proteins to form a coupled hapten that can stimulate an antibody response, as we will learn in Chapter 14.

The requirement for linked recognition helps to ensure self tolerance, because it means that an autoimmune response will occur only if both a self-reactive T cell and a self-reactive B cell are present at the same time. This is discussed further in Chapter 15. Vaccine design can take advantage of linked recognition, as in the vaccine used to immunize infants against *Haemophilus influenzae* type b. This bacterial pathogen can infect the covering of the brain, called the meninges, causing meningitis. In adults, protective immunity to *H. influenzae* is due to a strong thymus-independent antibody response to the capsular polysaccharide. Infants, however, make weak responses to these polysaccharide antigens. To make a vaccine that is effective in infants, the polysaccharide is linked chemically to tetanus toxoid, a foreign protein against which infants are routinely and successfully vaccinated (see Chapter 16). B cells that bind the polysaccharide component of the vaccine are activated by helper T cells specific for peptides of the linked toxoid (Fig. 10.5).

10-4 T cells make membrane-bound and secreted molecules that activate B cells.

Recognition of peptide:MHC class II complexes on B cells triggers helper T cells to synthesize both cell-bound and secreted effector molecules that synergize in activating the B cell. As described in Section 9-13, CD40 ligand is expressed by helper T cells and binds to CD40 expressed by B cells; this interaction sustains T-cell growth and differentiation. Reciprocally, the engagement of CD40 on B cells increases B-cell proliferation, immunoglobulin class switching, and somatic hypermutation. Binding of CD40 by CD40 ligand helps to drive the resting B cell into the cell cycle and is essential for B-cell responses to thymus-dependent antigens. It also causes the B cell to increase its expression of co-stimulatory molecules, especially those of the B7 family.

T cells provide additional signals to B cells in the form of secreted cytokines that regulate B-cell proliferation and antibody production. In the B-cell follicle, it is the follicular helper T cell (T_{FH}) (see Section 9-17) that secretes these cytokines. The IL-4 produced by T cells is an important cytokine in B-cell activation. It is made by some T_{FH} cells and by T_H2 cells when they recognize their specific ligand on the B-cell surface, and IL-4 and CD40 ligand are thought to synergize in driving the clonal expansion of B cells that precedes antibody

Petitioner Merck, Ex. 1170, p. 135
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

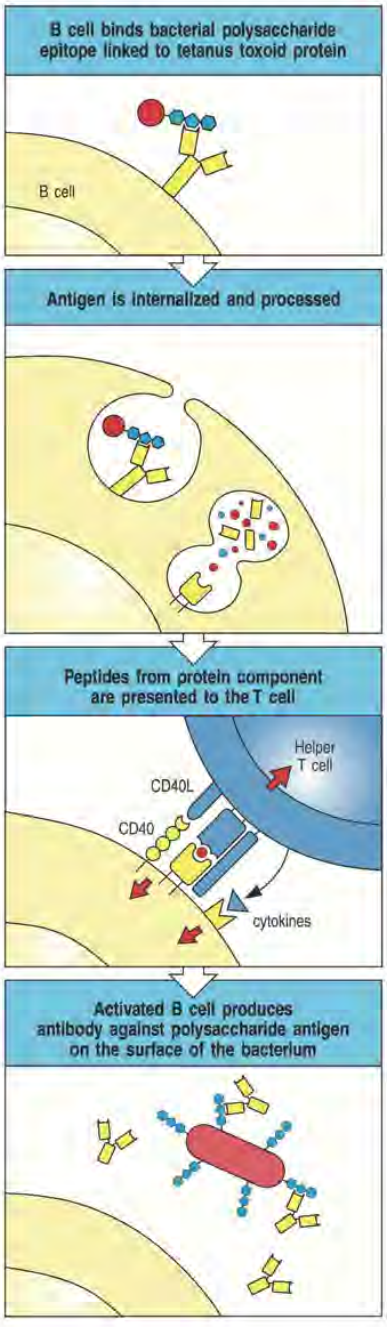


Fig. 10.5 Linked recognition can be exploited in the design of vaccines that boost B-cell responses against polysaccharide antigens. The Hib vaccine against *Haemophilus influenzae* type b is a conjugate of bacterial polysaccharide and the tetanus toxoid protein. The B cell recognizes and binds the polysaccharide, internalizes and degrades the whole conjugate and then displays toxoid-derived peptides on surface MHC class II molecules. Helper T cells generated in response to earlier vaccination against the toxoid recognize the complex on the B-cell surface and activate the B cell to produce anti-polysaccharide antibody. This antibody can then protect against infection with *H. influenzae* type b.

production *in vivo*. IL-4 is secreted in a polar fashion by T_H2 cells and is focused at the site of contact of the T cell with its target B cell (Fig. 10.6), so that it acts selectively on the antigen-specific target cell. Once a T-cell response is under way, however, the cytokines abundantly secreted by helper T cells can also help to activate nearby B cells not in contact with the T cell. After several rounds of proliferation, B cells differentiate into antibody-secreting plasma cells. Two additional cytokines, IL-5 and IL-6, both secreted by helper T cells, contribute to these later stages of B-cell activation.

Members of the TNF receptor/TNF family other than the CD40–CD40 ligand pair are involved in B-cell activation. Activated T cells express **CD30 ligand** (**CD30L**), which binds to **CD30** present on B cells and has been shown to promote B-cell activation. Mice lacking CD30 show reduced proliferation of activated B cells in lymphoid follicles and weaker secondary humoral responses than normal, both of which could be due to the inability of their B cells to respond to signals from T cells expressing CD30 ligand. The soluble TNF-family cytokine BAFF is secreted by dendritic cells and macrophages and acts as a survival factor for differentiating B cells (see Section 8-27).

10-5 B cells that encounter their antigens migrate toward the boundaries between B-cell and T-cell areas in secondary lymphoid tissues.

The frequency of naive lymphocytes specific for any given antigen is estimated to be between 1 in 10,000 and 1 in 1,000,000. Thus, the chance of an encounter between a T lymphocyte and a B lymphocyte that recognize the same antigen should be between 1 in 10^8 and 1 in 10^{12}. Also, as T cells and B cells mostly occupy two quite distinct zones in peripheral lymphoid tissues—the **T-cell areas** and the **primary lymphoid follicles** (also called B-cell areas or B-cell zones), respectively (see Figs 1.18–1.20), it is remarkable that B cells are able to find and interact with T cells of similar antigen specificity. Thus, linked recognition requires precise regulation of the migration of activated B and T cells into specific locations within the lymphoid tissues.

When circulating naive B cells migrate into lymphoid tissues, they enter the primary lymphoid follicles, attracted by the chemokine CXCL13 (Fig. 10.7, first panel). Within the follicle, stromal cells and a specialized cell type, the **follicular dendritic cell** (**FDC**), secrete CXCL13, while naive B cells express CXCR5, the receptor for this chemokine. The FDC is a nonphagocytic cell of nonhematopoietic origin that bears numerous long processes (see Section 8-23). Antigens derived from microorganisms are transported to lymph nodes via the lymph, and to the spleen via the blood.

Complement activation and the deposition of C3b onto microbial and viral antigens contribute to their efficient transport into and accumulation in the follicles. Antigens coated with C3b or C3dg can enter the follicle directly and be trapped there by the complement receptors CR1 and CR2, which are present on the dense network of FDC processes. In mice, intravital two-photon microscopy has indicated that opsonized particulate antigen entering lymph nodes in the afferent lymph (or entering the spleen via the blood), can also

Petitioner Merck, Ex. 1170, p. 136
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

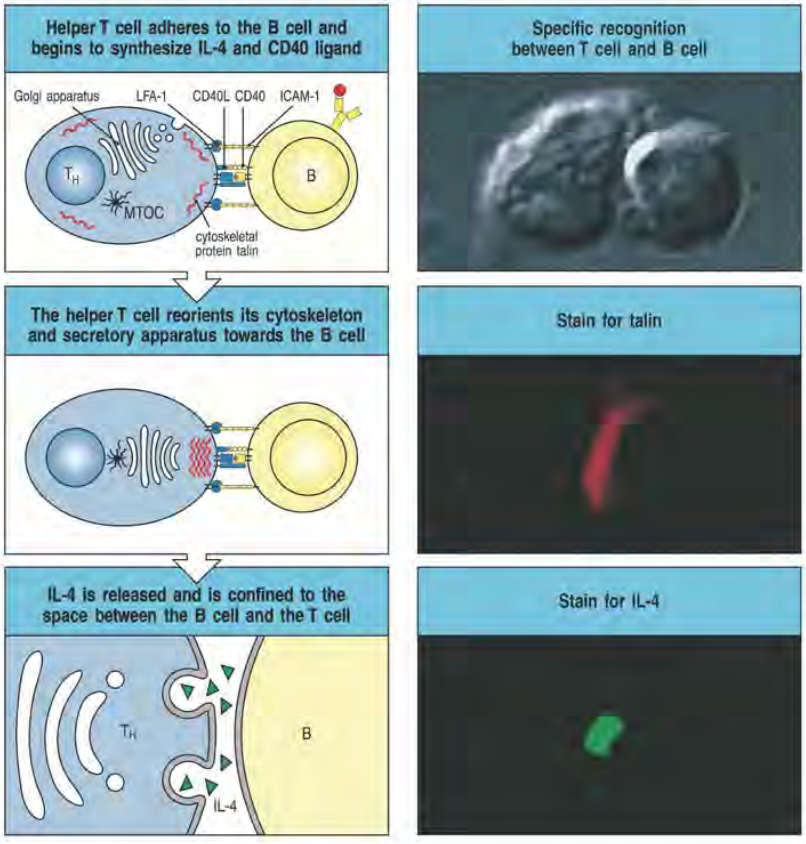


Fig. 10.6 Helper T cells stimulate B cells through binding of CD40 and directed cytokine secretion. When the T-cell receptor binds antigen presented by a B cell, CD40 ligand is induced on the T cell, which then binds to CD40 on the B cell (upper panels). The integrin LFA-1 on the T cell interacts with the adhesion molecule ICAM-1 on the B cell in the periphery of this immunological synapse (see Fig. 9.31). The cytoskeletal protein talin (stained red in the right center panel) becomes relocated to the point of cell–cell contact, and the secretory apparatus (the Golgi apparatus) is reoriented by the cytoskeleton toward the point of contact with the B cell. Cytokines are released at the point of contact (bottom panels). In this example, IL-4 (stained green) is confined to the space between the B cell and the helper T cell. MTOC, microtubule-organizing center. Photographs courtesy of A. Kupfer.

be taken up by specialized macrophages residing in the subcapsular sinus of lymph nodes and the marginal sinus of the spleen, parts of which are adjacent to the follicles (Fig. 10.8). These macrophages seem to retain the antigen on their surface rather than ingesting it and breaking it down. The antigen can then be sampled and picked up by antigen-specific follicular B cells. B cells of any antigen specificity could also acquire antigen from these macrophages via their complement receptors and transport it within the follicle.

When a naive B cell in the follicle encounters its specific antigen, either on specialized macrophages or displayed by FDCs, this induces expression of the chemokine receptor CCR7, and expression of CXCR5 is also retained. The B cell then moves toward the boundary with the T-cell area, where chemokine ligands for CCR7, such as CCL21, are highly expressed by stromal cells and dendritic cells (see Section 9-3). As with T cells, activation of B cells leads to a decrease in the sphingosine receptor $S1P_1$ on the B-cell surface, which retains the cells in the lymphoid tissues. Naive T cells express CCR7, but not CXCR5, and so are localized to the T-cell areas. When a naive T cell encounters its cognate peptide antigen presented by a dendritic cell, expression of CXCR5 is induced as the T cell begins to proliferate. Some T cells differentiate into effector cells and exit the lymphoid tissue, but others become T_{FH} cells and migrate to the boundary between the T-cell area and a follicle, where they can encounter activated B cells (see Fig. 10.7, second panel). When a T_{FH} cell recognizes a peptide displayed by MHC class II molecules on the surface of an activated B cell, it increases the expression of cell-surface molecules and secreted cytokines that promote B-cell activation—this is the basis for the phenomenon of linked recognition described earlier (see Section 10-3).

Petitioner Merck, Ex. 1170, p. 137
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

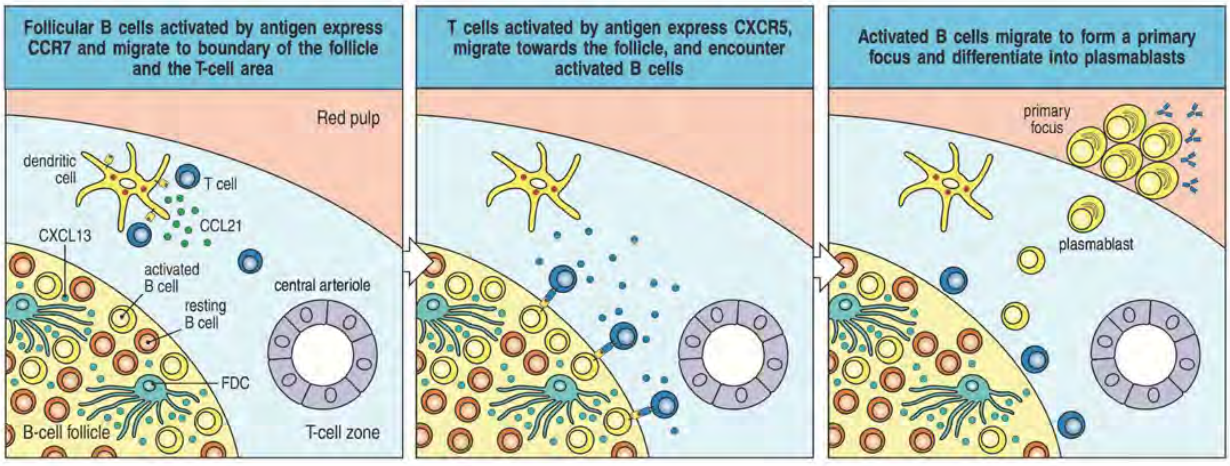


Fig. 10.7 Antigen-binding B cells meet T cells at the border between the T-cell area and a B-cell follicle in secondary lymphoid tissue. B-cell activation in the spleen is shown here. On entry into the spleen from the blood through the marginal sinus (not shown), naive CCR7-positive T cells and CXCR5-positive B cells home to distinct regions where the chemokines CCL21 and CXCL13, respectively, are being produced (first panel). If a B cell encounters its antigen, either on a follicular dendritic cell (FDC) or a macrophage, it migrates toward the border between the follicle and the T-cell area. There it may encounter a T cell that has migrated to this border after being activated by its antigen on the surface of an antigen-presenting dendritic cell in the T-cell area (second panel). Through linked recognition, the T-cell–B-cell interaction produces an initial proliferation of B cells (third panel). In the spleen, the activated lymphocytes then migrate to the border of the T-cell zone and the red pulp, where they continue to proliferate and where some of the B cells differentiate into plasmablasts, forming a so-called primary focus. These plasmablasts then undergo terminal differentiation into antibody-secreting plasma cells.

The coordinated migration of activated B cells and T cells toward the same location in the peripheral lymphoid organ helps solve the problem of getting B cells together with their appropriate helper T cells. Activated B cells bearing peptide:MHC complexes end up in precisely the location to maximize

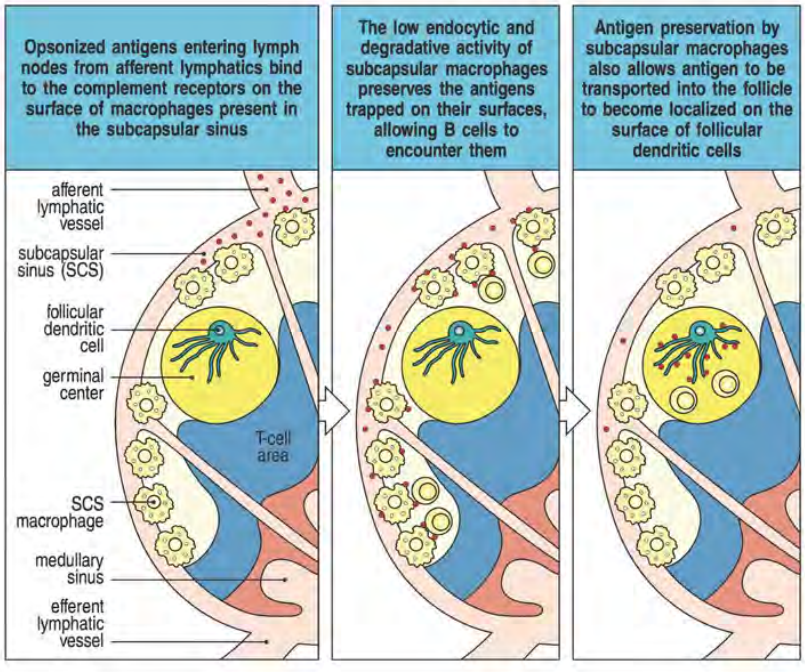


Fig. 10.8 Opsonized antigens are captured and preserved by subcapsular sinus macrophages. Macrophages residing in the lymph node subcapsular sinus (SCS) express complement receptors 1 (CR1) and CR2, are poorly endocytic and have reduced levels of lysosomal enzymes compared with macrophages in the medulla. Opsonized antigen arriving from the afferent lymphatics binds to CR1 and CR2 on the surface of SCS macrophages. Instead of being degraded by these macrophages, it is retained on the cell surface, where it can be presented and transferred to the surface of follicular B cells. B cells are then able to transport it into the follicle, where it can be trapped on the surfaces of follicular dendritic cells.

Petitioner Merck, Ex. 1170, p. 138
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

their chance of encountering helper T cells that can activate them. Antigen-stimulated B cells that fail to interact with T cells that recognize the same antigen die within 24 hours. After their initial encounter, B cells that have received T-cell help migrate from the follicle border to continue their proliferation and differentiation. In the spleen, these cells move to the border between the T-cell area and the red pulp. Here they establish a **primary focus** of clonal expansion (see Fig. 10.7, third panel). In lymph nodes, the primary focus is located in the medullary cords, where lymph drains out of the node. Primary foci appear about 5 days after an infection or immunization with an antigen not previously encountered, which correlates with the time needed for helper T cells to differentiate.

10-6 Antibody-secreting plasma cells differentiate from activated B cells.

Both T cells and B cells proliferate in the primary focus for several days, and this constitutes the first phase of the primary humoral immune response. Some of these proliferating B cells differentiate into antibody-synthesizing **plasmablasts** in the primary focus. Others may migrate into the lymphoid follicle and differentiate further there before becoming plasma cells, as we will describe later. Plasmablasts are cells that have begun to secrete antibody, yet are still dividing and express many of the characteristics of activated B cells that allow their interaction with T cells. After a few more days, the plasmablasts in the primary focus stop dividing and either die or undergo terminal differentiation into plasma cells. Some of the plasma cells remain in the lymphoid organs, where they are short lived, while others migrate to the bone marrow and continue antibody production there.

The properties of resting B cells, plasmablasts, and plasma cells are compared in Fig. 10.9. The differentiation of a B cell into a plasma cell is accompanied by many morphological changes that reflect its commitment to the production of large amounts of secreted antibody, which can make up to 20% of all the protein synthesized by a plasma cell. Plasmablasts and plasma cells have a prominent perinuclear Golgi apparatus and an extensive rough endoplasmic reticulum that is rich in immunoglobulin molecules being synthesized and exported into the lumen of the endoplasmic reticulum for secretion (see Fig. 1.23). Plasmablasts still express B7 co-stimulatory molecules and MHC class II molecules, but plasma cells do not, and so can no longer present antigen to helper T cells. Nevertheless, T cells still provide important signals for plasma-cell differentiation and survival, such as IL-6 and CD40 ligand. Plasmablasts also bear relatively large numbers of B-cell receptors on the cell surface,

	Intrinsic properties			Inducible by antigen stimulation		
B-lineage cell	Surface Ig	Surface MHC class II	High-rate Ig secretion	Growth	Somatic hyper-mutation	Class switch
Resting B cell	High	Yes	No	Yes	Yes	Yes
Plasmablast	High	Yes	Yes	Yes	Unknown	Yes
Plasma cell	Low	No	Yes	No	No	No

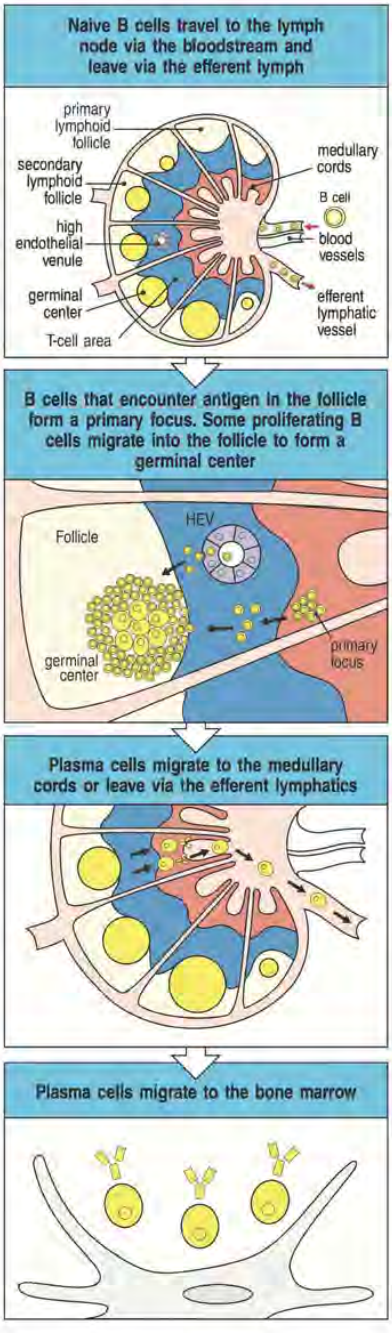
Fig. 10.9 Plasma cells secrete antibody at a high rate but can no longer respond to antigen or helper T cells. Resting naive B cells have membrane-bound immunoglobulin (usually IgM and IgD) and MHC class II molecules on their surface. Although their V genes do not carry somatic mutations, B cells can take up antigen and present it to helper T cells. The T cells in return induce the B cells to proliferate and to undergo isotype switching and somatic hypermutation, but B cells do not secrete significant amounts of antibody during this period. Plasmablasts have an intermediate phenotype. They secrete antibody but retain substantial surface immunoglobulin and MHC class II molecules and so can continue to take up and present antigen to T cells. Plasmablasts early in the immune response and those activated by T-independent antigens have usually not undergone somatic hypermutation and class switching, and therefore secrete IgM. Plasma cells are terminally differentiated plasmablasts that secrete antibodies. They can no longer interact with helper T cells because they have very low levels of surface immunoglobulin and lack MHC class II molecules. Early in the immune response they differentiate from unswitched plasmablasts and secrete IgM; later in the response they derive from plasmablasts that have undergone class switching and somatic hypermutation. Plasma cells have lost the ability to change the class of their antibody or undergo further somatic hypermutation.

Petitioner Merck, Ex. 1170, p. 139
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

whereas plasma cells have many fewer. These low levels of surface immunoglobulin may, however, be physiologically important, because the survival of plasma cells seems to be determined in part by their ability to continue to bind antigen. Plasma cells have a range of life spans. Some survive for only days to a few weeks after their final differentiation, whereas others are very long lived and account for the persistence of antibody responses.

Movie 10.1

10-7 The second phase of a primary B-cell immune response occurs when activated B cells migrate into follicles and proliferate to form germinal centers.



Some of the B cells that are activated and start to proliferate early in the immune response take a more circuitous route before they become plasma cells. Together with their associated T cells, they move into a primary lymphoid follicle (Fig. 10.10), where they continue to proliferate and ultimately form a **germinal center**; follicles with germinal centers are called **secondary lymphoid follicles**.

Germinal centers are composed mainly of proliferating B cells, but antigen-specific T cells make up about 10% of germinal center lymphocytes and provide indispensable help to the B cells. The germinal center is essentially an island of cell division that sets up amid a sea of resting B cells in the primary follicle. Proliferating germinal center B cells displace the resting B cells toward the periphery of the follicle, forming a **mantle zone** of resting cells around the two distinguishable areas of activated B cells, called the **light zone** and the **dark zone** (Fig. 10.11, top panel). The germinal center grows in size as the immune response proceeds, and then shrinks and finally disappears when the infection is cleared. Germinal centers are present for about 3–4 weeks after initial antigen exposure.

The events in the primary focus lead to the prompt secretion of specific antibody, mostly of the IgM isotype, that immediately protects the infected individual. In contrast, the germinal center reaction provides for a more effective humoral response later, should the pathogen establish a chronic infection or the host become reinfected. B cells undergo several important modifications in the germinal center that produce a more effective antibody response. These include somatic hypermutation, which alters the V regions of immunoglobulin genes (see Section 5-18) and enables a process called affinity maturation, which selects for the survival of mutated B cells with high affinity for the antigen. In addition, class switching allows the selected B cells to express a variety of effector functions in the form of antibodies of different classes (see Section 5-19). These B cells will differentiate either into memory B cells,

Fig. 10.10 Activated B cells form germinal centers in lymphoid follicles. Activation of B cells in a lymph node is shown here. Top panel: naive circulating B cells enter lymph nodes from the blood via high endothelial venules and are attracted by chemokines into the primary lymphoid follicle; if they do not encounter antigen in the follicle, they leave via the efferent lymphatic vessel. Second panel: B cells that have bound antigen move to the border with the T-cell area where they may encounter activated helper T cells specific for the same antigen, which interact with the B cells and activate them to start proliferation and differentiation into plasmablasts. Some B cells activated at the T-cell–B-cell border migrate to form a primary focus of antibody-secreting plasmablasts in the medullary cords, whereas others move back into the follicle, where they continue to proliferate and form a germinal center. Germinal centers are sites of sustained B-cell proliferation and differentiation. Follicles in which germinal centers have formed are known as secondary follicles. Within the germinal center, B cells begin their differentiation into either antibody-secreting plasma cells or memory B cells. Third and fourth panels: plasma cells leave the germinal center and migrate to the medullary cords, or leave the lymph node altogether via the efferent lymphatics and migrate to the bone marrow.

Petitioner Merck, Ex. 1170, p. 140
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

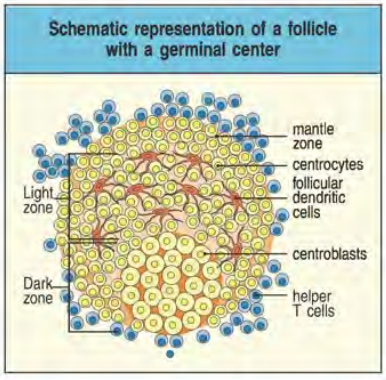
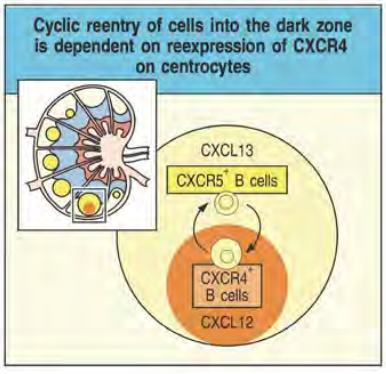
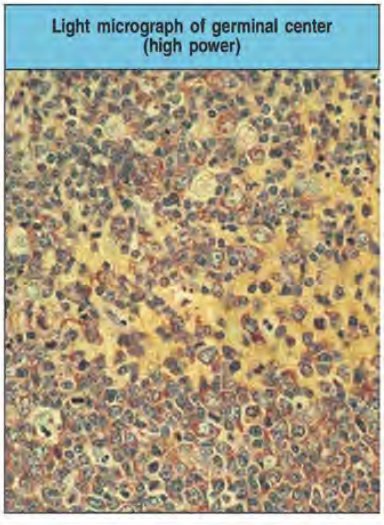


Fig. 10.11 The structure of a germinal center. The germinal center is a specialized microenvironment in which B-cell proliferation, somatic hypermutation, and selection for strength of antigen binding all occur. Closely packed centroblasts, which express CXCR4 and CXCR5, form the so-called 'dark zone' of the germinal center; the less densely packed 'light zone' contains centrocytes, which express only CXCR5. Cells in the dark zone produce CXCL12, which attracts the CXCR4-expressing centroblasts. Cyclic reentry describes the process by which B cells can lose and gain expression of CXCR4 and thus move from the light zone to the dark zone and back again, a process described in detail in Section 10-8.

whose function is described in Chapter 11, or into plasma cells that secrete higher-affinity and class-switched antibody during the latter part of the primary immune response.

B cells in the germinal center proliferate, dividing every 6–8 hours. Initially, these rapidly proliferating B cells, called **centroblasts**, express the chemokine receptors CXCR4 and CXCR5 but markedly reduce their expression of surface immunoglobulin, particularly of IgD. Centroblasts proliferate in the dark zone of the germinal center, named for its densely packed appearance (Fig. 10.12). Stromal cells in the dark zone produce CXCL12 (SDF-1), a ligand for CXCR4 that acts to retain centroblasts in this region. As time goes on, some centroblasts reduce their rate of cell division and enter the growth phase, while in the G_1 phase of the cell cycle they stop expressing CXCR4 and begin to produce higher levels of surface immunoglobulin. These B cells are termed





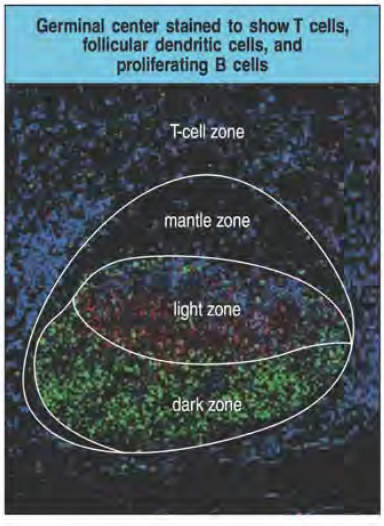


Fig. 10.12 Germinal centers are sites of intense cell proliferation and cell death. The photomicrograph (left panel) shows a high-power view of a section through a human tonsillar germinal center. Closely packed centroblasts seen in the lower part of this photomicrograph form the so-called dark zone of the germinal center. Above this region is the less densely packed light zone. The right panel shows immunofluorescent staining of a germinal center. B cells are found in the dark zone, light zone, and mantle zone. Proliferating cells are stained green for Ki67, an antigen expressed in nuclei of dividing cells, revealing the rapidly proliferating centroblasts in the dark zone. The dense network of follicular dendritic cells, stained red, mainly occupies the light zone. Centrocytes in the light zone proliferate to a lesser degree than centroblasts. Small recirculating B cells occupy the mantle zone at the edge of the B-cell follicle. Large masses of CD4 T cells, stained blue, can be seen in the T-cell zones, which separate the follicles. There are also significant numbers of T cells in the light zone of the germinal center; CD4 staining in the dark zone is associated mainly with CD4-positive phagocytes. Photographs courtesy of I. MacLennan.

Petitioner Merck, Ex. 1170, p. 141
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

centrocytes. The loss of CXCR4 allows centrocytes to move into the light zone, a less densely packed area containing abundant FDCs that produce the chemokine CXCL13 (BLC), a ligand for CXCR5 (see Fig. 10.11, bottom panels). The B cells proliferate in the light zone, but to a lesser extent than in the dark zone.

10-8 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected.

The original diversification of antigen receptors produced by DNA rearrangement generates clones of B cells with radically differing B-cell receptors, as described in Sections 5-1 to 5-6. In contrast, the secondary diversification of immunoglobulin genes by somatic hypermutation introduces individual point mutations that change a single, or just a few, amino acids in the resulting immunoglobulin, producing closely related clones of B cells that differ subtly from each other in their specificity and affinity for antigen. When undergoing somatic hypermutation, immunoglobulin V-region genes accumulate mutations at a rate of about one base pair change per 10^3 base pairs per cell division. These mutations are targeted by the action of the enzyme AID (activation-induced cytidine deaminase) to the rearranged V genes (see Section 5-18 for the molecular detail of this process). The mutation rate in the rest of the DNA is much lower: around one base pair change per 10^{10} base pairs per cell division. Somatic hypermutation also affects some DNA flanking the rearranged V gene, but the mutations generally do not extend into the C-region exons. Each of the expressed heavy-chain and light-chain V-region genes is encoded by about 360 base pairs, and about three out of every four base changes results in an altered amino acid. This means that there is about a 50% chance at each cell division that a B cell will acquire a mutation in its receptor.

The point mutations accumulate in a stepwise manner as the descendants of each B cell proliferate in the germinal center to form B-cell clones. An altered receptor can affect the ability of a B cell to bind antigen and thus will affect the fate of the B cell in the germinal center (Fig. 10.13). Most mutations have a negative impact on the ability of the B-cell receptor to bind the original antigen, by preventing the correct folding of the immunoglobulin molecule or by blocking the complementarity-determining regions from binding antigen. Cells that harbor such mutations are eliminated by apoptosis, either because they can no longer make a functional B-cell receptor or because they cannot compete with sibling cells that bind antigen more strongly (see Fig. 10.13, left panels). Germinal centers are filled with apoptotic B cells that are quickly engulfed by macrophages, giving rise to the characteristic **tingible body macrophages**. These contain dark-staining nuclear debris in their cytoplasm and are a long-recognized histologic feature of germinal centers. Less frequently, mutations improve the affinity of a B-cell receptor for antigen. Cells that harbor these mutations are efficiently selected and expanded (see Fig. 10.13, right panels). Expansion seems to be due to the prevention of apoptosis, and thus increased survival compared with low-affinity cells, rather than an increased rate of proliferation.

Selection occurs in increments. It is thought that somatic hypermutation occurs in the centroblasts in the dark zone; when a centroblast reduces its rate of proliferation and becomes a centrocyte, it increases the number of B-cell receptors on its surface and moves to the light zone, where there are abundant FDCs bearing antigen. There, the centrocyte's ability to bind antigen is tested, in competition with the other clonally related centrocytes harboring different mutations. After each round of mutation, the centrocyte begins to express the new antigen receptor. If the new receptor can bind antigen, the centrocyte receives a signal through its receptor, which stimulates it to undergo additional rounds of division and to reexpress CXCR4, in effect becoming a centroblast again. The B cell reenters the dark zone, where the process of mutation and

Petitioner Merck, Ex. 1170, p. 142
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

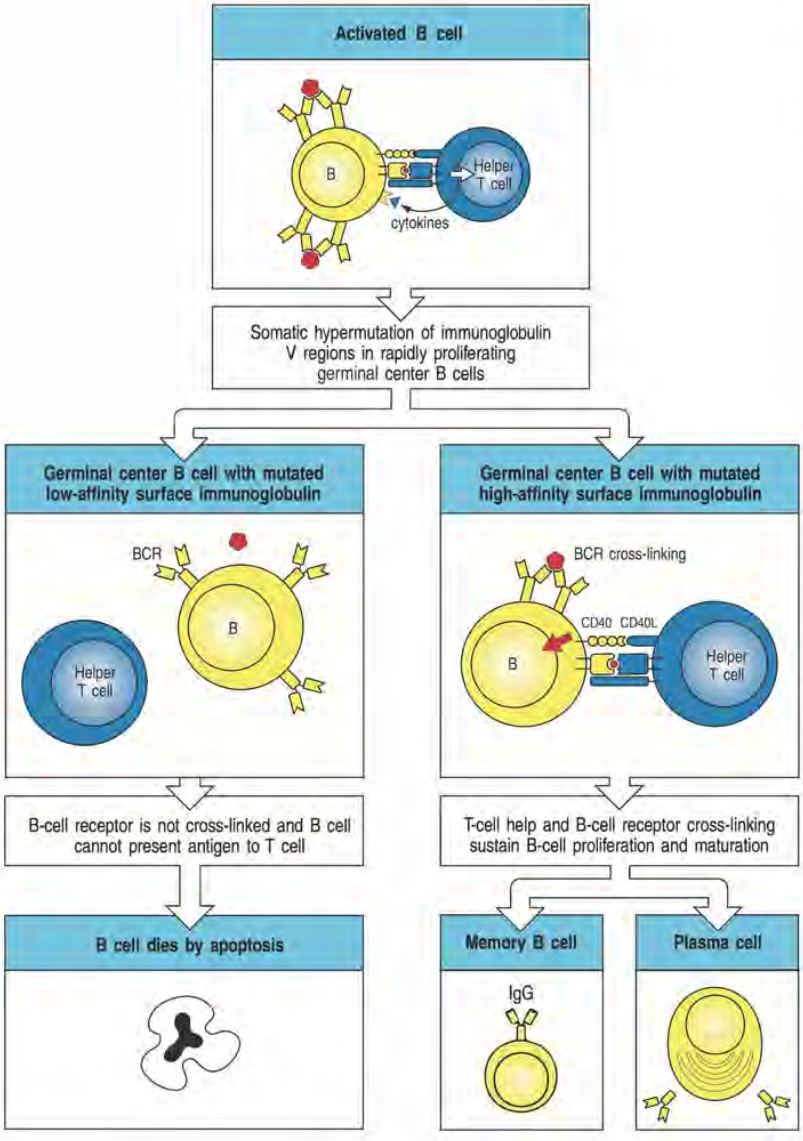


Fig. 10.13 Activated B cells undergo rounds of mutation and selection for higher-affinity mutants in the germinal center, resulting in high-affinity antibody-secreting plasma cells and high-affinity memory B cells. B cells are first activated at the follicle border by a combination of antigen and helper T cells (top panel). They migrate to germinal centers, where the remaining events occur. Somatic hypermutation can result in amino acid replacements in immunoglobulin V regions that affect the fate of the B cell. Mutations that result in a B-cell receptor (BCR) of lower or no affinity for the antigen (left panels) will prevent the B cell from being activated as efficiently, because both B-cell receptor cross-linking and the ability of the B cell to present peptide antigen to T cells are reduced. This results in such B cells dying by apoptosis, which purges low-affinity B cells from the germinal center. Most mutations are either negative or neutral, and thus the germinal center is a site of massive B-cell death as well as of proliferation. Some mutations, however, will improve the ability of the B-cell receptor to bind antigen. This increases the B cell's chance of interacting with T cells, and thus of proliferating and surviving (right panels). Surviving cells undergo repeated cycles of mutation and selection during which some of the progeny B cells undergo differentiation to either memory B cells or plasma cells (bottom right panels) and leave the germinal center. The signals that control these differentiation decisions are unknown.

selection is repeated. Being able to bind antigen will also allow the centrocyte to take up, process, and present antigen to T_{FH} cells, which will then provide survival signals to the centrocyte. This widely accepted model of the dynamics of B-cell migration within the germinal center is known as the **cyclic reentry model** (see Fig. 10.11, bottom right panel). The length of time that B cells can survive in the light zone is limited: they must reenter the dark zone or exit the germinal center within a few hours, or else they undergo apoptosis.

In this way, the affinity and specificity of positively selected B cells are continually refined during the germinal center response, the process known as **affinity maturation**. This leads to the average affinity of the population of responding B cells for its antigen increasing over time. The selection process can be quite stringent: although 50–100 B cells may seed the germinal center, most of them leave no progeny, and by the time the germinal center reaches maximum size, it is typically composed of the descendants of only one or a few B cells.

Petitioner Merck, Ex. 1170, p. 143
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Evidence of both positive and negative selection is seen in the pattern of somatic hypermutations in V regions of B cells that have survived passage through the germinal center (see Section 5-18). The existence of negative selection is shown by the relative scarcity of amino acid replacements in the framework regions, reflecting the loss of cells that had mutated any one of the many residues that are critical for immunoglobulin V-region folding. Negative selection is important, because it prevents rapidly dividing B cells from expanding to numbers that would overwhelm the lymphoid tissues. Positive selection is evident in the accumulation of numerous amino acid replacements in the complementarity-determining regions, which determine antibody specificity and affinity (see Fig. 5.24).

10-9 Class switching in thymus-dependent antibody responses requires expression of CD40 ligand by helper T cells and is directed by cytokines.

As well as undergoing somatic hypermutation, germinal center B cells undergo class switching. Antibodies are remarkable not only for the diversity of their antigen-binding sites but also for their versatility as effector molecules. The antigen specificity of an antibody is determined by the variable domains of the immunoglobulin chains—V_H and V_L. In contrast, the effector action of the antibody is determined by the isotype of its heavy-chain C region (see Section 4-1). A given heavy-chain V domain exon can become associated with a C-region exon of any isotype through the process of class, or isotype, switching (see Section 5-19). Class switching only starts after B cells become activated by helper T cells, and can occur in B cells in the primary foci as well as in a proportion of the B cells in the germinal center. The DNA rearrangements that underlie class switching and confer this functional diversity on the humoral immune response are directed by cytokines, especially those released by effector CD4 T cells. We will see later in this chapter how antibodies of each class contribute to the elimination of pathogens.

All naive B cells express cell-surface IgM and IgD. IgM is the first antibody secreted by activated B cells (see Section 5-15), but makes up less than 10% of the immunoglobulin found in plasma; IgG is the most abundant. Much of the antibody in plasma has therefore been produced by plasma cells derived from B cells that have undergone class switching. Little IgD antibody is produced at any time, so the early stages of the antibody response are dominated by IgM antibodies. Later, IgG and IgA are the predominant antibody classes, with IgE contributing a small but biologically important part of the response. The overall predominance of IgG is also due in part to its longer lifetime in the plasma (see Fig. 5.15).

CD40 Ligand Deficiency



Productive interactions between B cells and helper T cells are essential for class switching to occur. This is demonstrated by people who have a genetic deficiency of CD40 ligand, which is required for those interactions. Class switching is greatly reduced in such individuals and they have abnormally high levels of IgM in their plasma, a condition known as **hyper IgM syndrome**, which is characterized by a lack of antibodies of classes other than IgM and severe humoral immunodeficiency, shown by repeated infections with common bacterial pathogens. Other defects that interfere with class switching, such as a deficiency of CD40, or of the enzyme AID, which is essential for the class-switch recombination process, also result in forms of hyper IgM syndrome (discussed in Chapter 13). Much of the IgM in hyper IgM syndromes may be induced by thymus-independent antigens on the pathogens that chronically infect these patients. Nevertheless, people with CD40 ligand deficiency can make IgM antibodies in response to thymus-dependent antigens, which indicates that in the B-cell response, CD40L–CD40 interactions are most important in enabling a sustained response that includes class switching and affinity maturation, rather than in the initial activation of B cells.

Activation-induced Cytidine Deaminase Deficiency



Petitioner Merck, Ex. 1170, p. 144
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The molecular mechanisms that underlie class-switch recombination were outlined in Section 5-19. In this process, the rearranged V region of the heavy-chain gene is translocated from its original location upstream of the C_μ constant region and placed in front of a different C region, with deletion of the intervening chromosomal DNA (see Fig. 5.25). The selection of a particular C region for recombination is not random but is regulated by the cytokines being produced by helper T cells and other cells during the immune response; in this way it ensures that antibodies most useful in dealing with the particular infectious agent are produced. Much of what is known about the regulation of class switching by cytokines has come from experiments *in vitro* in which mouse B cells are exposed to various nonspecific stimuli, such as bacterial lipopolysaccharide (LPS), along with purified cytokines (Fig. 10.14). These experiments showed that different cytokines preferentially induce switching to different isotypes. In the mouse, IL-4 preferentially induces switching to IgG1 ($C\gamma_1$) and IgE ($C\varepsilon$), transforming growth factor (TGF)-β induces switching to IgG2b ($C\gamma_{2b}$) and IgA ($C\alpha$), IL-5 promotes switching to IgA, and interferon (IFN)-γ preferentially induces switching to IgG2a and IgG3 (Fig. 10.15). These cytokines can be produced by the CD4 T-cell subsets described in Section 9-17—for example, T_H2 cells make IL-4 and TGF-β, and T_H1 cells produce IFN-γ. However, T_{FH} cells are likely to regulate most B-cell switching in the germinal center, and they seem to be able to secrete cytokines characteristic of whichever T-cell subset is dominant within the effector T-cell response. Thus, helper T cells both regulate the production of antibody by B cells and also influence the heavy-chain isotype that determines the effector function of the antibody.

Cytokines induce class switching in part by inducing the production of RNA transcripts through the switch regions that lie 5′ to each heavy-chain C gene segment (see Fig. 10.14). When activated B cells are exposed to IL-4, for example, transcription from promoters that lie upstream of the switch regions of $C\gamma_1$ and $C\varepsilon$ can be detected a day or two before switching occurs. Interestingly, each of the cytokines that induces switching seems to induce transcription from the switch regions of two different heavy-chain C genes, but specific recombination occurs in only one of these genes.

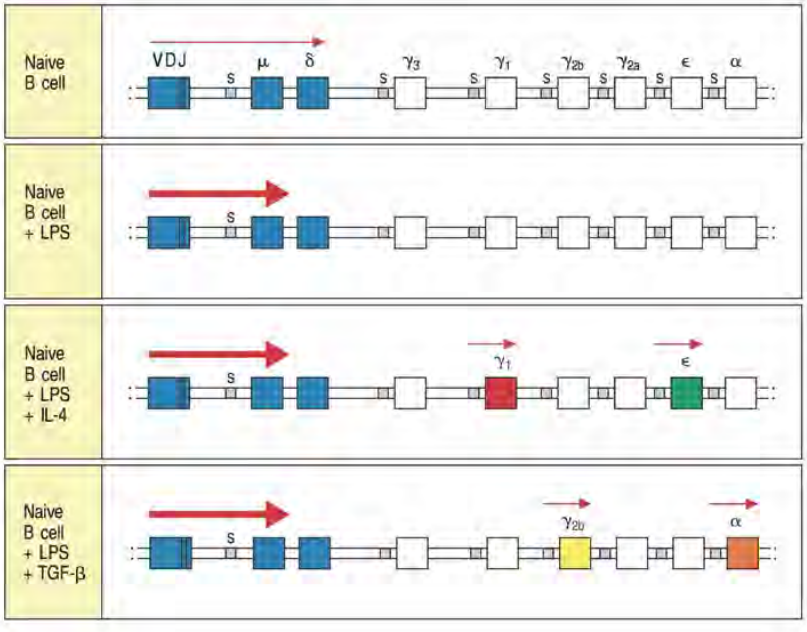


Fig. 10.14 Class switching is preceded by transcriptional activation of heavy-chain C-region genes. Resting naive B cells transcribe the genes for the heavy-chain isotypes μ and δ at a low rate, giving rise to surface IgM and IgD (first row). Bacterial lipopolysaccharide (LPS), which can activate B cells independently of antigen, induces IgM secretion (second row). In the presence of IL-4, however, transcripts of $C_{\gamma 1}$ and C_ε are initiated at a low rate from promoters that are located at the 5′ end of each switch region (third row). These do not code for a protein but drive switching to IgG1 or IgE, respectively (see Section 5-19). Similarly, TGF-β gives rise to $C_{\gamma 2b}$ and C_α transcripts and drives switching to IgG2b or IgA (fourth row). It is not known what determines which of the two transcriptionally activated heavy-chain C genes undergoes switching in a given event. Red arrows indicate transcription. The figure shows class switching in the mouse.

Petitioner Merck, Ex. 1170, p. 145
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

	Role of cytokines in regulating expression of antibody classes						
Cytokines	**IgM**	**IgG3**	**IgG1**	**IgG2b**	**IgG2a**	**IgE**	**IgA**
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces

Fig. 10.15 Different cytokines induce switching to different antibody classes. The individual cytokines induce (violet) or inhibit (red) the production of certain antibody classes. Much of the inhibitory effect is probably the result of directed switching to a different class. These data are drawn from experiments with mouse cells.

10-10 Ligation of CD40 and prolonged contact with T follicular helper cells is required to sustain germinal center B cells.

Germinal center B cells are inherently prone to dying, and to survive they must receive specific signals. It was originally discovered *in vitro* that resting B cells could be kept alive by simultaneously cross-linking their B-cell receptors and ligating their cell-surface CD40. *In vivo* these signals are delivered by antigen and by T_{FH} cells, respectively. The precise source of antigen in the germinal center has been a matter of some controversy. Antigen can be trapped and stored for long periods in the form of immune complexes on FDCs (Figs 10.16 and 10.17). It has been assumed that this is the antigen that sustains germinal center B-cell proliferation; other cells, however, may present antigen as well, and this issue remains an active area of research.

T_{FH} cells and germinal center B cells interact to deliver signals that are important for both cells. B cells express ICOS ligand (ICOSL), which co-stimulates T_{FH} cells through ICOS (see Section 9-13). Mice that lack ICOS are deficient in the germinal center reaction and have severely reduced switched antibody responses—in this case as a result of defective T_{FH}-cell function rather than any defect in the B cells. In turn, T_{FH} cells express CD40 ligand, which binds to CD40 on B cells, increasing their expression of a protein called Bcl-X_L, a relative of Bcl-2, which promotes B-cell survival by inhibiting apoptosis (see Section 7-23). Another important interaction involves receptors of the SLAM (signaling lymphocyte activation molecule) family, which are members of the immunoglobulin superfamily. SLAM-family receptors bind homotypically—that is, a SLAM receptor on one cell binds to a SLAM receptor on the other cell. Two members of the SLAM family, CD84 and Ly108, are involved in promoting prolonged contact between cognate T cells and B cells in the germinal center. Intravital microscopy has revealed that mice lacking CD84 have reduced numbers of conjugates between antigen-specific T cells and B cells in germinal centers, and these mice also have a reduced humoral response to antigen. Thus, it seems that T_{FH} cells make prolonged contact with B cells in germinal centers through several different receptor–ligand interactions that deliver signals in both directions. This cellular dialog is the basis for linked recognition.

10-11 Surviving germinal center B cells differentiate into either plasma cells or memory cells.

Some B cells eventually exit from the light zone and start to differentiate into plasma cells that produce large amounts of antibody. In B cells, the transcription factors Pax5 and Bcl6 inhibit the expression of transcription factors required for plasma-cell differentiation, and Pax5 and Bcl6 are downregulated

Petitioner Merck, Ex. 1170, p. 146
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

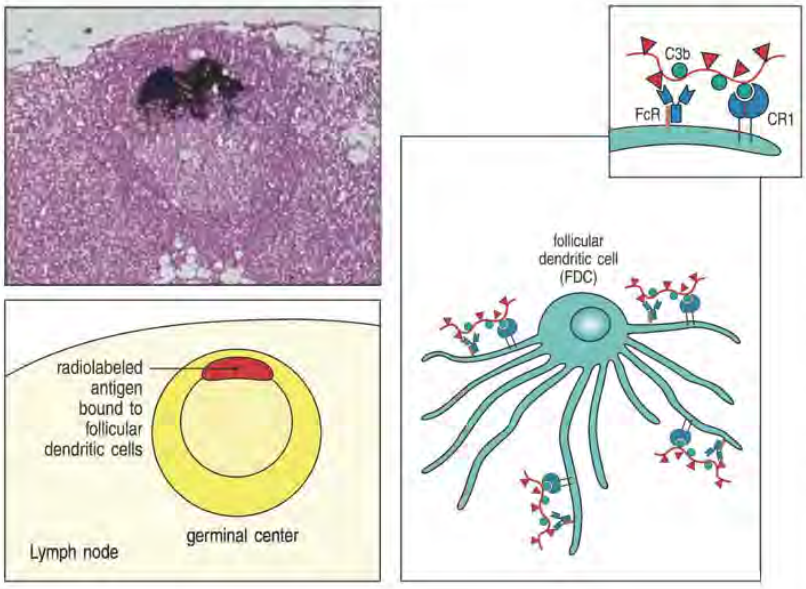


Fig. 10.16 Antigens are trapped in immune complexes that bind to the surface of follicular dendritic cells. Radiolabeled antigen localizes to, and persists in, lymphoid follicles of draining lymph nodes (see the light micrograph and the schematic representation below, showing a germinal center in a lymph node). Radiolabeled antigen has been injected 3 days previously and its localization in the germinal center is shown by the intense dark staining. The antigen is in the form of antigen:antibody:complement complexes bound to Fc receptors and to complement receptors CR1 or CR2 on the surface of the follicular dendritic cell (FDC), as depicted schematically in the right panel and inset. These complexes are not internalized. Antigen can persist in this form for long periods. Photograph courtesy of J. Tew.

when the B cell starts differentiation. The transcription factor IRF4 then induces the expression of BLIMP-1, a transcriptional repressor that switches off genes required for B-cell proliferation, class switching, and affinity maturation. B cells in which BLIMP-1 is induced become plasma cells; they cease proliferating, increase the synthesis and secretion of immunoglobulins, and change their cell-surface properties. They downregulate the chemokine receptor CXCR5, and upregulate CXCR4 and α_4:β_1 integrins, so that the plasma cells can now leave the germinal centers and home to peripheral tissues.

Some plasma cells deriving from germinal centers in lymph nodes or spleen migrate to the bone marrow, where a subset lives for a long period, whereas others migrate to the medullary cords in lymph nodes or splenic red pulp.

Fig. 10.17 Immune complexes bound to FDCs form iccosomes, which are released and can be taken up by B cells in the germinal center. FDCs have a prominent cell body and many dendritic processes. Immune complexes, bound to complement receptors and Fc receptors on the FDC surface, become clustered, forming prominent 'beads' along the dendrites (a). An intermediate form of FDC is shown, which has both straight filiform dendrites and others that are becoming beaded. These beads are shed from the cell as iccosomes (immune complex-coated bodies), which can bind to a B cell in the germinal center (b) and be taken up by it (c). In panels b and c, the iccosome has been formed with immune complexes containing horseradish peroxidase, which is electron-dense and therefore appears dark in the transmission electron micrographs. Photographs courtesy of A.K. Szakal.

Petitioner Merck, Ex. 1170, p. 147
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The transcription factor XBP1 (X-box binding protein 1) is expressed in plasma cells and helps to regulate their secretory capacity; XBP1 is also required for plasma cells to colonize bone marrow successfully. B cells that have been activated in germinal centers in mucosal tissues, and which are predominantly switched to IgA production, stay within the mucosal system (discussed in Chapter 12). Plasma cells in bone marrow receive signals from stromal cells that are essential for their survival and they can be very long lived, whereas plasma cells in the medullary cords or red pulp are not long lived. Plasma cells in the bone marrow are the source of long-lasting high-affinity class-switched antibody.

Other germinal center B cells differentiate into **memory B cells**. Memory B cells are long-lived descendants of cells that were once stimulated by antigen and had proliferated in the germinal center. They divide very slowly if at all; they express surface immunoglobulin but secrete no antibody, or do so only at a low rate. Because the precursors of memory B cells once participated in a germinal center reaction, memory B cells inherit the genetic changes that occur in germinal center cells, including somatic hypermutation and the gene rearrangements that result in a class switch. The signals that control which differentiation path a B cell takes, and even whether at any given point the B cell continues to divide instead of differentiating, are still being investigated. We discuss memory B cells in Chapter 11.

10-12 Some bacterial antigens do not require T-cell help to induce B-cell responses.

Although antibody responses to most protein antigens are dependent on helper T cells, humans and mice with T-cell deficiencies nevertheless make antibodies against many bacterial antigens. This is because some bacterial polysaccharides, polymeric proteins, and lipopolysaccharides are able to stimulate naive B cells in the absence of T-cell help. The nonprotein bacterial products cannot elicit classical T-cell responses, yet they induce antibody responses in normal individuals. Such antigens are known as thymus-independent antigens (TI antigens).

Thymus-independent antigens fall into two classes, which activate B cells by two different mechanisms. **TI-1 antigens** rely on activity that can directly induce B-cell division without T-cell help. We now understand that TI-1 antigens contain molecules that cause the proliferation and differentiation of most B cells regardless of their antigen specificity; this is known as **polyclonal activation** (Fig. 10.18, top panels). TI-1 antigens are therefore often called **B-cell mitogens**, a mitogen being a substance that induces cells to undergo mitosis. For example, LPS and bacterial DNA are both TI-1 antigens because they activate TLRs expressed by B cells (see Section 3-5) and can act as a mitogen. Naive murine B cells express most TLRs constitutively, but naive human B cells do not express high levels of most TLRs until they receive stimulation through the B-cell receptor. So, by the time a B cell has been stimulated by antigen through its B-cell receptor, it is likely to express several TLRs and be responsive to stimulation by TLR ligands that accompany the antigens. Thus, when B cells are exposed to concentrations of TI-1 antigens

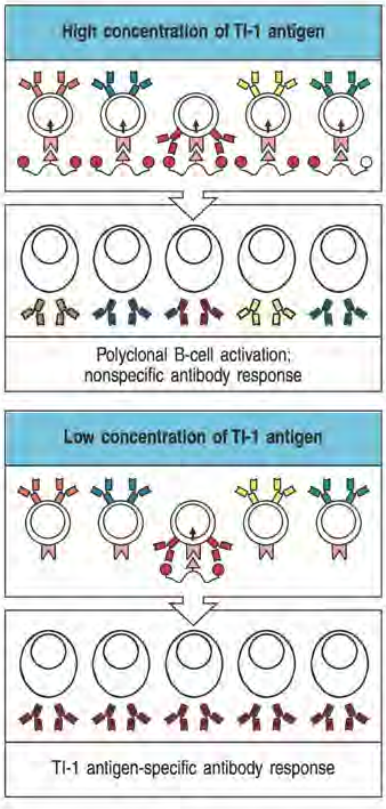


Fig. 10.18 TI-1 antigens induce polyclonal B-cell responses at high concentrations, and antigen-specific antibody responses at low concentrations. At high concentration, the signal delivered by the B-cell-activating moiety of TI-1 antigens is sufficient to induce proliferation and antibody secretion by B cells in the absence of specific antigen binding to surface immunoglobulin. Thus, all B cells respond (top panels). At low concentration, only B cells specific for the TI-1 antigen bind enough of it to focus its B-cell activating properties onto the B cell; this gives a specific antibody response to epitopes on the TI-1 antigen (lower panels).

Petitioner Merck, Ex. 1170, p. 148
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

that are 10^3–10^5 times lower than those used for polyclonal activation, only those B cells whose B-cell receptors specifically bind the TI-1 antigen become activated. At these low concentrations, amounts of TI-1 antigen sufficient for B-cell activation can only be concentrated on the B-cell surface with the aid of this specific binding (Fig. 10.18, bottom panels).

The small amounts of TI-1 antigens present during the early stages of an infection are therefore likely to activate antigen-specific B cells only and to induce antibodies specific for the TI-1 antigen. Such responses may be important in defense against several extracellular pathogens: they arise earlier than thymus-dependent responses because they do not require the previous priming and clonal expansion of helper T cells. However, TI-1 antigens are inefficient inducers of affinity maturation and memory B cells, both of which require antigen-specific T-cell help.

Responses to TI antigens can, however, be influenced by T cells and natural killer (NK) cells, if such cells become activated in the immune response. In particular, these cells secrete cytokines that can affect the isotype of the antibody secreted. iNKT cells (see Section 8-9) are particularly intriguing as cells that might influence the TI response to nonprotein antigens. Because the T-cell receptors on these cells recognize certain polysaccharides bound to unconventional MHC class I or class I-like molecules such as CD1 (see Section 6-19), they could become activated by the same TI antigens as those that are activating the B cell, and so provide B-cell help in a manner similar to linked recognition.

10-13 B-cell responses to bacterial polysaccharides do not require peptide-specific T-cell help.

The second class of thymus-independent antigens consists of molecules such as bacterial capsular polysaccharides that have highly repetitive structures. These thymus-independent antigens, called **TI-2 antigens**, contain no intrinsic B-cell-stimulating activity. Whereas TI-1 antigens can activate both immature and mature B cells, TI-2 antigens can activate only mature B cells; immature B cells, as we saw in Section 8-6, are inactivated by encounter with repetitive epitopes. Infants and young children up to about 5 years of age do not make fully effective antibody responses against polysaccharide antigens, and this might be because most of their B cells are immature.

Responses to several TI-2 antigens are made prominently by B-1 cells (also known as CD5 B cells), which comprise an autonomously replicating subpopulation of nonconventional B cells (see Sections 3-24 and 8-28), and by marginal zone B cells, another unique subset of nonrecirculating B cells that line the border of the splenic white pulp (see Section 8-28). Marginal zone B cells are rare at birth and accumulate with age; they might therefore be responsible for most physiological TI-2 responses, which increase in efficiency with age.

TI-2 antigens most probably act by simultaneously cross-linking a critical number of B-cell receptors on the surface of antigen-specific mature B cells (Fig. 10.19, left panels). There is also evidence that dendritic cells and macrophages provide co-stimulatory signals for the initial activation of B cells by TI-2 antigens, signals that are necessary for the survival of the antigen-specific B cell and its differentiation into a plasmablast secreting IgM. One of these co-stimulatory signals is the TNF-family cytokine BAFF, which is secreted by the dendritic cell and interacts with the receptor TACI on the B cell (Fig. 10.19, right panels).

Excessive cross-linking of B-cell receptors renders mature B cells unresponsive or anergic, just as it does immature B cells. Thus, the density of TI-2 antigen epitopes presented to the B cell is critical. If it is too low, receptor cross-linking is insufficient to activate the cell; if too high, the B cell becomes anergic.

Petitioner Merck, Ex. 1170, p. 149
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

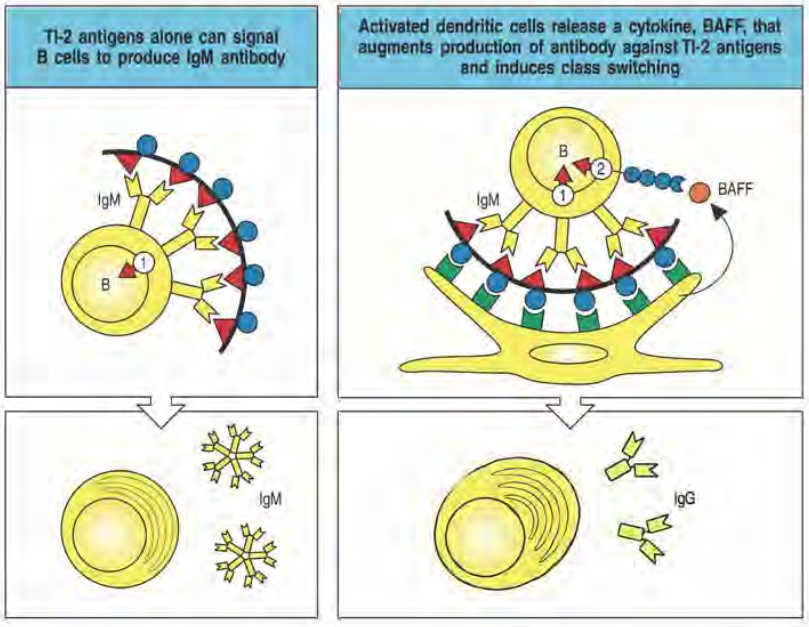


Fig. 10.19 B-cell activation by TI-2 requires, or is greatly enhanced by, cytokines. Multiple cross-linking of the B-cell receptor by TI-2 antigens can lead to IgM antibody production (left panels), but there is evidence that in addition, cytokines greatly augment these responses and lead to isotype switching as well (right panels). It is not clear where such cytokines are produced, but one possibility is that dendritic cells, which may be able to bind the antigen through innate immune system receptors on their surface and so present it to the B cells, secrete a soluble TNF-family cytokine called BAFF, which can activate class switching by the B cell.

B-cell responses to TI-2 antigens provide a prompt and specific response to an important class of pathogen—capsulated bacteria. Many common extracellular bacterial pathogens are surrounded by a polysaccharide capsule that enables them to resist ingestion by phagocytes. The bacteria not only escape direct destruction by phagocytes but also avoid stimulating T-cell responses in response to bacterial peptides presented by macrophages. IgM antibodies rapidly produced against the capsular polysaccharide independently of peptide-specific T-cell help will coat the bacteria, promoting their ingestion and destruction by phagocytes early in the infection.

As well as producing IgM, thymus-independent responses can include switching to certain other antibody classes, such as IgG3 in the mouse. This is probably the result of help from dendritic cells (see Fig. 10.19, right panels), which provide secreted cytokines such as BAFF and membrane-bound signals to proliferating plasmablasts as they respond to TI antigens.

Wiskott–Aldrich Syndrome

Not all antibodies against bacterial polysaccharides are produced strictly through this TI-2 mechanism. We mentioned earlier the importance of antibodies against the capsular polysaccharide of *Haemophilus influenzae* type b in protective immunity to this bacterium. The immunodeficiency disease Wiskott–Aldrich syndrome is caused by defects in T cells that impair their interaction with B cells (described in more detail in Chapter 13). Patients with Wiskott–Aldrich syndrome respond poorly to protein antigens, but, unexpectedly, also fail to make IgM and IgG antibody against polysaccharide antigens and are highly susceptible to infection with encapsulated bacteria such as *H. influenzae*. The failure to make IgM seems to be due in part to greatly reduced development of the marginal zone of the spleen, which contains B cells responsible for making much of the 'natural' IgM antibody against ubiquitous carbohydrate antigens. Thus, IgM and IgG antibodies induced by TI-2 antigens are likely to be an important part of the humoral immune response in many bacterial infections, and in humans at least, the production of class-switched antibodies to TI-2 antigens might normally rely on some degree of T-cell help. The distinguishing features of thymus-dependent, TI-1, and TI-2 antibody responses are summarized in Fig. 10.20.

Petitioner Merck, Ex. 1170, p. 150
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

	TD antigen	TI-1 antigen	TI-2 antigen
Antibody response in infants	Yes	Yes	No
Antibody production in congenitally athymic individual	No	Yes	Yes
Antibody response in absence of all T cells	No	Yes	No
Primes T cells	Yes	No	No
Polyclonal B-cell activation	No	Yes	No
Requires repeating epitopes	No	No	Yes
Examples of antigen	Diphtheria toxin Viral hemagglutinin Purified protein derivative (PPD) of *Mycobacterium tuberculosis*	Bacterial lipopoly-saccharide *Brucella abortus*	Pneumococcal polysaccharide *Salmonella* polymerized flagellin Dextran Hapten-conjugated Ficoll (polysucrose)

Fig. 10.20 Properties of different classes of antigen that elicit antibody responses.

Summary.

B-cell activation by many antigens requires both binding of the antigen by the B-cell surface immunoglobulin—the B-cell receptor—and interaction of the B cell with antigen-specific helper T cells. Helper T cells recognize peptide fragments derived from the antigen internalized by the B cell and displayed by the B cells as peptide:MHC class II complexes. Follicular helper T cells stimulate B cells by prolonged conjugation in germinal centers, with binding of CD40 ligand on the T cell to CD40 on the B cell, and by the directed release of cytokines. Activated B cells also provide signals to T cells, for example via B7-family molecules, that promote the T cells' continued activation. The initial interaction occurs at the border of the T-cell and B-cell areas of secondary lymphoid tissue, to which antigen-activated helper T cells and B cells migrate in response to chemokines. Further interactions between T cells and B cells continue after migration into the follicle and the formation of a germinal center.

Helper T cells induce a phase of vigorous B-cell proliferation in the germinal center reaction and direct the differentiation of clonally expanded B cells into either antibody-secreting plasma cells or memory B cells. The switching to different antibody isotypes is regulated by cytokines released from helper T cells. Somatic hypermutation and selection for high-affinity binding occur in the germinal centers. Helper T cells control these processes by selectively activating B cells that have retained their specificity for the antigen and by inducing proliferation and differentiation into plasma cells and memory B cells. Some nonprotein antigens stimulate B cells in the absence of linked recognition by peptide-specific helper T cells. Responses to these thymus-independent antigens are accompanied by only limited class switching and do not induce memory B cells. However, such responses have a crucial role in host defense against pathogens whose surface antigens cannot elicit peptide-specific T-cell responses.

Petitioner Merck, Ex. 1170, p. 151
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

The distributions and functions of immunoglobulin classes.

Extracellular pathogens can find their way to most sites in the body, and antibodies must be equally widely distributed to combat them. Most classes of antibodies are distributed by diffusion from their site of synthesis, but specialized transport mechanisms are required to deliver antibodies to epithelial surfaces lining the lumina of organs such as the lungs and intestine. The distribution of antibodies is determined by their heavy-chain isotype, which can limit their diffusion or enable them to engage specific transporters that deliver them across epithelia. In this part of the chapter we describe the mechanisms by which antibodies of different classes are directed to the compartments of the body in which their particular effector functions are appropriate, and discuss the protective functions of antibodies that result solely from their binding to pathogens. In the last part of the chapter we discuss the effector cells and molecules that are specifically engaged by different antibody classes.

10-14 Antibodies of different classes operate in distinct places and have distinct effector functions.

Pathogens most commonly enter the body across the epithelial barriers of the mucosa lining the respiratory, digestive, and urogenital tracts, or through damaged skin. Less often, insects, wounds, or hypodermic needles introduce microorganisms directly into the blood. Antibodies protect all the body's mucosal surfaces, tissues, and blood from such infections; these antibodies serve to neutralize the pathogen or promote its elimination before it can establish a significant infection. Antibodies of different classes are adapted to function in different compartments of the body. Because a given V region can become associated with any C region through class switching (see Section 10-9), the progeny of a single B cell can produce antibodies that share the same specificity yet provide all of the protective functions appropriate for each body compartment.

IgM antibodies are produced first in a humoral immune response, because they can be expressed without class switching (see Fig. 5.17). These early IgM antibodies tend to be of low affinity, being produced before B cells have undergone somatic hypermutation. However, IgM molecules form pentamers with 10 antigen-binding sites, conferring higher overall avidity when binding to multivalent antigens such as bacterial capsular polysaccharides, and so compensating for the low affinity of the IgM monomers. Because of the large size of the pentamers, IgM is found mainly in the bloodstream and, to a lesser extent, in the lymph, rather than in intercellular spaces within tissues. The pentameric structure of IgM makes it especially effective in activating the complement system, as we will see in the last part of this chapter. Infection of the bloodstream has serious consequences unless it is controlled quickly, and the rapid production of IgM and its efficient activation of the complement system are important in controlling such infections. Some IgM is produced in secondary and subsequent responses, and also from B cells that have not undergone class switching during somatic hypermutation, although other classes dominate the later phases of the antibody response. Much IgM is also produced by the nonconventional B-1 cells that reside in the peritoneal cavity and the pleural spaces and by the marginal zone B cells of the spleen. These cells secrete antibodies against commonly encountered carbohydrate antigens, including those of bacteria, and do not require T-cell help; they therefore provide a preformed repertoire of IgM antibodies in blood and body cavities that can recognize invading pathogens (see Sections 3-24 and 8-28).

Petitioner Merck, Ex. 1170, p. 152
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Antibodies of the other classes—IgG, IgA, and IgE—are smaller, and diffuse easily out of the blood into the tissues. IgA can form dimers, as we saw in Chapter 4, but IgG and IgE are always monomeric. The affinity of the individual antigen-binding sites for their antigen is therefore critical for the effectiveness of these antibodies, and most of the B cells expressing these classes have been selected in the germinal centers for their increased affinity for antigen after somatic hypermutation. IgG4 is the least abundant of the IgG subclasses, but has the unusual ability to form hybrid antibodies. One IgG4 heavy chain and attached light chain can split from the original heavy-chain dimer and reassociate with a different IgG4 heavy chain–light chain pair, forming a bivalent IgG4 antibody with two distinct antigen specificities.

IgG is the principal class of antibody in blood and extracellular fluid, whereas IgA is the principal class in secretions, the most important being those from the epithelia lining the intestinal and respiratory tracts. IgG efficiently opsonizes pathogens for engulfment by phagocytes and activates the complement system, but IgA is a less potent opsonin and a weak activator of complement. IgG operates mainly in the tissues, where accessory cells and molecules are available, whereas dimeric IgA operates mainly on epithelial surfaces, where complement and phagocytes are not normally present; therefore IgA functions chiefly as a neutralizing antibody. IgA is also produced by plasma cells that differentiate from class-switched B cells in lymph nodes and spleen, and acts as a neutralizing antibody in extracellular spaces and in the blood. This IgA is monomeric and is predominantly of the subclass IgA1; the ratio of IgA1 to IgA2 in the blood is 10:1. The IgA antibodies produced by plasma cells in the gut are dimeric and predominantly of subclass IgA2; the ratio of IgA2 to IgA1 in the gut is 3:2.

Finally, IgE antibody is present only at very low levels in blood or extracellular fluid, but is bound avidly by receptors on mast cells that are found just beneath the skin and mucosa and along blood vessels in connective tissue. Antigen binding to this cell-associated IgE triggers mast cells to release powerful chemical mediators that induce reactions, such as coughing, sneezing, and vomiting, that in turn can expel infectious agents, as discussed later in this chapter. The distribution and main functions of antibodies of the different classes are summarized in Fig. 10.21.

10-15 Transport proteins that bind to the Fc regions of antibodies carry particular isotypes across epithelial barriers.

In the mucosal immune system, IgA-secreting plasma cells are found predominantly in the lamina propria, which lies immediately below the basement membrane of many surface epithelia. From there the IgA antibodies can be transported across the epithelium to its external surface, for example to the lumen of the gut or of the bronchi (Fig. 10.22). IgA antibody synthesized in the lamina propria is secreted as a dimeric IgA molecule associated with a single J chain (see Fig. 5.19). This polymeric form of IgA binds specifically to a receptor called the **polymeric immunoglobulin receptor** (**pIgR**), which is present on the basolateral surfaces of the overlying epithelial cells. When the pIgR has bound a molecule of dimeric IgA, the complex is internalized and carried through the cytoplasm of the epithelial cell in a transport vesicle to its luminal surface. This process is called transcytosis. IgM also binds to the pIgR and can be secreted into the gut by the same mechanism. Upon reaching the luminal surface of the enterocyte, the antibody is released into the mucous layer covering the gut lining by proteolytic cleavage of the extracellular domain of the pIgR. The cleaved extracellular domain of the pIgR is known as secretory component (frequently abbreviated to SC) and remains associated with the antibody. Secretory component is bound to the part of the Fc region of IgA that contains the binding site for the Fcα receptor I, which

Petitioner Merck, Ex. 1170, p. 153
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Fig. 10.21 Each human immunoglobulin class has specialized functions and a unique distribution. The major effector functions of each class (+++) are shaded in dark red, whereas lesser functions (++) are shown in dark pink, and very minor functions (+) in pale pink. The distributions are marked similarly, with actual average levels in serum being shown in the bottom row. IgA has two subclasses, IgA1 and IgA2. The IgA column refers to both. *IgG2 can act as an opsonin in the presence of an Fc receptor of the appropriate allotype, found in about 50% of white people.

is why secretory IgA does not bind to this receptor. Secretory component serves several physiological roles. It binds to mucins in mucus, acting as 'glue' to bind secreted IgA to the mucous layer on the luminal surface of the gut epithelium, where the antibody binds and neutralizes gut pathogens and their toxins (see Fig. 10.22). Secretory component also protects the antibodies against cleavage by gut enzymes.

The principal sites of IgA synthesis and secretion are the gut, the respiratory epithelium, the lactating breast, and various other exocrine glands such as the salivary and tear glands. It is believed that the primary functional role of IgA antibodies is to protect epithelial surfaces from infectious agents, just as IgG antibodies protect the extracellular spaces inside tissues. By binding bacteria, virus particles, and toxins, IgA antibodies prevent the attachment of bacteria and viruses to epithelial cells and the uptake of toxins, and provide

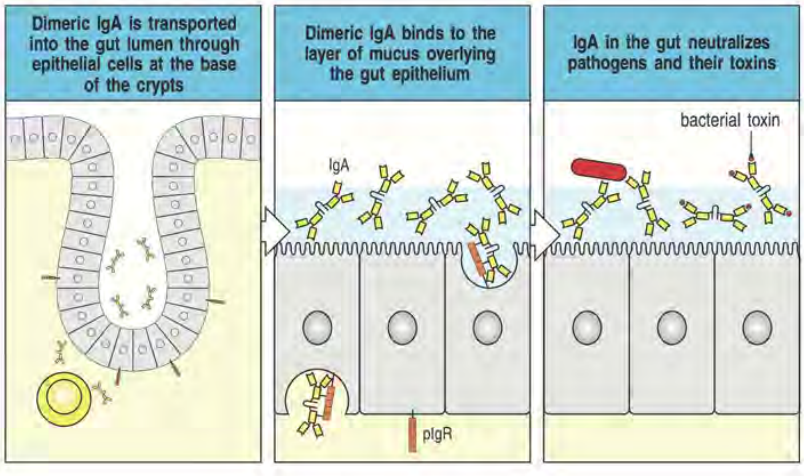


Fig. 10.22 The major class of antibody present in the lumen of the gut is secretory dimeric IgA. This is synthesized by plasma cells in the lamina propria and transported into the lumen of the gut through epithelial cells at the base of the crypts. Dimeric IgA binds to the layer of mucus overlying the gut epithelium and acts as an antigen-specific barrier to pathogens and toxins in the gut lumen.

Petitioner Merck, Ex. 1170, p. 154
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

the first line of defense against a wide variety of pathogens. IgA is also thought to have an additional role in the gut, that of regulating the gut microbiota (see Chapter 12).

Newborn infants are especially vulnerable to infection, having had no previous exposure to the microbes in the environment they enter at birth. IgA antibodies are secreted in breast milk and are thereby transferred to the gut of the newborn infant, where they provide protection from newly encountered bacteria until the infant can synthesize its own protective antibody. IgA is not the only protective antibody that a mother passes on to her baby. Maternal IgG is transported across the placenta directly into the bloodstream of the fetus during intrauterine life; human babies at birth have as high levels of plasma IgG as their mothers, and with the same range of antigen specificities. The selective transport of IgG from mother to fetus is due to an IgG transport protein in the placenta, **FcRn** (**neonatal Fc receptor**), which is closely related in structure to MHC class I molecules. Despite this similarity, FcRn binds IgG quite differently from the binding of peptide to MHC class I molecules, because its peptide-binding groove is occluded. It binds to the Fc portion of IgG molecules (Fig. 10.23). Two molecules of FcRn bind one molecule of IgG, bearing it across the placenta. In some rodents, FcRn also delivers IgG to the circulation of the neonate from the gut lumen. Maternal IgG is also ingested by the newborn animal in its mother's milk and colostrum, the protein-rich fluid secreted by the early postnatal mammary gland. In this case, FcRn transports the IgG from the lumen of the neonatal gut into the blood and tissues. Interestingly, FcRn is also found in adults in the gut and liver and on endothelial cells. Its function in adults is to maintain the levels of IgG in plasma, which it does by binding antibody, endocytosing it, and recycling it to the blood, thus preventing its excretion from the body.

By means of these specialized transport systems, mammals are supplied from birth with antibodies against pathogens common in their environments. As they mature and make their own antibodies of all isotypes, these are distributed selectively to different sites in the body (Fig. 10.24). Thus, throughout life, class switching and the distribution of antibody classes throughout the body provide effective protection against infection in extracellular spaces.

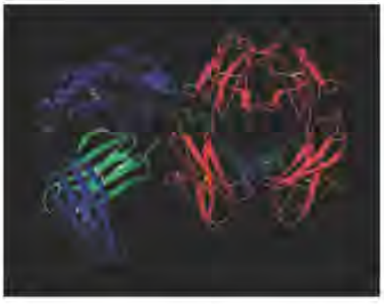


Fig. 10.23 The neonatal Fc receptor (FcRn) binds to the Fc portion of IgG. The structure of a molecule of FcRn (blue and green) is shown bound to one chain of the Fc portion of IgG (red), at the interface of the $C_{\gamma 2}$ and $C_{\gamma 3}$ domains, with the $C_{\gamma 2}$ region at the top. The β_2-microglobulin component of the FcRn is green. The dark-blue structure attached to the Fc portion of IgG is a carbohydrate chain, reflecting glycosylation. FcRn transports IgG molecules across the placenta in humans and also across the gut in rats and mice. It also has a role in maintaining the levels of IgG in adults. Although only one molecule of FcRn is shown binding to the Fc portion, it is thought that it takes two molecules of FcRn to capture one molecule of IgG. Courtesy of P. Björkman.

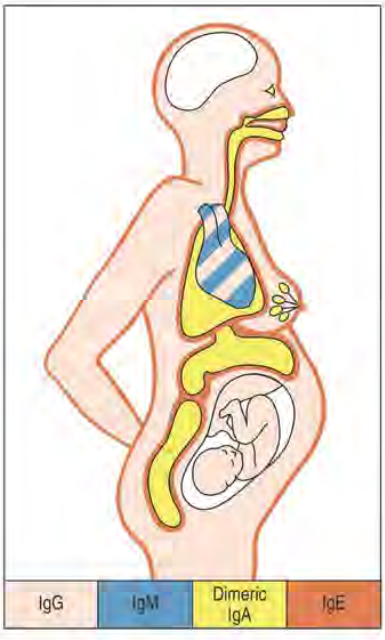


Fig. 10.24 Immunoglobulin classes are selectively distributed in the body. IgG and IgM predominate in blood (shown here for simplicity by IgM and IgG in the heart), whereas IgG and monomeric IgA are the major antibodies in extracellular fluid within the body. Dimeric IgA predominates in secretions across epithelia, including breast milk. The fetus receives IgG from the mother by transplacental transport. IgE is found mainly associated with mast cells just beneath epithelial surfaces (especially of the respiratory tract, gastrointestinal tract, and skin). The brain is normally devoid of immunoglobulin.

Petitioner Merck, Ex. 1170, p. 155
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

10-16 High-affinity IgG and IgA antibodies can neutralize bacterial toxins.

Many bacteria cause disease by secreting proteins called toxins, which damage or disrupt the function of the host's cells (Fig. 10.25). To have an effect, a toxin must interact specifically with a molecule that serves as a receptor on the surface of the target cell. In many toxins the receptor-binding domain is on one polypeptide chain but the toxic function is carried by a second chain. Antibodies that bind to the receptor-binding site on the toxin molecule can prevent the toxin from binding to the cell and thus protect the cell from attack (Fig. 10.26). Antibodies that act in this way to neutralize toxins are referred to as neutralizing antibodies.

Most toxins are active at nanomolar concentrations: a single molecule of diphtheria toxin can kill a cell. To neutralize toxins, therefore, antibodies must be able to diffuse into the tissues and bind the toxin rapidly and with high affinity. The ability of IgG antibodies to diffuse easily throughout the extracellular fluid, and their high affinity for antigen once affinity maturation has taken place, make them the principal antibodies that neutralize toxins in tissues. High-affinity IgA antibodies similarly neutralize toxins at the mucosal surfaces of the body.

Diphtheria and tetanus toxins are two bacterial toxins in which the toxic and receptor-binding functions are on separate protein chains. It is therefore possible to immunize individuals, usually as infants, with modified toxin molecules in which the toxic chain has been denatured. These modified toxins,

Fig. 10.25 Many common diseases are caused by bacterial toxins. These toxins are all exotoxins—proteins secreted by the bacteria. High-affinity IgG and IgA antibodies protect against these toxins. Bacteria also have nonsecreted endotoxins, such as lipopolysaccharide, which are released when the bacterium dies. The endotoxins are also important in the pathogenesis of disease, but there the host response is more complex because the innate immune system has receptors for some endotoxins (see Chapter 3).

Disease	Organism	Toxin	Effects *in vivo*
Tetanus	*Clostridium tetani*	Tetanus toxin	Blocks inhibitory neuron action, leading to chronic muscle contraction
Diphtheria	*Corynebacterium diphtheriae*	Diphtheria toxin	Inhibits protein synthesis, leading to epithelial cell damage and myocarditis
Gas gangrene	*Clostridium perfringens*	Clostridial toxin	Phospholipase activation, leading to cell death
Cholera	*Vibrio cholerae*	Cholera toxin	Activates adenylate cyclase, elevates cAMP in cells, leading to changes in intestinal epithelial cells that cause loss of water and electrolytes
Anthrax	*Bacillus anthracis*	Anthrax toxic complex	Increases vascular permeability, leading to edema, hemorrhage, and circulatory collapse
Botulism	*Clostridium botulinum*	Botulinum toxin	Blocks release of acetylcholine, leading to paralysis
Whooping cough	*Bordetella pertussis*	Pertussis toxin	ADP-ribosylation of G proteins, leading to lymphoproliferation
		Tracheal cytotoxin	Inhibits cilia and causes epithelial cell loss
Scarlet fever	*Streptococcus pyogenes*	Erythrogenic toxin	Vasodilation, leading to scarlet fever rash
		Leukocidin Streptolysins	Kill phagocytes, allowing bacterial survival
Food poisoning	*Staphylococcus aureus*	Staphylococcal enterotoxin	Acts on intestinal neurons to induce vomiting. Also a potent T-cell mitogen (SE superantigen)
Toxic-shock syndrome	*Staphylococcus aureus*	Toxic-shock syndrome toxin	Causes hypotension and skin loss. Also a potent T-cell mitogen (TSST-1 superantigen)

Petitioner Merck, Ex. 1170, p. 156
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

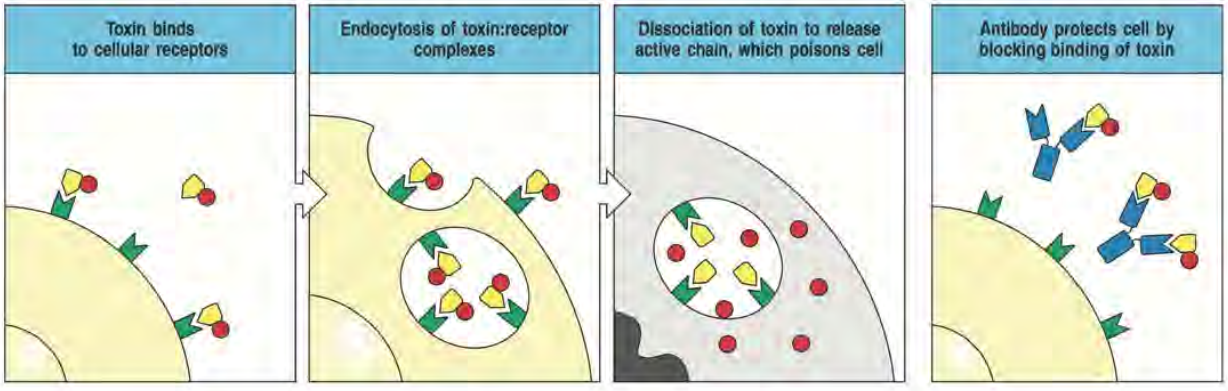


Fig. 10.26 Neutralization of toxins by IgG antibodies protects cells from their damaging action. The damaging effects of many bacteria are due to the toxins they produce (see Fig. 10.25). These toxins are usually composed of several distinct moieties. One part of the toxin molecule binds a cell-surface receptor, which enables the molecule to be internalized. Another part of the toxin molecule then enters the cytoplasm and poisons the cell. Antibodies that inhibit toxin binding can prevent, or neutralize, these effects.

called toxoids, lack toxic activity but retain the receptor-binding site. Thus, immunization with the toxoid induces neutralizing antibodies that protect against the native toxin.

Some insect or animal venoms are so toxic that a single exposure can cause severe tissue damage or death, and for these the adaptive immune response is too slow to be protective. Exposure to these venoms is a rare event, and protective vaccines have not been developed for use in humans. Instead, neutralizing antibodies are generated by immunizing other species, such as horses, with insect and snake venoms to produce anti-venom antibodies (antivenins). The antivenins are injected into exposed individuals to protect them against the toxic effects of the venom. Transfer of antibodies in this way is known as passive immunization (see Appendix I, Section A-36).

10-17 High-affinity IgG and IgA antibodies can inhibit the infectivity of viruses.

Animal viruses infect cells by binding to a particular cell-surface receptor, often a cell-type-specific protein that determines which cells they can infect. The hemagglutinin of influenza virus, for example, binds to terminal sialic acid residues on the carbohydrates of glycoproteins present on epithelial cells of the respiratory tract. It is known as hemagglutinin because it recognizes and binds to similar sialic acid residues on chicken red blood cells and agglutinates these red blood cells. Antibodies against the hemagglutinin can prevent infection by the influenza virus. Such antibodies are called virus-neutralizing antibodies and, as with the neutralization of toxins, high-affinity IgA and IgG antibodies are particularly important.

Many antibodies that neutralize viruses do so by directly blocking the binding of virus to surface receptors (Fig. 10.27). However, viruses are sometimes successfully neutralized when only a single molecule of antibody is bound to a virus particle that has many receptor-binding sites on its surface. In these cases, the antibody must cause some change in the virus that disrupts its structure. This might prevent it from interacting with its receptor, or it might interfere with the fusion of the virus with the plasma membrane after the virus has engaged its receptor.

10-18 Antibodies can block the adherence of bacteria to host cells.

Many bacteria have cell-surface molecules called **adhesins** that enable them to bind to the surfaces of host cells. This adherence is crucial to the ability of these bacteria to cause disease, whether they subsequently enter the cell, as do *Salmonella* species, or remain attached to the cell surface as extracellular

Petitioner Merck, Ex. 1170, p. 157
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

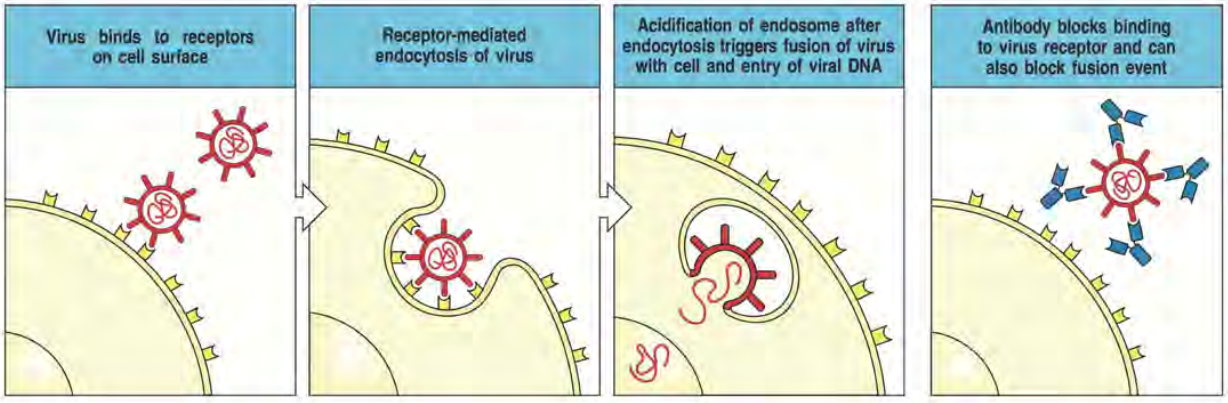
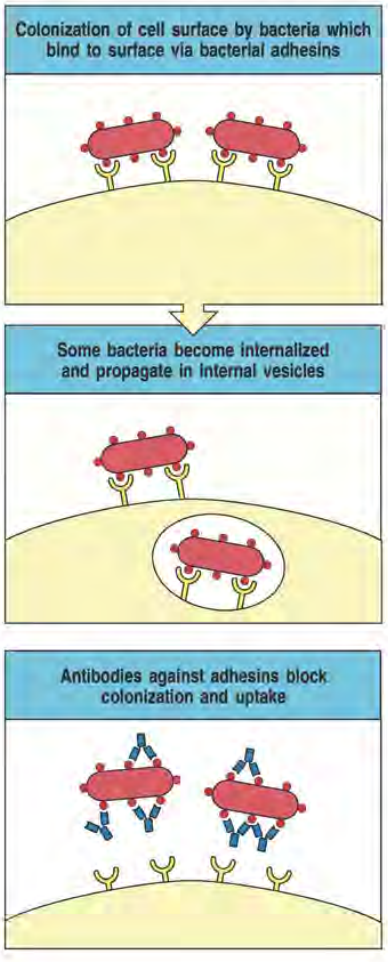


Fig. 10.27 Viral infection of cells can be blocked by neutralizing antibodies. For a virus to multiply within a cell, it must introduce its genes into the cell. The first step in entry is usually the binding of the virus to a receptor on the cell surface. For enveloped viruses, as shown in the figure, entry into the cytoplasm requires fusion of the viral envelope and the cell membrane. For some viruses this fusion event takes place on the cell surface (not shown); for others it can occur only within the more acidic environment of endosomes, as shown here. Non-enveloped viruses must also bind to receptors on cell surfaces, but they enter the cytoplasm by disrupting endosomes. Antibodies bound to viral surface proteins neutralize the virus, inhibiting either its initial binding to the cell or its subsequent entry.



pathogens (Fig. 10.28). *Neisseria gonorrhoeae*, the causative agent of the sexually transmitted disease gonorrhea, has a cell-surface protein known as pilin, which enables the bacterium to adhere to the epithelial cells of the urinary and reproductive tracts and is essential to its infectivity. Antibodies against pilin can inhibit this adhesive reaction and prevent infection.

IgA antibodies secreted onto the mucosal surfaces of the intestinal, respiratory, and reproductive tracts are particularly important in inhibiting the colonization of these surfaces by pathogens and in preventing infection of the epithelial cells. Adhesion of bacteria to cells within tissues can also contribute to pathogenesis, and IgG antibodies against adhesins protect tissues from damage in much the same way as IgA antibodies protect mucosal surfaces.

10-19 Antibody:antigen complexes activate the classical pathway of complement by binding to C1q.

Another way in which antibodies can protect against infection is by activation of complement. We described the complement system in Chapter 2 because it is first activated in the absence of antibody as part of an innate immune response. Complement activation proceeds via a cascade of proteolytic cleavage reactions, in which inactive complement proteins in the plasma are cleaved to form proteases that then cleave and activate the next enzyme in the series. Three pathways of complement activation converge to coat pathogen surfaces or antigen:antibody complexes with covalently attached complement fragment C3b, which acts as an opsonin to promote uptake and removal by phagocytes. In addition, the terminal complement components can form a membrane-attack complex that damages some bacteria.

Fig. 10.28 Antibodies can prevent the attachment of bacteria to cell surfaces. Many bacterial infections require an interaction between the bacterium and a cell-surface receptor. This is particularly true for infections of mucosal surfaces. The attachment process involves very specific molecular interactions between bacterial adhesins and their receptors on host cells; antibodies against bacterial adhesins can block such infections.

Petitioner Merck, Ex. 1170, p. 158
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Antibodies initiate complement activation by a pathway known as the classical pathway because it was the first pathway of complement activation to be discovered. The full details of this pathway, and of the other two known pathways of complement activation, are given in Chapter 2. There we focused on how the classical pathway can be activated in innate immunity in the absence of specific antibody, but here we describe how antibody formed in the adaptive immune response initiates the classical pathway.

The first component of the classical pathway of complement activation is C1, which is a complex of three proteins called C1q, C1r, and C1s (see Fig. 2.17). Recall that C1r and C1s are serine proteases, and two molecules each of C1r and C1s are bound to each molecule of C1q. Complement activation is initiated when antibodies attached to the surface of a pathogen bind C1 via C1q (Fig. 10.29). C1q can be bound by either IgM or IgG antibodies but, because of the structural requirements of binding to C1q, neither of these antibody classes can activate complement in solution; the complement reactions are initiated only when the antibodies are already bound to multiple sites on a cell surface, normally that of a pathogen.

Each globular head of a C1q molecule can bind to one Fc region, and binding of two or more heads activates the C1 complex. In plasma, the pentameric IgM molecule has a planar conformation that does not bind C1q (Fig. 10.30, left panel); however, binding to the surface of a pathogen deforms the IgM pentamer so that it looks like a staple (Fig. 10.30, right panel), and this distortion exposes binding sites for the C1q heads. Although C1q binds with low affinity to some subclasses of IgG in solution, the binding energy required for C1q activation is achieved only when a single molecule of C1q can bind two or more IgG molecules that are held within 30–40 nm of each other as a result of binding antigen. This requires that multiple molecules of IgG be bound to a single pathogen or to an antigen in solution. For this reason, IgM is much more efficient than IgG in activating complement. The binding of

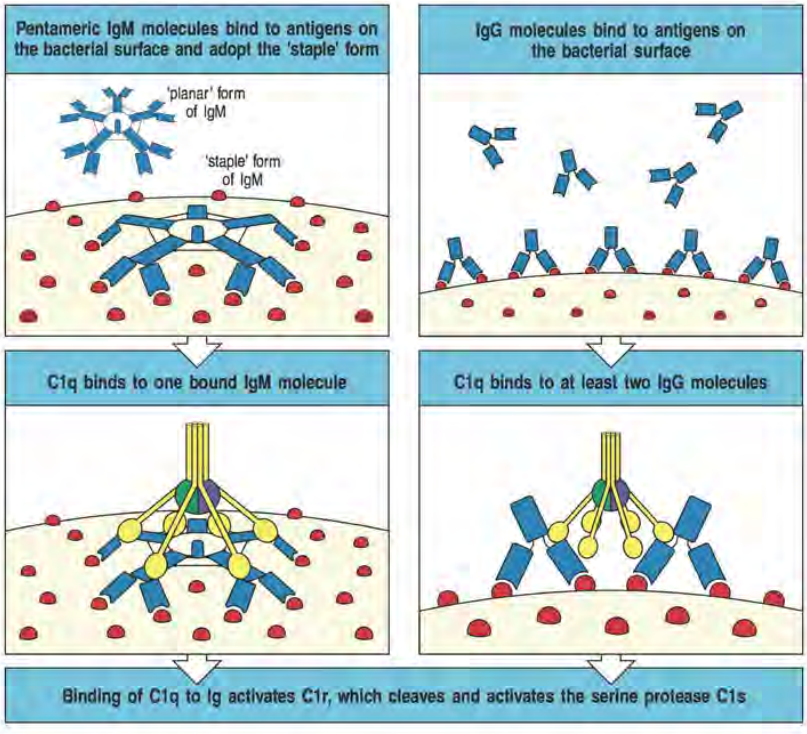


Fig. 10.29 The classical pathway of complement activation is initiated by the binding of C1q to antibody on a pathogen surface. When a molecule of IgM binds several identical epitopes on a pathogen surface, it is bent into the 'staple' conformation, which allows the globular heads of C1q to bind to its Fc pieces (left panels). Multiple molecules of IgG bound on the surface of a pathogen allow the binding of a single molecule of C1q to two or more Fc pieces (right panels). In both cases, the binding of C1q activates the associated C1r, which becomes an active enzyme that cleaves the pro-enzyme C1s, generating a serine protease that initiates the classical complement cascade (see Chapter 2).

Petitioner Merck, Ex. 1170, p. 159
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

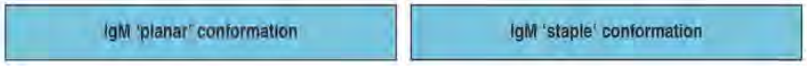


Fig. 10.30 The two conformations of IgM. The left panel shows the planar conformation of soluble IgM; the right panel shows the 'staple' conformation of IgM bound to a bacterial flagellum. Photographs (×760,000) courtesy of K.H. Roux.

C1q to a single bound IgM molecule, or to two or more bound IgG molecules (see Fig. 10.29), leads to activation of the protease activity of C1r, triggering the complement cascade (see Section 2-7 for a full description of the subsequent reactions). This translates antibody binding into the activation of the complement cascade.

10-20 Complement receptors are important in the removal of immune complexes from the circulation.

Many small soluble antigens form antibody:antigen complexes (immune complexes) that contain too few molecules of IgG to be efficiently bound by the Fcγ receptors that we discuss in the next part of the chapter. These antigens include toxins bound by neutralizing antibodies, and debris from dead host cells and microorganisms. Such immune complexes are found after most infections and are removed from the circulation through the action of complement. The soluble immune complexes trigger their own removal by activating complement, again through the binding of C1q, leading to the covalent binding of the activated complement fragments C4b and C3b to the complex, which is then cleared from the circulation by the binding of C4b and C3b to complement receptor 1 (CR1) on the surface of erythrocytes (see Section 2-13 for a description of the different types of complement receptors). The erythrocytes transport the bound complexes of antigen, antibody, and complement to the liver and spleen. Here, macrophages bearing CR1 and Fc receptors remove the complexes from the erythrocyte surface without destroying the cell, and then degrade them (Fig. 10.31). Even larger aggregates of particulate antigen, such as bacteria, viruses, and cell debris, can be coated with complement, picked up by erythrocytes, and transported to the spleen for destruction.

Complement-coated immune complexes that are not removed from the circulation tend to deposit in the basement membranes of small blood vessels, most notably those of the renal glomerulus, where the blood is filtered to form urine. Immune complexes that pass through the basement membrane of the glomerulus bind to CR1 present on the renal podocytes, cells that lie beneath the basement membrane. The functional significance of these receptors in the kidney is unknown; however, they have an important role in the pathology of some autoimmune diseases.

In the autoimmune disease systemic lupus erythematosus, which we describe in Chapter 15, excessive levels of circulating immune complexes lead to their deposition in large amounts on the podocytes, damaging the glomerulus; kidney failure is the principal danger in this disease. Antigen:antibody complexes can also be a cause of pathology in patients with deficiencies in the

Petitioner Merck, Ex. 1170, p. 160
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

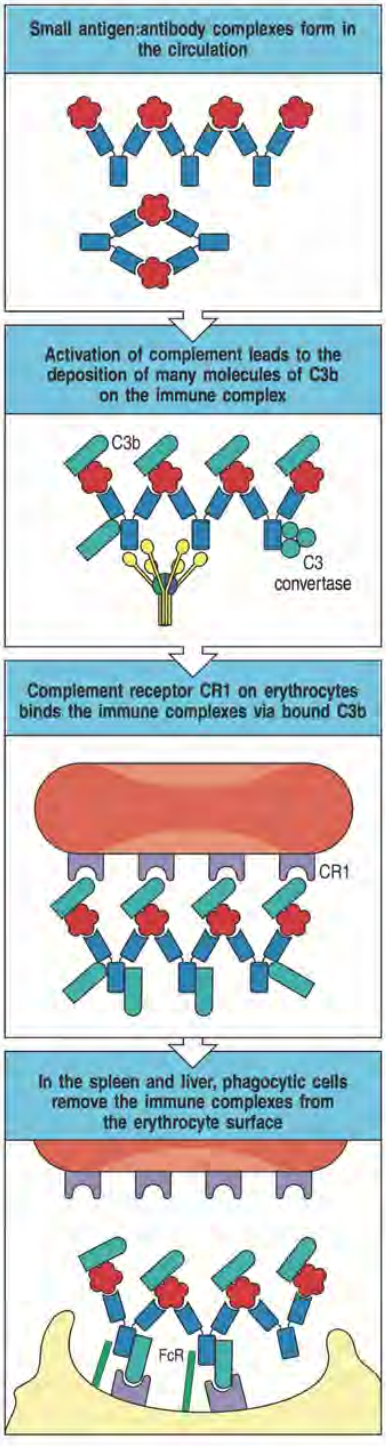


Fig. 10.31 Erythrocyte CR1 helps to clear immune complexes from the circulation. CR1 on the erythrocyte surface has an important role in the clearance of immune complexes from the circulation. Immune complexes bind to CR1 on erythrocytes, which transport them to the liver and spleen, where they are removed by macrophages expressing receptors for both Fc and bound complement components.

early components of complement (C1, C2, and C4). The classical complement pathway is not activated properly, and immune complexes are not cleared effectively because they do not become tagged with complement. These patients also suffer tissue damage as a result of immune-complex deposition, especially in the kidneys.

Summary.

The T-cell dependent antibody response begins with IgM secretion but quickly progresses to the production of additional antibody classes. Each class is specialized both in its localization in the body and in the functions it can perform. IgM antibodies are found mainly in blood; they are pentameric in structure. IgM is specialized to activate complement efficiently upon binding antigen and to compensate for the low affinity of a typical IgM antigen-binding site. IgG antibodies are usually of higher affinity and are found in blood and in extracellular fluid, where they can neutralize toxins, viruses, and bacteria, opsonize them for phagocytosis, and activate the complement system. IgA antibodies are synthesized as monomers, which enter blood and extracellular fluids, or as dimeric molecules by plasma cells in the lamina propria of various mucosal tissues. IgA dimers are selectively transported across the epithelial layer into sites such as the lumen of the gut, where they neutralize toxins and viruses and block the entry of bacteria across the intestinal epithelium. Most IgE antibody is bound to the surface of mast cells that reside mainly just below body surfaces; antigen binding to this IgE triggers local defense reactions. Antibodies can defend the body against extracellular pathogens and their toxic products in several ways. The simplest is by direct interactions with pathogens or their products, for example by binding to the active sites of toxins and neutralizing them or by blocking their ability to bind to host cells through specific receptors. When antibodies of the appropriate isotype bind to antigens, they can activate the classical pathway of complement, which leads to the elimination of the pathogen by the various mechanisms described in Chapter 2. Soluble immune complexes of antigen and antibody also fix complement and are cleared from the circulation via complement receptors on red blood cells.

The destruction of antibody-coated pathogens via Fc receptors.

The neutralization of toxins, viruses, or bacteria by high-affinity antibodies can protect against infection but does not, on its own, solve the problem of how to remove the pathogens and their products from the body. Moreover, many pathogens cannot be neutralized by antibody and must be destroyed by other means. Many pathogen-specific antibodies do not bind to neutralizing targets on pathogen surfaces and thus need to be linked to other effector mechanisms to play their part in host defense. We have already seen how the binding of antibody to antigen can activate complement. Another important defense mechanism is the activation of a variety of **accessory effector**

Petitioner Merck, Ex. 1170, p. 161
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

cells bearing receptors called **Fc receptors** because they are specific for the Fc portion of antibodies. These receptors facilitate the phagocytosis of antibody-bound extracellular pathogens by macrophages, dendritic cells, and neutrophils. Other, nonphagocytic, cells of the immune system—NK cells, eosinophils, basophils, and mast cells (see Fig. 1.4)—are triggered to secrete stored mediators when their Fc receptors are engaged by antibody-coated pathogens. These mechanisms maximize the effectiveness of all antibodies regardless of where they bind.

10-21 The Fc receptors of accessory cells are signaling receptors specific for immunoglobulins of different classes.

The Fc receptors are a family of cell-surface molecules that bind the Fc portion of immunoglobulins. Each member of the family recognizes immunoglobulin of one or a few closely related heavy-chain isotypes through a recognition domain on the α chain of the Fc receptor. Most Fc receptors are themselves members of the immunoglobulin gene superfamily. Different cell types bear different sets of Fc receptors, and the isotype of the antibody thus determines which types of cells will be engaged in a given response. The different Fc receptors, the cells that express them, and their specificities for different antibody classes are shown in Fig. 10.32.

Most Fc receptors function as part of a multisubunit complex. Only the α chain is required for antibody recognition; the other chains are required for transport of the receptor to the cell surface and for signal transduction when an Fc region is bound. Some Fcγ receptors, the Fcα receptor I, and the high-affinity receptor for IgE (FcεRI) all use a γ chain for signaling. This chain, which is closely related to the ζ chain of the T-cell receptor complex (see Section 7-7), associates noncovalently with the Fc-binding α chain. The human FcγRII-A is a single-chain receptor in which the cytoplasmic domain of the α chain replaces the function of the γ chain. FcγRII-B1 and FcγRII-B2 are also single-chain receptors but function as inhibitory receptors because

Fig. 10.32 Distinct receptors for the Fc region of the different immunoglobulin classes are expressed on different accessory cells. The subunit structure and binding properties of these receptors and the cell types expressing them are shown. The exact chain composition of any receptor can vary from one cell type to another. For example, FcγRIII in neutrophils is expressed as a molecule with a glycosylphosphatidylinositol membrane anchor, without γ chains, whereas in NK cells it is a transmembrane molecule associated with γ chains. The FcγRII-B1 differs from the FcγRII-B2 by the presence of an additional exon in the intracellular region. This exon prevents the FcγRII-B1 from being internalized after cross-linking. The binding affinities are taken from data on human receptors. *Only some allotypes of FcγRII-A bind IgG2. †In eosinophils, the molecular weight of the CD89α chain is 70–100 kDa. All are immunoglobulin superfamily members except FcεRII, which is a lectin and can form trimers.

Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)	FcεRI	FcεRII (CD23)	FcαRI (CD89)	Fcα/μR
Structure	α 72 kDa, γ	α 40 kDa, γ-like domain	ITIM	ITIM	α 50–70 kDa or γ, γ or ζ	α 45 kDa, β 33 kDa, γ 9 kDa	lectin domain, trimer, N	α 55–75 kDa, γ 9 kDa	α 70 kDa
Binding	IgG1	IgG1	IgG1	IgG1	IgG1	IgE	IgE	IgA1, IgA2	IgA, IgM
Order of affinity	$10^8\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	$2 \times 10^6\ M^{-1}$ 1) IgG1 2) IgG3=IgG2* 3) IgG4	$2 \times 10^6\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	$2 \times 10^6\ M^{-1}$ 1) IgG1=IgG3 2) IgG4 3) IgG2	$5 \times 10^5\ M^{-1}$ IgG1=IgG3	$10^{10}\ M^{-1}$	$2–7 \times 10^7\ M^{-1}$ (trimer) $2–7 \times 10^6\ M^{-1}$ (monomer)	$10^7\ M^{-1}$ IgA1=IgA2	$3 \times 10^9\ M^{-1}$ 1) IgM 2) IgA
Cell type	Macrophages Neutrophils Eosinophils Dendritic cells	Macrophages Neutrophils Eosinophils Platelets Langerhans cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells	Mast cells Basophils	Eosinophils B cells	Macrophages Eosinophils† Neutrophils	Macrophages B cells
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)	Secretion of granules	Degranulation	Uptake Induction of killing	Uptake

Petitioner Merck, Ex. 1170, p. 162
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

they contain an ITIM that engages the inositol 5′-phosphatase SHIP (see Section 7-18). The most prominent function of Fc receptors is the activation of accessory cells to attack pathogens, but they also contribute in other ways to immune responses. For example, FcγRII-B receptors negatively regulate the activities of B cells, mast cells, macrophages, and neutrophils by adjusting the threshold at which immune complexes will activate these cells. Fc receptors expressed by dendritic cells enable them to ingest antigen:antibody complexes efficiently and so be able to process these antigens and present their peptides to T cells.

10-22 Fc receptors on phagocytes are activated by antibodies bound to the surface of pathogens and enable the phagocytes to ingest and destroy pathogens.

The most important Fc-bearing cells in humoral immune responses are the phagocytic cells of the monocytic and myelocytic lineages, particularly macrophages and neutrophils. Many bacteria are directly recognized, ingested, and destroyed by phagocytes, and these bacteria are not pathogenic in normal individuals. Bacterial pathogens with polysaccharide capsules resist direct engulfment by phagocytes, however, and such bacteria only become susceptible to phagocytosis when they are coated with antibodies and complement that engage the Fcγ or Fcα receptors and the complement receptor CR1 on phagocytic cells, triggering bacterial uptake (Fig. 10.33). The stimulation of phagocytosis by complement-coated antigens binding to complement receptors is particularly important early in the immune response, before isotype-switched antibodies have been made. Capsular polysaccharides belong to the TI-2 class of thymus-independent antigens (see Section 10-13) and can therefore stimulate the early production of IgM antibodies, which are very effective at activating the complement system. IgM binding to encapsulated bacteria thus triggers the opsonization of these bacteria by complement and their prompt ingestion and destruction by phagocytes bearing complement receptors. Recently, Fcα/μR was discovered as a receptor that binds both IgA and IgM. Fcα/μR is expressed primarily on macrophages and B cells in the lamina propria of the intestine and in germinal centers. It is thought to have a role in the endocytosis of IgM antibody complexed with bacteria such as *Staphylococcus aureus*.

Phagocyte activation can initiate an inflammatory response that causes tissue damage, and so Fc receptors on phagocytes must be able to distinguish antibody molecules bound to a pathogen from the much larger number of free antibody molecules that are not bound to anything. This distinction is made possible by the aggregation of antibodies that occurs when they bind

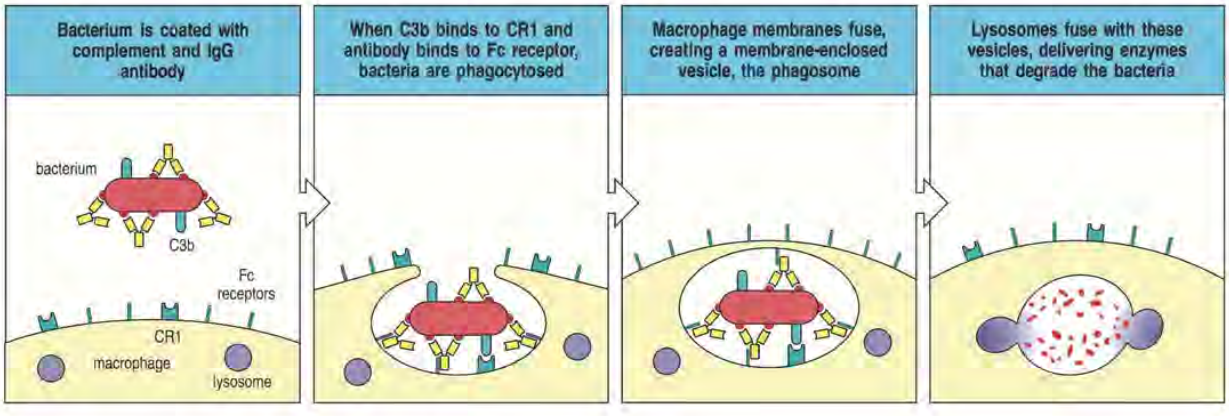


Fig. 10.33 Fc and complement receptors on phagocytes trigger the uptake and degradation of antibody-coated bacteria. Many bacteria resist phagocytosis by macrophages and neutrophils. Antibodies bound to these bacteria, however, enable them to be ingested and degraded through the interaction of the multiple Fc domains arrayed on the bacterial surface with Fc receptors on the phagocyte surface. Antibody coating also induces activation of the complement system and the binding of complement components to the bacterial surface. These can interact with complement receptors (for example CR1) on the phagocyte. Fc receptors and complement receptors synergize in inducing phagocytosis. Bacteria coated with IgG antibody and complement are therefore more readily ingested than those coated with IgG alone. Binding of Fc and complement receptors signals the phagocyte to increase the rate of phagocytosis, to fuse lysosomes with phagosomes, and to increase its bactericidal activity.

Petitioner Merck, Ex. 1170, p. 163
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

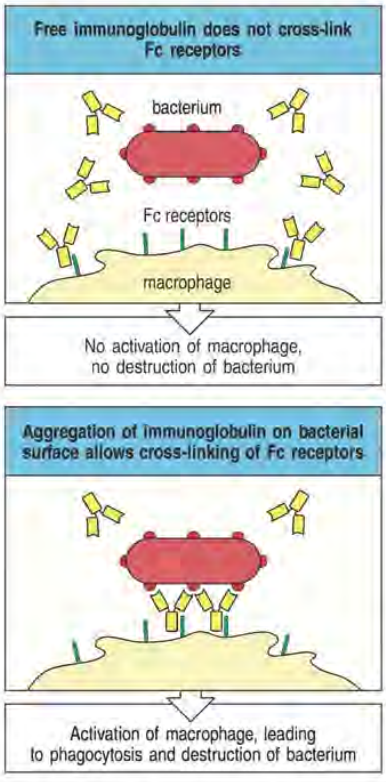


Fig. 10.34 Bound antibody is distinguishable from free immunoglobulin by its state of aggregation. Free immunoglobulin molecules bind most Fc receptors with very low affinity and cannot cross-link Fc receptors. Antigen-bound immunoglobulin, however, binds to Fc receptors with high avidity because several antibody molecules that are bound to the same surface bind to multiple Fc receptors on the surface of the accessory cell. This Fc receptor cross-linking sends a signal to activate the cell bearing it. With Fc receptors that have ITIMs, the result is inhibition.

to multimeric antigens or to multivalent particulate antigens such as viruses and bacteria. Individual Fc receptors on a cell surface bind monomers of free antibody with low affinity, but when presented with an antibody-coated particle, the simultaneous binding by multiple Fc receptors results in binding of high avidity, and this is the principal mechanism by which bound antibodies are distinguished from free immunoglobulin (Fig. 10.34). The result is that Fc receptors enable cells to detect pathogens via the antibody molecules bound to them. Fc receptors therefore give phagocytic cells that lack intrinsic specificity the ability to identify and remove pathogens and their products from the extracellular spaces.

Phagocytosis is greatly enhanced by interactions between the molecules coating an opsonized microorganism and receptors on the phagocyte surface. When an antibody-coated pathogen binds to Fcγ receptors, for example, the cell surface extends around the surface of the particle through successive binding of the Fcγ receptors to the antibody Fc regions bound to the pathogen. This is an active process that is triggered by the stimulation of the Fcγ receptors. Phagocytosis leads to enclosure of the particle in an acidified cytoplasmic vesicle—the phagosome. This then fuses with one or more lysosomes to generate a phagolysosome; lysosomal enzymes are released into the vesicle interior, where they destroy the bacterium (see Fig. 10.33). The process of intracellular killing by phagocytes is described in more detail in Chapter 2.

Some particles are too large for a phagocyte to ingest; parasitic worms are one example. In this case the phagocyte attaches to the surface of the antibody-coated parasite via its Fcγ, Fcα, or Fcε receptors, and the contents of the secretory granules or lysosomes of the phagocyte are released by exocytosis. The contents are discharged directly onto the surface of the parasite and damage it. Thus, stimulation of Fcγ and Fcα receptors can trigger either the internalization of external particles by phagocytosis or the externalization of internal vesicles by exocytosis. The principal leukocytes involved in the destruction of bacteria are macrophages and neutrophils, but large parasites such as helminths are more usually attacked by eosinophils (Fig. 10.35), nonphagocytic cells that can bind antibody-coated parasites via several different Fc receptors, including the low-affinity Fcε receptor for IgE, CD23 (Fig. 10.32). Cross-linking of these receptors by antibody-coated surfaces activates the eosinophil to release its granule contents, which include proteins toxic to parasites (see Fig. 14.13). Cross-linking by antigen of IgE bound to the high-affinity FcεRI on mast cells and basophils also results in exocytosis of their granule contents, as we describe later in this chapter.

10-23 Fc receptors activate NK cells to destroy antibody-coated targets.

Virus-infected cells are usually destroyed by T cells that recognize virus-derived peptides bound to cell-surface MHC molecules. Cells infected by some viruses also signal the presence of intracellular infection by expressing on their surfaces proteins, such as viral envelope proteins, that can be recognized by antibodies originally produced against the virus particle. Host cells with antibodies bound to them can be killed by a specialized non-T, non-B cell of the lymphoid lineage called a natural killer cell (NK cell), which we met in Chapter 3. NK cells are large cells with prominent intracellular granules and make up a small fraction of peripheral blood lymphocytes. Although belonging to the lymphoid linage, NK cells express a limited repertoire of invariant receptors recognizing a range of ligands that are induced on abnormal cells, such as those infected with viruses; they are considered as part of innate immunity (see Section 3-21). On recognition of a ligand, the NK cell kills the cell directly, without the need for antibody, as described in Chapter 3. Although first discovered for their ability to kill some tumor cells, NK cells play an important role in innate immunity in the early stages of virus infection.

Petitioner Merck, Ex. 1170, p. 164
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

As well as this innate function, NK cells can recognize and destroy antibody-coated target cells in a process called **antibody-dependent cell-mediated cytotoxicity** (**ADCC**). This is triggered when antibody bound to the surface of a cell interacts with Fc receptors on the NK cell (Fig. 10.36). NK cells express the receptor FcγRIII (CD16), which recognizes the IgG1 and IgG3 subclasses. The killing mechanism is analogous to that of cytotoxic T cells, involving the release of cytoplasmic granules containing perforin and granzymes (see Section 9-27). ADCC has been shown to have a role in the defense against infection by viruses, and represents another mechanism by which antibodies can direct an antigen-specific attack by an effector cell that itself lacks specificity for antigen.

Fig. 10.35 Eosinophils attacking a schistosome larva in the presence of serum from an infected patient. Large parasites, such as worms, cannot be ingested by phagocytes; however, when the worm is coated with antibody, eosinophils can attack it through binding via their Fc receptors for IgG and IgA. Similar attacks can be mounted by other Fc receptor-bearing cells on large targets. These cells release the toxic contents of their granules directly onto the target, a process known as exocytosis. Photograph courtesy of A. Butterworth.

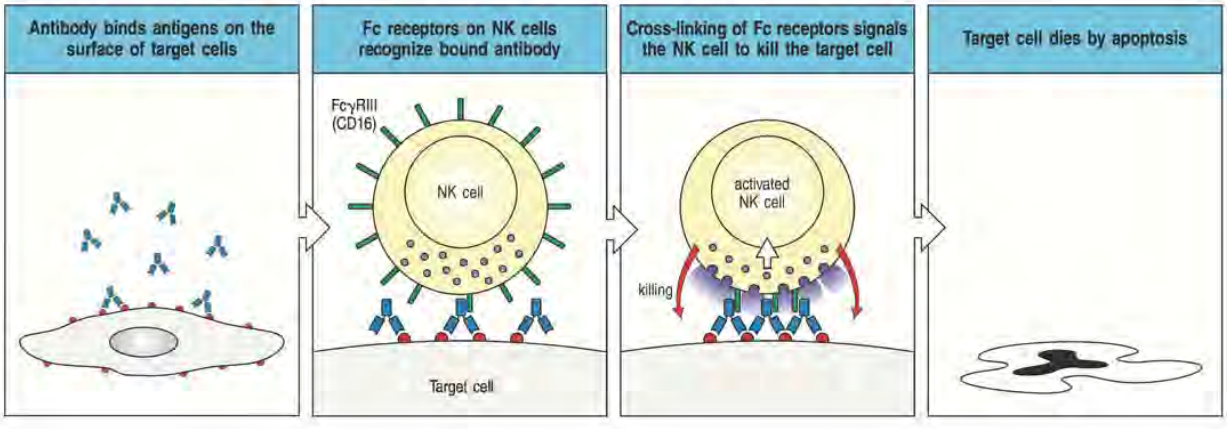
10-24 Mast cells and basophils bind IgE antibody via the high-affinity Fcε receptor.

When pathogens cross epithelial barriers and establish a local focus of infection, the host must mobilize its defenses and direct them to the site of pathogen growth. One way in which this is achieved is to activate the cells known as **mast cells**. Mast cells are large cells containing distinctive cytoplasmic granules that contain a mixture of chemical mediators, including histamine, that act rapidly to make local blood vessels more permeable. Mast cells have a distinctive appearance after staining with the dye toluidine blue that makes them readily identifiable in tissues (see Fig. 1.4). They are found in particularly high concentrations in vascularized connective tissues just beneath epithelial surfaces, including the submucosal tissues of the gastrointestinal and respiratory tracts and the dermis of the skin.

Mast cells have Fc receptors specific for IgE (FcεRI) and IgG (FcγRIII) and can be activated to release their granules, and to secrete lipid inflammatory mediators and cytokines, via antibody bound to these receptors. Most Fc receptors bind stably to the Fc regions of antibodies only when the antibodies have themselves bound antigen, and cross-linking of multiple Fc receptors is needed for strong binding. In contrast, FcεRI binds IgE antibody monomers with a very high affinity—approximately 10^{10} M^{-1}. Thus, even at the low levels of circulating IgE present in normal individuals, a substantial portion of the total IgE is bound to the FcεRI on mast cells in tissues and on circulating basophils.

Although mast cells are usually stably associated with bound IgE, this on its own does not activate them, nor will the binding of monomeric antigen to the IgE. Mast-cell activation occurs only when the bound IgE is cross-linked by multivalent antigens. This signal activates the mast cell to release the

Fig. 10.36 Antibody-coated target cells can be killed by NK cells in antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells (see Chapter 3) are large granular non-T, non-B lymphoid cells that have FcγRIII (CD16) on their surface. When these cells encounter cells coated with IgG antibody, they rapidly kill the target cell. ADCC is only one way in which NK cells can contribute to host defense.



contents of its granules, which occurs in seconds (Fig. 10.37), to synthesize and release lipid mediators such as prostaglandin D_2 and leukotriene C4, and to secrete cytokines such as TNF-α, thereby initiating a local inflammatory response. Degranulation releases stored histamine, which increases local blood flow and vascular permeability, which quickly leads to an accumulation of fluid and blood proteins, including antibodies, in the surrounding tissue. Shortly afterward there is an influx of blood-borne cells such as neutrophils and, later, macrophages, eosinophils, and effector lymphocytes. This influx can last from a few minutes to a few hours and produces an inflammatory response at the site of infection. Thus, mast cells are part of the front-line host defenses against pathogens that enter the body across epithelial barriers. They are also of additional medical importance because of their involvement in IgE-mediated allergic responses, which are discussed in Chapter 14. In allergic responses, mast cells are activated in the way described above by exposure to normally innocuous antigens (allergens) such as pollen, to which the individual has previously mounted a sensitizing immune response that produces allergen-specific IgE.

10-25 IgE-mediated activation of accessory cells has an important role in resistance to parasite infection.

Mast cells are thought to serve at least three important functions in host defense. First, their location near body surfaces allows them to recruit both pathogen-specific elements, such as antigen-specific lymphocytes, and nonspecific effector elements, such as neutrophils, macrophages, basophils, and eosinophils, to sites where infectious agents are most likely to enter the

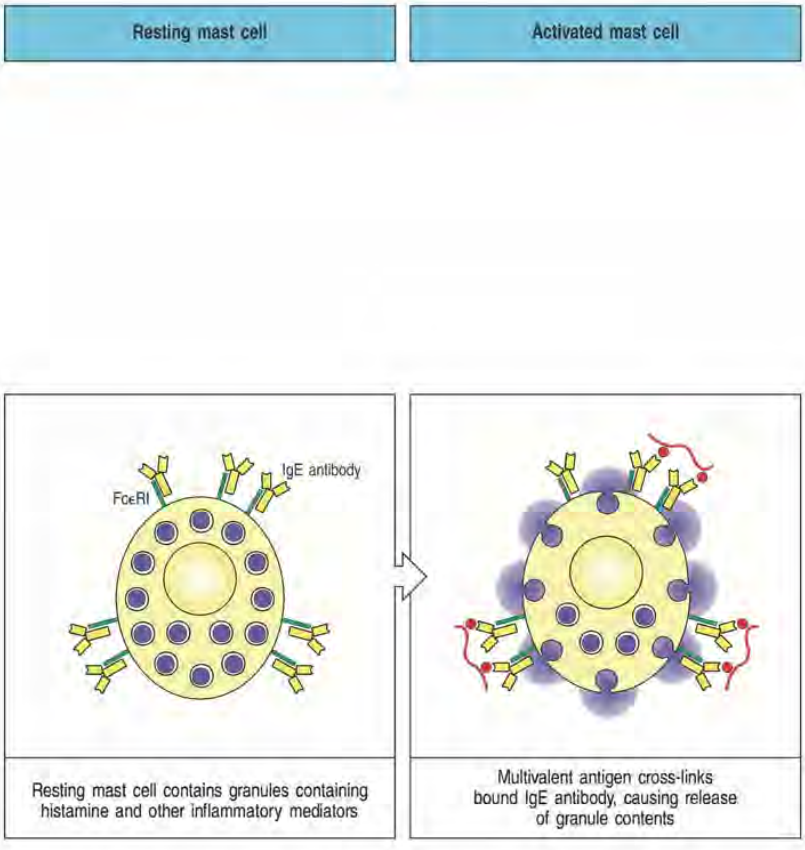


Fig. 10.37 IgE antibody cross-linking on mast-cell surfaces leads to a rapid release of inflammatory mediators. Mast cells are large cells found in connective tissue that can be distinguished by secretory granules containing many inflammatory mediators. They bind stably to monomeric IgE antibodies through the very high-affinity receptor FcεRI. Antigen cross-linking of the bound IgE antibody molecules triggers rapid degranulation, releasing inflammatory mediators into the surrounding tissue. These mediators trigger local inflammation, which recruits cells and proteins required for host defense to sites of infection. These cells are also triggered during allergic reactions when allergens bind to IgE on mast cells. Photographs courtesy of A.M. Dvorak.

Petitioner Merck, Ex. 1170, p. 166
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

internal milieu. Second, the inflammation they cause increases the flow of lymph from sites of antigen deposition to the regional lymph nodes, where naive lymphocytes are first activated. Third, the ability of mast-cell products to trigger muscular contraction can contribute to the physical expulsion of pathogens from the lungs or the gut. Mast cells respond rapidly to the binding of antigen to surface-bound IgE antibodies, and their activation leads to the initiation of an inflammatory response and the recruitment and activation of basophils and eosinophils, which contribute further to the inflammatory response (see Chapter 14). There is increasing evidence that such IgE-mediated responses are crucial to defense against parasite infestation.

A role for mast cells in the clearance of parasites is suggested by the accumulation of mast cells in the intestine, known as **mastocytosis**, that accompanies helminth infection, and by observations in W/W^V mutant mice, which have a profound mast-cell deficiency caused by mutation of the gene c-*kit*. These mutant mice show impaired clearance of the intestinal nematodes *Trichinella spiralis* and *Strongyloides* species. Clearance of *Strongyloides* is even more impaired in W/W^V mice that lack IL-3 and so also fail to produce basophils. Thus, both mast cells and basophils seem to contribute to defense against these helminth parasites.

Other evidence points to the importance of IgE antibodies and eosinophils in defense against parasites. Infection with certain types of multicellular parasites, particularly helminths, is strongly associated with the production of IgE antibodies and the presence of abnormally large numbers of eosinophils (eosinophilia) in blood and tissues. Furthermore, experiments in mice show that depletion of eosinophils by polyclonal anti-eosinophil antisera increases the severity of infection with the parasitic helminth *Schistosoma mansoni*. Eosinophils seem to be directly responsible for helminth destruction; examination of infected tissues shows degranulated eosinophils adhering to helminths, and experiments *in vitro* have shown that eosinophils can kill *S. mansoni* in the presence of anti-schistosome IgG or IgA antibodies (see Fig. 10.35).

The role of IgE, mast cells, basophils, and eosinophils can also be seen in resistance to the feeding of blood-sucking ixodid ticks. Skin at the site of a tick bite has degranulated mast cells and an accumulation of degranulated basophils and eosinophils, an indicator of recent activation. Subsequent resistance to feeding by these ticks develops after the first exposure, suggesting a specific immunological mechanism. Mice deficient in mast cells show no such acquired resistance to ticks, and in guinea pigs the depletion of either basophils or eosinophils by specific polyclonal antibodies also reduces resistance to tick feeding. Finally, experiments in mice showed that resistance to ticks is mediated by specific IgE antibody. Thus, many clinical studies and experiments support a role for this system of IgE bound to the high-affinity FcεRI in host resistance to pathogens that enter across epithelia or exoparasites such as ticks that breach it.

Summary.

Antibody-coated pathogens are recognized by effector cells through Fc receptors that bind to an array of constant regions (Fc portions) provided by the pathogen-bound antibodies. Binding activates the cell and triggers destruction of the pathogen, through either phagocytosis or granule release, or through both. Fc receptors comprise a family of proteins, each of which recognizes immunoglobulins of particular isotypes. Fc receptors on macrophages and neutrophils recognize the constant regions of IgG or IgA antibodies bound to a pathogen and trigger the engulfment and destruction of bacteria coated with IgG or IgA. Binding to the Fc receptor also induces the production of microbicidal agents in the intracellular vesicles of the

Petitioner Merck, Ex. 1170, p. 167
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

phagocyte. Eosinophils are important in the elimination of parasites too large to be engulfed: they bear Fc receptors specific for the constant region of IgG, as well as receptors for IgE; aggregation of these receptors triggers the release of toxic substances onto the surface of the parasite. NK cells, tissue mast cells, and blood basophils also release their granule contents when their Fc receptors are engaged. The high-affinity receptor for IgE is expressed constitutively by mast cells and basophils. It differs from other Fc receptors in that it can bind free monomeric antibody, thus enabling an immediate response to pathogens at their site of first entry into the tissues. When IgE bound to the surface of a mast cell is aggregated by binding to antigen, it triggers the release of histamine and many other mediators that increase the blood flow to sites of infection; it thereby recruits antibodies and effector cells to these sites. Mast cells are found principally below epithelial surfaces of the skin and beneath the basement membrane of the digestive and respiratory tracts. Their activation by innocuous substances is responsible for many of the symptoms of acute allergic reactions, as will be described in Chapter 14.

Summary to Chapter 10.

The humoral immune response to infection involves the production of antibody by plasma cells derived from B lymphocytes, the binding of this antibody to the pathogen, and the elimination of the pathogen by phagocytic cells and molecules of the humoral immune system. The production of antibody usually requires the action of helper T cells specific for a peptide fragment of the antigen recognized by the B cell, a phenomenon called linked recognition. An activated B cell first moves to the T-zone–B-zone boundary in secondary lymphoid tissues, where it may encounter its cognate T cell and begin to proliferate. Some B cells become plasmablasts, and others will move to the germinal center, where somatic hypermutation and class switch recombination take place. B cells generated there that bind antigen most avidly are selected for survival and further differentiation, leading to affinity maturation of the antibody response. Cytokines made by helper T cells direct class switching, leading to the production of antibody of various classes that can be distributed to various body compartments.

IgM antibodies are produced early in an infection by conventional, or B-2, cells, and is also made in the absence of infection by subsets of nonconventional B cells in particular locations (natural antibodies). IgM has a major role in protecting against infection in the bloodstream, whereas isotypes secreted later in an adaptive immune response, such as IgG, diffuse into the tissues. Antigens with highly repeating antigenic determinants and that contain mitogens—called TI antigens—can elicit IgM and some IgG independently of T-cell help, and this provides an early protective immune response. Multimeric IgA is produced in the lamina propria and is transported across epithelial surfaces, whereas IgE is made in small amounts and binds avidly to receptors on the surface of mast cells.

Antibodies that bind with high affinity to critical sites on toxins, viruses, and bacteria can neutralize them. However, pathogens and their products are destroyed and removed from the body largely through uptake into phagocytes and degradation inside these cells. Antibodies that coat pathogens bind to Fc receptors on phagocytes, which are thereby triggered to engulf and destroy the pathogen. Binding of antibody C regions to Fc receptors on other cells leads to the exocytosis of stored mediators; this is particularly important in parasite infections, in which Fcε-expressing mast cells are triggered by the binding of antigen to IgE antibody to release inflammatory mediators directly onto parasite surfaces. Antibodies can also initiate the destruction of pathogens by activating the complement system. Complement components

Petitioner Merck, Ex. 1170, p. 168
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

can opsonize pathogens for uptake by phagocytes, recruit phagocytes to sites of infection, and directly destroy pathogens by creating pores in their cell membrane. Receptors for complement components and Fc receptors often synergize in activating the uptake and destruction of pathogens and immune complexes. Thus, the humoral immune response is targeted to the infecting pathogen through the production of specific antibody; however, the effector actions of that antibody are determined by its heavy-chain isotype, which determines its class, and are the same for all pathogens bound by antibody of a particular class.

Questions.

10.1 Describe the requirements for the activation of naive B cells by a thymus-dependent antigen. By what mechanisms do T cells provide help to B cells in the humoral response?

10.2 What is meant by the term linked recognition? What are the advantages of this process for immune tolerance? What are the advantages for specificity?

10.3 Compare and contrast the properties and functions of antibodies of the IgM and IgG classes.

10.4 In the initiation of a thymus-dependent antibody response, B cells and T cells change their locations. What determined the location of T and B cells at different stages of their activation?

10.5 In the germinal center reaction, B cells (centroblasts and centrocytes) cycle between the light and dark zones. What factors regulate this movement and localization?

10.6 Describe the process responsible for the phenomenon of affinity maturation of the antibody response. Where does affinity maturation mainly take place?

10.7 Assuming a rate of one mutation in 10^3 base pairs within the V regions per cell division and that about three out of four changes will result in an altered amino acid, explain how the estimate was arrived at of a 50% chance of mutation in the B cell's antigen receptor per division during the germinal center reaction.

10.8 Which of the antibody classes mainly activates mast cells? How does it do so and what are the results? Which type of pathogen is this class of antibody mainly directed against? What unwanted reaction is this antibody also responsible for?

10.9 How do antibodies interact with the complement system to rid the body of pathogens?

10.10 Which classes of maternal antibodies would you expect to find in a breast-fed newborn infant and how have they got there?

Petitioner Merck, Ex. 1170, p. 169
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

General references.

Batista, F.D., and Harwood, N.E.: **The who, how and where of antigen presentation to B cells.** *Nat. Rev. Immunol.* 2009, **9**:15–27.

Nimmerjahn, F., and Ravetch, J.V.: **Fcγ receptors as regulators of immune responses.** *Nat. Rev. Immunol.* 2008, **8**:34–47.

Rajewsky, K.: **Clonal selection and learning in the antibody system.** *Nature* 1996, **381**:751–758.

Section references.

10-1 The humoral immune response is initiated when B cells that bind antigen are signaled by helper T cells or by certain microbial antigens alone.

Gulbranson-Judge, A., and MacLennan, I.: **Sequential antigen-specific growth of T cells in the T zones and follicles in response to pigeon cytochrome c.** *Eur. J. Immunol.* 1996, **26**:1830–1837.

10-2 B-cell responses are enhanced by co-ligation of the B-cell receptor and B-cell co-receptor by antigen and complement fragments on microbial surfaces.

Barrington, R.A., Zhang, M., Zhong, X., Jonsson, H., Holodick, N., Cherukuri, A., Pierce, S.K., Rothstein, T.L., and Carroll, M.C.: **CD21/CD19 coreceptor signaling promotes B cell survival during primary immune responses.** *J. Immunol.* 2005, **175**:2859–2867.

Fearon, D.T., and Carroll, M.C.: **Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex.** *Annu. Rev. Immunol.* 2000, **18**:393–422.

O'Rourke, L., Tooze, R., and Fearon, D.T.: **Co-receptors of B lymphocytes.** *Curr. Opin. Immunol.* 1997, **9**:324–329.

Rickert, R.C.: **Regulation of B lymphocyte activation by complement C3 and the B cell coreceptor complex.** *Curr. Opin. Immunol.* 2005, **17**:237–243.

10-3 Helper T cells activate B cells that recognize the same antigen.

Eskola, J., Peltola, H., Takala, A.K., Kayhty, H., Hakulinen, M., Karanko, V., Kela, E., Rekola, P., Ronnberg, P.R., Samuelson, J.S., *et al.*: **Efficacy of *Haemophilus influenzae* type b polysaccharide-diphtheria toxoid conjugate vaccine in infancy.** *N. Engl. J. Med.* 1987, **317**:717–722.

MacLennan, I.C.M., Gulbranson-Judge, A., Toellner, K.M., Casamayor-Palleja, M., Chan, E., Sze, D.M.Y., Luther, S.A., and Orbea, H.A.: **The changing preference of T and B cells for partners as T-dependent antibody responses develop.** *Immunol. Rev.* 1997, **156**:53–66.

McHeyzer-Williams, L.J., Malherbe, L.P., and McHeyzer-Williams, M.G.: **Helper T cell-regulated B cell immunity.** *Curr. Top. Microbiol. Immunol.* 2006, **311**:59–83.

Parker, D.C.: **T cell-dependent B-cell activation.** *Annu. Rev. Immunol.* 1993, **11**:331–340.

10-4 T cells make membrane-bound and secreted molecules that activate B cells.

Gaspal, F.M., Kim, M.Y., McConnell, F.M., Raykundalia, C., Bekiaris, V., and Lane, P.J.: **Mice deficient in OX40 and CD30 signals lack memory antibody responses because of deficient CD4 T cell memory.** *J. Immunol.* 2005, **174**:3891–3896.

Jaiswal, A.I., and Croft, M.: **CD40 ligand induction on T cell subsets by peptide-presenting B cells.** *J. Immunol.* 1997, **159**:2282–2291.

Kalled, S.L.: **Impact of the BAFF/BR3 axis on B cell survival, germinal center maintenance and antibody production.** *Semin. Immunol.* 2006, **18**:290–296.

Mackay, F., and Browning, J.L.: **BAFF: a fundamental survival factor for B cells.** *Nat. Rev. Immunol.* 2002, **2**:465–475.

Shanebeck, K.D., Maliszewski, C.R., Kennedy, M.K., Picha, K.S., Smith, C.A., Goodwin, R.G., and Grabstein, K.H.: **Regulation of murine B cell growth and differentiation by CD30 ligand.** *Eur. J. Immunol.* 1995, **25**:2147–2153.

Yoshinaga, S.K., Whoriskey, J.S., Khare, S.D., Sarmiento, U., Guo, J., Horan, T., Shih, G., Zhang, M., Coccia, M.A., Kohno, T. *et al.*: **T-cell co-stimulation through B7RP-1 and ICOS.** *Nature* 1999, **402**:827–832.

10-5 B cells that encounter their antigens migrate toward the boundaries between B-cell and T-cell areas in secondary lymphoid tissues.

Cahalan, M.D., and Parker, I.: **Close encounters of the first and second kind: T-DC and T-B interactions in the lymph node.** *Semin. Immunol.* 2005, **17**:442–451.

Fang, Y., Xu, C., Fu, Y.X., Holers, V.M., and Molina, H.: **Expression of complement receptors 1 and 2 on follicular dendritic cells is necessary for the generation of a strong antigen-specific IgG response.** *J. Immunol.* 1998, **160**:5273–5279.

Garside, P., Ingulli, E., Merica, R.R., Johnson, J.G., Noelle, R.J., and Jenkins, M.K.: **Visualization of specific B and T lymphocyte interactions in the lymph node.** *Science* 1998, **281**:96–99.

Okada, T., and Cyster, J.G.: **B cell migration and interactions in the early phase of antibody responses.** *Curr. Opin. Immunol.* 2006, **18**:278–285.

Pape, K.A., Kouskoff, V., Nemazee, D., Tang, H.L., Cyster, J.G., Tze, L.E., Hippen, K.L., Behrens, T.W., and Jenkins, M.K.: **Visualization of the genesis and fate of isotype-switched B cells during a primary immune response.** *J. Exp. Med.* 2003, **197**:1677–1687.

Phan, T.G., Gray, E.E., and Cyster, J.G.: **The microanatomy of B cell activation.** *Curr. Opin. Immunol.* 2009, **21**:258–265.

10-6 Antibody-secreting plasma cells differentiate from activated B cells.

Moser, K., Tokoyoda, K., Radbruch, A., MacLennan, I., and Manz, R.A.: **Stromal niches, plasma cell differentiation and survival.** *Curr. Opin. Immunol.* 2006, **18**:265–270.

Radbruch, A., Muehlinghaus, G., Luger, E.O., Inamine, A., Smith, K.G., Dorner, T., and Hiepe, F.: **Competence and competition: the challenge of becoming a long-lived plasma cell.** *Nat. Rev. Immunol.* 2006, **6**:741–750.

Sciammas, R., and Davis, M.M.: **Blimp-1; immunoglobulin secretion and the switch to plasma cells.** *Curr. Top. Microbiol. Immunol.* 2005, **290**:201–224.

Shapiro-Shelef, M, and Calame, K.: **Regulation of plasma-cell development.** *Nat. Rev. Immunol.* 2005, **5**:230–242.

10-7 The second phase of a primary B-cell immune response occurs when activated B cells migrate into follicles and proliferate to form germinal centers.

Allen, C.D., Okada, T., and Cyster, J.G.: **Germinal-center organization and cellular dynamics.** *Immunity* 2007, **27**:190–202.

Cozine, C.L., Wolniak, K.L., and Waldschmidt, T.J.: **The primary germinal center response in mice.** *Curr. Opin. Immunol.* 2005, **17**:298–302.

Jacob, J., Przylepa, J., Miller, C., and Kelsoe, G.: **In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. III. The kinetics of V region mutation and selection in germinal center B cells.** *J. Exp. Med.* 1993, **178**:1293–1307.

Kelsoe, G.: **The germinal center: a crucible for lymphocyte selection.** *Semin. Immunol.* 1996, **8**:179–184.

Kunkel, E.J., and Butcher, E.C.: **Plasma-cell homing.** *Nat. Rev. Immunol.* 2003, **3**:822–829.

MacLennan, I.C.: **Germinal centers still hold secrets.** *Immunity* 2005, **22**:656–657.

10-8 Germinal center B cells undergo V-region somatic hypermutation, and cells with mutations that improve affinity for antigen are selected.

Anderson, S.M., Khalil, A., Uduman, M., Hershberg, U., Louzoun, Y., Haberman, A.M., Kleinstein, S.H., and Shlomchik, M.J.: **Taking advantage: high-affinity B cells in the germinal center have lower death rates, but similar rates of division, compared to low-affinity cells.** *J. Immunol.* 2009, **183**:7314–7325.

Jacob, J., Kelsoe, G., Rajewsky, K., and Weiss, U.: **Intracional generation of antibody mutants in germinal centres.** *Nature* 1991, **354**:389–392.

Petitioner Merck, Ex. 1170, p. 170
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Li, Z., Woo, C.J., Iglesias-Ussel, M.D., Ronai, D., and Scharff, M.D.: **The generation of antibody diversity through somatic hypermutation and class switch recombination.** *Genes Dev.* 2004, **18**:1–11.

Odegard, V.H., and Schatz, D.G.: **Targeting of somatic hypermutation.** *Nat. Rev. Immunol.* 2006, **6**:573–583.

Pereira, J.P., Kelly, L.M., and Cyster, J.G.: **Finding the right niche: B-cell migration in the early phases of T-dependent antibody responses.** *Int. Immunol.* 2010, **22**:413–419.

10-9 Class switching in thymus-dependent antibody responses requires expression of CD40 ligand by helper T cells and is directed by cytokines.

Francke, U., and Ochs, H.D.: **The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome.** *Cell* 1993, **72**:291–300.

Jumper, M., Splawski, J., Lipsky, P., and Meek, K.: **Ligation of CD40 induces sterile transcripts of multiple Ig H chain isotypes in human B cells.** *J. Immunol.* 1994, **152**:438–445.

Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P., and Cerutti, A.: **DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL.** *Nat. Immunol.* 2002, **3**:822–829.

MacLennan, I.C., Toellner, K.M., Cunningham, A.F., Serre, K., Sze, D.M., Zuniga, E., Cook, M.C., and Vinuesa, C.G.: **Extrafollicular antibody responses.** *Immunol. Rev.* 2003, **194**:8–18.

Snapper, C.M., Kehry, M.R., Castle, B.E., and Mond, J.J.: **Multivalent, but not divalent, antigen receptor cross-linkers synergize with CD40 ligand for induction of Ig synthesis and class switching in normal murine B cells.** *J. Immunol.* 1995, **154**:1177–1187.

Stavnezer, J.: **Immunoglobulin class switching.** *Curr. Opin. Immunol.* 1996, **8**:199–205.

10-10 Ligation of CD40 and prolonged contact with T follicular helper cells is required to sustain germinal center B cells.

Banchereau, J., de Paoli, P., Vallé, A., Garcia, E., and Rousset, F.: **Long-term human B cell lines dependent on interleukin-4 and antibody to CD40.** *Science* 1991, **251**:70–72.

Cannons, J.L., Qi, H., Lu, K.T., Dutta, M., Gomez-Rodriguez, J., Cheng, J., Wakeland, E.K., Germain, R.N., and Schwartzberg, P.L.: **Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84**. *Immunity* 2010, **32**:253–265.

Hannum, L.G., Haberman, A.M., Anderson, S.M., and Shlomchik, M.J.: **Germinal center initiation, variable gene region hypermutation, and mutant B cell selection without detectable immune complexes on follicular dendritic cells.** *J. Exp. Med.* 2000, **192**:931–942.

Liu, Y.J., Joshua, D.E., Williams, G.T., Smith, C.A., Gordon, J., and MacLennan, I.C.M.: **Mechanism of antigen-driven selection in germinal centres.** *Nature* 1989, **342**:929–931.

Wang, Z., Karras, J.G., Howard, R.G., and Rothstein, T.L.: **Induction of bcl-x by CD40 engagement rescues sIg-induced apoptosis in murine B cells.** *J. Immunol.* 1995, **155**:3722–3725.

10-11 Surviving germinal center B cells differentiate into either plasma cells or memory cells.

Hu, C.C., Dougan, S.K., McGehee, A.M., Love, J.C., and Ploegh, H.L.: **XBP-1 regulates signal transduction, transcription factors and bone marrow colonization in B cells.** *EMBO J.* 2009, **28**:1624–1636.

Nera, K.P., and Lassila, O.: **Pax5—a critical inhibitor of plasma cell fate**. *Scand. J. Immunol.* 2006, **64**:190–199.

Omori, S.A., Cato, M.H., Anzelon-Mills, A., Puri, K.D., Shapiro-Shelef, M., Calame, K., and Rickert, R.C.: **Regulation of class-switch recombination and plasma cell differentiation by phosphatidylinositol 3-kinase signaling.** *Immunity* 2006, **25**:545–557.

Radbruch, A., Muehlinghaus, G., Luger, E.O., Inamine, A., Smith, K.G., Dorner, T., and Hiepe, F.: **Competence and competition: the challenge of becoming a long-lived plasma cell.** *Nat. Rev. Immunol.* 2006, **6**:741–750.

Schebesta, M., Heavey, B., and Busslinger, M.: **Transcriptional control of B-cell development.** *Curr. Opin. Immunol.* 2002, **14**:216–223.

10-12 Some bacterial antigens do not require T-cell help to induce B-cell responses.

Anderson, J., Coutinho, A., Lernhardt, W., and Melchers, F.: **Clonal growth and maturation to immunoglobulin secretion in vitro of every growth-inducible B lymphocyte.** *Cell* 1977, **10**:27–34.

Bekeredjian-Ding, I., and Jego, G.: **Toll-like receptors—sentries in the B-cell response**. *Immunology* 2009, **128**:311–323.

Garcia De Vinuesa, C., Gulbranson-Judge, A., Khan, M., O'Leary, P., Cascalho, M., Wabl, M., Klaus, G.G., Owen, M.J., and MacLennan, I.C.: **Dendritic cells associated with plasmablast survival.** *Eur. J. Immunol.* 1999, **29**:3712–3721.

Ruprecht, C.R., and Lanzavecchia, A.: **Toll-like receptor stimulation as a third signal required for activation of human naive B cells.** *Eur. J. Immunol.* 2006, **36**:810–816.

10-13 B-cell responses to bacterial polysaccharides do not require peptide-specific T-cell help.

Balazs, M., Martin, F., Zhou, T., and Kearney, J.: **Blood dendritic cells interact with splenic marginal zone B cells to initiate T-independent immune responses.** *Immunity* 2002, **17**:341–352.

Craxton, A., Magaletti, D., Ryan, E.J., and Clark, E.A.: **Macrophage- and dendritic cell-dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF.** *Blood* 2003, **101**:4464–4471.

Fagarasan, S., and Honjo, T.: **T-independent immune response: new aspects of B cell biology.** *Science* 2000, **290**:89–92.

MacLennan, I., and Vinuesa, C.: **Dendritic cells, BAFF, and APRIL: innate players in adaptive antibody responses.** *Immunity* 2002, **17**:341–352.

Mond, J.J., Lees, A., and Snapper, C.M.: **T cell-independent antigens type 2.** *Annu. Rev. Immunol.* 1995, **13**:655–692.

Snapper, C.M., Shen, Y., Khan, A.Q., Colino, J., Zelazowski, P., Mond, J.J., Gause, W.C., and Wu, Z.Q.: **Distinct types of T-cell help for the induction of a humoral immune response to *Streptococcus pneumoniae*.** *Trends Immunol.* 2001, **22**:308–311.

10-14 Antibodies of different classes operate in distinct places and have distinct effector functions.

Clark, M.R.: **IgG effector mechanisms.** *Chem. Immunol.* 1997, **65**:88–110.

Herrod, H.G.: **IgG subclass deficiency.** *Allergy Proc.* 1992, **13**:299–302.

Rispens, T., den Bleker, T.H., and Aalberse, R.C.: **Hybrid IgG4/IgG4 Fc antibodies form upon 'Fab-arm' exchange as demonstrated by SDS-PAGE or size-exclusion chromatography.** *Mol. Immunol.* 2010, **47**:1592–1594.

Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., and Fagarasan, S.: **Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut.** *Proc. Natl Acad. Sci. USA* 2004, **101**:1981–1986.

Ward, E.S., and Ghetie, V.: **The effector functions of immunoglobulins: implications for therapy.** *Ther. Immunol.* 1995, **2**:77–94.

10-15 Transport proteins that bind to the Fc regions of antibodies carry particular isotypes across epithelial barriers.

Burmeister, W.P., Gastinel, L.N., Simister, N.E., Blum, M.L., and Bjorkman, P.J.: **Crystal structure at 2.2 Å resolution of the MHC-related neonatal Fc receptor.** *Nature* 1994, **372**:336–343.

Corthesy, B., and Kraehenbuhl, J.P.: **Antibody-mediated protection of mucosal surfaces.** *Curr. Top. Microbiol. Immunol.* 1999, **236**:93–111.

Ghetie, V., and Ward, E.S.: **Multiple roles for the major histocompatibility complex class I-related receptor FcRn.** *Annu. Rev. Immunol.* 2000, **18**:739–766.

Lamm, M.E.: **Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense.** *Am. J. Physiol.* 1998, **274**:G614–G617.

Petitioner Merck, Ex. 1170, p. 171
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Mostov, K.E.: **Transepithelial transport of immunoglobulins.** *Annu. Rev. Immunol.* 1994, **12**:63–84.

10-16 High-affinity IgG and IgA antibodies can neutralize bacterial toxins.
&
10-17 High-affinity IgG and IgA antibodies can inhibit the infectivity of viruses.

Brandtzaeg, P.: **Role of secretory antibodies in the defence against infections.** *Int. J. Med. Microbiol.* 2003, **293**:3–15.

Mandel, B.: **Neutralization of polio virus: a hypothesis to explain the mechanism and the one hit character of the neutralization reaction.** *Virology* 1976, **69**:500–510.

Roost, H.P., Bachmann, M.F., Haag, A., Kalinke, U., Pliska, V., Hengartner, H., and Zinkernagel, R.M.: **Early high-affinity neutralizing anti-viral IgG responses without further overall improvements of affinity.** *Proc. Natl Acad. Sci. USA* 1995, **92**:1257–1261.

Sougioultzis, S., Kyne, L., Drudy, D., Keates, S., Maroo, S., Pothoulakis, C., Giannasca, P.J., Lee, C.K., Warny, M., Monath, T.P., *et al.*: ***Clostridium difficile* toxoid vaccine in recurrent *C. difficile*-associated diarrhea.** *Gastroenterology* 2005, **128**:764–770.

10-18 Antibodies can block the adherence of bacteria to host cells.

Fischetti, V.A., and Bessen, D.: **Effect of mucosal antibodies to M protein in colonization by group A streptococci.** In Switalski, L., Hook, M., and Beachery, E. (eds): *Molecular Mechanisms of Microbial Adhesion.* New York, Springer, 1989.

Wizemann, T.M., Adamou, J.E., and Langermann, S.: **Adhesins as targets for vaccine development.** *Emerg. Infect. Dis.* 1999, **5**:395–403.

10-19 Antibody:antigen complexes activate the classical pathway of complement by binding to C1q.

Cooper, N.R.: **The classical complement pathway. Activation and regulation of the first complement component.** *Adv. Immunol.* 1985, **37**:151–216.

Perkins, S.J., and Nealis, A.S.: **The quaternary structure in solution of human complement subcomponent $C1r_2C1s_2$.** *Biochem. J.* 1989, **263**:463–469.

10-20 Complement receptors are important in the removal of immune complexes from the circulation.

Nash, J.T., Taylor, P.R., Botto, M., Norsworthy, P.J., Davies, K.A., and Walport, M.J.: **Immune complex processing in C1q-deficient mice.** *Clin. Exp. Immunol.* 2001, **123**:196–202.

Nash, J.T., Taylor, P.R., Botto, M., Norsworthy, P.J., Davies, K.A., Walport, M.J., Schifferli, J.A., and Taylor, J.P.: **Physiologic and pathologic aspects of circulating immune complexes.** *Kidney Int.* 1989, **35**:993–1003.

Schifferli, J.A., Ng, Y.C., and Peters, D.K.: **The role of complement and its receptor in the elimination of immune complexes.** *N. Engl. J. Med.* 1986, **315**:488–495.

Walport, M.J., Davies, K.A., and Botto, M.: **C1q and systemic lupus erythematosus.** *Immunobiology* 1998, **199**:265–285.

10-21 The Fc receptors of accessory cells are signaling receptors specific for immunoglobulins of different classes.

Kinet, J.P., and Launay, P.: **Fc α/microR: single member or first born in the family?** *Nat. Immunol.* 2000, **1**:371–372.

Ravetch, J.V., and Bolland, S.: **IgG Fc receptors.** *Annu. Rev. Immunol.* 2001, **19**:275–290.

Ravetch, J.V., and Clynes, R.A.: **Divergent roles for Fc receptors and complement *in vivo*.** *Annu. Rev. Immunol.* 1998, **16**:421–432.

Shibuya, A., Sakamoto, N., Shimizu, Y., Shibuya, K., Osawa, M., Hiroyama, T., Eyre, H.J., Sutherland, G.R., Endo, Y., Fujita, T., *et al.*: **Fc α/μ receptor mediates endocytosis of IgM-coated microbes.** *Nat. Immunol.* 2000, **1**:441–446.

Stefanescu, R.N., Olferiev M., Liu, Y., and Pricop, L.: **Inhibitory Fc gamma receptors: from gene to disease.** *J. Clin. Immunol.* 2004, **24**:315–326.

10-22 Fc receptors on phagocytes are activated by antibodies bound to the surface of pathogens and enable the phagocytes to ingest and destroy pathogens.

Dierks, S.E., Bartlett, W.C., Edmeades, R.L., Gould, H.J., Rao, M., and Conrad, D.H.: **The oligomeric nature of the murine Fc epsilon RII/CD23. Implications for function.** *J. Immunol.* 1993, **150**:2372–2382.

Hogan, S.P., Rosenberg, H.F., Moqbel, R., Phipps, S., Foster, P.S., Lacy, P., Kay, A.B., and Rothenberg, M.E.: **Eosinophils: biological properties and role in health and disease.** *Clin. Exp. Allergy* 2008, **38**:709–750.

Karakawa, W.W., Sutton, A., Schneerson, R., Karpas, A., and Vann, W.F.: **Capsular antibodies induce type-specific phagocytosis of capsulated *Staphylococcus aureus* by human polymorphonuclear leukocytes.** *Infect. Immun.* 1986, **56**:1090–1095.

10-23 Fc receptors activate NK cells to destroy antibody-coated targets.

Chung, A.W., Rollman, E., Center, R.J., Kent, S.J., and Stratov, I.: **Rapid degranulation of NK cells following activation by HIV-specific antibodies.** *J. Immunol.* 2009, **182**:1202–1210.

Lanier, L.L., and Phillips, J.H.: **Evidence for three types of human cytotoxic lymphocyte.** *Immunol. Today* 1986, **7**:132.

Leibson, P.J.: **Signal transduction during natural killer cell activation: inside the mind of a killer.** *Immunity* 1997, **6**:655–661.

Sulica, A., Morel, P., Metes, D., and Herberman, R.B.: **Ig-binding receptors on human NK cells as effector and regulatory surface molecules.** *Int. Rev. Immunol.* 2001, **20**:371–414.

Takai, T.: **Multiple loss of effector cell functions in FcR γ-deficient mice.** *Int. Rev. Immunol.* 1996, **13**:369–381.

10-24 Mast cells and basophils bind IgE antibody via the high-affinity Fcε receptor.

Beaven, M.A., and Metzger, H.: **Signal transduction by Fc receptors: the FcεRI case.** *Immunol. Today* 1993, **14**:222–226.

Kalesnikoff, J., Huber, M., Lam, V., Damen, J.E., Zhang, J., Siraganian, R.P., and Krystal, G.: **Monomeric IgE stimulates signaling pathways in mast cells that lead to cytokine production and cell survival.** *Immunity* 2001, **14**:801–811.

Sutton, B.J., and Gould, H.J.: **The human IgE network.** *Nature* 1993, **366**:421–428.

10-25 IgE-mediated activation of accessory cells has an important role in resistance to parasite infection.

Capron, A., Riveau, G., Capron, M., and Trottein, F.: **Schistosomes: the road from host-parasite interactions to vaccines in clinical trials.** *Trends Parasitol.* 2005, **21**:143–149.

Grencis, R.K.: **Th2-mediated host protective immunity to intestinal nematode infections.** *Phil. Trans. R. Soc. Lond. B* 1997, **352**:1377–1384.

Grencis, R.K., Else, K.J., Huntley, J.F., and Nishikawa, S.I.: **The in vivo role of stem cell factor (c-kit ligand) on mastocytosis and host protective immunity to the intestinal nematode *Trichinella spiralis* in mice.** *Parasite Immunol.* 1993, **15**:55–59.

Kasugai, T., Tei, H., Okada, M., Hirota, S., Morimoto, M., Yamada, M., Nakama, A., Arizono, N., and Kitamura, Y.: **Infection with *Nippostrongylus brasiliensis* induces invasion of mast cell precursors from peripheral blood to small intestine.** *Blood* 1995, **85**:1334–1340.

Ushio, H., Watanabe, N., Kiso, Y., Higuchi, S., and Matsuda, H.: **Protective immunity and mast cell and eosinophil responses in mice infested with larval *Haemaphysalis longicornis* ticks.** *Parasite Immunol.* 1993, **15**:209–214.

Petitioner Merck, Ex. 1170, p. 172
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

11 Dynamics of Adaptive Immunity

Throughout this book we have examined the separate ways in which the innate and the adaptive immune responses protect the individual from invading microorganisms. In this chapter, we consider how the cells and molecules of the immune system work as an integrated defense system to eliminate or control an infectious agent and how the adaptive immune system provides long-lasting protective immunity. This is the first of several chapters that consider how the immune system functions as a whole in health and disease. The next chapter describes the role and specializations of the mucosal immune system, which forms the front-line defense against most pathogens. Subsequent chapters examine how immune defenses can fail (Chapter 13) or unwanted immune responses occur (Chapters 14 and 15), and how the immune response can be manipulated to benefit the individual (Chapter 16).

In Chapters 2 and 3, we saw how innate immunity is brought into play in the earliest phases of an infection and is probably sufficient to prevent colonization of the body by most of the microorganisms encountered in the environment. However, pathogenic microorganisms, by definition, have developed strategies that allow them to elude or overcome innate immune defenses and to establish a focus of infection from which they can spread. In these circumstances, the innate immune response sets the scene for the induction of an adaptive immune response. In the **primary immune response**, which occurs against a pathogen encountered for the first time, several days are required for the clonal expansion and differentiation of naive lymphocytes into effector T cells and antibody-secreting B cells, as described in Chapters 9 and 10. In most cases, these cells and antibodies will effectively target the pathogen for elimination (Fig. 11.1).

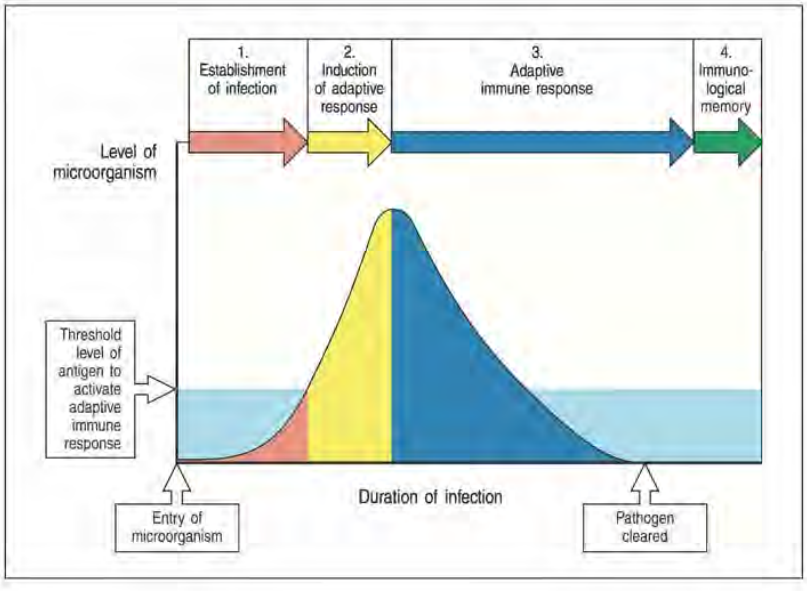


Fig. 11.1 The course of a typical acute infection that is cleared by an adaptive immune reaction. 1. The level of infectious agent increases as the pathogen replicates. 2. When numbers of the pathogen exceed the threshold dose of antigen required for an adaptive response, the response is initiated; the pathogen continues to grow, retarded only by responses of the innate immune system. At this stage, immunological memory also starts to be induced. 3. After 4–7 days, effector cells and molecules of the adaptive response start to clear the infection. 4. When the infection has been cleared and the dose of antigen has fallen below the response threshold, the response ceases, but antibody, residual effector cells, and immunological memory provide lasting protection against reinfection in most cases.

Petitioner Merck, Ex. 1170, p. 173
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

During this period, specific **immunological memory** is also established. This ensures a rapid reinduction of antigen-specific antibody and effector T cells on subsequent encounters with the same pathogen, thus providing long-lasting and often lifelong protection against it. Immunological memory is discussed in the last part of the chapter. Memory responses differ in several ways from primary responses. We discuss the reasons for this, and what is known about how immunological memory is maintained.

The course of the immune response to infection.

The immune response is a dynamic process, and both its nature and intensity change over time. It starts with the relatively nonspecific responses of innate immunity and becomes both more focused on the pathogen and more powerful as the adaptive immune response is initiated and rapidly develops. In this part of the chapter, we discuss how the different phases of an immune response are orchestrated in space and time, how the response develops in both strength and precision, how changes in specialized cell-surface molecules and chemokines guide effector lymphocytes to the appropriate site of action, and how these cells are regulated during the different stages.

An innate immune response is an essential prerequisite to a primary adaptive immune response, because the co-stimulatory molecules induced on cells of the innate immune system during their interaction with microorganisms are essential for the activation of the antigen-specific lymphocytes (see Chapter 9). Cells of the innate immune system hand on other important signals in the form of secreted cytokines that influence the characteristics of the adaptive response and tailor it to the type of pathogen encountered. For this to happen, cells from different locations must engage to coordinate the specific activation of naive T cells and B cells, and the migration of cells to precise locations within lymphoid tissues is thus critical for the coordination of an adaptive response.

11-1 The course of an infection can be divided into several distinct phases.

Movie 11.1 

An infection can be broken down into various stages (see Fig. 2.5), but in Chapters 2 and 3 we considered in detail only the responses of innate immunity. In this chapter, we return to the various stages of an infection but will now integrate the adaptive immune response into the picture.

In the first stage of infection with a pathogen, a new host is exposed to infectious particles either shed by an infected individual or present in the environment. The numbers, route, mode of transmission, and stability of an infectious agent outside the host determine its infectivity. Some pathogens, such as the anthrax bacterium, are spread by spores that are highly resistant to heat and drying, whereas others, such as the human immunodeficiency virus (HIV), are spread only by the exchange of bodily fluids or tissues because they are unable to survive as infectious agents outside the body.

The first contact with a new host occurs through an epithelial surface, such as the skin or the mucosal surfaces of the respiratory, gastrointestinal, or urogenital tracts. As most pathogens gain entry to the body through mucosal surfaces, the immune responses that occur in this specialized compartment of the immune system are of great importance and are considered in detail in Chapter 12. After making contact, an infectious agent must establish a focus of infection. It must either adhere to the epithelial surface and colonize it, or penetrate it to replicate in the tissues (Fig. 11.2, first two panels). Wounds

Petitioner Merck, Ex. 1170, p. 174
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

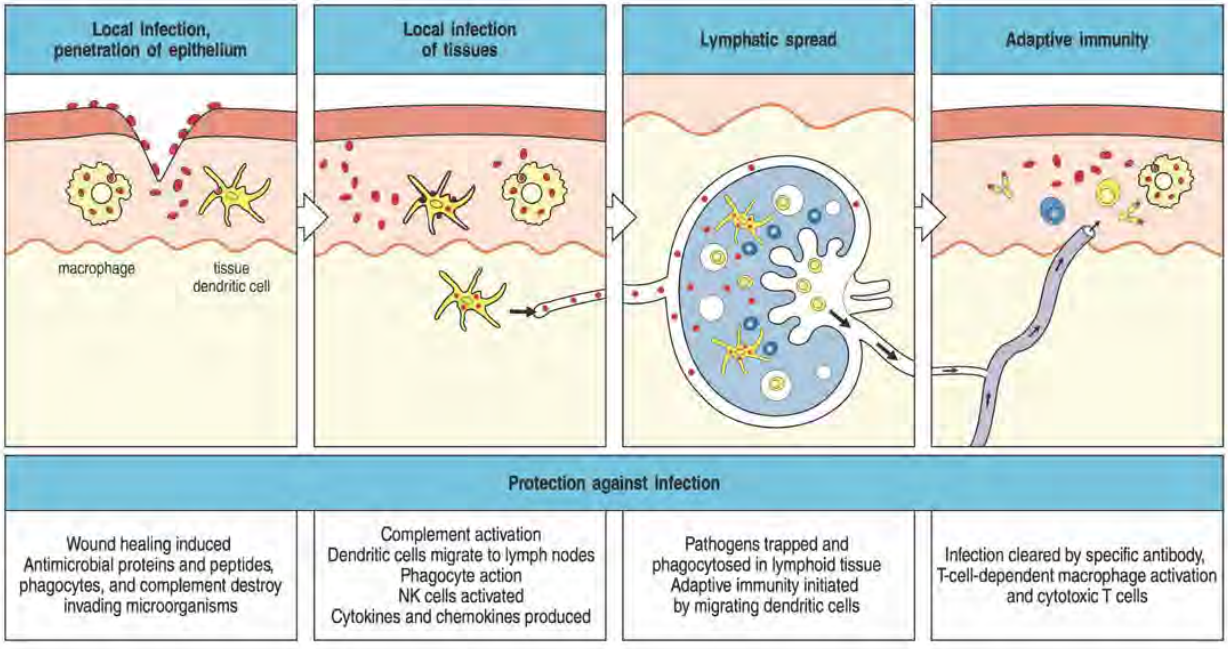


Fig. 11.2 Infections and the responses to them can be divided into a series of stages. These are illustrated here for a pathogenic microorganism (red) entering across a wound in an epithelium. The microorganism first adheres to epithelial cells and then invades beyond the epithelium into underlying tissues (first panel). A local innate immune response helps to contain the infection, and delivers antigen and antigen-loaded dendritic cells to lymphatics (second panel) and thence to local lymph nodes (third panel). This leads to an adaptive immune response in the lymph node that involves the activation and further differentiation of B cells and T cells with the eventual production of antibody and effector T cells, which clear the infection (fourth panel).

and insect and tick bites that breach the epidermal barrier help some microorganisms to get through the skin. Many microorganisms are repelled or kept in check at this stage by innate defenses, which include the induced innate immune response triggered by stimulation of the various germline-encoded receptors (such as TLRs and NK-cell receptors) that discriminate between foreign microbial and self host-cell surfaces or between infected and normal cells (see Chapters 2 and 3). These responses are not as effective as adaptive immune responses, which can afford to be more powerful because they are antigen specific and thus target the pathogen precisely. However, they can prevent an infection from being established or, failing that, can contain it and prevent the spread of a pathogen into the bloodstream while an adaptive immune response develops.

Only when a microorganism has successfully established a focus of infection in the host does disease occur. With the possible exception of lung infections such as tuberculosis, and diarrhea-inducing gut infections such as cholera, in which the primary infection can cause life-threatening disease, little damage will be caused unless the agent spreads from the original focus or secretes toxins that can spread to other parts of the body. Extracellular pathogens spread by direct extension of the infection through the lymphatics or the bloodstream. Usually, spreading into the bloodstream occurs only after the lymphatic system has been overwhelmed. Obligate intracellular pathogens spread from cell to cell; they do so either by direct transmission from one cell to the next or by release into the extracellular fluid and reinfection of both adjacent and distant cells. In contrast, some of the bacteria that cause gastroenteritis exert their effects without spreading into the tissues. They establish a site of infection on the luminal surface of the epithelium lining the gut and cause no direct pathology themselves, but secrete toxins that cause damage either *in situ* or after crossing the epithelial barrier and entering the circulation.

Most infectious agents show a significant degree of host specificity, causing disease in only one or a few related species. What determines host specificity for every agent is not known, but the requirement for attachment to

Petitioner Merck, Ex. 1170, p. 175
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

a particular cell-surface molecule is one critical factor. As other interactions with host cells are also commonly needed to support replication, most pathogens have a limited host range. The molecular mechanisms of host specificity comprise an area of research known as molecular pathogenesis, which is outside the scope of this book.

Adaptive immunity is triggered when an infection eludes or overwhelms the innate defense mechanisms and generates a threshold level of antigen (see Fig. 11.1). Adaptive immune responses are then initiated in the local lymphoid tissue, in response to antigens presented by dendritic cells activated during the course of the innate immune response (Fig. 11.2, second and third panels). Antigen-specific effector T cells and antibody-secreting B cells are generated by clonal expansion and differentiation over several days, as described in greater detail in Chapters 9 and 10. During this time, the induced responses of innate immunity, such as the acute-phase responses and interferon production (see Sections 3-7 and 3-18), continue to function. Eventually, antigen-specific T cells and then antibodies are released into the blood and from there can enter the site of infection (Fig. 11.2, fourth panel). Resolution of the infection involves the clearance of extracellular infectious particles by opsonizing antibodies and phagocytes (see Chapter 10) and the clearance of intracellular residues of infection through the actions of effector T cells (see Chapter 9).

After many types of infection, little or no residual pathology follows an effective primary adaptive response. In some cases, however, the infection or the response to it causes significant tissue damage. In yet other cases, such as infection with cytomegalovirus or *Mycobacterium tuberculosis*, the pathogen is contained but not eliminated, and can persist in a latent form. If the adaptive immune response is later weakened, as it is in acquired immune deficiency syndrome (AIDS), these pathogens may resurface to cause virulent systemic infections. We will focus on the strategies used by certain pathogens to evade or subvert adaptive immunity, and thereby establish a persistent, or chronic, infection, in the first part of Chapter 13. In addition to clearing the infectious agent, an effective adaptive immune response prevents reinfection. For some infectious agents, this protection is essentially absolute, whereas for others infection is only reduced or attenuated on reexposure to the pathogen.

It is not known how many infections are dealt with solely by the nonadaptive mechanisms of innate immunity, because such infections are eliminated early and produce little in the way of symptoms or pathology. Naturally occurring deficiencies in nonadaptive defenses are rare, so it has seldom been possible to study their consequences. Innate immunity does, however, seem to be essential for effective host defense, as shown by the progression of infection in mice that lack components of innate immunity but have an intact adaptive immune system (Fig. 11.3). In humans, for example, mutations in the Toll-like receptor TLR-3 have been associated with increased susceptibility to encephalitis due to the herpes simplex virus, which more usually causes self-limiting cold sores on the skin. Adaptive immunity is also essential, as shown by the immunodeficiency syndromes associated with defects in various components of the adaptive immune response (discussed in Chapter 13).

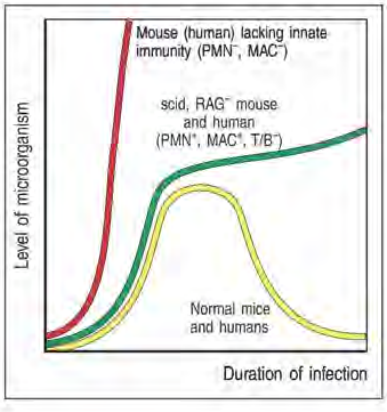


Fig. 11.3 The time course of infection in normal and immunodeficient mice and humans. The red curve shows the rapid growth of microorganisms in the absence of innate immunity, when macrophages (MAC) and polymorphonuclear leukocytes (PMN) are lacking. The green curve shows the course of infection in mice and humans that have innate immunity but have no T or B lymphocytes and so lack adaptive immunity. The yellow curve shows the normal course of an infection in immunocompetent mice or humans.

11-2 The nonspecific responses of innate immunity are necessary for an adaptive immune response to be initiated.

The establishment of a focus of infection in tissues and the response of the innate immune system produce changes in the immediate environment. Many of these changes have been described in earlier chapters, but we review them briefly here to provide a cohesive framework for the induction of adaptive immunity.

Petitioner Merck, Ex. 1170, p. 176
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

In a bacterial infection, the first thing that usually happens is that the infected tissue becomes inflamed. This is initially the result of the activation of the resident macrophages by bacterial components such as lipopolysaccharide (LPS) acting through Toll-like receptors (TLRs) on the macrophage. The cytokines and chemokines secreted by the activated macrophages, especially the cytokine tumor necrosis factor-α (TNF-α), induce numerous changes in the endothelial cells of nearby blood capillaries, a process known as endothelial cell activation. Inflammation also results from the activation of complement, resulting in the production of the anaphylatoxins C3a and C5a, which are able to activate vascular endothelium. In a primary infection, complement is activated mainly via the alternative and lectin pathways (see Fig. 2.12).

Activation of the vascular endothelium causes the release of the contents of Weibel–Palade bodies (the cell adhesion molecule P-selectin and von Willebrand factor) within the endothelial cells to the cell surface (see Section 3-15). Activation also induces the expression of E-selectin, which then also appears on the endothelial cell surface. These two selectins cause neutrophils, monocytes, and other leukocytes to adhere to and roll on the endothelial surface. Cytokine activation of the endothelium also induces the production of the adhesion molecule ICAM-1. By binding to adhesion molecules, such as LFA-1, on neutrophils and monocytes, ICAM-1 strengthens the interaction of these cells with the endothelium and aids their entry in large numbers into the infected tissue to form an inflammatory focus (see Fig. 3.26). As monocytes mature into tissue macrophages and become activated in their turn, additional inflammatory cells are attracted into the infected tissue, and the inflammatory response is maintained and reinforced. The inflammatory response can be thought of as putting up a flag on the endothelial cells to signal the presence of infection, but as yet the response is nonspecific for the pathogen.

A second crucial effect of infection is the activation of specialized antigen-presenting cells, the dendritic cells residing in most tissues, as described in Sections 9-4 to 9-6. Dendritic cells take up antigen in the infected tissues and, like macrophages, they are activated through innate immune receptors that respond to common constituents of pathogens, such as TLRs (Section 3-7) and NOD proteins (Section 3-8). Activated dendritic cells increase their synthesis of MHC class II molecules and, most importantly, begin to express the co-stimulatory molecules B7.1 and B7.2 on their surface. As described in Chapter 9, these antigen-presenting cells migrate away from the infected tissue through the lymphatics, along with their antigen cargo, to enter peripheral lymphoid tissues, where they initiate the adaptive immune response. They arrive in large numbers at the draining lymph nodes, or other nearby lymphoid tissue, attracted by the chemokines CCL19 and CCL21 produced by the lymph-node stroma (see Section 9-3).

When dendritic cells arrive in the lymphoid tissues, they seem to have reached their final destination. They activate antigen-specific naive T lymphocytes in these tissues, after which they die. Naive lymphocytes are continually passing through the lymph nodes, which they enter from the blood across the walls of high endothelial venules (see Fig. 9.4). Those naive T cells that recognize antigen on the surface of dendritic cells are activated, and divide and mature into effector cells that reenter the circulation. Where there is a local infection, the changes induced by inflammation in the walls of nearby venules induce these effector T cells to leave the blood vessel and migrate into the site of infection.

Thus, the local release of cytokines and chemokines at the site of infection has far-reaching consequences. In addition to recruiting neutrophils and macrophages, which are not specific for antigen, the changes induced in the blood vessel walls also enable newly activated effector T lymphocytes to enter infected tissue, as discussed in more detail later in this chapter.

Petitioner Merck, Ex. 1170, p. 177
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

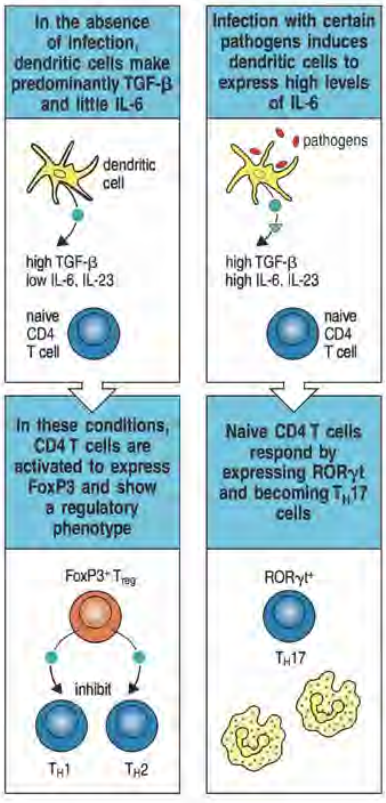


Fig. 11.4 Cytokines produced by dendritic cells regulate the balance of regulatory T-cell development and T_H17 differentiation. The balance between TGF-β and IL-6 production acts to induce either the transcription factor FoxP3, which is characteristic of regulatory T cells, or RORγt (an 'orphan' member of the nuclear receptor family), which is characteristic of T_H17 cells. In the absence of infection, IL-6 production by dendritic cells is low, and TGF-β production dominates. In these conditions, T cells that do encounter their cognate antigen will be induced to express FoxP3 and predominantly acquire a regulatory phenotype, whereas those that do not encounter antigen remain naive. Infection by certain bacteria and fungi induce dendritic cells to produce abundant IL-6 and IL-23, but less IL-12 (see Fig. 11.5); under these conditions, naive T cells will express RORγt and become T_H17 cells. The cytokines produced by this T-cell subset, IL-17 and IL-17F, induce cells such as epithelium to secrete chemokines that attract inflammatory cells such as neutrophils.

11-3 Cytokines made during infection can direct differentiation of CD4 T cells toward the T_H17 subset.

In Chapter 9 we described transcriptional mechanisms that control how specific cytokines direct the differentiation of naive CD4 T cells into distinct classes of CD4 effector T cells—T_H17, T_H1, or T_H2, or regulatory subsets (see Fig. 9.29). The cytokines that are produced during the progression of an infection depend on how the microorganism influences the behavior of innate immune cells and antigen-presenting cells. The conditions produced by these interactions have a major impact on how T cells differentiate during their initial contact with antigen-presenting cells, thus determining the types of T cells that are generated (see Chapter 9). In turn, these T-cell subsets influence the nature of the effector responses that are recruited, such as the extent of macrophage activation, neutrophil and eosinophil recruitment to the site of infection, and which classes of antibody will predominate.

The subset of effector T cells generated in response to infection by extracellular bacteria and fungi is often T_H17. At the beginning of an infection, dendritic cells are not yet fully activated; in this state they produce TGF-β, but little IL-6 or other cytokines that direct T-cell differentiation. Upon encountering such pathogens, dendritic cells become activated and are induced to synthesize IL-6 along with IL-23. As there is no source of IL-4 or IL-12 at this time, naive CD4 T cells will differentiate into T_H17 cells rather than T_H1 or T_H2 cells (Fig. 11.4). For example, activation of dendritic cells through Dectin-1, a receptor that recognizes carbohydrates common to yeast and other fungi, induces dendritic cells to produce abundant IL-23 but little IL-12. The effect is that dendritic cells activated by these organisms will tend to promote T_H17 differentiation.

When the T_H17 cells leave the lymph node and migrate to the sites of infection, they encounter pathogen antigens and are stimulated to synthesize and release cytokines, which include various members of the IL-17 family such as IL-17A and IL-17E (also known as IL-25). The receptor for IL-17 is expressed ubiquitously on cells such as fibroblasts, epithelial cells, and keratinocytes. IL-17 induces these cells to secrete various cytokines, including IL-6, the chemokines CXCL8 and CXCL2, and the hematopoietic factors granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF). These chemokines can act directly to recruit neutrophils, whereas G-CSF and GM-CSF act to augment neutrophil and macrophage production in the bone marrow or increase the differentiation of local monocytes into macrophages.

Thus, one important action of IL-17 at sites of infection is to induce local cells to secrete cytokines and chemokines that attract neutrophils. T_H17 cells also produce IL-22, a cytokine related to IL-10. IL-22 acts cooperatively with IL-17 to induce the expression of antimicrobial peptides, such as β-defensins, by the keratinocytes of the epidermis. In this way, the presence of pathogen-specific T_H17 cells serves as an efficient amplifier of an acute inflammatory response by the innate immune system at sites of early infection. CD4 T cells that acquire the T_H17 phenotype are not the only cells that can produce IL-17 in response to infections. CD8 T cells have also been shown to produce abundant IL-17.

The cytokine environment is also influential in preventing the immune system from making inappropriate responses to self antigens or those of commensal microorganisms, the microorganisms that normally inhabit the body. Even in the absence of infection, dendritic cells take up self and environmental antigens and eventually carry them to peripheral lymphoid tissues, where they may meet antigen-specific naive T cells. In such circumstances, the pro-inflammatory signals are not present; dendritic cells are not activated and so produce the cytokine TGF-β but not the other cytokines that can affect CD4 T-cell differentiation. In this setting, dendritic cells seem to actively

Petitioner Merck, Ex. 1170, p. 178
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

generate tolerance to the antigens that the naive T cells encounter (Fig. 11.4, left panels). TGF-β on its own inhibits the proliferation and differentiation of T_H17, T_H1, and T_H2 cells. When a naive CD4 T cell encounters its cognate peptide:MHC ligand in the presence of TGF-β alone, it acquires the phenotype of a regulatory T cell, in that it can inhibit the activation of other T cells. Regulatory T cells induced in this way outside the thymus are called adaptive regulatory T cells and some express the transcription factor FoxP3 (see Section 9-18). The regulatory T cells, in theory, should not be specific for pathogen antigens—which they have not yet encountered—but should instead be specific for either self antigens or peptides from commensal organisms. Other FoxP3-expressing regulatory CD4 T cells, the natural regulatory T cells, seem to acquire their regulatory phenotype in the thymus (see Section 8-18).

The reciprocal pathways for the development of T_H17 cells and regulatory T cells seem to be based on an evolutionarily ancient system of activation and inactivation, because proteins similar to TGF-β and IL-17 are present in invertebrates that possess primitive intestinal immune systems. This might suggest that the dichotomy between T_H17 cells and regulatory T cells is largely concerned with maintaining the lymphocyte balance in tissues exposed to large numbers of potential pathogens, such as the mucosae of the gut and the lungs, where a rapid response to infection is critical. For example, IL-17-producing T cells have an important role in mice in resistance to infections of the lung by Gram-negative bacteria such as *Klebsiella pneumoniae*. Mice lacking the receptor for IL-17 are significantly more susceptible than normal mice to lung infection by this pathogen, and they show decreased production of G-CSF and CXCL2 and poorer recruitment of neutrophils to infected lungs. T_H17 cells also promote resistance to the gut nematode *Nippostrongylus brasiliensis*. This effect seems to be due to the induction or recruitment by IL-17E of a population of non-T non-B leukocytes, perhaps similar to basophils, that secrete the T_H2 cytokines IL-4, IL-5, and IL-13. These cytokines, particularly IL-13, promote resistance to *N. brasiliensis* by, for example, inducing its expulsion from the gut by augmenting the production of mucus (see Chapter 12).

11-4 T_H1 and T_H2 cells are induced by cytokines generated in response to different pathogens.

T_H1 responses tend to be induced by viruses and by bacterial and protozoan pathogens that can survive inside macrophage intracellular vesicles. In the case of viruses, the T_H1 response is generally involved in helping to activate the CD8 cytotoxic T cells that will recognize virus-infected cells and destroy them (see Chapter 9). T_H1 cells also induce the production of some subclasses of IgG antibodies, which neutralize virus particles in the blood and extracellular fluid. In the case of mycobacteria, and of protozoa such as *Leishmania* and *Toxoplasma*, which all take up residence inside macrophages, the role of T_H1 cells is to activate macrophages to a degree that will destroy the invaders.

Experiments *in vitro* have shown that naive CD4 T cells initially stimulated in the presence of IL-12 and IFN-γ tend to develop into T_H1 cells (Fig. 11.5, left panels). In part this is because these cytokines induce or activate the transcription factors leading to T_H1 development, and in part it is because IFN-γ inhibits the proliferation of T_H2 cells, as described in Chapter 9. NK cells and CD8 cells are also both activated in response to infections with viruses and some other intracellular pathogens, as discussed in Chapters 3 and 9, and both produce abundant IFN-γ. Dendritic cells and macrophages produce IL-12. Thus, CD4 T-cell responses in these infections tend eventually to be dominated by T_H1 cells.

Toll-like receptor (TLR) signaling is of major importance in driving dendritic cells to produce IL-12. This has been shown in mice lacking the adaptor

Petitioner Merck, Ex. 1170, p. 179
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

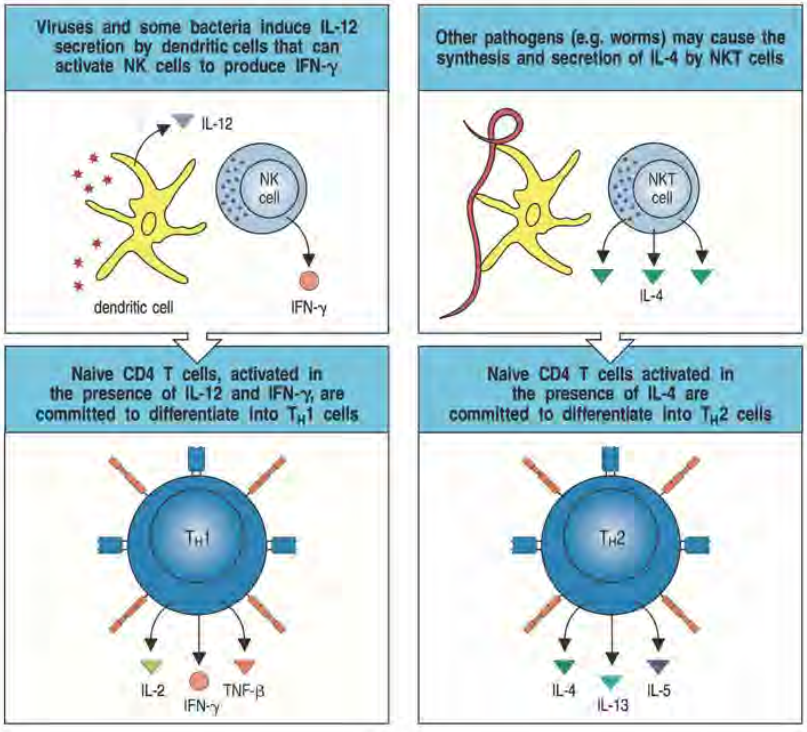


Fig. 11.5 The differentiation of naive CD4 T cells into subclasses of effector T cells is influenced by cytokines elicited by the pathogen. Left panels: many pathogens, especially viruses and intracellular bacteria, activate dendritic cells to produce IL-12 and NK cells to produce IFN-γ. These cytokines cause proliferating CD4 T cells to differentiate into T_H1 cells. NK cells can be induced by certain stimuli and adjuvants to migrate into lymph nodes, where they could promote T_H1 responses. Right panels: IL-4, which can be produced by various cells, is made in response to parasitic worms and some other pathogens, and acts on proliferating CD4 T cells to cause them to become T_H2 cells. An iNKT cell is shown as a source of IL-4 here, but these cells are not the only source of IL-4 that can promote T_H2 responses (see the text). The mechanisms by which these cytokines induce the selective differentiation of CD4 T cells are discussed in Section 9-18 and Fig. 9.29. Selective induction of transcription factors induced by cytokine binding to cytokine receptors leads to the activation of these two different fates.

protein MyD88, a component of an intracellular signaling pathway activated by the stimulation of some TLRs (see Section 3-7). Mice deficient in MyD88 do not survive a challenge with *Toxoplasma gondii*, which normally elicits a strong T_H1 response. Dendritic and other cells from mice lacking MyD88 failed to produce IL-12 in response to parasite antigens, and the animals failed to mount a T_H1 response (Fig. 11.6). In contrast to dendritic cells stimulated through Dectin-1, dendritic cells stimulated through TLR-9 produce abundant IL-12 and support T_H1 differentiation. It is important to note that many immune responses to pathogens produce a mixed response, with both T_H17 and T_H1 cells being generated. The basis for such mixed responses, and for potential plasticity between these subsets, is an area of active research.

The killer lymphocytes of innate immunity, the NK cells, may contribute to T_H1 development (see Fig. 11.5). NK cells are not normally found within lymph nodes, but injection of mice with certain adjuvants, or with mature dendritic cells, can induce their recruitment to lymph nodes via expression of the chemokine receptor CXCR3 by the NK cell. As NK cells produce abundant IFN-γ, but little IL-4, they may act in lymph nodes during infections to direct the development of T_H1 cells.

For T_H2 responses, the mechanisms linking innate immunity to regulation of the adaptive T_H2 response are somewhat less clear (see Section 9-18). Naive CD4 T cells activated in the presence of IL-4, especially when IL-6 is also present, tend to differentiate into T_H2 cells (see Fig. 11.5, right panels). Some pathogens, such as helminths and other extracellular parasites, consistently induce development of T_H2 responses *in vivo*, and do so in a manner that requires IL-4 signaling by T cells. But it is still uncertain how these pathogens are initially sensed by the immune system and how this triggers commitment of the naive T cell to the T_H2 subset. But once some T cells have differentiated into effector T_H2 cells, their production of IL-4 can strongly reinforce the development of more T_H2 cells. Many cells have been proposed to act as

Petitioner Merck, Ex. 1170, p. 180
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

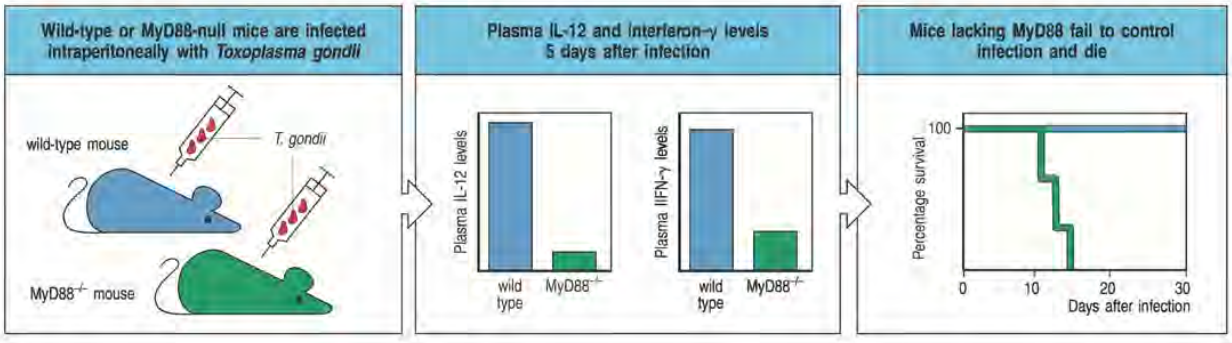


Fig. 11.6 Infection may trigger T_H1 polarization through Toll-like receptor signaling pathways. The adaptor protein MyD88 is a key component of Toll-like receptor signaling. Wild-type mice and mice deficient in MyD88 were infected intraperitoneally with the protozoan parasite *Toxoplasma gondii* (left panel). Five days after infection, mice lacking MyD88 showed a severe reduction in the amount of IL-12 in plasma compared with wild-type mice (center panel), and dendritic cells from the spleens of these animals failed to produce IL-12 on stimulation with antigens from *T. gondii*. The mice lacking MyD88 also failed to produce a large IFN-γ response (center panel) and a T_H1 response to the infection, and died about 2 weeks after infection (right panel, green line). In contrast, wild-type mice produced a strong IL-12, IFN-γ, and T_H1 response, controlled the infection, and survived (right panel, blue line). Sensing of *T. gondii* in mice may involve TLR-11 (see Section 3-5).

an initial and rapid source of IL-4, including invariant NKT cells (iNKT cells), mast cells, and basophils.

iNKT cells are a class of innate-like lymphocytes (see Section 3-24) that express the cell-surface marker NK1.1 normally associated with NK cells. Like other NKT cells (see Section 8-9), development of iNKT cells in the thymus does not involve selection by MHC class I or class II molecules, but instead depends on interactions with the MHC class IB molecules known as CD1 proteins (see Section 6-19), which bind self lipids. iNKT cells have a nearly invariant repertoire of α:β T-cell receptor composed of gene segments $V_\alpha14$–$J_\alpha28$ in mice, or the equivalent gene segments, $V_\alpha24$–$J_\alpha18$, in humans. Expression of CD1 proteins can be induced at sites of infection, which may allow the presentation of microbial lipids to T cells. At least some iNKT cells recognize specific glycolipid antigens presented by CD1d. On activation, iNKT cells secrete very large amounts of IL-4 and IFN-γ, and may provide an initial source of cytokines that polarize a T-cell response, particularly in the direction of T_H2 cells. iNKT cells are not the only T cells that recognize antigens presented by CD1 molecules. CD1b presents the bacterial lipid mycolic acid to α:β T cells, and other CD1 molecules are recognized by γ:δ T cells. However, a particular role for iNKT cells in T_H2 responses has emerged from studies of mice in which the genes encoding $J_\alpha18$ or CD1d have been inactivated, and so iNKT cells fail to develop. These mice also fail to develop antigen-induced airway hypersensitivity that mimics a condition similar to asthma in humans and which is strongly associated with a T_H2 response.

Mast cells and basophils are also potent IL-4 producers and can migrate to peripheral lymphoid organs, making them possible candidates for an early source of IL-4 in response to infection. However, transgenic mice engineered to lack basophils still developed T_H2 responses and IgE production when infected by the helminth *Nippostrongylus brasiliensis*, but basophils were required for protection against secondary infection. This suggests that their role is mainly as an effector, not an initiator, of T_H2-type responses. Some evidence suggests that certain TLR ligands can cause dendritic cells to produce cytokines favoring T_H2 development rather than T_H1. For example, dendritic cells make more IL-10 and less IL-12 when stimulated by some ligands for TLR-2, including bacterial lipoproteins, peptidoglycans, and zymosan, a carbohydrate derivative of yeast cell walls, compared with other TLR ligands, but these can also stimulate production of IL-23, which favors T_H17 development.

11-5 CD4 T-cell subsets can cross-regulate each other's differentiation.

The various subsets of CD4 T cells—regulatory T cells (T_{reg}), T_H17, T_H1, and T_H2—each have very different functions. T_{reg} cells maintain tolerance and limit immunopathology. T_H17 cells amplify acute inflammation at sites of infection. T_H1 cells are crucial for cell-mediated immunity due to phagocytes.

Petitioner Merck, Ex. 1170, p. 181
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

T_H2 cells promote allergic responses and protection against parasites by augmenting barrier immunity at epithelial surfaces. Cytokines mediate many of these distinct functions, but some can also influence the development of these T-cell subsets themselves. Thus, there is a complex pattern of cross-regulation during the development of CD4 T-cell subsets.

For example, T_H17 cells are induced by IL-6 and TGF-β, but IFN-γ (produced by T_H1 cells) or IL-4 (produced by T_H2 cells) can inhibit T_H17 development, promoting T_H1 or T_H2 development, respectively (Fig. 11.7). Furthermore, there is some degree of plasticity between these subsets, at least *in vitro*. Treatment with IFN-γ and IL-12 can convert established T_H17 cells into IFN-γ-producing T_H1 cells, and the combination of IFN-α, IFN-γ, and IL-12 can even induce IFN-γ production from T_H2 cells. There is also cross-regulation between T_H1 and T_H2 cells. IL-4 and IL-10, which are products of T_H2 cells, can inhibit the production of IL-12 by dendritic cells, and thereby inhibit T_H1 development. Conversely, IFN-γ, a product of T_H1 cells, can inhibit the proliferation of T_H2 cells (see Fig. 11.7). IL-10 is not made exclusively by T_H2 cells: it can also be made by T_H1 cells under conditions of strong stimulation of the T-cell receptor. In this way, antigen concentration can potentially modulate T_H1 and T_H2 differentiation by changing the overall balance of different cytokines such as IL-10.

It is sometimes possible to shift the balance between T_H1 and T_H2 cells by administering appropriate cytokines or antibodies, although studying their effects on human CD4 T-cells poses obvious difficulties. IL-2 and IFN-γ have been used to stimulate cell-mediated immunity in human diseases such as lepromatous leprosy and can cause both a local resolution of the lesion and a systemic change in T-cell responses. However, the links between cytokine action and disease have been explored mainly in mouse models. Such studies indicate that the appropriate choice of the CD4 T cell can sometimes be crucial for clearance of infection, and show that subtle differences in CD4 T-cell responses can have a significant impact on the outcome of infection.

One clear example of this is the murine model of infection by the protozoan parasite *Leishmania major*, which requires a T_H1 response and activation of

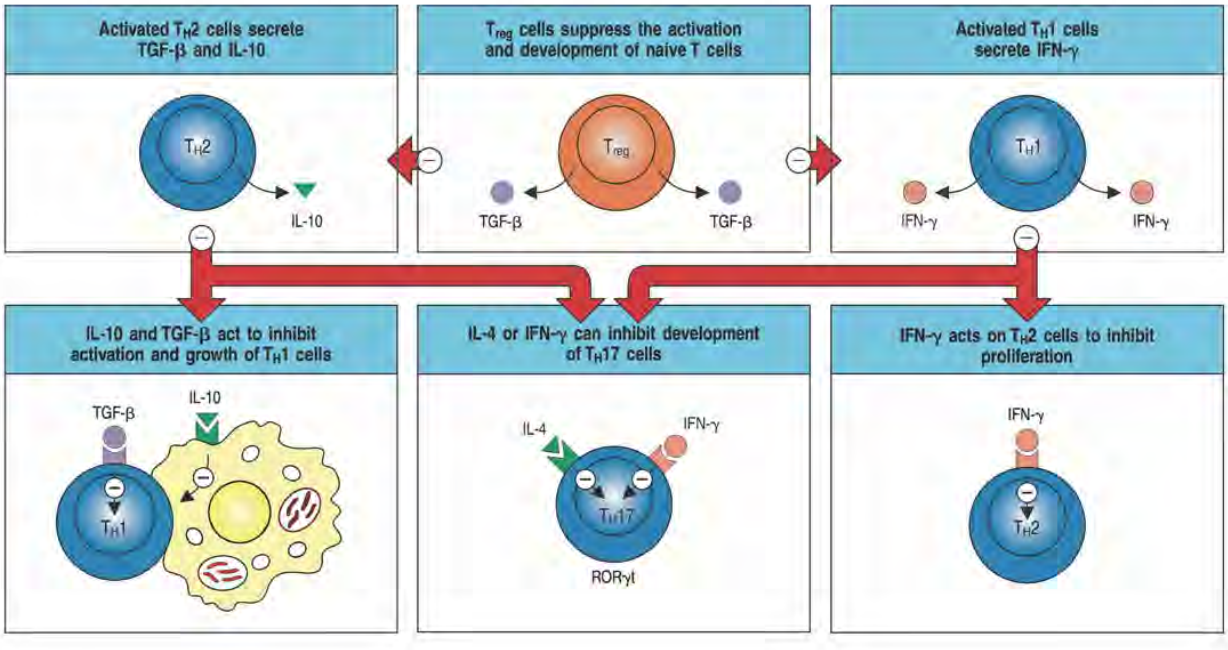


Fig. 11.7 The subsets of CD4 T cells each produce cytokines that can negatively regulate the development or effector activity of other subsets. In the absence of infection, in homeostatic conditions, the TGF-β produced by T_{reg} cells can inhibit the activation of naive T cells, thus preventing the development of a T_H17, T_H1, or T_H2 response (upper panels). During an infection, development of T_H17 cells occurs in response to IL-6 produced by dendritic cells. However, if signals are present to induce T_H1 or T_H2 cells, the cytokines IFN-γ or IL-4 produced by them can override the effect of IL-6 and inhibits T_H17 development (lower center panel). T_H2 cells can produce IL-10, which inhibits the production of IL-12 by macrophages, reducing T_H1 cells, and TGF-β, which inhibits T_H1 growth (left panels). T_H1 cells produce IFN-γ, which blocks the growth of T_H2 cells (right panels).

Petitioner Merck, Ex. 1170, p. 182
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

macrophages for clearance. C57BL/6 mice produce T_H1 cells that protect the animal by activating infected macrophages to kill *L. major*. In BALB/c mice infected with *L. major*, however, CD4 T cells fail to differentiate into T_H1 cells; instead, they become T_H2 cells, which are unable to activate macrophages to inhibit leishmanial growth. This difference seems to result from a population of memory T cells that are specific for gut-derived antigens but cross-react with an antigen, LACK (*Leishmania* analog of the receptors of activated C kinase), expressed by the *Leishmania* parasite. These memory cells are present in both strains of mice, but for unknown reasons they produce IL-4 in BALB/c mice but not in C57BL/6 mice. In BALB/c mice, the small amount of IL-4 secreted by these memory cells that are activated during *Leishmania* infection drives new *Leishmania*-specific CD4 T cells to become T_H2 cells, which leads eventually to a failure to eliminate the pathogen, and to death. The preferential development of T_H2 rather than T_H1 cells in BALB/c mice can be reversed if IL-4 is blocked in the first days of infection by injecting anti-IL-4 antibody, but this treatment is ineffective after a week or so of infection, demonstrating the crucial importance of the early exposure to cytokines for decisions by naive T cells (Fig. 11.8).

CD8 T cells are also able to regulate the immune response by producing cytokines. Effector CD8 T cells can, in addition to their familiar cytotoxic function, also respond to antigen by secreting cytokines typical of either T_H1 or T_H2 cells. Such CD8 T cells, called T_C1 or T_C2 by analogy with the T_H subsets, seem to be responsible for the development of leprosy in its lepromatous rather than its tuberculoid form. As we saw in Chapter 9, lepromatous leprosy is due to the predominance of a T_H2 cell response, which does not clear the bacteria. Patients with the less destructive tuberculoid leprosy make T_C1 cells whose cytokines induce T_H1 cells, which can activate macrophages to rid the body of its burden of leprosy bacilli. Patients with lepromatous leprosy have CD8 T cells that suppress the T_H1 response by making IL-10 and TGF-β. The expression of these cytokines could explain the suppression of CD4 T cells by CD8 T cells that has been observed in various situations.

Another factor that may possibly influence the differentiation of CD4 T cells into various subsets is the amount, or sequence, of the antigenic peptide that initiates the response. In some cases, large amounts of antigenic peptide, or peptides that interact strongly with the T-cell receptor, favor T_H1 responses, whereas a low density of peptide or peptides that bind weakly tend to elicit T_H2 responses. These effects do not seem to be due to differences in signaling through the T-cell receptor but may involve changes in the overall balance of different cytokines produced by the cells involved in activating naive T cells. It is not clear whether this effect is universal, but it could be important in certain circumstances. For instance, allergic reactions are caused by the production of IgE antibody, which requires high levels of IL-4 but does not occur in the presence of IFN-γ, a powerful inhibitor of IL-4-driven class switching to IgE. Antigens that elicit IgE-mediated allergy are generally delivered in minute doses and elicit T_H2 cells that make IL-4 and no IFN-γ. Also, it is possible that many allergens do not activate cytokine production from innate immune responses, which might override the effects of antigen dose. Finally, allergens are delivered to humans in minute doses across a thin mucosa, such as that of the lung, and something about this route of sensitization seems to favor T_H2 responses.

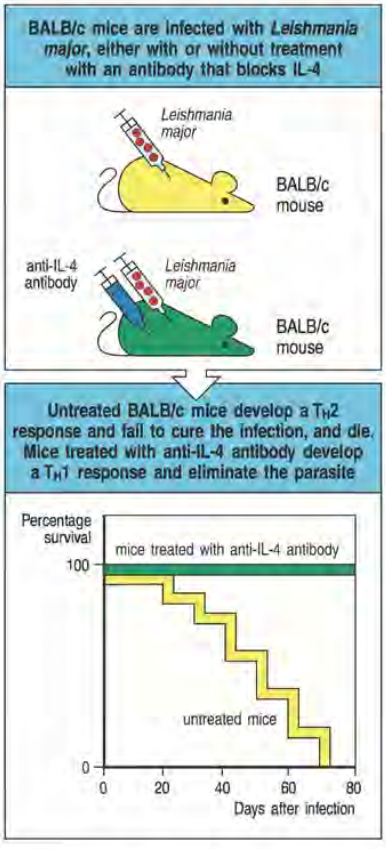


Fig. 11.8 The development of CD4 T-cell subsets can be manipulated by altering the cytokines acting during the early stages of infection. Elimination of infection with the intracellular protozoan parasite *Leishmania major* requires a T_H1 response, because IFN-γ is needed to activate the macrophages that provide protection. BALB/c mice are normally susceptible to *L. major* because they generate a T_H2 response to the pathogen. This is because they produce IL-4 early during infection and this induces naive T cells into the T_H2 lineage (see the text). Treatment of BALB/c mice with neutralizing anti-IL-4 antibodies at the beginning of infection inhibits this IL-4 and prevents the diversion of naive T cells toward the T_H2 lineage; these mice develop a protective T_H1 response.

11-6 Effector T cells are guided to sites of infection by chemokines and newly expressed adhesion molecules.

The full activation of naive T cells takes 4–5 days and is accompanied by marked changes in their homing behavior. Effector cytotoxic CD8 T cells must travel from the peripheral lymphoid tissue in which they have been activated to attack and destroy infected cells. Effector CD4 T_H1 cells must also leave

Petitioner Merck, Ex. 1170, p. 183
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

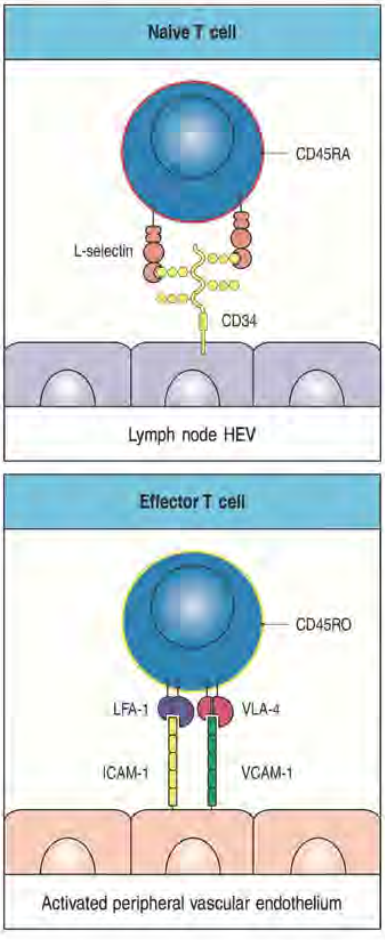


Fig. 11.9 Effector T cells change their surface molecules, allowing them to home to sites of infection. Naive T cells home to lymph nodes through the binding of L-selectin to sulfated carbohydrates displayed by various proteins, such as CD34 and GlyCAM-1, on the high endothelial venule (HEV, upper panel). After encounter with antigen, many of the differentiated effector T cells lose expression of L-selectin, leave the lymph node about 4–5 days later, and now express the integrin VLA-4 and increased levels of LFA-1. These bind to VCAM-1 and ICAM-1, respectively, on peripheral vascular endothelium at sites of inflammation (lower panel). On differentiating into effector cells, T cells also alter their splicing of the mRNA encoding the cell-surface protein CD45. The CD45RO isoform expressed by effector T cells lacks one or more exons that encode extracellular domains present in the CD45RA isoform expressed by naive T cells, and somehow makes effector T cells more sensitive to stimulation by specific antigen.

the lymphoid tissues to activate macrophages at the site of infection. Exit of effector T cells from lymphoid tissues is regulated by changes in their responsiveness to the chemotactic lipid sphingosine 1-phosphate, as described in Section 9-3. Most effector T cells cease production of L-selectin, which mediates homing to the lymph nodes, whereas the expression of other adhesion molecules is increased (Fig. 11.9). One important change is a marked increase in synthesis of the integrin α_4:β_1, also known as VLA-4. This binds to the adhesion molecule VCAM-1, a member of the immunoglobulin superfamily that is induced on activated endothelial cell surfaces and initiates the extravasation of the effector T cells. Thus, if the innate immune response has already activated the endothelium at the site of infection, as described in Section 11-2, effector T cells will be rapidly recruited.

In the early stage of an immune response, only a few of the effector T cells that enter the infected tissue will be expected to be specific for pathogen, because any effector T cell specific for any antigen will also be able to enter. Specificity of the reaction is maintained, however, because only those effector T cells that recognize pathogen antigens will carry out their function, destroying infected cells or specifically activating pathogen-loaded macrophages. By the peak of an adaptive immune response, after several days of clonal expansion and differentiation, most of the recruited T cells will be specific for the infecting pathogen.

Not all infections trigger innate immune responses that activate local endothelial cells, and it is not so clear how effector T cells are guided to the sites of infection in these cases. However, activated T cells seem to enter all tissues in very small numbers, perhaps via adhesive interactions such as the binding of P-selectin on the endothelial cells to its ligand **P-selectin glycoprotein ligand-1** (**PSGL-1**), which is expressed by activated T cells, and so effector T cells could encounter their antigens even in the absence of a previous inflammatory response.

By the means just described, one or a few specific effector T cells encountering antigen in a tissue can initiate or augment a potent local inflammatory response that recruits many more effector lymphocytes and nonspecific inflammatory cells to that site. Effector T cells that recognize pathogen antigens in the tissues produce cytokines such as TNF-α, which activates endothelial cells to express E-selectin, VCAM-1, and ICAM-1, and chemokines such as CCL5, which acts on effector T cells to activate their adhesion molecules. VCAM-1 and ICAM-1 on endothelial cells bind VLA-4 and LFA-1, respectively, on effector T cells, recruiting more of these cells into tissues that contain the antigen. At the same time, monocytes and polymorphonuclear leukocytes are recruited by adhesion to E-selectin. TNF-α and IFN-γ released by activated T cells also act synergistically to change the shape of endothelial cells, allowing increased blood flow, increased vascular permeability, and increased emigration of leukocytes, fluid, and protein into the infected tissue. Thus, at the later stages of an infection, the protective effects of macrophages secreting TNF-α and other pro-inflammatory cytokines at the infected site (see Section 3-17) are reinforced by the actions of effector T cells.

By contrast, effector T cells that enter tissues but do not recognize their antigen are rapidly lost. They either enter the lymph from the tissues and eventually return to the bloodstream, or undergo apoptosis. Most of the T cells in the afferent lymph that drains tissues are memory or effector T cells, which characteristically express the CD45RO isoform of the cell-surface molecule CD45 and lack L-selectin (see Fig. 11.9). Effector T cells and memory T cells have similar phenotypes, as we discuss later, and both seem to be committed to migration through potential sites of infection. In addition to allowing effector T cells to clear all sites of infection, this pattern of migration allows them to contribute, along with memory cells, to protecting the host against reinfection with the same pathogen.

Petitioner Merck, Ex. 1170, p. 184
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

Expression of particular adhesion molecules can direct different subsets of effector T cells to specific sites. As we shall see in Chapter 12, the peripheral immune system is compartmentalized, such that different populations of lymphocytes migrate through different lymphoid compartments and—after activation—through the different tissues they serve. This is achieved by the selective expression of adhesion molecules that bind selectively to tissue-specific addressins. In this context, the adhesion molecules are often known as **homing receptors**. For example, some activated T cells specifically populate the skin. They are induced during activation to express the adhesion molecule **cutaneous lymphocyte antigen (CLA)** (Fig. 11.10). This is a specifically glycosylated isoform of PSGL-1 that binds to E-selectin on cutaneous vascular endothelium. CLA-expressing T lymphocytes also produce the chemokine receptor CCR4. This binds the chemokine CCL17 (TARC), which is present at high levels on the endothelium of cutaneous blood vessels. Interaction of CLA with E-selectin causes the T cell to roll against the wall of the vessel, and the signal delivered by endothelial CCL17 is thought to cause the arrest of lymphocytes and to bring about their adhesion to the wall, probably by inducing tight binding of integrin, as described for the action of CCL21 on naive T cells (see Section 9-3). The condition known as leukocyte adhesion deficiency is found in people who lack integrin β subunits, and such people suffer from recurrent infections with pyogenic bacteria and problems with wound healing. In addition to CCR4, skin-homing T cells carry the chemokine receptor CCR10 (GPR-2), which binds the chemokine CCL27 (CTACK) expressed by keratinocytes, the epithelial cells of the skin. Different combinations of chemokines and receptors are used by T cells that home to the mucosal immune system of the gut, and these are discussed in Chapter 12.



Leukocyte Adhesion Deficiency

11-7 Differentiated effector T cells are not a static population but continue to respond to signals as they carry out their effector functions.

The commitment of CD4 T cells to become distinct lineages of effector cells begins in peripheral lymphoid tissues, such as lymph nodes, as described in Sections 11-3 and 11-4. However, the effector activities of these cells once they enter sites of infection are not defined simply by the signals received in the lymphoid tissues. Instead, evidence suggests that there is continuous regulation of the expansion and the effector activities of differentiated CD4 cells, in particular of T_H17 and T_H1 cells.

As noted in Chapter 9, commitment of naive T cells to become T_H17 cells is triggered by exposure to TGF-β and IL-6; commitment to T_H1 cells is initially triggered by IFN-γ. These initial conditions are not, however, sufficient to

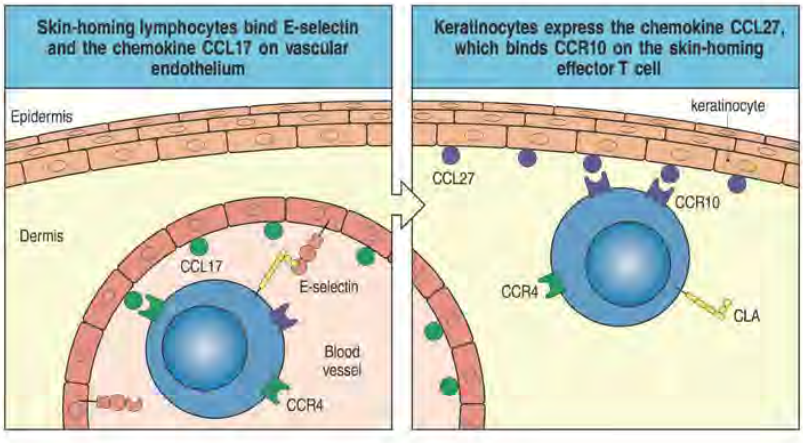


Fig. 11.10 Skin-homing T cells use specific combinations of integrins and chemokines to migrate specifically to the skin. Left panel: a skin-homing lymphocyte binds to the endothelium lining a cutaneous blood vessel by interactions between cutaneous lymphocyte antigen (CLA) and constitutively expressed E-selectin on the endothelial cells. The adhesion is strengthened by an interaction between lymphocyte chemokine receptor CCR4 and the endothelial chemokine CCL17. Right panel: once through the endothelium, effector T lymphocytes are attracted to keratinocytes of the epidermis by the chemokine CCL27, which binds to the receptor CCR10 on lymphocytes.

Petitioner Merck, Ex. 1170, p. 185
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

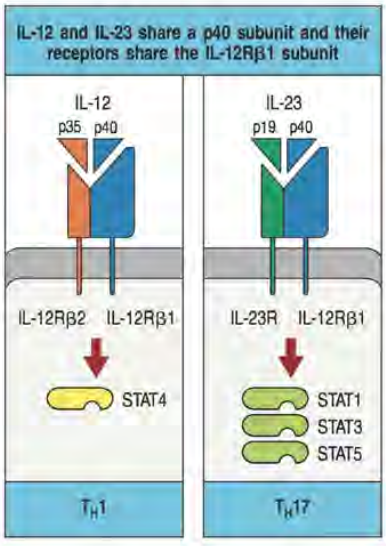


Fig. 11.11 The cytokines IL-12 and IL-23 share subunits, and their receptors have a common component. The dimeric cytokines IL-12 and IL-23 share the p40 subunit, and the receptors for IL-12 and IL-23 have the IL-12Rβ1 subunit in common. IL-12 signaling activates the transcriptional activators STAT1, STAT3, and STAT4, but its action in increasing IFN-γ production is due to STAT4. IL-23 activates several other STATs, but activates STAT4 only weakly. Both cytokines augment the activity and proliferation of the CD4 subsets that express receptors for them; T_H1 cells express IL-12R, and T_H17 cells express IL-23R. Mice deficient in p40 lack expression of both of these cytokines, and manifest immune defects as a result of deficiencies in both T_H1 and T_H17 activities.

generate complete or effective T_H17 or T_H1 responses. In addition, each T-cell subset also requires stimulation by another cytokine: IL-23 in the case of T_H17 cells, and IL-12 in the case of T_H1 cells. IL-23 and IL-12 are closely related in structure; each is a heterodimer and they share a subunit. IL-23 is composed of one p40 and one p19 subunit, whereas IL-12 has the p40 subunit and a unique p35 subunit. Committed T_H17 cells express a receptor for IL-23, whereas T_H1 cells express a receptor for IL-12. The receptors for IL-12 and IL-23 are also related, having a common subunit (Fig. 11.11).

IL-23 and IL-12 amplify the activities of T_H17 and T_H1 cells, respectively. Like many other cytokines, they both act through the JAK–STAT intracellular signaling pathway (see Fig. 7.29). IL-23 signaling activates the intracellular transcriptional activators STAT1, STAT3, and STAT5, but activates STAT4 very weakly. In contrast, IL-12 activates STAT1 and STAT3 and also strongly activates STAT4. IL-23 does not initiate the commitment of naive CD4 T cells to T_H17 cells, but it does stimulate their expansion. Many *in vivo* responses that depend on IL-17 are diminished in the absence of IL-23. For example, mice lacking the IL-23-specific subunit p19 show decreased production of IL-17 and IL-17F in the lung after infection by *Klebsiella pneumoniae*.

Mice lacking the p40 subunit, which is shared by IL-12 and IL-23, are deficient in both IL-23 and IL-12. This was the source of some confusion before the separate role of IL-23 in T_H17 activity was understood. It had been assumed that inflammation in the brains of mice with experimental autoimmune encephalomyelitis (EAE) was due to T_H1 cells and IFN-γ, because EAE could not be induced in p40-deficient mice. But later it was found that EAE could be induced in p35-deficient mice, which lack IL-12 but maintain IL-23, and that EAE cannot be induced in p19-deficient mice. It turns out that the brain inflammation in EAE is largely a result of the activity of T_H17 cells.

IL-12 regulates the effector activity of committed T_H1 cells at sites of infection, but other cytokines such as IL-18 may also be involved. Studies of two different pathogens have shown that the initial differentiation of T_H1 cells is not sufficient for protection, and that continuous signals are required. Mice deficient in p40 can resist initial infection by *T. gondii* as long as IL-12 is administered continuously. If IL-12 is administered during the first 2 weeks of infection, p40-deficient mice survive the initial infection and establish a latent chronic infection characterized by cysts containing the pathogen. When IL-12 administration is stopped, however, these mice gradually reactivate the latent cysts and the animals eventually die of toxoplasmic encephalitis. IFN-γ production by pathogen-specific T cells decreases in the absence of IL-12 but could be restored by IL-12 administration. Similarly, the adoptive transfer of differentiated T_H1 cells from mice cured of *L. major* protects RAG-deficient mice infected by *L. major*, but cannot protect p40-deficient mice (Fig. 11.12). Together, these experiments suggest that T_H1 cells continue to respond to signals during an infection, and that continuous IL-12 is needed to sustain the effectiveness of differentiated T_H1 cells against at least some pathogens.

11-8 Primary CD8 T-cell responses to pathogens can occur in the absence of CD4 T-cell help.

Many CD8 T-cell responses require help from CD4 T cells (see Section 9-16). This is typically the case where the antigen recognized by the CD8 T cells is derived from an agent that does not cause inflammation on initial infection. In such circumstances, CD4 T-cell help is required to activate dendritic cells to become able to stimulate a complete CD8 T-cell response, an activity that has been described as licensing of the antigen-presenting cell (see Section 9-6). Licensing involves the induction of co-stimulatory molecules such as B7, CD40, and 4-1BBL on the dendritic cell, which can then deliver signals that

Petitioner Merck, Ex. 1170, p. 186
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

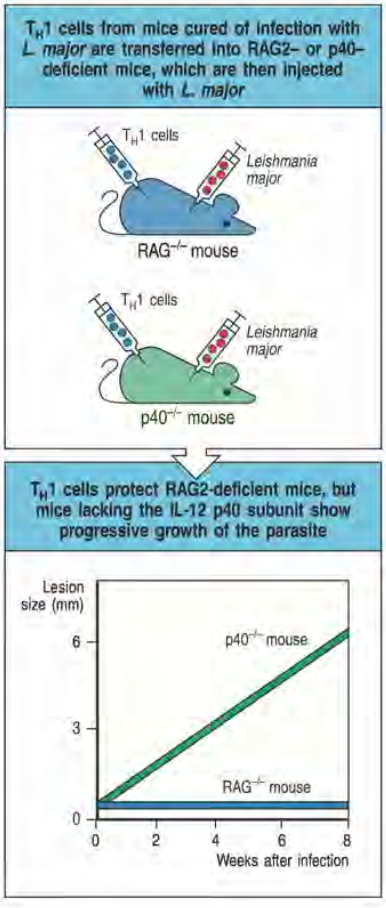


Fig. 11.12 Continuous IL-12 is required for resistance to pathogens requiring T_H1 responses. Mice that have eliminated an infection with *Leishmania major* and have generated T_H1 cells specific to the pathogen were used as a source of T cells that were adoptively transferred into either RAG2-deficient mice, which lack T cells and B cells and cannot control *L. major* infection but can produce IL-12, or into mice lacking p40, which cannot produce IL-12. On subsequent infection of the RAG2-deficient mice, lesions did not enlarge because the transferred T_H1 cells conferred immunity. But despite the fact that the transferred cells were already differentiated T_H1 cells, they did not confer resistance to IL-12 p40-deficient mice in which a continuous source of IL-12 was not present.

fully activate naive CD8 T cells (see Fig. 9.27). Licensing enforces a requirement for dual recognition of an antigen by the immune system by both CD4 and CD8 T cells, which provides a useful safeguard against autoimmunity. Dual recognition is also seen in the cooperation between T cells and B cells for antibody generation (see Chapter 10). However, not all CD8 T-cell responses require such help.

Some infectious agents, such as the intracellular Gram-positive bacterium *Listeria monocytogenes* and the Gram-negative bacterium *Burkholderia pseudomallei*, provide the inflammatory environment required to license dendritic cells and thus can induce primary CD8 T-cell responses without help from CD4 T cells. These pathogens carry a number of immunostimulatory signals, such as ligands for TLRs, and so can directly activate antigen-presenting cells to express the co-stimulatory molecules B7 and CD40. Thus, fully activated dendritic cells presenting *Listeria* or *Burkholderia* antigens can activate naive antigen-specific CD8 T cells without the help of CD4 T cells, and can induce them to undergo clonal expansion (Fig. 11.13). The activated dendritic cell also secretes cytokines such as IL-12 and IL-18, which act on naive CD8 T cells in a so-called 'bystander' effect to induce them to produce IFN-γ, which in turn induces other protective effects (see Fig. 11.13).

Primary CD8 T-cell responses to *L. monocytogenes* were examined in mice that were genetically deficient in MHC class II molecules and thus lacked CD4 T cells (see Section 8-18). The numbers of CD8 T cells specific for a particular antigen expressed by the pathogen were measured by using MHC tetramers (see Appendix I, Section A-28). On day 7 after infection, wild-type mice and mice lacking CD4 T cells show equivalent expansion, and equivalent cytotoxic capacity, of pathogen-specific CD8 T cells. Mice lacking CD4 T cells cleared the initial infection by *L. monocytogenes* as effectively as wild-type mice. These experiments clearly show that protective responses can be generated by pathogen-specific CD8 T cells without CD4 T-cell help. However, as we will see later, the nature of the CD8 memory response is different and is diminished in the absence of CD4 T-cell help.

Naive CD8 T cells can also undergo 'bystander' activation by IL-12 and IL-18 to produce IFN-γ very early during infection (see Fig. 11.13). Mice infected with *L. monocytogenes* or *B. pseudomallei* rapidly produce a strong IFN-γ response, which is essential for their survival. The source of this IFN-γ seems to be both the NK cells of innate immunity and naive CD8 T cells, which begin to secrete it within the first few hours after infection. This is believed to be too soon for any significant expansion of pathogen-specific CD8 T cells, which would initially be too rare to contribute in an antigen-specific manner. The production of IFN-γ by both NK and CD8 T cells at this early time can be blocked experimentally by antibodies against IL-12 and IL-18, suggesting that these cytokines are responsible. The source of the IL-12 and IL-18 was not identified in this experiment, but they are produced by macrophages and dendritic cells in response to activation via TLRs. These experiments indicate that naive CD8 T cells can contribute nonspecifically in a kind of innate defense, not requiring CD4 T cells, in response to early signals of infection.

Petitioner Merck, Ex. 1170, p. 187
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

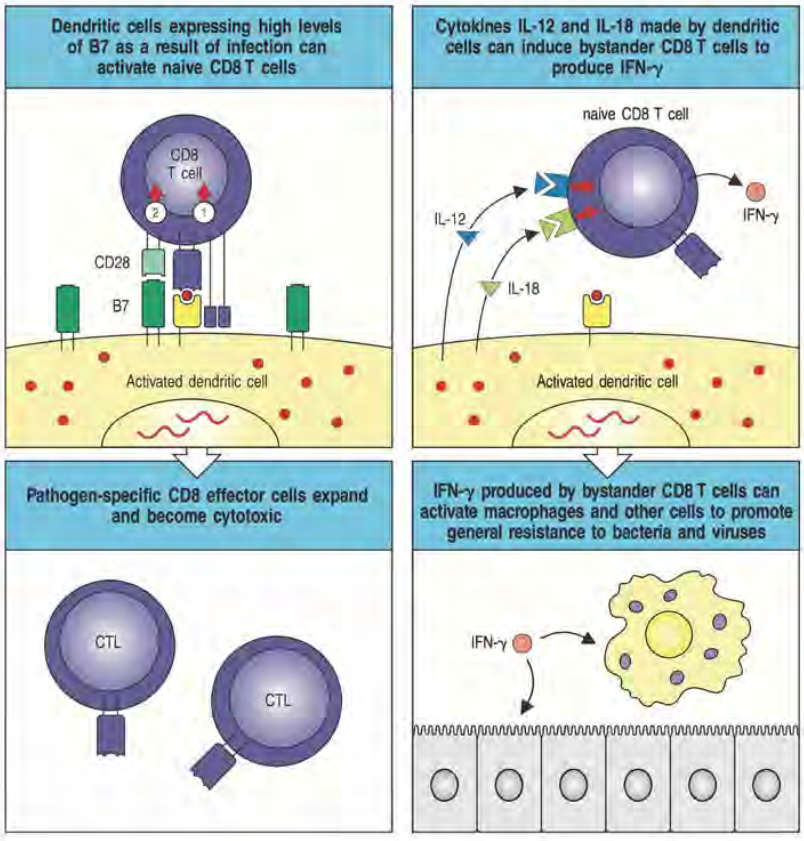


Fig. 11.13 Naive CD8 T cells can be activated directly by potent antigen-presenting cells through their T-cell receptor or through the action of cytokines. Left panels: naive CD8 T cells that encounter peptide:MHC class I complexes on the surface of dendritic cells expressing high levels of co-stimulatory molecules as a result of the inflammatory environment produced by some pathogens (upper left panel) are activated to proliferate in response, eventually differentiating into cytotoxic CD8 T cells (lower left panel). Right panels: activated dendritic cells also produce the cytokines IL-12 and IL-18, whose combined effect on CD8 T cells rapidly induces the production of IFN-γ (upper right panel). This activates macrophages for the destruction of intracellular bacteria and can promote antiviral responses in other cells (lower right panel).

11-9 Antibody responses develop in lymphoid tissues under the direction of T_{FH} cells.

As described in Chapter 10, B cells specific for a protein antigen cannot be activated to proliferate, form germinal centers, or differentiate into plasma cells until they encounter a helper T cell that is specific for one of the peptides derived from that antigen. Recall that naive antigen-binding B cells first enter the follicle, but when they encounter antigen, CCR7 is expressed and they move toward the boundary of the B-cell and T-cell zones (see Section 10-5). Similarly, in the T-cell zones, some naive T cells that are activated by antigen-bearing dendritic cells become follicular T helper (T_{FH}) cells that express CXCR5; these move toward the boundary of the B-cell and T-cell zones, where they can interact with recently activated B cells (see Fig. 10.5). Through linked recognition (see Section 10-3), this initial T cell–B cell interaction gives rise to the primary focus after about 5 days, and later can generate a germinal center reaction in the follicle that facilitates B cells to undergo isotype switching and affinity maturation (see Sections 5-18 and 10-10). Some B cells activated in the primary focus migrate to the medullary cords of the lymph node, or to those parts of the red pulp that are next to the T-cell zones of the spleen, where they become plasma cells and secrete specific antibody for a few days (see Fig. 10.7). The final products of the germinal center response are high-affinity memory B cells and long-lived plasma cells, which are important for the maintenance of serum antibody levels and long-lasting immunity.

Antigen is retained for very long periods in lymphoid follicles in the form of antigen:antibody complexes on the surface of the local follicular dendritic

Petitioner Merck, Ex. 1170, p. 188
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

cells. The antigen:antibody complexes, which become coated with fragments of C3, are held on the cell by receptors for the complement fragments (CR1, CR2, and CR3) as well as by a nonphagocytic Fc receptor (see Fig. 10.16). The function of this long-lived antigen is unclear, because there is evidence that it is not absolutely required for the stimulation of B cells in the germinal center (see Section 10-10), but it may regulate the long-term antibody response.

11-10 Antibody responses are sustained in medullary cords and bone marrow.

The B cells activated in primary foci migrate either to adjacent follicles or to local extrafollicular sites of proliferation. B cells grow exponentially in these sites for 2–3 days and undergo six or seven cell divisions before the progeny come out of the cell cycle and form antibody-producing plasma cells *in situ* (Fig. 11.14, upper panel). Most of these plasma cells have a life-span of 2–4 days, after which they undergo apoptosis. About 10% of plasma cells in these extrafollicular sites live longer; their origin and ultimate fate are unknown. Surviving B cells that have undergone class switching and affinity maturation in the germinal center will either become memory cells or can leave the germinal center as plasmablasts (pre-plasma cells) to become relatively long-lived antibody-producing cells in other locations (see Sections 10-7 to 10-9).

Plasmablasts originating in the follicles of Peyer's patches and mesenteric lymph nodes migrate via lymph to the blood and then enter the lamina propria of the gut and other epithelial surfaces. Those originating in peripheral lymph node or splenic follicles migrate to the bone marrow (Fig. 11.14, lower panel). In these distant sites of antibody production, the plasmablasts differentiate into plasma cells that have a life-span of months to years. These cells are thought to provide the antibody that can be present in the blood for years after the initial immune response. Whether this supply of plasma cells is replenished by the continual but occasional differentiation of memory cells is not yet known. Studies of responses to nonreplicating antigens show that germinal centers are present for only 3–4 weeks after initial exposure to antigen. However, small numbers of B cells continue to proliferate in the follicles for months and may be the precursors of antigen-specific plasma cells in the mucosa and bone marrow throughout the subsequent months and years.

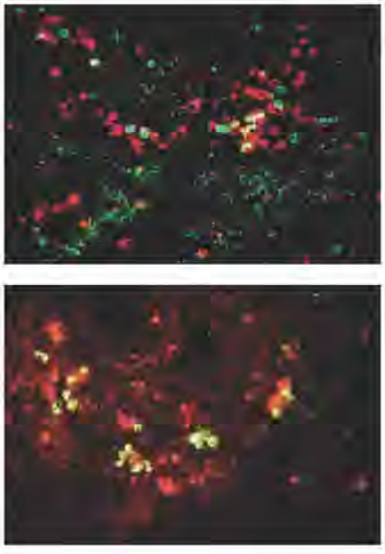


Fig. 11.14 Plasma cells are dispersed in medullary cords and bone marrow. In these sites they secrete antibody at high rates directly into the blood for distribution to the rest of the body. In the upper micrograph, plasma cells in lymph node medullary cords are stained green (with fluorescein anti-IgA) if they are secreting IgA, and red (with rhodamine anti-IgG) if they are secreting IgG. The plasma cells in these local extrafollicular sites are short lived (2–4 days). The lymphatic sinuses are outlined by green granular staining selective for IgA. In the lower micrograph, longer-lived plasma cells (3 weeks to 3 months or more) in the bone marrow are revealed with antibodies specific for light chains (fluorescein anti-λ and rhodamine anti-κ stain). Plasma cells secreting immunoglobulins containing λ light chains show on this micrograph as yellow. Those secreting immunoglobulins containing κ light chains stain red. Photographs courtesy of P. Brandtzaeg.

11-11 The effector mechanisms used to clear an infection depend on the infectious agent.

Most infections engage both the cell-mediated and the humoral aspects of immunity, and in many cases both are helpful in clearing or containing the pathogen and setting up protective immunity, as shown in Fig. 11.15, although the relative importance of the different effector mechanisms, and the effective classes of antibody involved, vary with different pathogens. As we learned in Chapter 9, cytotoxic T cells are important in destroying virus-infected cells, and in some viral diseases they are the predominant class of lymphocytes present in the blood during a primary infection. Nevertheless, the role of antibodies in clearing viruses from the body and preventing them from getting a hold should not be forgotten. Ebola virus causes a hemorrhagic fever and is one of the most lethal viruses known, but some patients do survive and some people even become infected but remain asymptomatic. In both cases, a strong antiviral IgG response early in infection seems to be essential for survival. The antibody response seems to clear the virus from the bloodstream and gives the patient time to activate cytotoxic T cells. In contrast, this antibody response did not occur in infections that proved fatal; the virus continued to replicate, and even though there was some activation of T cells, the disease progressed.

Petitioner Merck, Ex. 1170, p. 189
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

	Pathological agent	Disease	Humoral immunity				Cell-mediated immunity	
			IgM	IgG	IgE	IgA	CD4 T cells (macrophages)	CD8 killer T cells
Viruses	Herpes zoster	Chickenpox						
	Epstein–Barr virus	Mononucleosis						
	Influenza virus	Influenza						
	Polio virus	Poliomyelitis						
Intracellular bacteria	*Rickettsia prowazekii*	Typhus						
	Mycobacteria	Tuberculosis, leprosy						
Extracellular bacteria	*Staphylococcus aureus*	Boils						
	Streptococcus pneumoniae	Pneumonia						
	Neisseria meningitidis	Meningitis						
	Corynebacterium diphtheriae	Diphtheria						
	Vibrio cholerae	Cholera						
Fungi	*Candida albicans*	Candidiasis						
Protozoa	*Plasmodium* spp.	Malaria						
	Trypanosoma spp.	Trypanosomiasis						
Worms	Schistosome	Schistosomiasis						
Toxins	*Corynebacterium diphtheriae*	Diphtheria						
	Clostridium tetani	Tetanus						

Fig. 11.15 Different effector mechanisms are used to clear primary infections with different classes of pathogens and to protect against reinfection. The defense mechanisms used to clear a primary infection are identified by red shaded boxes. Yellow shading indicates a role in protective immunity. Paler shades indicate less well established mechanisms. It is clear that classes of pathogens elicit similar protective immune responses, reflecting similarities in their lifestyles. The CD4 responses indicated on this diagram refer only to those involved in macrophage activation. In addition, in virtually all diseases, helper CD4 T-cell responses will be involved in stimulating antibody production, class switching, and the production of memory cells.

Cytotoxic T cells are also required for the destruction of cells infected with some intracellular bacterial pathogens, such as *Rickettsia*, the causative agent of typhus. In contrast, mycobacteria, which live inside macrophage vesicles, are mainly kept in check by CD4 T_H1 cells, which activate infected macrophage to kill the bacteria. Antibodies are the principal immune reactants that clear primary infections with common extracellular bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. The IgM and IgG antibodies produced against components of the bacterial surface coat opsonize the bacteria and make them more susceptible to phagocytosis.

Figure 11.15 also indicates the mechanisms involved in immunity to reinfection, or protective immunity, against the pathogens listed. Inducing protective immunity is the goal of vaccine development, which we discuss more detail in Chapter 16. To achieve protective immunity requires a vaccine to induce an adaptive immune response that has both the antigen specificity and the appropriate functional elements to combat the particular pathogen concerned. Pathogens carry multiple epitopes for both B cells and T cells, and thus generate diverse antibody and T-cell responses, but not all of these will be equally effective in clearing the disease. Protective immunity consists of two components—immune reactants, such as antibody or effector T cells generated in the initial infection or by vaccination, and long-lived immunological memory (Fig. 11.16).

The type of antibody or effector T cell that offers protection depends on the infectious strategy and lifestyle of the pathogen. Thus, when opsonizing

Petitioner Merck, Ex. 1170, p. 190
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

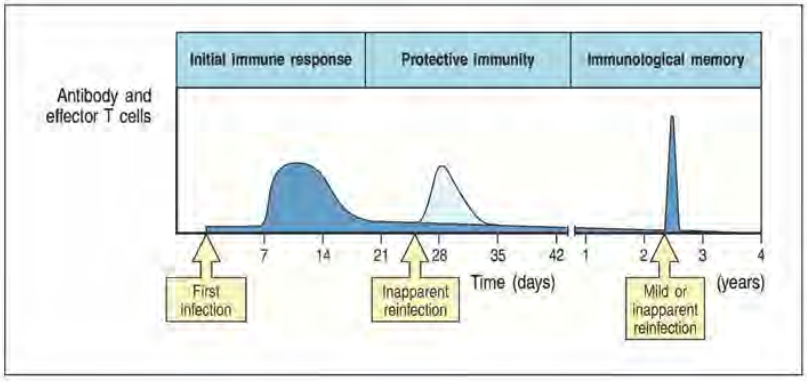


Fig. 11.16 Protective immunity consists of preformed immune reactants and immunological memory. The first time a particular pathogen is encountered, pathogen-specific antibody and effector T cells are produced. After the infection has been cleared, their levels gradually decline. An early reinfection with the same pathogen is rapidly cleared by these preformed immune reactants. There are few symptoms, but levels of immune reactants are found to increase temporarily (light blue peak). Reinfection years afterward leads to an immediate and rapid increase in pathogen-specific antibody and effector T cells as a result of immunological memory, and disease symptoms are mild or inapparent.

antibodies such as IgG1 are present (see Section 10-14), opsonization and phagocytosis of extracellular pathogens will be more efficient. If specific IgE is present, pathogens will trigger activation of mast cells, rapidly initiating an inflammatory response through the release of histamine and leukotrienes. In many cases the most efficient protective immunity is mediated by neutralizing antibody that can prevent pathogens from establishing an infection, and most of the established vaccines against acute childhood viral infections work primarily by inducing protective antibodies. Effective immunity against polio virus, for example, requires preexisting antibody (see Fig. 11.15), because the virus rapidly infects motor neurons and destroys them unless it is immediately neutralized by antibody and prevented from spreading within the body. In polio, specific IgA on mucosal epithelial surfaces also neutralizes the virus before it enters the tissues. Thus, protective immunity can involve effector mechanisms (IgA in this case) that do not operate in the elimination of the primary infection.

When a primary adaptive immune response is successful in halting an infection, it will often clear the primary infection from the body by the effector mechanisms discussed in Chapters 9 and 10. However, as we discuss in Chapter 13, many pathogens evade complete clearance and persist for the life of the host. The virus herpes zoster, which causes chickenpox on primary infection, then lies latent in the body for years without causing disease, but can later in life, or if the body is stressed, become reactivated and cause shingles.

11-12 Resolution of an infection is accompanied by the death of most of the effector cells and the generation of memory cells.

When an infection is effectively repelled by the adaptive immune system, two things occur. The actions of effector cells remove the specific stimulus that originally recruited them. In the absence of this stimulus, the cells then undergo 'death by neglect,' removing themselves by apoptosis. The dying cells are rapidly cleared by phagocytes and other cells, which recognize the membrane lipid phosphatidylserine. This lipid is normally found only on the inner surface of the plasma membrane, but in apoptotic cells it rapidly redistributes to the outer surface, where it can be recognized by specific receptors on many cells. Thus, not only does the ending of infection lead to the removal of the pathogen, it also leads to the loss of most of the pathogen-specific effector cells.

However, some of the effector cells are retained, and these provide the raw material for memory T-cell and B-cell responses. These are crucially important to the operation of the adaptive immune system. Memory T cells, in particular, are retained virtually forever. The mechanisms underlying the decision to induce apoptosis in most effector cells and to retain only a few are only now being discovered and are still not well understood. It seems likely that

Petitioner Merck, Ex. 1170, p. 191
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003

the answers will lie in the cytokines produced by the environment and by the T cells themselves, and in the affinity of the T-cell receptors for their antigens.

Summary.

The adaptive immune response is required for effective protection of the host against pathogenic microorganisms. The response of the innate immune system to pathogens helps to initiate the adaptive immune response. Pathogens cause the activation of dendritic cells to full antigen-presenting cell status, and interactions with other cells of the innate immune system lead to the production of cytokines that direct the quality of the CD4 T-cell response. Pathogen antigens are transported to local lymphoid organs by the migrating dendritic cells and are presented to antigen-specific naive T cells that continuously recirculate through the lymphoid organs. T-cell priming and the differentiation of effector T cells occur here, and the effector T cells either leave the lymphoid organ to provide cell-mediated immunity in sites of infection in the tissues or remain in the lymphoid organ to participate in humoral immunity by activating antigen-binding B cells. Distinct types of CD4 T cells develop in response to infection by different types of pathogens. T_H17 responses provide for the generation of acute inflammation at sites of infection by robust recruitment of neutrophils. T_H1 responses activate the phagocytic pathways to protect against intracellular pathogens. T_H2 responses are directed against infections by parasites such as helminths by promoting barrier and mucosal immunity, IgE production, and recruitment of eosinophils to sites of infection. CD8 T cells have an important role in protective immunity, especially in protecting the host against infection by viruses and intracellular infections by *Listeria* and other microbial pathogens that have special means for entering the host cell's cytoplasm. Primary CD8 T-cell responses to pathogens usually require CD4 T-cell help, but can occur in response to some pathogens without such help. CD4-independent responses can lead either to the generation and expansion of antigen-specific cytotoxic T cells or to the nonspecific activation of naive CD8 T cells to secrete IFN-γ, which in turn contributes to host protection. Ideally, the adaptive immune response eliminates the infectious agent and provides the host with a state of protective immunity against reinfection with the same pathogen.

Immunological memory.

Having considered how an appropriate primary immune response is mounted, we now turn to how long-lasting protective immunity is generated. The establishment of immunological memory is perhaps the most important consequence of an adaptive immune response, because it enables the immune system to respond more rapidly and effectively to pathogens that have been encountered previously, and prevents them from causing disease. Memory responses, which are called **secondary immune responses**, **tertiary immune responses**, and so on, depending on the number of exposures to antigen, also differ qualitatively from primary responses. This is particularly clear in the antibody response, in which the characteristics of antibodies produced in secondary and subsequent responses are distinct from those produced in the primary response to the same antigen. Memory T-cell responses can also be distinguished qualitatively from the responses of naive or effector T cells. The principal focus of this part of the chapter is the altered character of memory responses, although we also discuss emerging explanations of how immunological memory persists after exposure to antigen.

Petitioner Merck, Ex. 1170, p. 192
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00003