Janeway's

immuno biology

SEVENTH EDITION

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Preface

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This book is intended as an introductory text for use in immunology courses for medical students, advanced undergraduate biology students, graduate students, and scientists in other fields who want to know more about the immune system. It presents the field of immunology from a consistent viewpoint, that of the host's interaction with an environment containing many species of potentially harmful microbes. The justification for this approach is that the absence of one or more components of the immune system is virtually always made clear by an increased susceptibility to one or more specific infections. Thus, first and foremost, the immune system exists to protect the host from infection, and its evolutionary history must have been shaped largely by this challenge. Other aspects of immunology, such as allergy, autoimmunity, graft rejection, and immunity to tumors, are treated as variations on this basic protective function. In these cases the nature of the antigen is the major variable.

In this seventh edition, all chapters have been updated to incorporate new observations that extend our knowledge and understanding of the immune system. Examples include new work on NK receptors, the deeper understanding of the role of activation-induced cytidine deaminase (AID) in the generation of antibody diversity, viral immunoevasins, cross-presentation of antigen to T cells, dendritic cell and T-cell subsets, and new innate receptors that recognize pathogens, to name but a few. Our chapter on evolution includes fascinating new insights into alternative forms of adaptive immunity in both invertebrates and higher organisms. The clinical chapters contain new sections on celiac disease and its mechanism, Crohn's disease, and immunological approaches to cancer treatment. New discussion questions have been provided at the end of each chapter. These questions can be used for review, or as the basis for discussion in class or in informal study groups. The accompanying CD-ROM, *Janeway's Immunobiology 7 Interactive*, has been expanded and enhanced.

Following a comprehensive overview of the immune system in Chapter 1, innate immunity is treated in Chapter 2 as an important protective system in its own right and a necessary precursor to the adaptive immune response. The coverage of Toll receptors and other pathogen-sensing mechanisms has been updated to reflect the rapid progress in this field in the past three years and the description of the different families of activating and inhibitory NK receptors has been revised to reflect the growing understanding of these. The material on pathogens at the beginning of Chapter 10 in previous editions has been moved to Chapter 2 to provide a more complete introduction to infection earlier in the book. After the discussion of innate immunity, the emphasis shifts to adaptive immunity, because we know far more about this subject, thanks to the efforts of the vast majority of immunologists. The central theme of the subsequent text is clonal selection of lymphocytes.

As in the sixth edition, we consider the two lymphoid lineages, B lymphocytes and T lymphocytes, together for most of the book, because it seems to us that essentially common mechanisms work in both cell types. An example is the rearrangement of gene segments to generate the receptors by which lymphocytes recognize antigen (Chapter 4). Chapter 5, on recognition of antigen, has been updated to include the cross-presentation of antigen by MHC class I molecules, and the interference by viral immunoevasins in antigen presentation. Chapter 6, on signaling, has been revised to describe the T-cell signaling pathways in more detail, with an extended and updated discussion of co-stimulatory signaling. We have substantially reorganized Chapter 7 so that the development of B cells and T cells are considered in separate sections.

Chapters 8 and 9 elaborate the effector functions of T cells and B cells separately, since different mechanisms are involved. We have updated and extended the treatment of dendritic cells and included recent research findings on the T_H17 and regulatory T-cell subsets (Chapter 8). We have taken the opportunity to refocus Chapter 10 on the dynamic nature of the immune response to infection, from innate immunity to the generation of immunological memory. We include recent advances in our understanding of the temporal changes in T-cell subsets during an immune response and of the nature of immunological memory. Because of its increasingly recognized role in immune defense, we have created a new chapter devoted entirely to mucosal immunity (Chapter 11).

The subsequent three chapters (Chapters 12-14) deal mainly with how diseases such as HIV/AIDS, autoimmunity, or allergy can be caused by inherited or acquired immunodeficiencies or by failure or malfunction of immunological mechanisms. As our understanding of the causes of disease grows, these chapters have been extended with descriptions of syndromes for which underlying gene defects have been newly identified. These chapters, which describe the failures of the immune system to maintain health, are followed by a chapter (Chapter 15) on how the immune response can be manipulated by vaccination and other means in attempts to combat not only infectious diseases, but also transplant rejections and cancer. These four chapters have been extensively revised and updated, especially in respect of the new generation of 'biological' therapeutics that are starting to enter medical practice.

The book ends with an updated chapter (Chapter 16) on the evolution of animal immune systems. The analysis of the genome sequences of both invertebrates and lower vertebrates has given us a new appreciation of the sophistication of invertebrate immune defenses and the discovery that our antibody and T-cell-based adaptive immune system is not the only way that adaptive immunity can be generated.

Diseases and immunological deficiencies are cross-referenced to the fifth edition of the clinical companion text *Case Studies in Immunology* by Raif Geha and Fred Rosen (ISBN: 9780815341451). This text presents major topics in immunology as background to a selection of real clinical cases that serve to reinforce and extend the basic science. There are five new cases in the fifth edition, bringing the total number to 47. A marginal icon in *Janeway's Immunobiology* provides the reader with a link to the relevant Case Study, where the science is applied in a clinical setting.

The seventh edition of *Janeway's Immunobiology* includes a CD-ROM with original immunological animations based on figures in the book and videos selected from visually compelling experiments. The animations have been revised and updated for this edition, and there are five new animations covering HIV Infection, DTH Response, Viral Evasins, Innate Recognition of Pathogens, and Pathogen Receptors. All the animations and videos are accompanied by a voice-over narration read by Julie Theriot, Stanford University School of Medicine. The CD-ROM also contains the figures and tables from

the book pre-loaded into PowerPoint® presentations. There is also a JPEG archive of all these images. Instructors who adopt *Janeway's Immunobiology* for their course will have access to Garland Science Classwire™. The Classwire course management system allows instructors to build websites for their courses easily. It also serves as an online archive for instructors' resources. After registering for Classwire, instructors will be able to download all of the figures from *Immunobiology*, which are available in JPEG and PowerPoint formats, and all of the videos and animations on the CD. Instructors may also download resources from other Garland Science textbooks. Please visit the Garland Science website at www.garlandscience.com or e-mail science@garland.com for additional information on Classwire.

This edition has been retitled *Janeway's Immunobiology* in memory of Charles A. Janeway who originated this textbook and was its continual inspiration until his death in 2003. Andrey Shaw, Washington University School of Medicine, St. Louis, completely revised and updated Chapter 6 on signaling; Allan Mowat, University of Glasgow, did the same for Chapter 11 on mucosal immunity; and Claudia Mauri (Chapters 12 and 14) and Michael Ehrenstein (Chapters 13 and 15), University College London, revised and updated the clinical chapters. Appendix III, Cytokines and their Receptors, was updated and reorganized by Robert Schreiber, Washington University School of Medicine, St. Louis. Joost Oppenheim, National Cancer Institute, Washington, D.C., updated Appendix IV, Chemokines and their Receptors. We owe them all a great debt of gratitude for bringing their particular expertise to the book and for the care and effort they put into these revisions.

The editors, illustrators, and publishers are the essential glue that binds this book together. We have benefited from the editorial skills of Eleanor Lawrence, the book's developmental editor from its beginning, and from the creativity and artistic talent of Matt McClements, our illustrator since the second edition. Their 'institutional memory' sustains the overall coherence of this heavily updated edition. At Garland, Mike Morales has managed the creation of compelling animations that bring important concepts to life. None of these efforts would have borne fruit without the skillful (but patient) coordination of Sigrid Masson and the insightful suggestions and continuous support of our publisher, Denise Schanck. Kenneth Murphy would like to thank Theresa Murphy and Paul, Mike, Mark and Jason for their encouragement and support. Paul Travers would like to thank Rose Zamoyska for her infinite patience and support. Mark Walport would like to thank his wife, Julia, and children, Louise, Robert, Emily, and Fiona, for their unstinting support.

We would like to give great thanks to all those people who read parts or all of the chapters of the sixth edition, as well as drafts of the seventh edition, and advised us on how to improve them. They are listed by chapter on page ix. Every effort has been made to write a book that is error-free. Nonetheless, you may find them here and there, and it would be of great benefit if you took the trouble to communicate them to us.

Kenneth Murphy

Paul Travers

Mark Walport

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Immunobiology Interactive

The *Immunobiology Interactive* CD-ROM contains the figures and tables from the book in PowerPoint® and JPEG formats. The videos and animations play in a self-contained interface and dynamically illustrate important concepts from the book. Videos are indicated with a (V).

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2.4 Complement System
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Section II

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12.1 HIV Infection
13.1 DTH Response

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PART I AN INTRODUCTION TO IMMUNOBIOLOGY AND INNATE IMMUNITY

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1 Basic Concepts in Immunology

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 level.

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 early 1890s, **Emil von Behring** and **Shibasaburo Kitasato** discovered that



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

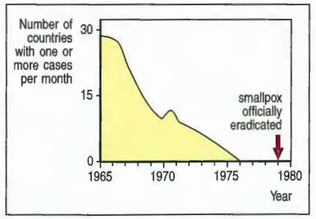


Fig. 1.2 The eradication of smallpox by vaccination. After a period of 3 years in which no cases of smallpox were recorded, the World Health Organization was able to announce in 1979 that smallpox had been eradicated, and vaccination stopped. A few laboratory stocks have been retained, however, and some fear that these are a source from which the virus might reemerge.

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 frontline 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. Innate and adaptive immune responses together provide a remarkably effective defense system. Many infections are handled successfully by innate immunity and cause no disease; those that cannot be resolved trigger an adaptive immune response and, if overcome, are often followed by lasting immunological memory, which prevents disease if reinfection occurs.

The main focus of this book will be on the diverse mechanisms of adaptive immunity, by which the specialized white blood cells known as **lymphocytes** recognize and target pathogenic microorganisms or infected cells. We shall see, however, that the actions of the innate immune system are a prerequisite for the development of adaptive immunity and that cells involved in innate immune responses also participate in adaptive immune responses. Indeed, most of the ways in which the adaptive immune response destroys invading microorganisms depend upon linking antigen-specific recognition of the pathogen to the activation of the same destructive mechanisms that are used in innate immunity.

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.

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 Functions of the immune response.

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, antibodies, 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 that prevent microbes from entering the body; these are not generally considered as part of the immune system proper and it is only when these barriers are overcome or evaded that the immune system comes into play. The first cells that respond are phagocytic white blood cells, such as macrophages, that form part of the innate immune system. These cells are able to ingest and kill microbes by producing a variety of 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. Overlapping with the innate immune response, but taking days rather than hours to develop, the adaptive immune system is capable of eliminating infections more efficiently than the innate immune response. It is present only in vertebrates and depends on the exquisitely specific recognition functions of lymphocytes, which have the ability to distinguish the particular pathogen and focus the immune response more strongly on it. These cells can recognize and respond to individual antigens by means of highly specialized **antigen receptors** on the lymphocyte surface. The billions of lymphocytes

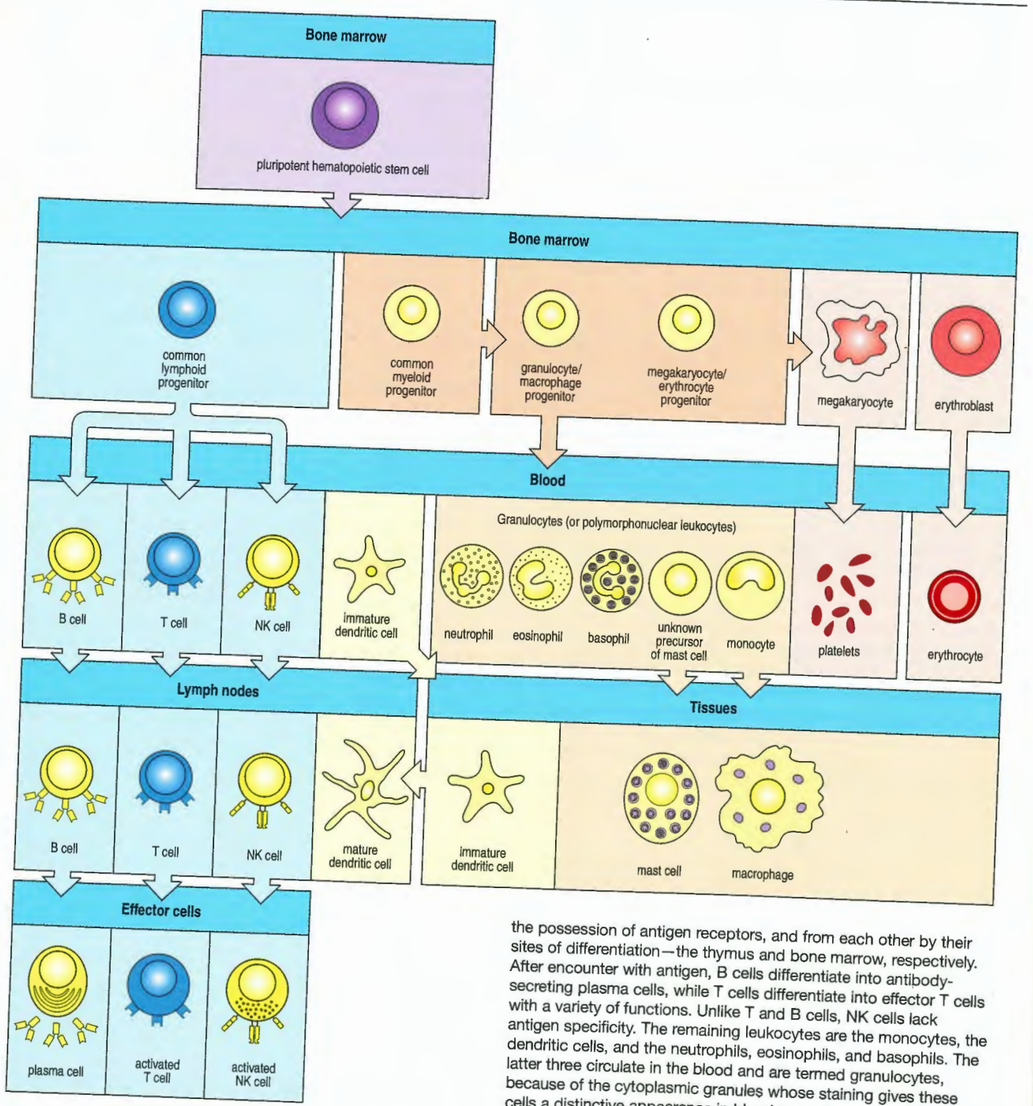


Fig. 1.3 All the cellular elements of the blood, including the cells of the immune system, arise from pluripotent hematopoietic stem cells in the bone marrow. These pluripotent cells divide to produce two types of stem cells. A common lymphoid progenitor gives rise to the lymphoid lineage (blue background) 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 latter three 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.

present in the body collectively possess a vast repertoire of antigen receptors, which enables the immune system to recognize and respond to virtually any antigen a person is likely to be exposed to. By making recognition and response specific for a particular pathogen, the adaptive immune response focuses the resources of the immune system on combating that pathogen, enabling the body to overcome pathogens that have evaded or overwhelmed innate immunity. Antibodies and activated lymphocytes produced in this phase of the response also persist after the original infection has been eliminated and prevent immediate reinfection. Lymphocytes are also responsible for the long-lasting immunity that is generated after a successful adaptive immune response to many pathogens, so that the response to a second exposure to the same microbe is both faster and greater in magnitude, 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. They then migrate to guard the peripheral tissues—some of them residing within tissues, others circulating 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. As 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 stem 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.

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

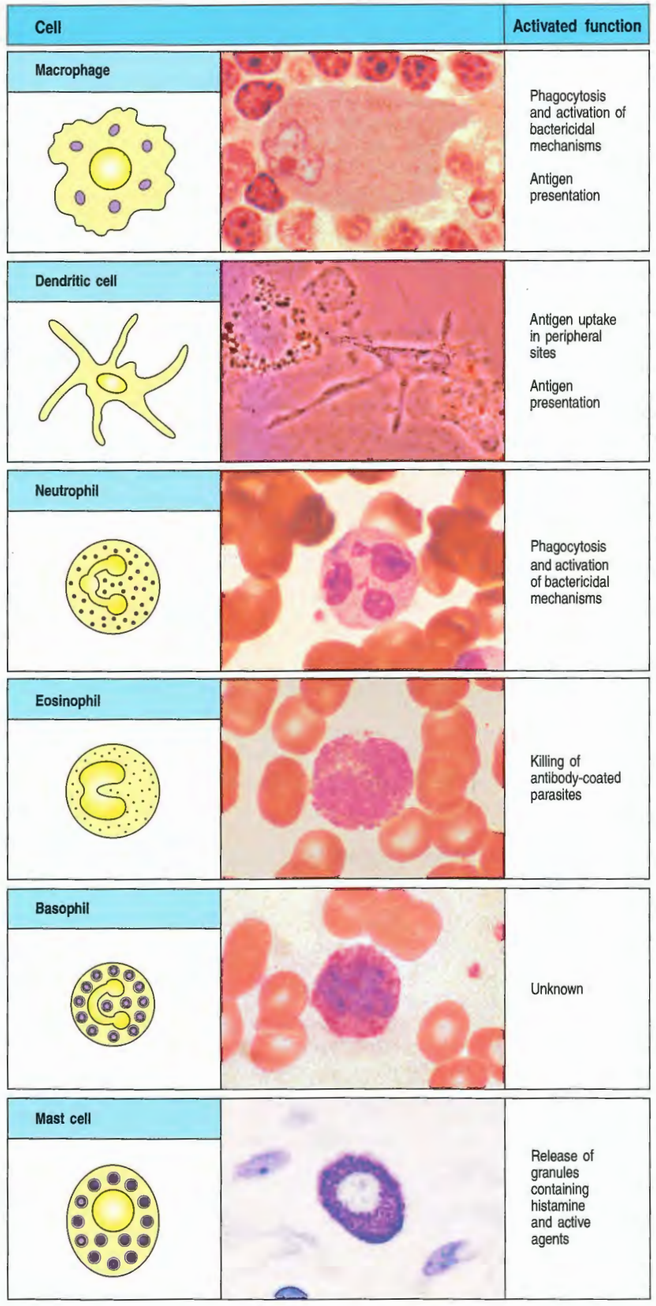


Fig. 1.4 Myeloid cells in innate and adaptive immunity. Cells of the myeloid lineage perform various important functions in the immune response. In the rest of the book these cells will be represented in the schematic form shown on the left. A photomicrograph of each cell type is shown in the center 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, whereas the function of basophils is less clear. Mast cells are tissue cells that trigger a local inflammatory response to antigen by releasing substances that act on local blood vessels; they are also important in allergic responses. Photographs courtesy of N. Rooney, R. Steinman, and D. Friend.

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. As well as 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 2. Hereditary deficiencies in neutrophil function lead to overwhelming bacterial infection, which is fatal if untreated.

The protective functions of **eosinophils** and **basophils** are less well understood. Their granules contain a variety of enzymes and toxic proteins, which are released when the cell is 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, but their main medical importance is their involvement in allergic inflammatory reactions, in which their effects are damaging rather than protective. We discuss the functions of these cells in Chapter 9 and their role in allergic inflammation in Chapter 13.

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 13, 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.

The **dendritic cells** are the third class of phagocytic cell of the immune system. They have long finger-like processes, like the dendrites of nerve cells, which gives them their name. Immature dendritic cells migrate through the bloodstream from the bone marrow to enter tissues. They both take up particulate matter by phagocytosis and 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, dendritic cells that have encountered invading microorganisms mature into cells capable of activating a particular class of lymphocytes—the T lymphocytes—which are described below. Dendritic cells do this by displaying pathogen antigens on their surface in such a way that they can be recognized and responded to by this type of lymphocyte. As we discuss later in the chapter, recognition of antigen alone is not sufficient to activate a T lymphocyte that has never encountered its antigen before. Mature dendritic cells, however, have additional properties that enable them to activate T lymphocytes. Cells that can present antigens to inactive T lymphocytes and activate them for the very first time are known as **antigen-presenting cells** (**APCs**), and such cells form a crucial link between the innate immune response and the adaptive immune response. Macrophages can also

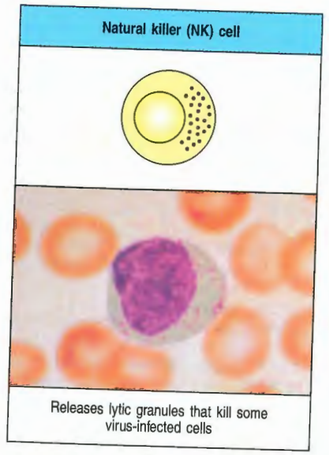


Fig. 1.5 Natural killer (NK) cells. These are large granular lymphoid-like cells with important functions in innate immunity, especially against intracellular infections, being able to kill other cells. Unlike lymphocytes, they lack antigen-specific receptors. Photograph courtesy of B. Smith.

act as antigen-presenting cells, and they are important in particular situations. Dendritic cells, however, are the cells that specialize in presenting antigens to lymphocytes and initiating adaptive immune responses.

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. 1.5). These cells are able to recognize and kill some abnormal cells, for example some tumor cells and cells infected with herpes viruses. Their functions in innate immunity are described in Chapter 2.

We come finally to the antigen-specific lymphocytes, with which most of this book will be concerned: 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. 1.6). 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**.

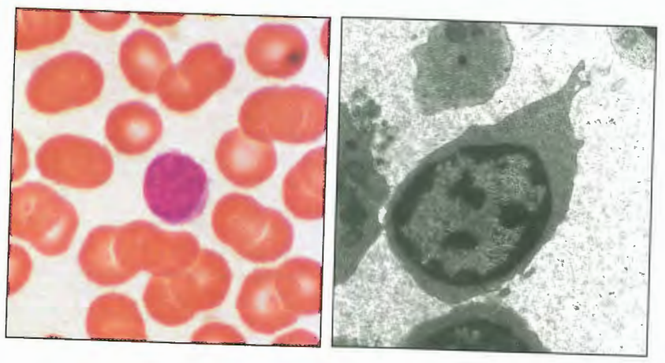


Fig. 1.6 Lymphocytes are mostly small and inactive cells. The left panel shows a light micrograph of a small lymphocyte in which the nucleus has been stained purple by the hematoxylin and eosin dye, surrounded by red blood cells (which have no nuclei). Note the darker purple patches of condensed chromatin of the lymphocyte nucleus, indicating little transcriptional activity, the relative absence of cytoplasm, and the small size. The right 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.

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 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 is activated by its first encounter with antigen it proliferates and differentiates into one of several different functional types of **effector T lymphocytes**. 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 activate antigen-stimulated B cells to differentiate and produce antibody; some of these T cells can also activate macrophages 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 8 and 10. **Regulatory T cells** suppress the activity of other lymphocytes and help control immune responses; they are discussed in Chapters 8, 10, and 14.

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 10.

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 **central** or **primary lymphoid organs**, where lymphocytes are generated, and **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. 1.7, and we shall 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

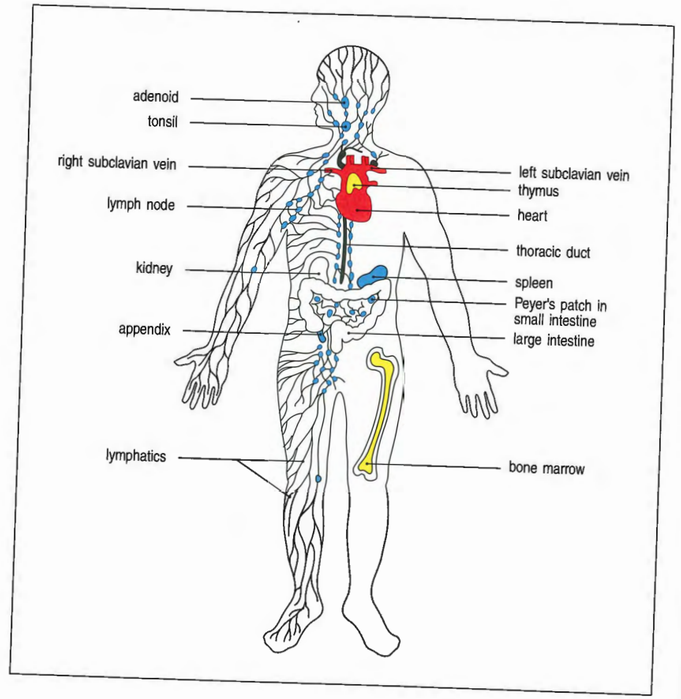


Fig. 1.7 The distribution of lymphoid tissues in the body. Lymphocytes arise from stem cells in bone marrow and differentiate in the central lymphoid organs (yellow)—B cells in the bone marrow and T cells in the thymus. They migrate from these tissues and are carried in the bloodstream to the peripheral 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 and 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).

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.

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 are the first line of defense against bacteria, for example, which they recognize 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, as well as other biologically active molecules. 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 attract cells bearing chemokine receptors, such as neutrophils and monocytes, out of the bloodstream and into the infected tissue (Fig. 1.8). The cytokines and chemokines

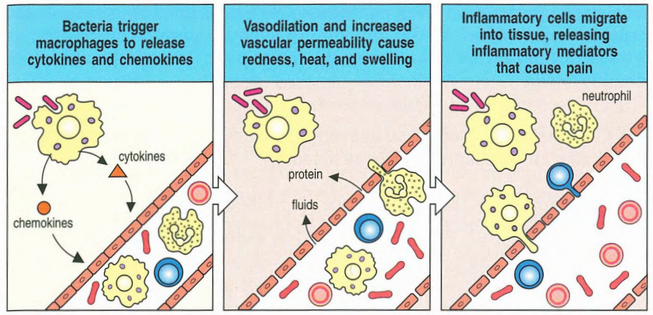


Fig. 1.8 Infection triggers an inflammatory response. Macrophages encountering bacteria or other types of microorganisms in tissues are triggered to release cytokines that increase the permeability of blood vessels, allowing fluid and proteins to pass into the tissues. They also produce chemokines, which 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.

released by activated macrophages initiate the process known as **inflammation**. Inflammation of an infected tissue has several beneficial effects in combating infection. It recruits cells and molecules of innate immunity out of the blood and into the tissue where they are needed to destroy the pathogen directly. In addition, it increases the flow of lymph bearing microbes and antigen-bearing cells to nearby lymphoid tissues, where they will activate lymphocytes and initiate the adaptive immune response. Finally, once the adaptive immune response has been triggered, inflammation also recruits the effectors of the adaptive immune system—antibody molecules and effector T cells—to the site of infection.

Local inflammation and the phagocytosis of invading bacteria may 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 traditionally defined by the four Latin words *calor*, *dolor*, *rubor*, and *tumor*, meaning heat, pain, redness, and swelling, all of which reflect the effects of cytokines and other inflammatory mediators on the local blood vessels. Dilation and increased permeability of blood vessels during inflammation lead to increased local blood flow and the leakage of fluid into the tissues, and account for the heat, redness, and swelling. Cytokines and complement fragments have important effects on the endothelium lining blood vessels; endothelial cells themselves also produce cytokines in response to infection. The 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 monocytes, which rapidly differentiate into macrophages, thus reinforcing and sustaining the innate immune response. More slowly, eosinophils also migrate into inflamed tissues and also contribute to the destruction of the invading microorganisms.

As well as directly destroying pathogens, the innate immune response has crucial consequences for the initiation of adaptive immune responses, as we see in the next section. It does this principally through the agency of dendritic cells.

1-7 Activation of specialized antigen-presenting cells is a necessary first step for induction of adaptive immunity.

The induction of an adaptive immune response begins when a pathogen is ingested by an immature dendritic cell in the infected tissue. These specialized phagocytic cells are resident in most tissues and, like macrophages, are long-lived compared with other white blood cells. They originate in the bone marrow (see Section 1-3), and while still not fully mature they migrate through the bloodstream to their peripheral stations, where they survey the local environment for pathogens.

Like macrophages and neutrophils, the immature dendritic cell carries receptors on its surface that recognize common features of many pathogens, such as **bacterial lipopolysaccharide**. Microbial components binding to these receptors stimulate the dendritic cell to engulf the pathogen and degrade it intracellularly. Immature dendritic cells are also continually taking up extracellular material, including virus particles and bacteria, by the receptor-independent mechanism of macropinocytosis, and are thus even able to internalize and degrade pathogens that their cell-surface receptors do not detect. The function of dendritic cells, however, is not primarily to destroy pathogens but to carry pathogen antigens to peripheral lymphoid organs and there present them to T lymphocytes. On taking up pathogens and their components, the dendritic cell migrates to peripheral lymphoid tissues, where it matures into a highly effective antigen-presenting cell. It displays fragments of pathogen antigens on its surface and also starts to produce cell-surface proteins known as **co-stimulatory molecules**, which, as their name suggests, provide signals that act together with antigen to stimulate the T lymphocyte to proliferate and differentiate into its final fully functional form (Fig. 1.9). Because B cells are not activated by most antigens without 'help' from activated helper T cells, the activation of naive T lymphocytes is an essential first stage in virtually all adaptive immune responses.

Activated dendritic cells also secrete cytokines that influence both innate and adaptive immune responses, making these cells essential gatekeepers that determine whether and how the immune system responds to the presence of infectious agents. We consider the maturation of dendritic cells and their central role in presenting antigens to naive T cells in Chapter 8.

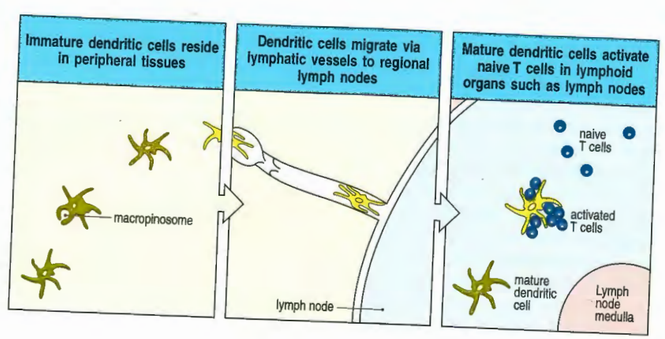


Fig. 1.9 Dendritic cells initiate adaptive immune responses. Immature dendritic cells resident in a tissue take up pathogens and their antigens by macropinocytosis and by receptor-mediated endocytosis. They are stimulated by recognition of the presence of pathogens to migrate through the lymphatics to 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.

1-8 The innate immune system provides an initial discrimination between self and nonself.

The defense systems of innate immunity are effective in combating many pathogens. They are constrained, however, by relying on a limited and invariant repertoire of receptors to 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 body's own cells (Fig. 1.10). These receptors 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. The innate immune system is thus able broadly to distinguish self (the body) from nonself (pathogen) and to mount an attack against invaders. By becoming activated through their pattern recognition receptors, immature dendritic cells, which form part of the innate immune system, become able in their turn to activate naive T lymphocytes, as discussed in the previous section. Thus, the adaptive immune response is essentially initiated by an explicit recognition of nonself by the innate immune system.

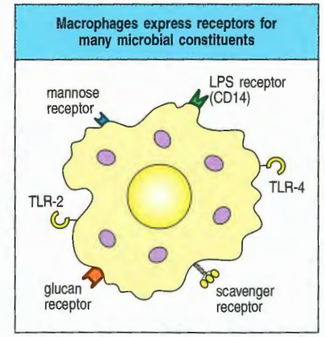


Fig. 1.10 Macrophages express a number of receptors that allow them to recognize different pathogens. Macrophages express a variety of receptors, each of which is able to recognize specific components of microbes. Some, like the mannose and glucan receptors and the scavenger receptor, bind cell-wall 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 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.

The common pathogen constituents recognized by pattern recognition receptors are usually quite distinct from the pathogen-specific antigens that are recognized by lymphocytes. The requirement for microbial constituents other than the antigen to initiate an adaptive immune response was recognized experimentally long before the discovery of dendritic cells and their mode of activation. It was found 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, it was essential to add killed bacteria or bacterial extracts to the antigen. This additional material was termed an **adjuvant**, as 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; we describe modern adjuvant formulations in Appendix I.

Microorganisms can evolve more rapidly than their hosts, and this may explain why the cells and molecules of the innate immune system recognize only molecular structures that have remained unchanged during evolution. As we see next, the recognition mechanism used by the lymphocytes of the adaptive immune response has evolved to overcome the constraints faced by the innate immune system. It enables the recognition of an almost infinite diversity of antigens, so that each different pathogen can be specifically targeted.

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

Instead of bearing several different receptors, each recognizing a different feature shared by many pathogens, a naive lymphocyte bears antigen receptors specific for a single chemical structure. However, each lymphocyte emerging from the central lymphoid organs differs from the others in its receptor specificity. The diversity 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**

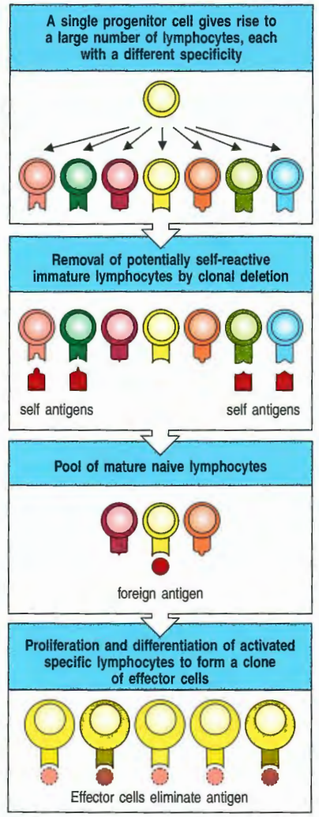


Fig. 1.11 Clonal selection. Each lymphoid progenitor gives rise to a large number of lymphocytes, each bearing a distinct antigen receptor. Lymphocytes with receptors that bind ubiquitous self antigens are eliminated before they become fully mature, ensuring tolerance to such self antigens. When a foreign 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.

Fig. 1.12 The four basic principles of clonal selection.

repertoire of the individual. These 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 is 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. 1.11). 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 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

Postulates of the clonal selection hypothesis
Each lymphocyte bears a single type of receptor with a unique specificity
Interaction between a foreign molecule and a lymphocyte receptor capable of binding that molecule with high affinity leads to lymphocyte activation
The differentiated effector cells derived from an activated lymphocyte will bear receptors of identical specificity to those of the parental cell from which that lymphocyte was derived
Lymphocytes bearing receptors specific for ubiquitous self molecules are deleted at an early stage in lymphoid cell development and are therefore absent from the repertoire of mature lymphocytes

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 7.

Clonal selection of lymphocytes is the single most important principle in adaptive immunity. Its four basic postulates are listed in Fig. 1.12. 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 an apparently 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. 1.13, 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 disposes of the 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. 1.13,

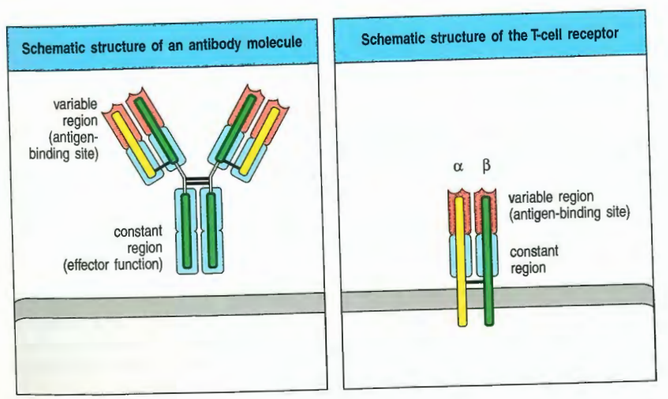


Fig. 1.13 Schematic structure of antigen receptors. Left panel: an antibody molecule, which is secreted by activated B cells as an antigen-binding effector molecule. A membrane-bound version of this molecule acts as the B-cell antigen receptor (not shown). An antibody is composed of two identical heavy 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. Right panel: a T-cell antigen receptor. This is also composed of two chains, an α chain (yellow) and a β chain (green), which each have 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.

where the heavy chains are in green and the light chains in yellow). Heavy and light chains both have variable and constant regions; the variable regions of a heavy 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 structure of antibody molecules is described in detail in Chapter 3, and the functional properties of antibodies conferred by their constant regions are considered in Chapters 4 and 9. For the time being, we are concerned only with the properties of antibody molecules as antigen receptors, and how the diversity of the variable regions is generated.

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; in fact, the T-cell receptor closely resembles a part of the antibody molecule. There are, however, important differences between the two molecules that, as we shall see, relate to their different roles within the immune system. The T-cell receptor, as shown in Fig. 1.13, 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. This structure is described in detail in Chapter 3, and the way in which diversity is introduced into the variable regions is discussed in Chapter 4. As we will see, the organization of the genes for the antigen receptors and the way in which diversity is introduced to create a unique antigen-binding site is essentially the same for both the B-cell receptor and the T-cell receptor. 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 5. 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 8.

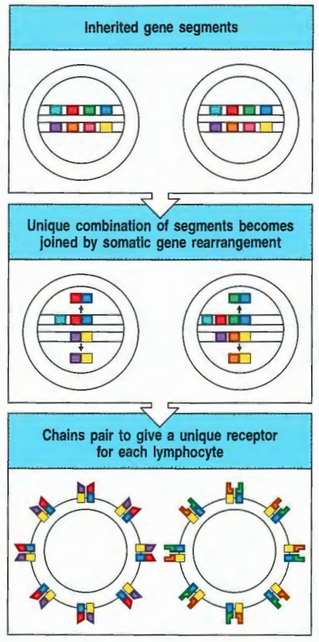


Fig. 1.14 The diversity of lymphocyte antigen receptors is generated by somatic gene-segment rearrangements. Different parts of the variable regions of antigen receptors are encoded by sets of gene segments. During a lymphocyte's development, one member of each set of gene segments is joined randomly to the others by an irreversible process of DNA recombination. The juxtaposed gene segments make up a complete gene that encodes the variable part of one chain of the receptor and is unique to that cell. This random rearrangement is repeated for the set of gene segments encoding the other chain. The rearranged genes are expressed to produce the two types of polypeptide chains. These come together to form a unique antigen receptor on the lymphocyte surface. Each lymphocyte bears many copies of its unique receptor.

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 (Fig. 1.14). 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. Because there are many different gene segments in each set, and different gene segments are joined together in different cells, each cell generates unique genes for the variable regions of the heavy and light chains of the immunoglobulin molecule. Once these recombination events have succeeded in producing a functional receptor, further rearrangement is prohibited. Thus each lymphocyte expresses only one receptor specificity.

This mechanism has three important consequences. First, it enables a limited number of gene segments to generate a vast number of different proteins. Second, because each cell assembles a different set of gene segments, each cell expresses a unique 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.

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 are adapted to recognize antigen in two different ways, which reflects the roles their effector cells will eventually play in destroying pathogens. B cells are specialized to recognize the surface antigens on pathogens living outside cells and to differentiate into effector plasma cells that secrete antibodies to target these pathogens. B-cell receptors and their antibody counterparts are thus able to bind a wide variety of molecular structures.

Effector T cells, in contrast, have to deal with pathogens that have entered host cells, and also have to help activate B cells. To carry out these functions, the T-cell receptor is specialized to recognize antigens that have been generated inside cells and are being displayed on their surface. The recognition properties of the T-cell receptor reflect this: it recognizes only one type of antigen—peptides that have been produced in another host cell by the breakdown of proteins and are then displayed on the cell's surface. In addition, peptides are recognized only if they are bound to a particular type of cell-surface protein. These are the membrane glycoproteins known as **MHC molecules**, which are encoded in a cluster of genes called the **major histocompatibility complex**, which is abbreviated to **MHC**. The antigen recognized by T-cell receptors is thus a complex of a foreign peptide antigen and an MHC molecule (Fig. 1.16). We shall see how these compound antigens are recognized by T-cell receptors, and how they are generated, in Chapters 3 and 5, respectively.

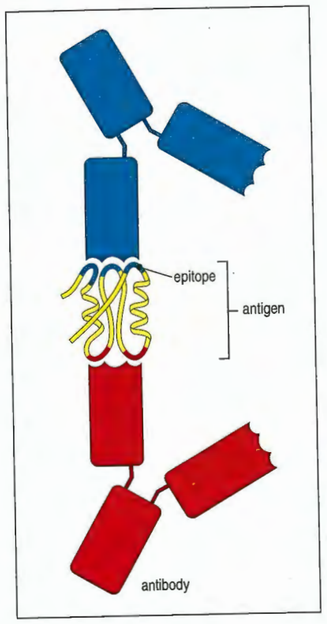


Fig. 1.15 Antigens are the molecules recognized by the immune response, while epitopes are sites within antigens to which antigen receptors bind. Antigens can be complex macromolecules such as proteins, as shown in yellow. Most antigens are larger than the sites on the antibody or antigen receptor to 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.

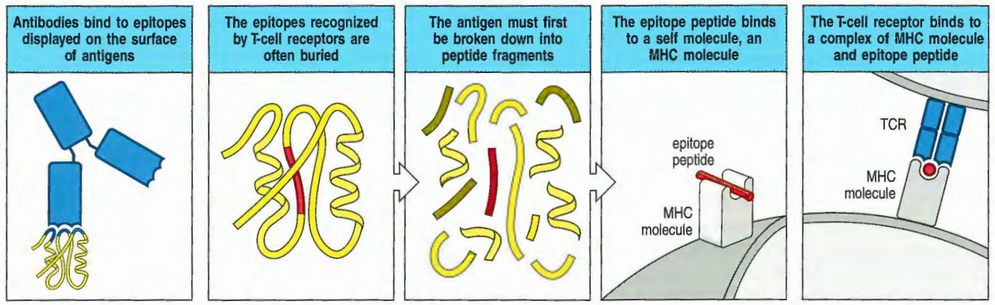


Fig. 1.16 An antibody binds an antigen directly, whereas a T-cell receptor binds a complex of antigen fragment and self molecule. Antibodies (first panel) bind directly to their antigens and recognize epitopes that form surface features of the antigen. In contrast, T-cell receptors can recognize epitopes that are buried within antigens and cannot be recognized directly (second panel). These antigens must first be degraded by proteinases (third panel), and the peptide epitope delivered to a self molecule, called an MHC molecule (fourth panel). It is in this form, as a complex of peptide and MHC molecule, that antigens are recognized by T-cell receptors (fifth panel).

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

Equally amazing as the generation of millions of different antigen receptors is the shaping of this repertoire during lymphocyte development and the maintenance of an extensive repertoire in the periphery. How are potentially useful receptors maintained while those that could react against an individual's own antigens—their **self antigens**—are eliminated? How are the numbers of peripheral lymphocytes, and the percentages of B cells and T cells, kept relatively constant? The answer seems to be that throughout its lifetime, from its development in the central lymphoid organs onwards, the survival of a lymphocyte depends on signals received through its antigen receptor. If a lymphocyte fails to receive such survival signals, it dies by a form of cell suicide called **apoptosis** or **programmed cell death**. Lymphocytes that react strongly to self antigens are removed during development by clonal deletion, as predicted by Burnet's clonal selection theory, before they mature to a stage at which they could do damage. In contrast, a complete absence of signals from the antigen receptor during development can also lead to cell death. In addition, if a 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 they are potentially functional. The mechanisms that shape and maintain the lymphocyte receptor repertoire are examined in Chapter 7.

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. Each 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.

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

Pathogens can enter the body by many routes and set up an infection anywhere in the tissues, whereas lymphocytes are normally found only in the blood, lymph, and lymphoid organs. How, then, are they to meet? Antigen and lymphocytes eventually encounter each other in the peripheral lymphoid organs—the lymph nodes, spleen, and the mucosal lymphoid tissues

(see Fig. 1.7). Mature naive lymphocytes are continually recirculating through these tissues, to which pathogen antigens are carried from sites 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 non-leukocyte 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. 1.7), which then carries them to the tissues where they will act. This whole process takes about 4–6 days from the time that antigen is recognized, which means that an adaptive immune response to an antigen not encountered before does not become effective until about a week after infection. 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.

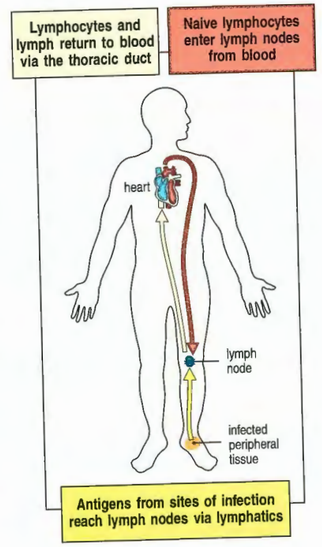


Fig. 1.17 Circulating lymphocytes encounter antigen in peripheral lymphoid organs. Naive lymphocytes recirculate constantly through peripheral lymphoid tissue, here illustrated as a popliteal lymph node—a lymph node situated behind the knee. In the case of an infection in the foot, this will be the draining lymph node, where lymphocytes may encounter their specific antigens and become activated. Both activated and non-activated 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. 1.7). 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 under the influence of chemotactic 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, since 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 creates an ideal environment in the T-cell zone in which naive T cells can bind their antigen and thus become activated.

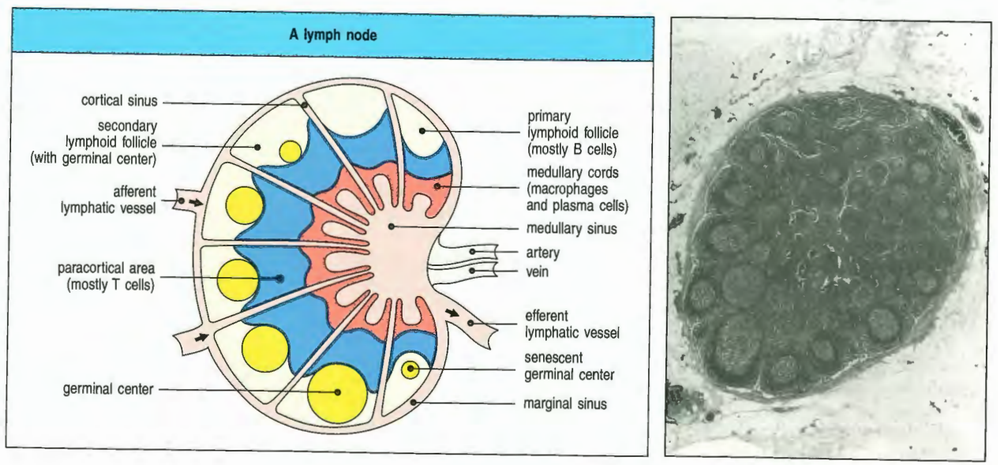
As noted earlier, activation of B cells usually requires not only antigen, which binds to the B-cell receptor, but also the cooperation of helper T cells, a type of effector T cell (see Section 1-4). The organization of the lymph node ensures that before entering the follicles, naive B cells pass through the T-cell

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.

zones, where they may encounter both their antigen and their cooperating helper T cells and become activated. Some of the B-cell follicles include **germinal centers**, where activated B cells are undergoing intense proliferation and differentiation into plasma cells.

In humans, the **spleen** is a fist-sized organ situated just behind the stomach (see Fig. 1.7). 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, non-circulating population of B cells known as **marginal zone B cells**, about which little is known; they are discussed in Chapter 7. Blood-borne microbes, soluble antigens and antigen:antibody complexes are filtered from the blood by macrophages and immature dendritic cells within the marginal zone. As with the migration of immature dendritic cells from peripheral tissues to the T-cell areas of lymph nodes, when dendritic cells in the splenic marginal zones take up antigens and become activated they migrate into the T-cell areas of the spleen, where 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 called **Peyer's patches** in the



small intestine, 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 **microfold** or **M cells** (Fig. 1.20). The lymphocytes form

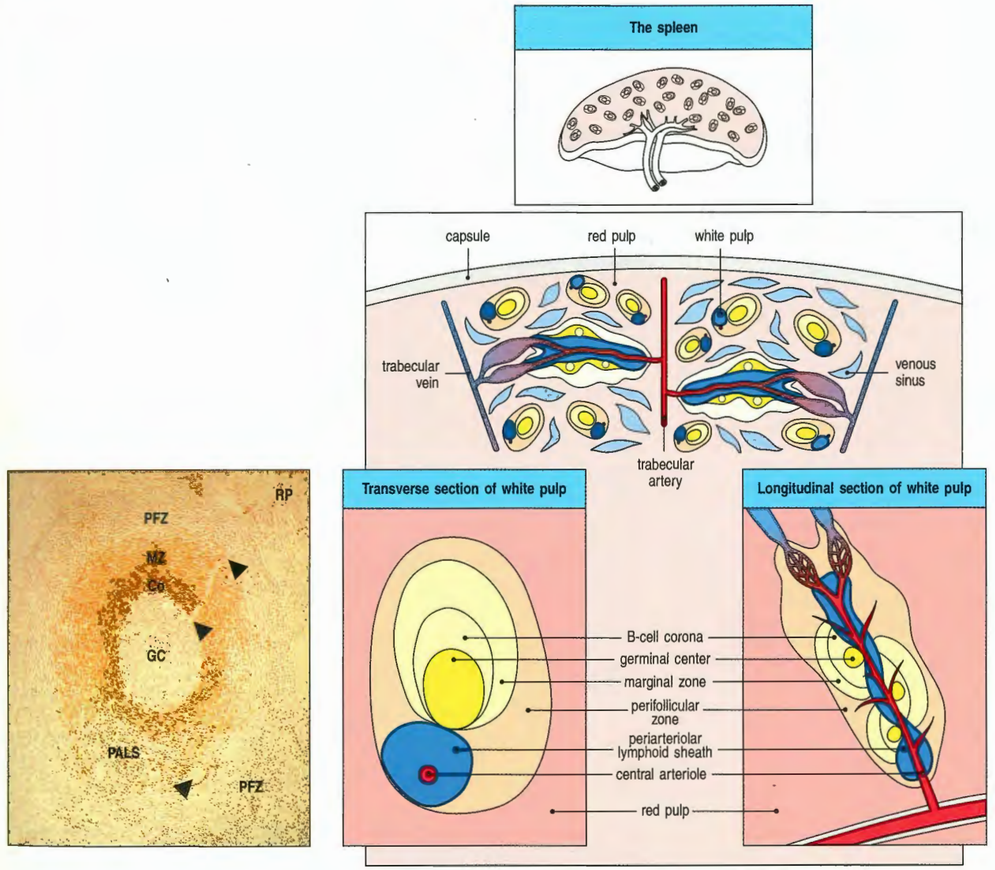


Fig. 1.19 Organization of the lymphoid tissues of the spleen. The schematic at top right shows that the spleen consists of red pulp (pink areas in the top panel), which is a site of red blood cell destruction, interspersed with the lymphoid white pulp. An enlargement of a small section of a human spleen (center) 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.

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.

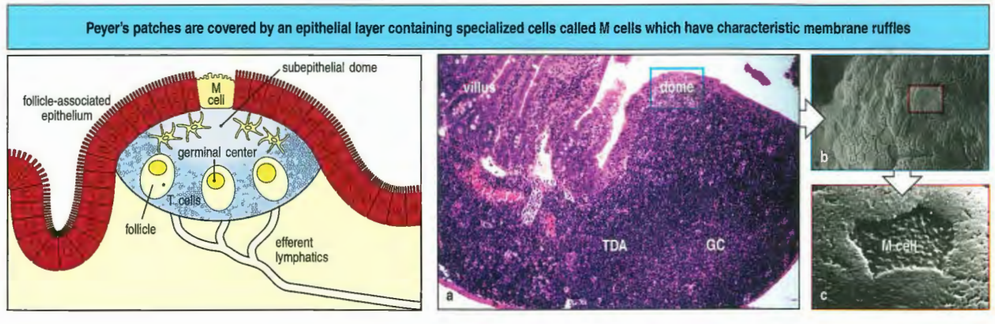
Similar but more diffuse aggregates of lymphocytes are present in the respiratory tract and other mucosa: **nasal-associated lymphoid tissue** (**NALT**) and **bronchus-associated lymphoid tissue** (**BALT**) are present in the respiratory tract. Like the Peyer's patches, these mucosal lymphoid tissues 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 11.

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 dramatically, 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 play an important role in protecting these tissues from infection, and are described further in Chapters 7 and 11.

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 (a), showing the M cells, which lack the microvilli and the mucous 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 (b), showing the characteristic ruffled surface of an M cell. M cells are the portal of entry for many pathogens and other particles. (a) Hematoxylin and eosin stain. Magnification ×100; (b) ×5000; (c) ×23,000. Source: Mowat, A., Viney, J.: *Immunol. Rev.* 1997, **156**:145—166.



1-16 Interaction with other cells as well as with antigen is necessary for lymphocyte activation.

As we have seen in Sections 1-3 and 1-6, peripheral lymphoid tissues are specialized not only to trap phagocytic cells that have ingested antigen but also to promote their interactions with lymphocytes that are needed to initiate an adaptive immune response.

All lymphocyte responses to antigen require not only the signal that results from antigen binding to lymphocyte receptors but also a second signal, which is delivered by another cell by means of cell-surface molecules known generally as co-stimulatory molecules (see Section 1-7). Naive T cells are usually stimulated by activated dendritic cells (Fig. 1.21, left panel), but for naive B cells (Fig. 1.21, right panel) the second signal is delivered by an activated helper T cell. Macrophages and B cells presenting foreign antigen on their surface can also be induced to express co-stimulatory molecules and thus can activate naive 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 this respect, with a central role in the initiation of adaptive immune responses.

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 shall return to this topic in Chapter 7. 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.

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

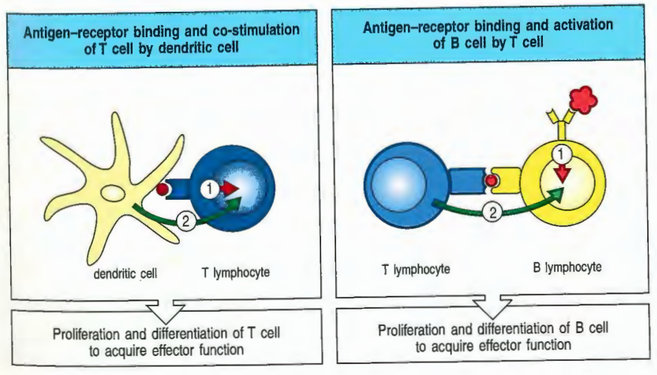


Fig. 1.21 Two signals are required for lymphocyte activation. As well as receiving a signal through their antigen receptor (signal 1), mature naive lymphocytes must also receive a second signal (signal 2) to become activated. For T cells (left panel), this second signal is delivered by an antigen-presenting 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.

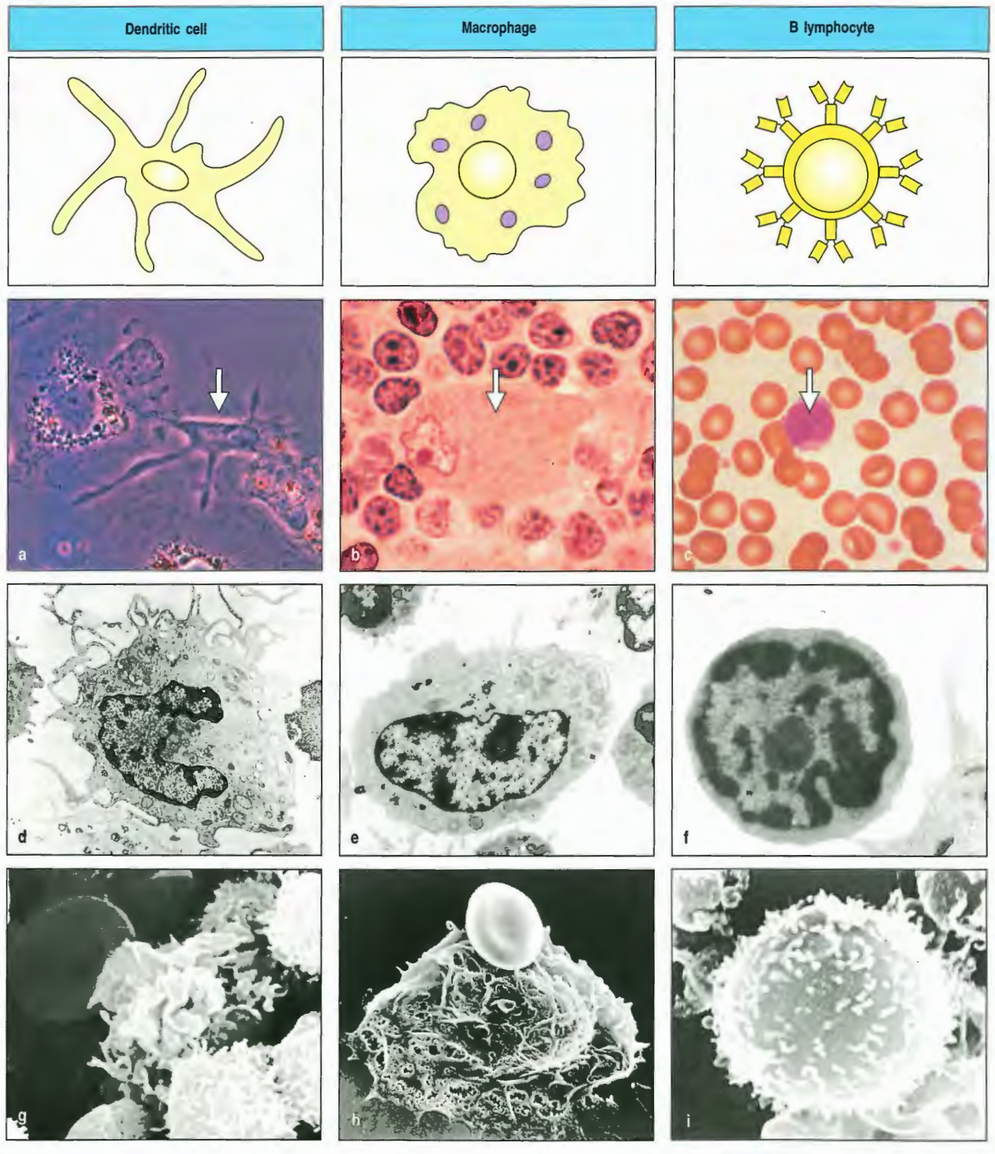


Fig. 1.22 The antigen-presenting cells. The three types of antigen-presenting cells are shown in the form in which they will be depicted throughout this book (top row), as they appear in the light microscope (second row; the relevant cell is indicated by an arrow), by transmission electron microscopy (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).

activated first to proliferate. Only when a large clone of identical cells has been produced do these finally differentiate into effector cells. This clonal expansion is a feature common to all adaptive immune responses. On recognizing its specific antigen on an activated antigen-presenting cell, a naive lymphocyte stops migrating and enlarges. The chromatin in its nucleus becomes less dense, nucleoli appear, the volume of both the nucleus and the 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** (Fig. 1.23).

The lymphoblasts now begin to divide, normally duplicating themselves two to four times every 24 hours for 3–5 days, so that one naive lymphocyte gives rise to 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

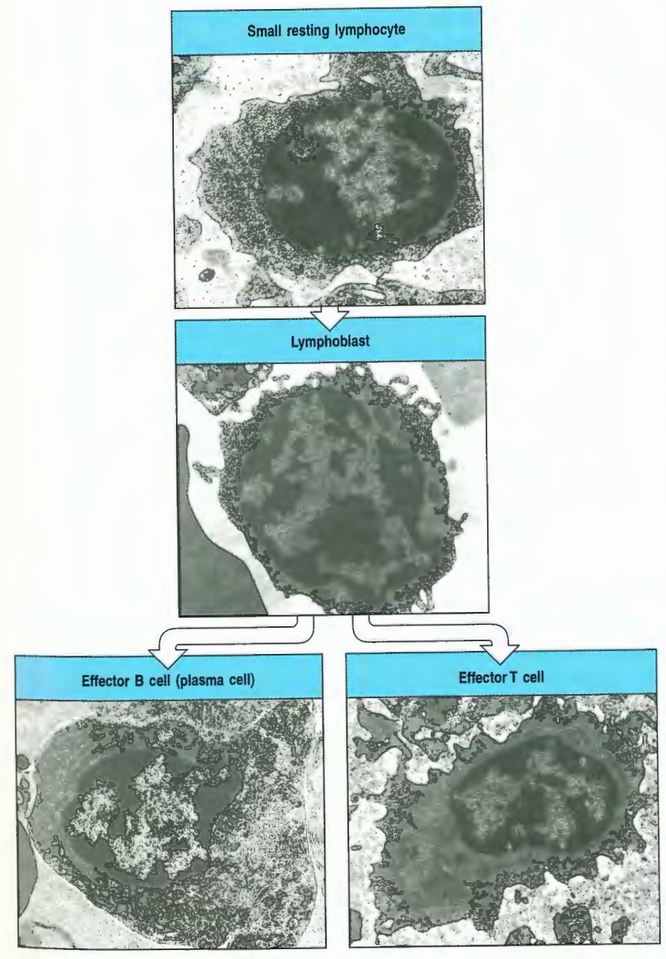


Fig. 1.23 Transmission electron micrographs of lymphocytes at various stages of activation to effector function. Small resting lymphocytes (top panel) have not yet encountered antigen. Note the scanty cytoplasm, the absence of rough endoplasmic reticulum, and the condensed chromatin, all indicative of an inactive cell. This could be either a T cell or a B cell. Small circulating lymphocytes are trapped in lymph nodes when their receptors encounter antigen on antigen-presenting cells. Stimulation by antigen induces the lymphocyte to become an active lymphoblast (center panel). Note the larger size, enlarged nucleus and more diffuse chromatin; again, T and B lymphoblasts are similar in appearance. This cell undergoes repeated division, which is followed by differentiation to effector function. The bottom panels show effector T and B lymphocytes. Note the large amount of cytoplasm, abundant mitochondria, and the presence of rough endoplasmic reticulum, all hallmarks of active cells. The rough endoplasmic reticulum is especially prominent in plasma cells (effector B cells), which are synthesizing and secreting very large amounts of protein in the form of antibody. Photographs courtesy of N. Rooney.

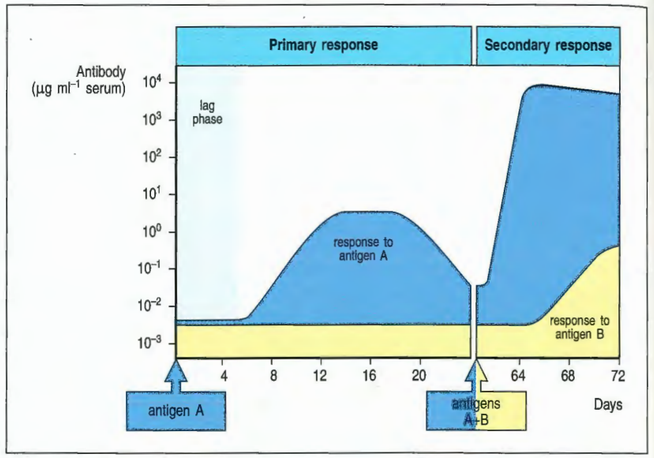


Fig. 1.24 The course of a typical antibody response. The first encounter with an antigen produces a primary response. Antigen A introduced at time zero encounters little specific antibody in the serum. After a lag phase (light blue), antibody against antigen A (dark blue) appears; its concentration rises 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.

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 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.

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. The first adaptive immune response to a pathogen thus 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 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. 1.24, 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 4, 9, 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 an adaptive immune response. Immunological memory is the most important biological consequence of adaptive immunity, although its cellular and molecular basis is still not fully understood, as we shall see in Chapter 10.

Summary.

The early innate systems of defense, which depend on invariant receptors recognizing common features of pathogens, 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. This process produces a large collection of lymphocytes each bearing a distinct receptor, so that the total receptor repertoire can recognize virtually any antigen. If the receptor is specific for a ubiquitous self antigen, the lymphocyte is eliminated by encountering the antigen early in its development, while survival signals received through the antigen receptor select and maintain a repertoire of potentially useful lymphocytes. 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 progeny then 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 require different responses for both their recognition and their destruction (Fig. 1.25). B-cell receptors recognize antigens from the extracellular environment and

Fig. 1.25 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 carinii*	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

differentiate into effector plasma cells that secrete antibody back into that environment. T-cell receptors are specialized to detect antigens that have been generated inside the body's cells, 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.

Most of the other effector mechanisms that dispose of pathogens targeted by an adaptive immune response 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. This is discussed in Chapter 16. 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 deal with extracellular forms of 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. 1.13, 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 4 and 9.

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

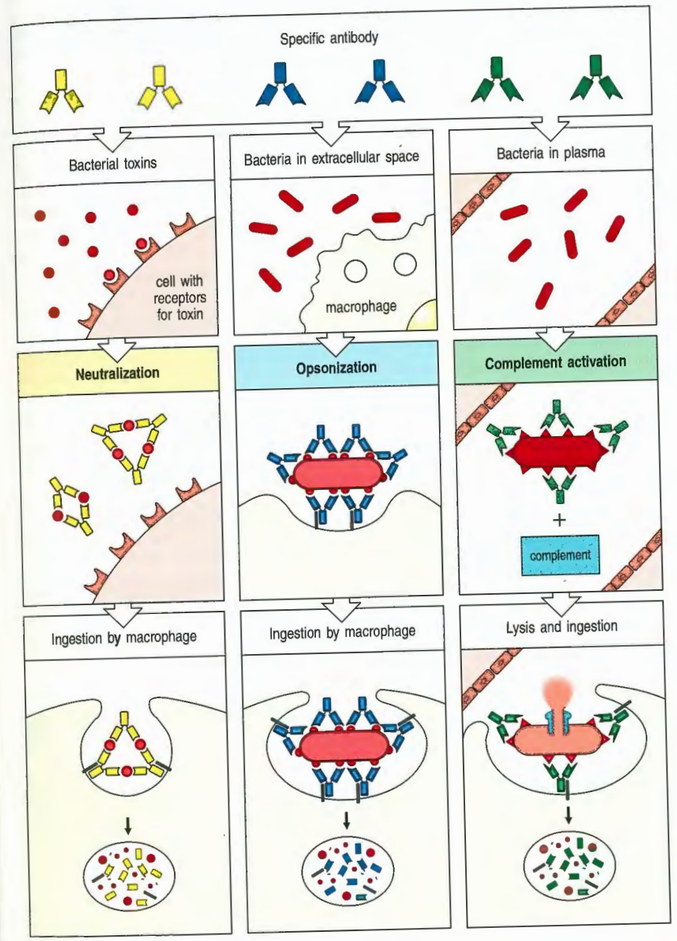


Fig. 1.26 Antibodies can participate in host defense in three main ways. The left panels show antibodies binding to and neutralizing a bacterial toxin, thus preventing it from interacting with host cells and causing pathology. Unbound toxin can react with receptors on the host cell, whereas the toxin:antibody 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 middle 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.

access to cells that they might infect or destroy (Fig. 1.26, 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 bacterium, leading to phagocytosis (see Fig. 1.26, middle 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 the constant regions of antibodies bound to bacterial surfaces form receptors for the first protein of the complement system, so that once antibodies are produced, complement activation increases. Complement components bound to the bacterial surface can directly destroy certain bacteria, and this is important in a few bacterial infections (see Fig. 1.26, right panels). The main function of complement, however, like that of antibodies, is to coat the pathogen surface and enable 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.

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. 1.26, 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 are needed to control intracellular pathogens and to activate B-cell responses to most antigens.

Pathogens are accessible to antibodies 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 these

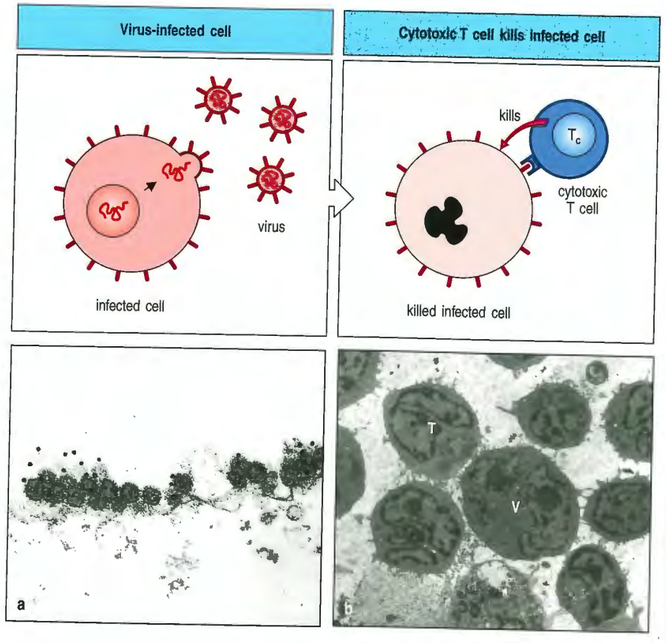


Fig. 1.27 Mechanism of host defense against intracellular infection by viruses. Cells infected by viruses are recognized by specialized T cells called cytotoxic T cells, which kill the infected cells directly. The killing mechanism involves the activation of enzymes known as caspases, which contain cysteine 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.

invaders is the function of the T lymphocytes, which are responsible for the **cell-mediated immune responses** of adaptive immunity.

The action of cytotoxic T cells is the most direct. These effector T cells 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 killing the infected cell before viral replication is complete and new viruses are released (Fig. 1.27).

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, as they help to determine the interactions the T cell makes with other cells. Cytotoxic T cells carry CD8, while the class of T cells involved in activating the cells they recognize, rather than killing them, 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 different types of effector T cells after their initial activation by antigen. The two major types of CD4 effector T cells are called $\mathbf{T_H1}$ and $\mathbf{T_H2}$ cells, although more have been described, as we shall see in Chapter 8. These two types are both involved in combating bacterial infections, but in quite different ways. T_H1 cells have a dual function. The first is to control certain intracellular bacterial infections. Some bacteria grow only in the intracellular membrane-bounded vesicles of macrophages; important examples are *Mycobacterium tuberculosis* and *M. leprae*, the pathogens that cause tuberculosis and leprosy, respectively. Bacteria phagocytosed by macrophages are usually destroyed in the lysosomes, which contain a variety of enzymes and antimicrobial substances. Mycobacteria and some other bacteria survive intracellularly because they prevent the vesicles they occupy from fusing with lysosomes (Fig. 1.28). These infections can be controlled by T_H1 cells that recognize bacterial antigens displayed on the

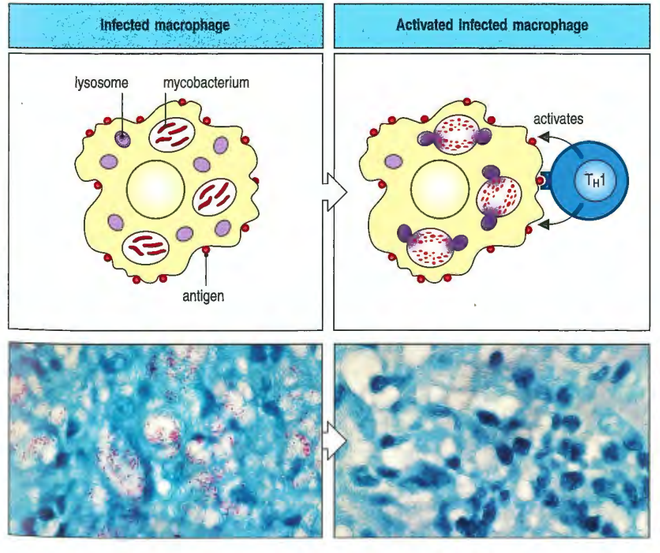


Fig. 1.28 Mechanism of host defense against intracellular infection by mycobacteria. Mycobacteria are engulfed by macrophages but resist being destroyed by preventing the intracellular vesicles in which they reside from fusing with lysosomes containing bactericidal agents. Thus the bacteria are protected 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.

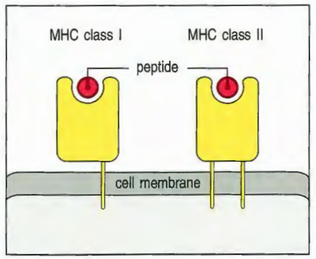


Fig. 1.29 MHC molecules on the cell surface display peptide fragments of antigens. MHC molecules are membrane proteins whose outer extracellular domains form a cleft in which a peptide fragment is bound. These fragments are derived from proteins degraded inside the cell, including both self and foreign 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.

macrophage surface. T_H1 cells activate the infected macrophages, inducing the fusion of their lysosomes with the vesicles containing the bacteria and stimulating the macrophage's antibacterial mechanisms (Fig. 1.28). The second role of T_H1 cells is as helper T cells to stimulate the production of antibodies by producing co-stimulatory signals and interacting with B lymphocytes. We shall see in Chapter 9, when we discuss the humoral immune response in detail, that only a few antigens with special properties can activate naive B lymphocytes on their own; an accompanying co-stimulatory signal from T cells is usually required (see Fig. 1.21).

Whereas T_H1 cells have a dual role, helper T_H2 cells are entirely dedicated to the activation of naive B cells to produce antibody. The term 'helper T cell' is sometimes used by researchers to describe all CD4 T cells. It was originally coined, however, to describe T cells that 'help' B cells produce antibody, before the existence of two subtypes of CD4 T cells was recognized. When the macrophage-activating role of CD4 T cells was discovered, the designation 'helper' was extended to cover these as well (hence the H in T_H1). We consider this blanket usage confusing, and throughout this book we will only use the term 'helper T cell' in connection with the activation of B cells for antibody production, whether by T_H1 or T_H2 cells.

Naive T lymphocytes recognize their corresponding antigens on specialized antigen-presenting cells, which can also activate them. Effector T cells similarly recognize peptide antigens bound to MHC molecules, but in this case the T cell is already activated and thus does not need co-stimulatory signals.

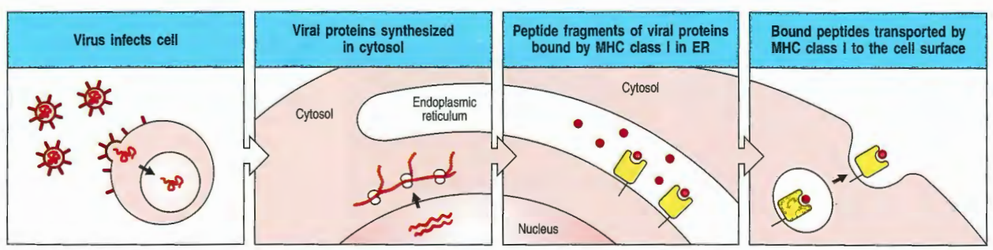
1-20 CD4 and CD8 T cells recognize peptides bound to two different classes of MHC molecules.

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** 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. 1.29).

The most important differences between the two classes of MHC molecule lie not in their structure but in the source of the peptides that they trap and carry to the cell surface. **MHC class I molecules** collect peptides derived from proteins

Fig. 1.30 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.



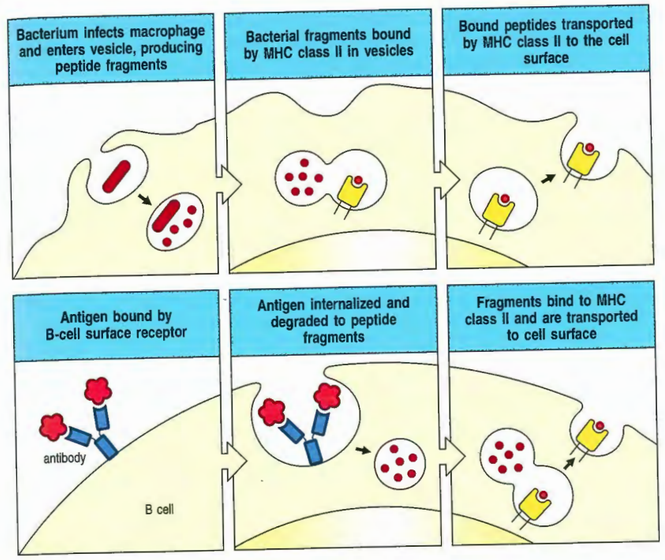


Fig. 1.31 MHC class II molecules present antigen originating in intracellular vesicles. Some bacteria infect cells and grow in intracellular vesicles. Peptides derived from such bacteria are bound by MHC class II molecules and transported to the cell surface (top row). MHC class II molecules also bind and transport peptides derived from antigen that has been bound and internalized by B-cell antigen receptor-mediated uptake into intracellular vesicles (bottom row).

synthesized in the cytosol and are thus able to display fragments of viral proteins on the cell surface (Fig. 1.30). **MHC class II molecules** bind peptides derived from proteins in intracellular vesicles, and thus display peptides derived from pathogens living in macrophage vesicles or internalized by phagocytic cells and B cells (Fig. 1.31). We shall see in Chapter 5 exactly how peptides from these different sources are made available to the two types of MHC molecule.

Having reached the cell surface with their peptide cargo, the two classes of MHC molecule are recognized by different functional classes of T cell. This occurs because the CD8 molecule binds preferentially to MHC class I molecules, whereas CD4 binds preferentially to MCH class II molecules. Thus, MHC class I molecules bearing viral peptides are recognized by CD8-bearing cytotoxic T cells, which then kill the infected cell (Fig. 1.32); MHC class II molecules bearing peptides derived from pathogens taken up into vesicles are recognized by CD4-bearing T cells (Fig. 1.33). CD4 and CD8 are thus known as **co-receptors**, as they are inextricably involved in signaling to the T cell that the receptor has bound the correct antigen. Useful interactions are further ensured by the fact that all cells express MHC class I molecules, and thus any virus-infected

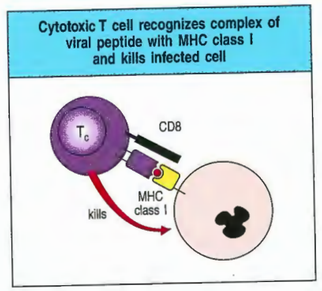


Fig. 1.32 Cytotoxic CD8 T cells recognize antigen presented by MHC class I molecules and kill the cell. The peptide:MHC class I complex on virus-infected cells is detected by antigen-specific cytotoxic T cells. Cytotoxic T cells are preprogrammed to kill the cells they recognize.

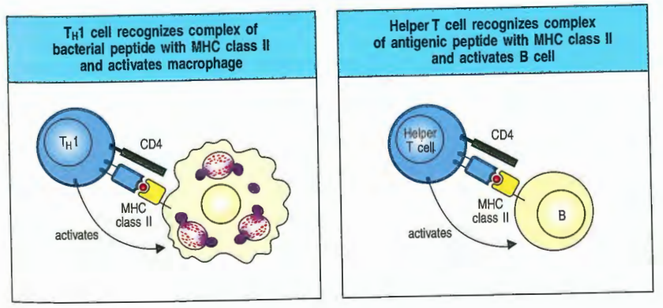


Fig. 1.33 CD4 T cells recognize antigen presented by MHC class II molecules. On recognition of their specific antigen on infected macrophages, T_H1 cells activate the macrophage, leading to the destruction of the intracellular bacteria (left panel). When T_H2 or T_H1 helper T cells recognize antigen on B cells, they activate these cells to proliferate and differentiate into antibody-producing plasma cells (right panel).

cell can be recognized and killed by a CD8 cytotoxic T cell, while the only cells that normally express MHC class II molecules are dendritic cells, macrophages, and B cells—the cells that must activate, or be activated by, CD4 T cells.

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 lymphocyte development, and naive T cells emerge from the central lymphocyte organs bearing the correct combination of receptors and co-receptors. The maturation of T cells into either CD8 or CD4 T cells reflects a testing of T-cell receptor specificity that occurs during development. Exactly how this selective process works and how it maximizes the usefulness of the T-cell repertoire is a central question in immunology, and is a major topic of Chapter 7.

On recognizing their targets, the various types of effector T cells are stimulated to release different sets of effector molecules. These can directly affect their target cells or help to recruit other effector cells in ways we discuss in Chapter 8. These effector molecules include many cytokines, which have a crucial role in the clonal expansion of lymphocytes as well as in innate immune responses and in the effector actions of most cells of the immune system. Thus, understanding the actions of cytokines is central to understanding the various behaviors of the immune system. The actions of all the known cytokines are summarized in Appendix III, some are introduced in Chapter 2, and the T-cell-derived cytokines are discussed in Chapter 8.

1-21 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 pathogen, 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.

More than 25 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 12 together with the inherited immunodeficiencies.

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

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 non-infectious 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. 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. The MHC was originally recognized in mice as a gene locus, the **H2 locus**, that controlled the acceptance or rejection of transplanted tissues, while 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. 1.34).

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 to treat these responses is with immunosuppressive drugs, which inhibit all immune responses, desirable and undesirable. 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. There is hope that this dream of antigen-specific immunoregulation to control unwanted immune responses could become a reality, as antigen-specific suppression of immune responses can be induced experimentally, although the molecular basis of this suppression is not fully understood. We shall discuss the present state of understanding of allergies, autoimmune disease, graft rejection, and immunosuppressive drugs in Chapters 13–15, and we shall see in Chapter 14 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.

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. 1.34 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.

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

Although the specific suppression of immune responses must await advances in basic research on immune regulation and its application, 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. 1.35). 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 or polio 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 15. The prospect of controlling these important diseases is tremendously exciting.

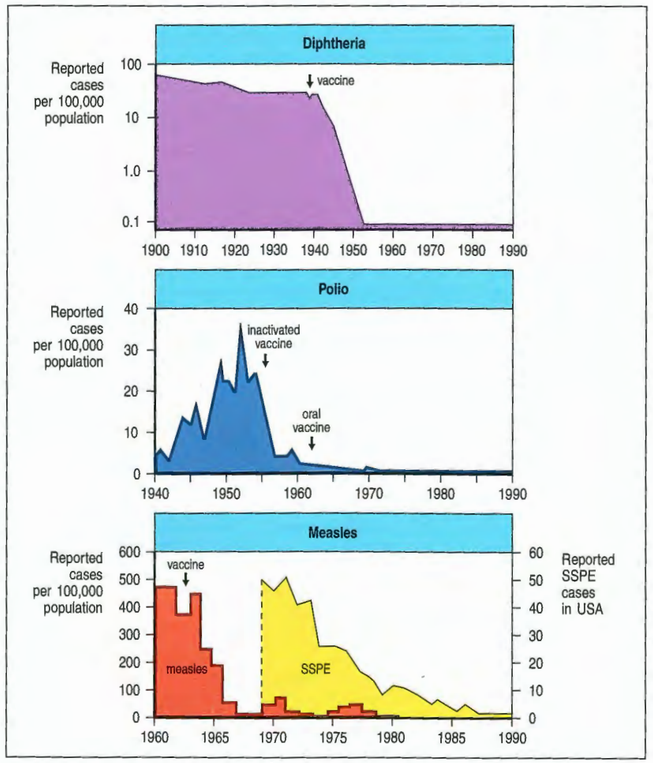


Fig. 1.35 Successful vaccination campaigns. Diphtheria, polio, and measles and its consequences have been virtually eliminated in the United States, as shown in these three graphs. SSPE stands for subacute sclerosing panencephalitis, a brain disease that is a late consequence of measles infection in a few patients. When measles was prevented, SSPE disappeared 15–20 years later. However, as these diseases have not been eradicated worldwide, immunization must be maintained in a very high percentage of the population to prevent their reappearance.

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 used by successful pathogens in Chapter 12. 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 CD4 T cells that mainly activate macrophages and B cells. Thus, T cells are crucially important for both the humoral and cell-mediated responses of adaptive immunity. The adaptive immune response seems to have engrafted 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. 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. 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.

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2 Innate Immunity

Throughout most of this book we will examine the ways in which the adaptive immune response protects the host from microorganisms that otherwise would cause disease. In this chapter, however, we will examine the role of the innate, nonadaptive defenses that form the earliest barriers to infection. The microorganisms that are encountered daily in the life of a healthy individual only occasionally cause perceptible disease. Most are detected and destroyed within minutes or hours by defense mechanisms that do not rely on the clonal expansion of antigen-specific lymphocytes (see Section 1-9) and thus do not require a prolonged period of induction: these are the mechanisms of **innate immunity**.

The time course and different phases of an encounter with a new pathogen are summarized in Fig. 2.1. Some innate immune mechanisms start acting immediately on encounter with infectious agents. Others are activated and amplified in the presence of infection and then return to baseline levels after the infection is finished. Innate immune mechanisms do not generate long-term protective immunological memory. Only if an infectious organism can breach these lines of defense will an adaptive immune response ensue, with the generation of antigen-specific effector cells that target the specific pathogen, and memory cells that provide long-lasting immunity against reinfection with the same microorganism. The power of adaptive immune responses is due to their antigen specificity, which we will be studying in the following chapters. However, they harness, and also depend upon, many of the effector mechanisms used by the innate immune system to remove pathogens, which we will describe in this chapter.

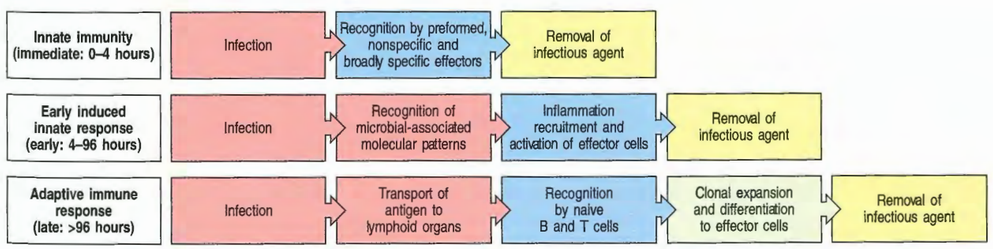


Fig. 2.1 The response to an initial infection occurs in three phases. These are the innate phase, the early induced innate response, and the adaptive immune response. The first two phases rely on the recognition of pathogens by germline-encoded receptors of the innate immune system, whereas adaptive immunity uses variable antigen-specific receptors that are produced as a result of gene segment rearrangements. Adaptive immunity occurs late, because the rare B and T cells specific for the invading pathogen must first undergo clonal expansion before they differentiate into effector cells that can clear the infection. The effector mechanisms that remove the infectious agent are similar or identical in each phase.

Whereas the adaptive immune system uses a large repertoire of receptors encoded by rearranging gene segments to recognize a huge variety of antigens (see Section 1-12), innate immunity depends upon germline-encoded receptors to recognize features that are common to many pathogens. In fact, the mechanisms of innate immunity discriminate very effectively between host cells and pathogens, and this ability to discriminate between self and nonself, and to recognize broad classes of pathogens, contributes to the induction of an appropriate adaptive immune response.

In the first part of the chapter we will consider the fixed defenses of the body: the epithelia that line the internal and external surfaces of the body, and the phagocytes that lie beneath all epithelial surfaces and that engulf and digest invading microorganisms. As well as killing microorganisms directly, these phagocytes induce the next phase of the innate immune response, inducing an inflammatory response that recruits new phagocytic cells and circulating effector molecules to the site of infection. Second, we will take a closer look at the ancient system of pattern-recognition receptors used by the phagocytic cells of the innate immune system to identify pathogens and distinguish them from self antigens. We shall see how, as well as directing the immediate destruction of pathogens, stimulation of some of these receptors on macrophages and dendritic cells leads to their becoming cells that can effectively present antigen to T lymphocytes, thus initiating an adaptive immune response. The third part of the chapter is devoted to a system of plasma proteins known as the complement system. This important element of so-called humoral innate immunity interacts with microorganisms to promote their removal by phagocytic cells. In the last part of the chapter we shall describe how the cytokines and chemokines produced by activated phagocytes and dendritic cells induce the later phases of the innate immune response, such as the so-called acute-phase response. We shall also meet another cell of the innate immune system, the natural killer cell (NK cell), which contributes to innate host defenses against viruses and other intracellular pathogens. In this phase, the first steps towards initiating an adaptive immune response take place, so that if the infection is not cleared by the innate responses, a full immune response will ensue.

The front line of host defense.

Microorganisms that cause disease in humans and animals enter the body at different sites and produce disease symptoms by a variety of mechanisms. Many different infectious agents can cause disease and damage to tissues, or pathology, and are referred to as **pathogenic microorganisms** or **pathogens**. In vertebrates, microbial invasion is initially countered by innate defenses that preexist in all individuals and begin to act within minutes of encounter with the infectious agent. Only when the innate host defenses are bypassed, evaded, or overwhelmed is an adaptive immune response required. Although innate immunity is obviously sufficient to prevent the body from being routinely overwhelmed by the vast number of microorganisms that live on and in it, pathogens, almost by definition, are microorganisms that have evolved ways of overcoming the body's innate defenses more effectively than other microorganisms. Once they have gained a hold, they require the concerted efforts of both innate and adaptive immune responses to clear them from the body. Even in these cases, however, the innate immune system usually performs a valuable delaying function, keeping pathogen numbers in check while the adaptive immune system gears up for action. In the first part of this chapter we will describe briefly the different types of pathogens and their

invasive strategies, and then examine the innate defenses that, in most cases, prevent microorganisms from establishing an infection. We will examine the defense functions of the epithelial surfaces of the body, the role of antimicrobial peptides and proteins, and the defense of body tissues by phagocytic cells—the macrophages and neutrophils—which bind to and ingest invading microorganisms.

2-1 Infectious diseases are caused by diverse living agents that replicate in their hosts.

The agents that cause disease fall into five groups: viruses, bacteria, fungi, protozoa, and helminths (worms). Protozoa and worms are usually grouped together as parasites, and are the subject of the discipline of parasitology, whereas viruses, bacteria, and fungi are the subject of microbiology. In Fig. 2.2, the classes of microorganisms and parasites that cause disease are listed, with typical examples of each. The characteristic features of each pathogen are its mode of transmission, its mechanism of replication, its mechanism of **pathogenesis**—the means by which it causes disease—and the response it elicits from the host. The distinct habitats and life cycles of different pathogens mean that a range of different innate and adaptive immune mechanisms have to be deployed for their destruction.

Infectious agents can grow in all body compartments, as shown schematically in Fig. 2.3. We saw in Chapter 1 that two major compartments can be defined—extracellular and intracellular. Both innate and adaptive immune responses have different ways of dealing with pathogens found in these two compartments. Many bacterial pathogens live and replicate in extracellular spaces, either within tissues or on the surface of the epithelia that line body cavities. Extracellular bacteria are usually susceptible to killing by phagocytes, an important arm of the innate immune system, but some pathogens, such as *Staphylococcus* and *Streptococcus* species, are protected by a polysaccharide capsule that resists engulfment. This can be overcome to some extent by the help of another component of innate immunity—complement—which renders the bacteria more susceptible to phagocytosis. In the adaptive immune response, bacteria are rendered more susceptible to phagocytosis by a combination of antibodies and complement.

Obligate intracellular pathogens, such as all viruses, must invade host cells to replicate, whereas facultative intracellular pathogens, such as mycobacteria, can replicate either intracellularly or outside the cell. Intracellular pathogens must either be prevented from entering cells or be detected and eliminated once they have done so. They can be subdivided further into those that replicate free in the cell, such as viruses and certain bacteria (for example, *Chlamydia*, *Rickettsia*, and *Listeria*), and those, such as the mycobacteria, that replicate inside intracellular vesicles. Infectious agents that live intracellularly frequently cause disease by damaging or killing the cells that house them. The innate immune system has two general means of defense against this type of pathogen. Phagocytes may take up the pathogen before it enters cells, while NK cells can directly recognize and kill cells infected with some intracellular pathogens. NK cells are instrumental in keeping some viral infections in check until an adaptive immune response has been generated, after which cytotoxic T cells are able to take over the role of killing virus-infected cells. Pathogens that live inside macrophage vesicles may become susceptible to killing after activation of the macrophage as a result of NK cell or T cell actions (see Fig. 2.3).

Once pathogens have overcome the defenses of innate immunity, they grow and replicate in the body, causing markedly different diseases that reflect the diverse ways in which they damage tissues (Fig. 2.4). Many of the most

Fig. 2.2 A variety of microorganisms can cause disease. Pathogenic organisms are of five main types: viruses, bacteria, fungi, protozoa, and worms. Some well-known pathogens in each group are listed.

Some common causes of disease in humans			
Viruses	DNA viruses	Adenoviruses	Human adenoviruses (e.g., types 3, 4, and 7)
		Herpesviruses	Herpes simplex, varicella zoster, Epstein–Barr virus, cytomegalovirus, HHV8
		Poxviruses	Variola, vaccinia virus
		Parvoviruses	Human parvovirus
		Papovaviruses	Papilloma virus
		Hepadnaviruses	Hepatitis B virus
	RNA viruses	Orthomyxoviruses	Influenza virus
		Paramyxoviruses	Mumps, measles, respiratory syncytial virus
		Coronaviruses	Cold viruses, SARS
		Picornaviruses	Polio, coxsackie, hepatitis A, rhinovirus
		Reoviruses	Rotavirus, reovirus
		Togaviruses	Rubella, arthropod-borne encephalitis
		Flaviviruses	Arthropod-borne viruses, (yellow fever, dengue fever)
		Arenaviruses	Lymphocytic choriomeningitis, Lassa fever
		Rhabdoviruses	Rabies
		Retroviruses	Human T-cell leukemia virus, HIV
Bacteria	Gram +ve cocci	Staphylococci	*Staphylococcus aureus*
		Streptococci	*Streptococcus pneumoniae, Strep. pyogenes*
	Gram –ve cocci	Neisseriae	*Neisseria gonorrhoeae, N. meningitidis*
	Gram +ve bacilli		*Corynebacterium diphtheriae, Bacillus anthracis, Listeria monocytogenes*
	Gram –ve bacilli		*Salmonella typhi, Shigella flexneri, Campylobacter jejuni, Vibrio cholerae, Yersinia pestis, Pseudomonas aeruginosa, Brucella melitensis, Haemophilus influenzae, Legionella pneumophilus, Bordetella pertussis*
	Firmicutes	Clostridia	*Clostridium tetani, C. botulinum, C. perfringens*
	Spirochaetes	Spirochetes	*Treponema pallidum, Borrelia burgdorferi, Leptospira interrogans*
	Actinobacteria	Mycobacteria	*Mycobacterium tuberculosis, M. leprae, M. avium*
	Protobacteria	Rickettsias	*Rickettsia prowazekii*
	Chlamydiae	Chlamydias	*Chlamydia trachomatis*
	Mollicutes	Mycoplasmas	*Mycoplasma pneumoniae*
Fungi	Ascomycetes		*Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Histoplasma capsulatum, Coccidioides immitis, Pneumocystis carinii*
Protozoa			*Entamoeba histolytica, Giardia intestinalis, Leishmania donovani, Plasmodium falciparum, Trypanosoma brucei, Toxoplasma gondii, Cryptosporidium parvum*
Worms	Nematodes	Intestinal	*Trichuris trichura, Trichinella spiralis, Enterobius vermicularis, Ascaris lumbricoides, Ancylostoma duodenale, Strongyloides stercoralis*
		Tissues	*Onchocerca volvulus, Loa loa, Dracuncula medinensis*
	Flukes	Blood, liver	*Schistosoma mansoni, Clonorchis sinensis*

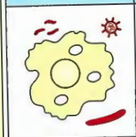
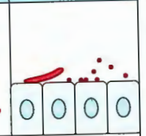
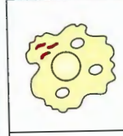
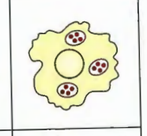
	Extracellular		Intracellular	
	Interstitial spaces, blood, lymph	Epithelial surfaces	Cytoplasmic	Vesicular
Site of infection				
Organisms	Viruses Bacteria Protozoa Fungi Worms	*Neisseria gonorrhoeae* *Mycoplasma* spp. *Streptococcus pneumoniae* *Vibrio cholerae* *Escherichia coli* *Helicobacter pylori* *Candida albicans* Worms	Viruses *Chlamydia* spp. *Rickettsia* spp. *Listeria monocytogenes* Protozoa	*Mycobacterium* spp. *Salmonella typhimurium* *Yersinia pestis* *Listeria* spp. *Legionella pneumophila* *Cryptococcus neoformans* *Leishmania* spp. *Trypanosoma* spp. *Histoplasma*
Protective immunity	Complement Phagocytosis Antibodies	Antimicrobial peptides Antibodies, especially IgA	NK cells Cytotoxic T cells	T-cell and NK-cell dependent macrophage activation

Fig. 2.3 Pathogens can be found in various compartments of the body, where they must be combated by different host defense mechanisms. Virtually all pathogens have an extracellular phase in which they are vulnerable to the circulating molecules and cells of innate immunity and to the antibodies of the adaptive immune response. All these clear the microorganism primarily by promoting its phagocytosis by the phagocytes of the immune system. Intracellular phases of pathogens such as viruses are not accessible to these mechanisms; instead the infected cell is attacked by the NK cells of innate immunity or by the cytotoxic T cells of adaptive immunity. Activation of macrophages as a result of NK-cell or T-cell activity can induce the macrophage to kill pathogens that are living inside macrophage vesicles.

dangerous extracellular bacterial pathogens cause disease by releasing protein toxins, against which the innate immune system has little defense. The highly specific antibodies produced by the adaptive immune system are required to neutralize the action of such toxins (see Fig. 1.26). The damage caused by a particular infectious agent also depends on the site in which it grows; *Streptococcus pneumoniae* in the lung causes pneumonia, for example, whereas in the blood it causes a rapidly fatal systemic illness, pneumococcal sepsis.

As we will see in the following sections, for a microorganism to invade the body, it must first bind to or cross an epithelium. Intestinal pathogens such as *Salmonella typhi*, the causal agent of typhoid fever, or *Vibrio cholerae*, which causes cholera, are spread through fecally contaminated food and water, respectively. Immune responses to this type of pathogen occur in the specialized mucosal immune system once they have breached the epithelial barrier, as described in Chapter 11. The first defense against microorganisms invading through the gut consists of a healthy intestinal epithelium and intestinal flora, which competes with the pathogens for nutrients and epithelial attachment sites.

Most pathogenic microorganisms have evolved to be able to overcome innate immune responses and to continue to grow, making us ill. An adaptive immune response is required to eliminate them and to prevent subsequent reinfection. Other pathogens are never entirely eliminated by the immune system, and persist in the body for years. But most pathogens are not universally lethal. Those that have lived for thousands of years in the human population are highly evolved to exploit their human hosts; they cannot alter their pathogenicity without upsetting the compromise they have achieved with the human immune system. Rapidly killing every host it infects is no better for the long-term survival of a pathogen than being wiped out by the immune response before the microbe has had time to infect someone else. In short, we have adapted to live with our enemies, and they with us. Nevertheless, the

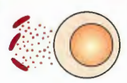
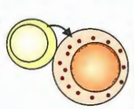
	Direct mechanisms of tissue damage by pathogens			Indirect mechanisms of tissue damage by pathogens		
	Exotoxin production	Endotoxin	Direct cytopathic effect	Immune complexes	Anti-host antibody	Cell-mediated immunity
Pathogenic mechanism						
Infectious agent	*Streptococcus pyogenes* *Staphylococcus aureus* *Corynebacterium diphtheriae* *Clostridium tetani* *Vibrio cholerae*	*Escherichia coli* *Haemophilus influenzae* *Salmonella typhi* *Shigella* *Pseudomonas aeruginosa* *Yersinia pestis*	Variola Varicella-zoster Hepatitis B virus Polio virus Measles virus Influenza virus Herpes simplex virus Human herpes virus 8 (HHV8)	Hepatitis B virus Malaria *Streptococcus pyogenes* *Treponema pallidum* Most acute infections	*Streptococcus pyogenes* *Mycoplasma pneumoniae*	*Mycobacterium tuberculosis* *Mycobacterium leprae* Lymphocytic choriomeningitis virus *Borrelia burgdorferi* *Schistosoma mansoni* Herpes simplex virus
Disease	Tonsilitis, scarlet fever Boils, toxic shock syndrome, food poisoning Diphtheria Tetanus Cholera	Gram-negative sepsis Meningitis, pneumonia Typhoid fever Bacillary dysentery Wound infection Plague	Smallpox Chickenpox, shingles Hepatitis Poliomyelitis Measles, subacute sclerosing panencephalitis Influenza Cold sores Kaposi's sarcoma	Kidney disease Vascular deposits Glomerulonephritis Kidney damage in secondary syphilis Transient renal deposits	Rheumatic fever Hemolytic anemia	Tuberculosis Tuberculoid leprosy Aseptic meningitis Lyme arthritis Schistosomiasis Herpes stromal keratitis

Fig. 2.4 Pathogens can damage tissues in a variety of different ways. The mechanisms of damage, representative infectious agents, and the common names of the diseases associated with each are shown. Exotoxins are released by microorganisms and act at the surface of host cells, for example by binding to receptors. Endotoxins, which are intrinsic components of microbial structure, trigger phagocytes to release cytokines that produce local or systemic symptoms. Many pathogens are cytopathic, directly damaging the cells they infect. Finally, an adaptive immune response to the pathogen can generate antigen:antibody complexes that activate neutrophils and macrophages, antibodies that can cross-react with host tissues, or T cells that kill infected cells. All of these have some potential to damage the host's tissues. In addition, neutrophils, the most abundant cells early in infection, release many proteins and small-molecule inflammatory mediators that both control infection and cause tissue damage (see Fig. 2.9).

recent concern about highly pathogenic strains of avian influenza, and the episode in 2002–2003 of SARS (severe acute respiratory syndrome), caused by a corona virus from bats that caused severe pneumonia in humans, remind us that new and deadly infections can transfer to humans from animal reservoirs. These are known as **zoonotic** infections—and we must be on the alert at all times for the emergence of new pathogens and new threats to health. The human immunodeficiency virus that causes AIDS (discussed in Chapter 12) serves as a warning that we remain constantly vulnerable.

2-2 Infectious agents must overcome innate host defenses to establish a focus of infection.

Our bodies are constantly exposed to microorganisms present in our environment, including infectious agents that have been shed from other infected individuals. Contact with these microorganisms may occur through external or internal epithelial surfaces: the respiratory tract mucosa provides a route of entry for airborne microorganisms, and the gastrointestinal mucosa does the same for microorganisms in food and water. Insect bites and wounds allow microorganisms to penetrate the skin, and direct contact between individuals offers opportunities for infection of the skin, the gut, and the mucosa of the reproductive tract (Fig. 2.5).

In spite of this exposure, infectious disease is fortunately quite infrequent. The epithelial surfaces of the body serve as an effective barrier against most microorganisms, and they are rapidly repaired if wounded. Furthermore, most of the microorganisms that do succeed in crossing an epithelial surface are efficiently removed by innate immune mechanisms that function in the underlying tissues. Thus, in most cases these defenses prevent infection from

Routes of infection for pathogens			
Route of entry	Mode of transmission	Pathogen	Disease
Mucosal surfaces			
Airway	Inhaled droplet	Influenza virus	Influenza
	Spores	*Neisseria meningitidis*	Meningococcal meningitis
		Bacillus anthracis	Inhalation anthrax
Gastrointestinal tract	Contaminated water or food	*Salmonella typhi*	Typhoid fever
		Rotavirus	Diarrhea
Reproductive tract	Physical contact	*Treponema pallidum*	Syphilis
		HIV	AIDS
External epithelia			
External surface	Physical contact	*Trichophyton*	Athlete's foot
Wounds and abrasions	Minor skin abrasions	*Bacillus anthracis*	Cutaneous anthrax
	Puncture wounds	*Clostridium tetani*	Tetanus
	Handling infected animals	*Francisella tularensis*	Tularemia
Insect bites	Mosquito bites (*Aedes aegypti*)	Flavivirus	Yellow fever
	Deer tick bites	*Borrelia burgdorferi*	Lyme disease
	Mosquito bites (*Anopheles*)	*Plasmodium* spp.	Malaria

Fig. 2.5 Pathogens infect the body through a variety of routes.

becoming established. It is difficult to know how many infections are repelled in this way, because they cause no symptoms and pass undetected. It is clear, however, that the microorganisms that a normal human being inhales or ingests, or that enter through minor wounds, are mostly held at bay or eliminated, because they seldom cause clinical disease.

Disease occurs when a microorganism succeeds in evading or overwhelming innate host defenses to establish a local site of infection, and then replicates there to allow its further transmission within our bodies. In some cases, such as the fungal disease athlete's foot, the initial infection remains local and does not cause significant pathology. In other cases, the infectious agent causes significant damage and serious illness as it spreads through the lymphatics or the bloodstream, invades and destroys tissues, or disrupts the body's workings with its toxins, as in the case of the agent of tetanus (*Clostridium tetani*), which secretes a powerful neurotoxin.

The spread of a pathogen is often initially countered by an inflammatory response that recruits more effector cells and molecules of the innate immune system from local blood vessels (Fig. 2.6.), while inducing clotting further downstream so that the microbe cannot spread through the blood. The induced responses of innate immunity act over several days, during which time an adaptive immune response gets under way in response to pathogen antigens delivered to local lymphoid tissue by dendritic cells (see Section 1-15). An adaptive immune response differs from innate immunity in its ability to target structures that are specific to particular strains and variants

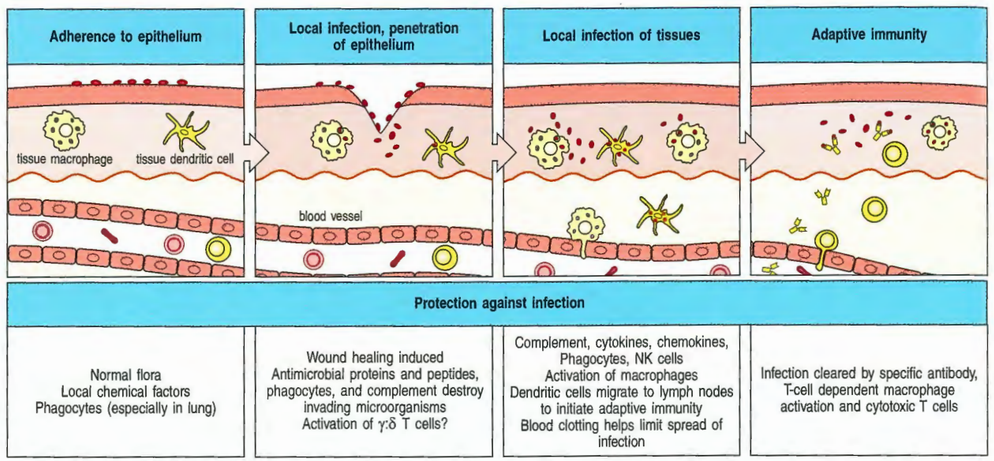


Fig. 2.6 An infection and the response to it can be divided into a series of stages. These are illustrated here for an infectious microorganism entering through a wound in the skin. The infectious agent must first adhere to the epithelial cells and then cross the epithelium. A local immune response may prevent the infection from becoming established. If not, it helps to contain the infection and also delivers the infectious agent, carried in lymph and inside dendritic cells, to local lymph nodes. This initiates the adaptive immune response and eventual clearance of the infection. The role of γ:δ T cells is uncertain, as we shall see in Section 2-34, and this is indicated by the question mark.

of pathogens. This response will usually clear the infection, and protect the host against reinfection with the same pathogen, by producing effector cells and antibodies against the pathogen and by generating immunological memory of that pathogen.

2-3 The epithelial surfaces of the body make up the first lines of defense against infection.

Our body surfaces are defended by epithelia, which provide a physical barrier between the internal milieu and the external world that contains pathogens (Fig. 2.7). Epithelial cells are held together by tight junctions, which effectively form a seal against the external environment. Epithelia comprise the skin and the linings of the body's tubular structures—the gastrointestinal, respiratory, and urogenital tracts. Infections occur only when the pathogen can colonize or cross through these barriers, and because the dry, protective layers of the skin present a more formidable barrier, pathogen entry most often occurs through the internal epithelial surfaces, which make up the vast majority of epithelial surfaces of our bodies. The importance of epithelia in protection against infection is obvious when the barrier is breached, as in wounds, burns, and loss of the integrity of the body's internal epithelia, where

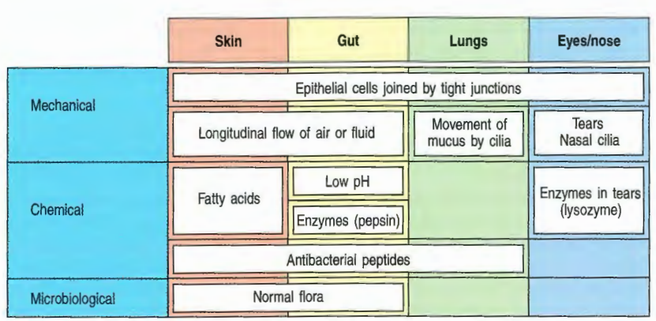


Fig. 2.7 Many barriers prevent pathogens from crossing epithelia and colonizing tissues. Surface epithelia provide mechanical, chemical, and microbiological barriers to infection.

infection is a major cause of mortality and morbidity. In the absence of wounding or disruption, pathogens normally cross epithelial barriers by binding to molecules on the epithelial surfaces of our internal organs, or establish an infection by adhering to and colonizing these surfaces. This specific attachment allows the pathogen to infect the epithelial cell, to damage the epithelium so it can be crossed, or, in the case of colonizing pathogens, to avoid being dislodged by the flow of air or fluid across the epithelial surface.

The internal epithelia are known as **mucosal epithelia** because they secrete a viscous fluid called mucus, which contains many glycoproteins called mucins. Microorganisms coated in mucus may be prevented from adhering to the epithelium, and in mucosal epithelia such as that of the respiratory tract, microorganisms can be expelled in the flow of mucus driven by the beating of epithelial cilia. The efficacy of mucus flow in clearing infection is illustrated by people with defective mucus secretion or inhibition of ciliary movement, as occurs in the inherited disease cystic fibrosis. Such individuals frequently develop lung infections caused by bacteria that colonize the epithelial surface but do not cross it. In the gut, peristalsis is an important mechanism for keeping both food and infectious agents moving through the body. Failure of peristalsis is typically accompanied by the overgrowth of pathogenic bacteria within the lumen of the gut.

Our surface epithelia are more than mere physical barriers to infection; they also produce chemical substances that are microbicidal or that inhibit microbial growth. For example, the antibacterial enzymes lysozyme and phospholipase A are secreted in tears and saliva, and saliva contains several histatins—histidine-rich peptides with antimicrobial properties. The acid pH of the stomach and the digestive enzymes, bile salts, fatty acids, and lysolipids found in the upper gastrointestinal tract create a substantial chemical barrier to infection. Further down the intestinal tract, antibacterial and antifungal peptides called **cryptdins** or **α-defensins** are made by Paneth cells, which are resident in the base of the crypts in the small intestine beneath the epithelial stem cells. Related antimicrobial peptides, the **β-defensins**, are made by other epithelia, primarily in the respiratory and urogenital tracts, skin, and tongue. Antimicrobial peptides have a role in the immune defenses of many organisms, even in humans and other vertebrates that can make an adaptive immune response. Even more striking is resistance to infection in insects and other invertebrates, and even plants, in which innate immunity comprises the only system of host defense. In all these organisms, antimicrobial peptides are an important part of the defenses. Antimicrobial peptides such as the defensins are cationic peptides that are thought to kill bacteria by damaging the bacterial cell membrane.

Antimicrobial proteins that work by a different mechanism are secreted into the fluids that bathe the epithelial surfaces of the lung and gut. These proteins coat the surface of pathogens so that they are more easily phagocytosed by macrophages. They are members of a family of receptors able to recognize common features of microbial surfaces and are considered in detail later in this chapter.

In addition to these defenses, most epithelial surfaces are associated with a normal flora of nonpathogenic bacteria, known as **commensal** bacteria, that compete with pathogenic microorganisms for nutrients and for attachment sites on epithelial cells. This flora can also produce antimicrobial substances, such as the lactic acid produced by vaginal lactobacilli, some strains of which also produce antimicrobial peptides (bacteriocins). When the nonpathogenic bacteria are killed by antibiotic treatment, pathogenic microorganisms frequently replace them and cause disease. Under some circumstances commensal bacteria can cause disease. Their survival on our body surfaces is regulated by a balance between bacterial growth and elimination by the

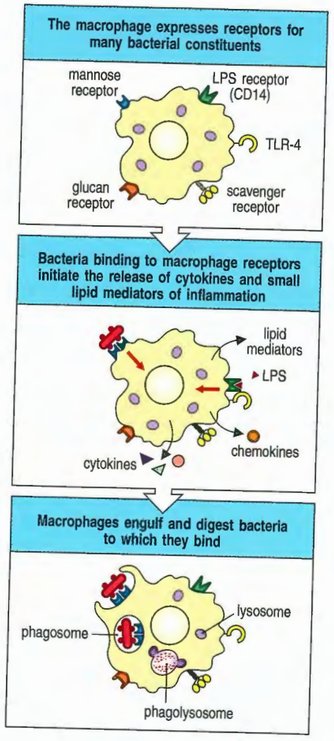


Fig. 2.8 Macrophages are activated by pathogens and both engulf them and initiate inflammatory responses. Macrophages derive from circulating monocytes. They have many of the same characteristics but acquire new functions and new receptors when they become resting cells in connective tissues throughout the body. Macrophages express receptors for many bacterial components, including bacterial carbohydrates (mannose and glucan receptors), lipids (LPS receptor) and other pathogen-derived components (Toll-like receptors (TLRs) and scavenger receptor). Binding of bacteria to macrophage receptors stimulates the phagocytosis and uptake of pathogens into intracellular vesicles, where they are destroyed. Signaling through some receptors, such as the Toll receptors, in response to bacterial components causes the secretion of 'pro-inflammatory cytokines' such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α).

mechanisms of innate immunity; failures in this regulation, such as those caused by the inherited deficiencies of proteins of innate immunity that we discuss in Chapter 12, can allow normally nonpathogenic bacteria to grow excessively and cause disease.

2-4 After entering tissues, many pathogens are recognized, ingested, and killed by phagocytes.

If a microorganism crosses an epithelial barrier and begins to replicate in the tissues of the host, in most cases it is immediately recognized by the mononuclear phagocytes, or **macrophages**, that reside in these tissues. Macrophages mature continuously from monocytes that leave the circulation to migrate into tissues throughout the body. Macrophages in different tissues were historically given different names, for example, microglial cells in neural tissue and **Kupffer cells** in the liver; generically these cells are referred to as mononuclear phagocytes. They are found in especially large numbers in connective tissue, in the submucosal layer of the gastrointestinal tract, in the lung (where they are also found in both the interstitium and the alveoli), along certain blood vessels in the liver, and throughout the spleen, where they remove senescent blood cells. The second major family of phagocytes—the **neutrophils**, or **polymorphonuclear neutrophilic leukocytes** (**PMNs** or polys)—are short-lived cells that are abundant in the blood but are not present in normal, healthy tissues. Both of these phagocytic cells have a key role in innate immunity because they can recognize, ingest, and destroy many pathogens without the aid of an adaptive immune response.

As most microorganisms enter the body through the mucosa of the gut and respiratory system, the macrophages located in the submucosal tissues are the first cells to encounter most pathogens, but they are soon reinforced by the recruitment of large numbers of neutrophils to sites of infection. Macrophages and neutrophils recognize pathogens by means of cell-surface receptors that can discriminate between the surface molecules displayed by pathogens and those of the host. These receptors, which we will examine in more detail later in this chapter, include the macrophage mannose receptor, which is found on macrophages but not on monocytes or neutrophils; scavenger receptors, which bind many negatively charged ligands such as lipoteichoic acids, which are cell-wall components of Gram-positive bacteria; and CD14, found predominantly on monocytes and macrophages (Fig. 2.8). This binds the lipopolysaccharide present on the surface of Gram-negative bacteria and allows it to be recognized by other receptors called Toll-like receptors. In many cases, binding of a pathogen to these cell-surface receptors leads to **phagocytosis**, followed by the death of the pathogen inside the phagocyte. Phagocytosis is an active process, in which the bound pathogen is first surrounded by the phagocyte membrane and then internalized in a membrane-enclosed vesicle known as a **phagosome** or endocytic vacuole. The phagosome then becomes acidified, which kills most pathogens. In addition to being phagocytic, macrophages and neutrophils have membrane-enclosed granules, called **lysosomes**, that contain enzymes, proteins, and peptides that can attack the microbe. The phagosome fuses with one or more lysosomes to generate a **phagolysosome** in which the lysosomal contents are released to destroy the pathogen (see Fig. 2.8).

Upon phagocytosis, macrophages and neutrophils produce a variety of other toxic products that help kill the engulfed microorganism (Fig. 2.9). The most important of these are the antimicrobial peptides and nitric oxide (NO), the superoxide anion (O_2^-), and hydrogen peroxide (H_2O_2), which are directly toxic to bacteria. Nitric oxide is produced by a high-output form of nitric oxide synthase, iNOS2. Superoxide is generated by a multicomponent,

Class of mechanism	Specific products
Acidification	pH=~3.5–4.0, bacteriostatic or bactericidal
Toxic oxygen-derived products	Superoxide O_2^-, hydrogen peroxide H_2O_2, singlet oxygen $^1O_2$, hydroxyl radical $^{\bullet}OH$, hypohalite OCl^-
Toxic nitrogen oxides	Nitric oxide NO
Antimicrobial peptides	Defensins and cationic proteins
Enzymes	Lysozyme—dissolves cell walls of some Gram-positive bacteria. Acid hydrolases—further digest bacteria
Competitors	Lactoferrin (binds Fe) and vitamin B_{12}-binding protein

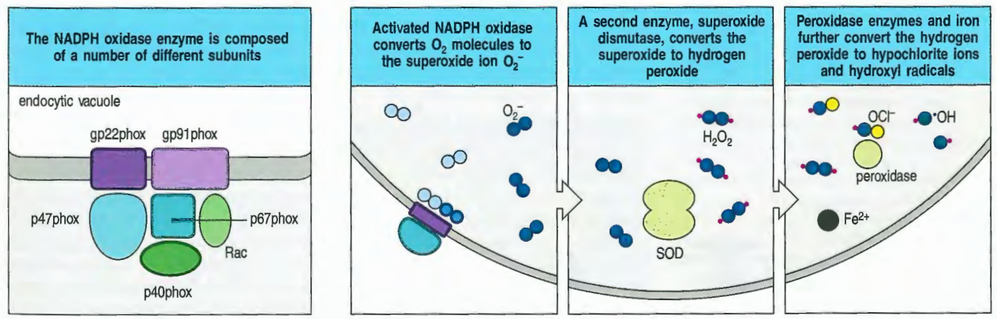
Fig. 2.9 Bactericidal agents produced or released by phagocytes on the ingestion of microorganisms. Most of these agents are made by both macrophages and neutrophils. Some of them are toxic; others, such as lactoferrin, work by binding essential nutrients and preventing their uptake by the bacteria. The same substances can be released by phagocytes interacting with large antibody-coated surfaces such as parasitic worms or host tissues. As these agents are also toxic to host cells, phagocyte activation can cause extensive tissue damage during an infection.

membrane-associated NADPH oxidase in a process known as the **respiratory burst** because it is accompanied by a transient increase in oxygen consumption; the superoxide is converted by the enzyme superoxide dismutase into H_2O_2 (Fig. 2.10). Further chemical and enzymatic reactions produce a range of toxic chemicals from H_2O_2, including the hydroxyl radical ($^{\bullet}OH$) and hypochlorite (OCl^-) and hypobromite (OBr^-). Neutrophils are short-lived cells, dying soon after they have accomplished a round of phagocytosis. Dead and dying neutrophils are a major component of the **pus** that forms in some infections by extracellular bacteria, which are thus known as **pus-forming** or **pyogenic bacteria**. Macrophages, in contrast, are long-lived and continue to generate new lysosomes. Patients with a disease called chronic granulomatous disease have a genetic deficiency of NADPH oxidase, which means that their phagocytes do not produce the toxic oxygen derivatives characteristic of the respiratory burst and so are less able to kill ingested microorganisms and clear an infection. People with this defect are unusually susceptible to bacterial and fungal infections, especially in infancy.

Macrophages can phagocytose pathogens and produce the respiratory burst immediately on encountering an infecting microorganism, and this can be sufficient to prevent an infection from becoming established. In the 19th century the cellular immunologist **Elie Metchnikoff** believed that the innate response of macrophages encompassed all host defense and, indeed, it is now clear that invertebrates, such as the sea star that he was studying, rely entirely

Chronic Granulomatous Disease

Fig. 2.10 The respiratory burst in macrophages and neutrophils is caused by a transient increase in oxygen consumption during the production of microbicidal oxygen metabolites. Ingestion of microorganisms activates the phagocyte to assemble the multisubunit enzyme NADPH oxidase from its components. The active enzyme converts molecular oxygen to the superoxide ion O_2^-, and other oxygen free radicals. Superoxide ion is then converted by the enzyme superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2), which can kill microorganisms and is also converted by other enzymes and by chemical reactions with ferrous (Fe^{2+}) ions to microbicidal hypochlorite (OCl^-) and hydroxyl ($^{\bullet}OH$) radical.



on innate immunity to overcome infection. Although this is not the case in humans and other vertebrates, the innate response of macrophages still provides an important front line of defense that must be overcome if a microorganism is to establish an infection that can be passed on to a new host.

A key feature that distinguishes pathogenic from nonpathogenic microorganisms is their ability to overcome innate immune defenses. Pathogens have developed a variety of strategies to avoid immediate destruction by macrophages. As noted earlier, many extracellular pathogenic bacteria coat themselves with a thick polysaccharide capsule that is not recognized by any phagocyte receptor. Other pathogens, for example mycobacteria, have evolved ways to grow inside macrophage phagosomes by inhibiting their acidification and fusion with lysosomes. Without such devices, a microorganism must enter the body in sufficient numbers to simply overwhelm the immediate innate host defenses and to establish a focus of infection.

A second important effect of the interaction between pathogens and tissue macrophages is the activation of macrophages to release small proteins called cytokines and chemokines (chemoattractant cytokines), and other chemical mediators that set up a state of inflammation in the tissue and attract neutrophils and plasma proteins to the infection site. It is thought that the pathogen induces cytokine and chemokine secretion by signals delivered through some of the receptors to which it binds, and we will see later how this occurs in response to bacterial lipopolysaccharide. Receptors that signal the presence of pathogens and induce cytokines also have another important role. This is to induce the expression of so-called co-stimulatory molecules on macrophages and on **dendritic cells**, another type of phagocytic cell present in tissues, thus enabling these antigen-presenting cells to initiate an adaptive immune response (see Section 1-7).

The cytokines released by macrophages make an important contribution both to local inflammation and to other induced innate responses that occur in the first few days of a new infection. We will describe these induced innate responses and the role of individual cytokines in the last part of this chapter. However, because an inflammatory response is usually initiated within hours of infection or wounding, we will outline here how it occurs and how it contributes to host defense.

2-5 Pathogen recognition and tissue damage initiate an inflammatory response.

Inflammation has three essential roles in combating infection. The first is to deliver additional effector molecules and cells to sites of infection, to augment the killing of invading microorganisms by the front-line macrophages. The second is to induce local blood clotting, which provides a physical barrier to the spread of the infection in the bloodstream. The third is to promote the repair of injured tissue, a nonimmunological role that we will not discuss further. Inflammation at the site of infection is initiated by the response of macrophages to pathogens.

Hereditary Periodic Fever Syndromes

Inflammatory responses are operationally characterized by pain, redness, heat, and swelling at the site of an infection, reflecting four types of change in the local blood vessels, as shown in Fig. 2.11. The first is an increase in vascular diameter, leading to increased local blood flow—hence the heat and redness—and a reduction in the velocity of blood flow, especially along the inner walls of small blood vessels. The second change is that the endothelial cells lining the blood vessel are activated to express **cell-adhesion molecules** that promote the binding of circulating leukocytes. The combination of slowed blood flow and adhesion molecules allows leukocytes to attach to the

Leukocyte Adhesion Deficiency

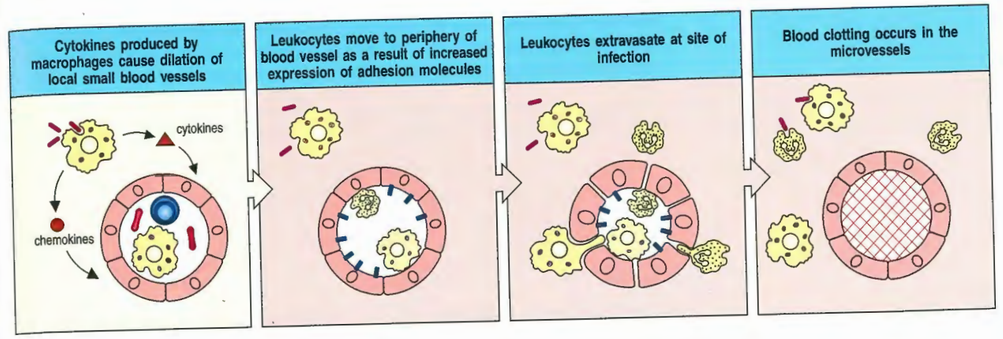


Fig. 2.11 Infection stimulates macrophages to release cytokines and chemokines that initiate an inflammatory response. Cytokines produced by tissue macrophages at the site of infection cause the dilation of local small blood vessels and changes in the endothelial cells of their walls. These changes lead to the movement of leukocytes, such as neutrophils and monocytes, out of the blood vessel (extravasation) and into the infected tissue, guided by chemokines produced by the activated macrophages. The blood vessels also become more permeable, allowing plasma proteins and fluid to leak into the tissues. Together, these changes cause the characteristic inflammatory signs of heat, pain, redness, and swelling at the site of infection.

endothelium and migrate into the tissues, a process known as **extravasation**. All these changes are initiated by the cytokines and chemokines produced by activated macrophages.

Once inflammation has begun, the first white blood cells attracted to the site are neutrophils. These are followed by monocytes, which differentiate into tissue macrophages (Fig. 2.12). Monocytes are also able to give rise to dendritic cells in the tissues, depending on the precise signals that they receive from their environment; for example, the cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF), together with interleukin 4 (IL-4), will induce the monocyte to differentiate into a dendritic cell, whereas the cytokine macrophage colony-stimulating factor (M-CSF) induces differentiation into macrophages.

In the later stages of inflammation, other leukocytes such as eosinophils (see Section 1-3) and lymphocytes also enter the infected site. The third major change in local blood vessels is an increase in vascular permeability. Thus, instead of being tightly joined together, the endothelial cells lining the blood vessel walls become separated, leading to an exit of fluid and proteins from the blood and their local accumulation in the tissue. This accounts for the swelling, or **edema**, and pain—as well as the accumulation of plasma proteins that aid in host defense. The changes that occur in endothelium as a

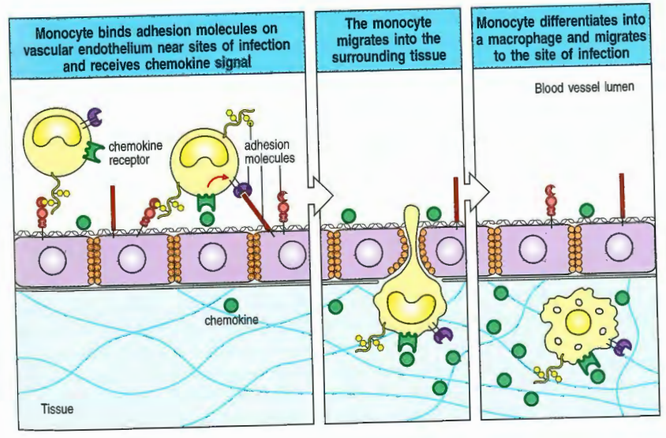


Fig. 2.12 Monocytes circulating in the blood leave the bloodstream to migrate toward sites of infection and inflammation. Adhesion molecules on the endothelial cells of the blood vessel wall first capture the monocyte and cause it to adhere to the vascular endothelium. Chemokines bound to the vascular endothelium then signal the monocyte to migrate across the endothelium into the underlying tissue. The monocyte, now differentiating into a macrophage, continues to migrate, under the influence of chemokines released during inflammatory responses, toward the site of infection. Monocytes leaving the blood in this way are also able to differentiate into dendritic cells (not shown), depending on the signals that they receive from their environment.

result of inflammation are known generally as **endothelial activation**. The fourth change, clotting in microvessels in the site of infection, prevents the spread of the pathogen via the blood.

These changes are induced by a variety of inflammatory mediators released as a consequence of the recognition of pathogens by macrophages. They include the lipid mediators of inflammation—**prostaglandins**, **leukotrienes**, and **platelet-activating factor** (**PAF**)—which are rapidly produced by macrophages through enzymatic pathways that degrade membrane phospholipids. Their actions are followed by those of the chemokines and cytokines that are synthesized and secreted by macrophages in response to pathogens. The cytokine **tumor necrosis factor-α** (**TNF-α**), for example, is a potent activator of endothelial cells.

As we will see in the third part of this chapter, another way in which pathogen recognition rapidly triggers an inflammatory response is through activation of complement. One of the cleavage products of the complement pathway is a peptide called C5a. C5a is a potent mediator of inflammation, with several different activities. In addition to increasing vascular permeability and inducing the expression of some adhesion molecules, it acts as a powerful chemoattractant for neutrophils and monocytes. C5a also activates phagocytes and local **mast cells** (see Section 1-3), which are in turn stimulated to release their granules containing the small inflammatory molecule histamine and the cytokine TNF-α.

If wounding has occurred, the injury to blood vessels immediately triggers two protective enzyme cascades. One is the **kinin system** of plasma proteases that is triggered by tissue damage to produce several inflammatory mediators, including the vasoactive peptide **bradykinin**. The kinin system is an example of a protease cascade, also known as a triggered-enzyme cascade, in which the enzymes are initially in an inactive, or **pro-enzyme** form. After the system is activated, one active protease cleaves and activates the next protease in the series, and so on. Bradykinin causes an increase in vascular permeability that promotes the influx of plasma proteins to the site of tissue injury. It also causes pain, which, although unpleasant to the victim, draws attention to the problem and leads to immobilization of the affected part of the body, which helps to limit the spread of the infection.

The **coagulation system** is another protease cascade that is triggered in the blood after damage to blood vessels. Its activation leads to the formation of a fibrin clot, whose normal role is to prevent blood loss. In regard to innate immunity, however, the clot physically bars the entry of infectious microorganisms to the bloodstream. The kinin cascade and the blood coagulation cascade are also triggered by activated endothelial cells, and so can have important roles in the inflammatory response to pathogens even if wounding or gross tissue injury has not occurred.Thus, within minutes of the penetration of tissues by a pathogen, the inflammatory response causes an influx of proteins and cells that may control the infection. It also forms a physical barrier in the form of blood clots to limit the spread of infection and makes the host fully aware of the local infection.

Summary.

The mammalian body is susceptible to infection by many pathogens, which must first make contact with the host and then establish a focus of infection in order to cause disease. These pathogens differ greatly in their lifestyles, the structures of their surfaces, and their mechanisms of pathogenesis, so an equally diverse set of defensive responses from the host immune system is required. The first phase of host defense consists of those mechanisms that

are present and ready to resist an invader at any time. The epithelial surfaces of the body keep pathogens out and protect against colonization and against viruses and bacteria that enter the body through specialized cell-surface interactions. Their defense mechanisms include the prevention of pathogen adherence and the secretion of antimicrobial enzymes and peptides. Bacteria, viruses, and parasites that overcome these barriers are faced immediately by tissue macrophages equipped with surface receptors that can bind and phagocytose many different types of pathogen. This, in turn, leads to an inflammatory response, which causes the accumulation of phagocytic neutrophils and macrophages at the site of infection, which ingest and destroy the invading microorganisms.

Pattern recognition in the innate immune system.

Although the innate immune system lacks the fine specificity of adaptive immunity that is necessary to produce immunological memory, it can distinguish self from nonself. We have already seen how this occurs in the response of macrophages to pathogenic microbes. In this part of the chapter we will look more closely at the receptors that activate the innate immune response, including those that recognize pathogens directly and signal for a cellular innate immune response. Regular patterns of molecular structure are present on many microorganisms but not on the body's own cells. Proteins that recognize these features occur as receptors on macrophages, neutrophils, and dendritic cells, and as secreted molecules. Their general characteristics are contrasted with the antigen-specific receptors of adaptive immunity in Fig. 2.13. Unlike the antigen receptors described in Chapter 1, the receptors of the innate immune system are not clonally distributed; instead, a given set of receptors is present on all the cells of the same cell type. The binding of pathogen components by these receptors gives rise to very rapid responses, which are put into effect without the delay imposed by the need for activated

Receptor characteristic	Innate immunity	Adaptive immunity
Specificity inherited in the genome	Yes	No
Expressed by all cells of a particular type (e.g. macrophages)	Yes	No
Triggers immediate response	Yes	No
Recognizes broad classes of pathogens	Yes	No
Interacts with a range of molecular structures of a given type	Yes	No
Encoded in multiple gene segments	No	Yes
Requires gene rearrangement	No	Yes
Clonal distribution	No	Yes
Able to discriminate between even closely related molecular structures	No	Yes

Fig. 2.13 Comparison of the characteristics of recognition molecules of the innate and adaptive immune systems. The innate immune system uses receptors that are encoded by complete genes inherited through the germline. In contrast, the adaptive immune system uses antigen receptors encoded in gene segments that are assembled into complete T-cell and B-cell receptor genes during lymphocyte development, a process that leads to each individual cell expressing a receptor of unique specificity. Receptors of the innate immune system are deployed nonclonally (that is, by all the cells of a given cell type), whereas the antigen receptors of the adaptive immune system are clonally distributed on individual lymphocytes and their progeny.

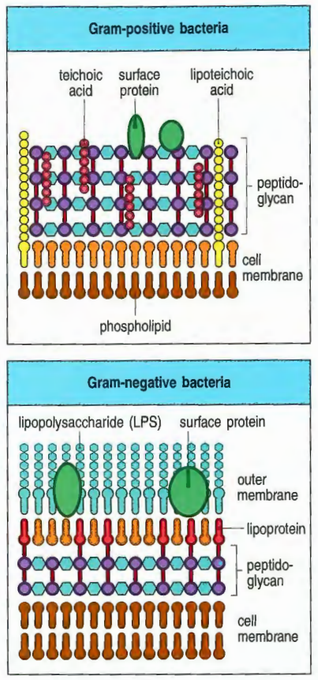


Fig. 2.14 The organization of the cell walls of Gram-positive and Gram-negative bacteria. Gram-positive bacteria (upper panel) have a cell wall composed of an outer layer of a repeating matrix of peptidoglycan molecules in which repeating *N*-acetylglucosamine (light blue hexagons) and *N*-acetylmuramic acid (purple circles) are cross-linked by peptide bridges into a dense three-dimensional network. Bacterial surface proteins and other molecules, such as teichoic acid, are embedded within this peptidoglycan layer, and lipoteichoic acids link the peptidoglycan layer to the bacterial cell membrane itself. The cell wall of Gram-negative bacteria (lower panel) is composed of an inner thin matrix of peptidoglycan and an outer lipid membrane, in which are embedded proteins and the lipopolysaccharide (LPS) characteristic of Gram-negative bacteria.

lymphocytes to divide and differentiate during the development of an adaptive immune response.

The pattern recognition receptors of the innate immune system have several different functions. Many are phagocytic receptors that stimulate ingestion of the pathogens they recognize. Some are chemotactic receptors, which guide cells to sites of infection. A third function is to induce the production of effector molecules that contribute to the later, induced responses of innate immunity, and also to induce proteins that influence the initiation and nature of any subsequent adaptive immune response. In this part of the chapter we will first look at the recognition properties of some receptors that bind pathogens directly. We will then focus on an evolutionarily ancient pathogen recognition and signaling system, mediated by receptors called Toll-like receptors, that has a key role in defense against infection in plants, adult insects, and vertebrates, including mammals.

2-6 Receptors with specificity for pathogen molecules recognize patterns of repeating structural motifs.

Microorganisms typically bear repeating patterns of molecular structure on their surface. The cell walls of Gram-positive and Gram-negative bacteria, for example, are composed of a matrix of proteins, carbohydrates and lipids in a repetitive array, as shown in Figure 2.14. The lipoteichoic acids of Gram-positive bacterial cell walls and the lipopolysaccharide of the outer membrane of Gram-negative bacteria are, as we will see, important in the recognition of bacteria by the innate immune system. Other microbial components also have a repetitive structure. Bacterial flagella are made of repeated protein subunits, and bacterial DNA contains unmethylated repeats of the dinucleotide CpG. Viruses almost invariably express double-stranded RNA as part of their life cycles. These repetitive structures are known generally as **pathogen-associated molecular patterns** (**PAMPs**) and the receptors that recognize them as **pattern recognition receptors** (**PRRs**).

One such receptor is the **mannose-binding lectin** (**MBL**), which is present as a free protein in blood plasma. As we shall see in the next part of this chapter, it can initiate the lectin pathway of complement activation, but we shall discuss it briefly here as a good example of the recognition of molecular patterns. As illustrated in Fig. 2.15, pathogen recognition and discrimination from self by MBL is due to the recognition of a particular orientation of certain sugar residues, as well as their spacing, which is found only on microbes and not on host cells. Once formed, the MBL–pathogen complex is bound by phagocytes, either through interactions with MBL or through the phagocyte's receptors for complement, which also becomes bound to the pathogen. The outcome is the phagocytosis and killing of the pathogen (see Section 2-4) and the induction of other cellular responses, such as chemokine production. Coating of a particle with proteins that facilitate its phagocytosis is known as **opsonization**, and we will meet other examples of this defense strategy in this and later chapters.

MBL is a member of the collectin family of proteins, so called because they contain both collagen-like and lectin (sugar-binding) domains. Other members of this family are the **surfactant proteins A** and **D** (**SP-A** and **SP-D**), which are present in the fluid that bathes the epithelial surfaces of the lung. There they bind to and coat the surfaces of pathogens, making them more susceptible to phagocytosis by macrophages that have left the subepithelial tissues to enter the alveoli of the lung.

Phagocytes are also equipped with several cell-surface receptors that recognize pathogen surfaces directly. Among these is the **macrophage mannose**

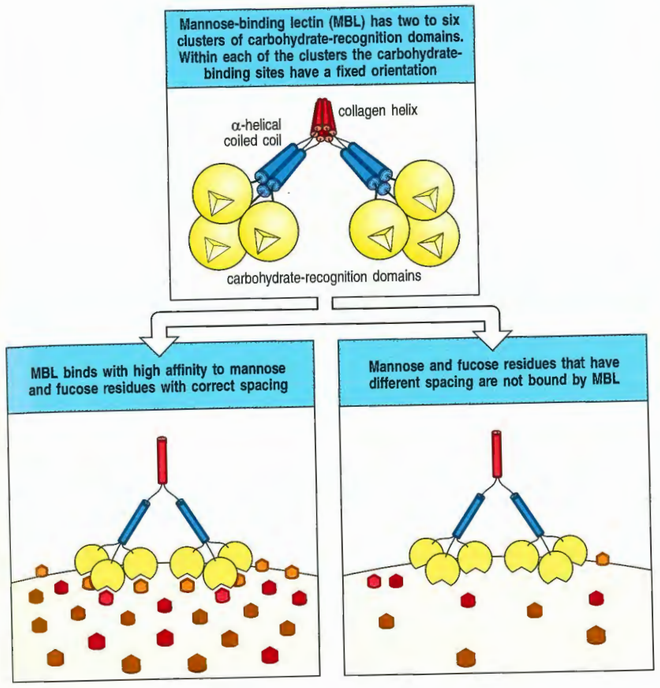


Fig. 2.15 Mannose-binding lectin recognizes bacterial surfaces by their particular spacing of carbohydrate residues. The plasma protein mannose-binding lectin (MBL) is part of the pathogen-recognition system of innate immunity. It binds to certain bacterial surfaces that display a particular spatial arrangement of mannose or fucose residues. The presence of these residues alone is not enough to ensure binding; the orientation of the binding sites in the MBL is fixed, and only if the mannose and fucose residues have the correct spacing will the MBL be able to bind. Once coated with MBL, bacteria are more susceptible to phagocytosis.

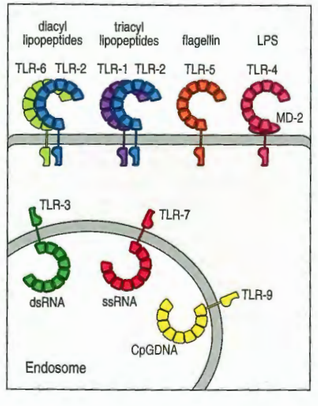
receptor (see Fig. 2.8). This receptor is a cell-bound C-type (calcium-dependent) lectin that binds certain sugars found on the surface of many bacteria and some viruses, including the human immunodeficiency virus (HIV). Its recognition properties are very similar to those of MBL (see Fig. 2.15) and, like that protein, it is a multipronged molecule with several carbohydrate-recognition domains. Because it is a transmembrane cell-surface receptor, however, it can function directly as a phagocytic receptor.

A second set of phagocytic receptors, called **scavenger receptors**, recognize various anionic polymers and acetylated low-density lipoproteins. These receptors are a structurally heterogeneous set of molecules, existing in at least six distinct molecular forms. Some scavenger receptors recognize structures that are shielded by sialic acid on normal host cells. There are other recognition targets, many of which still need to be characterized.

Not all receptors that recognize pathogen-specific molecules are phagocytic receptors. Bacterial polypeptides typically start with a formylated methionine residue, and the fMet-Leu-Phe (fMLP) receptor on macrophages and neutrophils binds these *N*-formylated peptides. This receptor is a chemotactic receptor and its ligation guides neutrophils to a site of infection. Binding of pathogens to some receptors on the macrophage surface not only stimulates phagocytosis but also sends signals to the cell that trigger the induced responses of innate immunity, as we will see later in this chapter. Stimulation of certain receptors by pathogen products also leads macrophages and dendritic cells to produce the cell-surface display of co-stimulatory molecules that enables them to act as antigen-presenting cells to T lymphocytes and initiate an adaptive immune response. The best-defined activation pathway of

Innate immune recognition by Toll-like receptors	
Toll-like receptor	**Ligand**
TLR-1:TLR-2 heterodimer	Peptidoglycan Lipoproteins Lipoarabinomannan (mycobacteria) GPI (*T. cruzi*) Zymosan (yeast)
TLR-2:TLR-6 heterodimer	
TLR-3	dsRNA
TLR-4 dimer (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)
TLR-5	Flagellin
TLR-7	ssRNA
TLR-8	G-rich oligonucleotides
TLR-9	Unmethylated CpG DNA

Fig. 2.16 Innate immune recognition by Toll-like receptors. Each of the TLRs whose specificity is known recognizes one or more microbial molecular patterns, generally by direct interaction with molecules on the pathogen surface. Although some Toll-like receptor proteins form heterodimers (e.g. TLR-1:TLR-2), this is not the rule; TLR-4, for instance, can form only homodimers. GPI, glycosylphosphatidylinositol; *T. cruzi*, the protozoan parasite *Trypanosoma cruzi*; dsRNA, double-stranded RNA; ssRNA, single-stranded RNA.



this type is triggered through a family of evolutionarily conserved transmembrane receptors called the Toll-like receptors that seem to function exclusively as signaling receptors, and which we shall describe next.

2-7 The Toll-like receptors are signaling receptors that distinguish different types of pathogen and help direct an appropriate immune response.

The mammalian **Toll-like receptors** (**TLRs**) belong to an evolutionarily ancient recognition and signaling system, originally discovered as a result of its role in embryonic development in the fruitfly *Drosophila melanogaster*. It was subsequently found to have a role in the defense against bacterial and fungal infections in the adult insect and is now known to have a key role in response to infection in plants, adult insects, and vertebrates, including mammals. The receptor mediating these functions in *Drosophila* is known as Toll, and the homologous proteins in mammals and other animals are therefore known as the Toll-like receptors.

There are 10 expressed *TLR* genes in mice and humans, and each of the 10 proteins they produce is devoted to recognizing a distinct set of molecular patterns that are not found in normal vertebrates. These patterns are characteristic of components of pathogenic microorganisms at one or other stage of infection. Because there are only 10 functional *TLR* genes, the Toll-like receptors have limited specificity compared with the antigen receptors of the adaptive immune system and have evolved to recognize certain microbe-associated molecular patterns. But although the diversity of the Toll-like receptors is limited, they can recognize elements of most pathogenic microbes, as illustrated in Fig. 2.16.

Some mammalian TLRs act as cell-surface receptors, whereas others act intracellularly and are located in the membranes of endosomes, where they sense the presence of pathogens and pathogen components taken into the cell by endocytosis or macropinocytosis (Fig. 2.17). An important Toll-like receptor in the response to common bacterial infections is **TLR-4** on macrophages, which signals the presence of bacterial lipopolysaccharide by associating with CD14, the macrophage receptor for lipopolysaccharide, and an additional cellular protein, MD-2. TLR-4 is also involved in the immune response to at least one virus, the respiratory syncytial virus, although in this case the exact nature of the stimulating ligand is not yet known. Another mammalian Toll-like receptor, **TLR-2**, signals the presence of a different set of microbial constituents, which include the lipoteichoic acid (LTA) of Gram-positive bacteria and lipoproteins of Gram-negative bacteria, although how it recognizes these is not known. The responses made by cells to stimulation of the various TLRs are directed to dealing with the particular type of pathogen present. For example, stimulation of **TLR-3** by virus-derived double-stranded RNA leads to the production of an antiviral cytokine, interferon, as discussed in more detail later in the chapter. TLR-4 and TLR-2 induce similar but distinct signals, as shown by the

Fig. 2.17 The cellular locations of the mammalian Toll-like receptors. Some TLRs are located on the cell surface of dendritic cells and macrophages, where they are able to detect extracellular pathogen molecules. TLRs are thought to act as dimers; only those that form heterodimers are shown in dimeric form here. The rest act as homodimers. TLRs located intracellularly, in the wall of the endosome, can recognize microbial components, such as DNA, that are accessible only after the microbe has been broken down. The diacyl and triacyl peptides recognized by the heterodimeric receptors TLR-6:TLR-2 and TLR-1:TLR-2, respectively, are derived from the lipoteichoic acid of Gram-positive bacterial cell walls and the lipoproteins of the Gram-negative bacterial surface.

distinct responses resulting from lipopolysaccharide signaling through TLR-4 and LTA signaling through TLR-2; for example, while both lipopolysaccharide and LTA induce the production of TNF-α, lipopolysaccharide is also able to induce the production of interferon (IFN)-β.

2-8 The effects of bacterial lipopolysaccharide on macrophages are mediated by CD14 binding to TLR-4.

Bacterial lipopolysaccharide (**LPS**) is a cell-wall component of Gram-negative bacteria such as *Salmonella* that has long been known to induce a reaction in the infected host. The systemic injection of LPS causes a collapse of the circulatory and respiratory systems, a condition known as **shock**. These dramatic effects of LPS are seen in humans as **septic shock**, which is caused by the overwhelming secretion of cytokines, particularly of TNF-α, as a result of an uncontrolled systemic bacterial infection, or **sepsis**. We will discuss the pathogenesis of septic shock later in this chapter and shall see that it is an undesirable consequence of the same effector actions of TNF-α that are important in containing local infections. LPS acts through TLR-4, and the benefits of TLR-4 signaling are clearly illustrated by mutant mice that lack TLR-4 function: although resistant to septic shock, they are highly sensitive to LPS-bearing pathogens such as *Salmonella typhimurium*, a natural pathogen of mice.

The same principles are seen in humans infected with *Salmonella typhi*, the cause of typhoid fever. This bacterium invades across the mucosal membranes (Fig. 2.18) but can be recognized by macrophages and other innate phagocytic cells because it expresses both LPS and flagellin, which allows it to activate two cell-surface TLRs—TLR-4 and **TLR-5** (see Fig. 2.16)—which leads to the production of TNF-α. Thus, systemic infection with *S. typhi* is capable of causing systemic shock in humans by the same mechanism provoked by *S. typhimurium* in mice, by inducing a systemic production of TNF-α.

However, *S. typhi*, in common with many pathogenic bacteria, has what is called a type III secretion system, a microhomolog of a syringe, which enables the bacterium to secrete molecules directly across the cell membrane of mammalian cells and into the cytosol. Using its type III secretion system, *S. typhi* is able to transfer into the macrophage cytosol protease molecules that inhibit the signaling pathway that leads to production of TNF-α; this may be a mechanism evolved by the bacterium to render the innate immune response less effective.

TLR-4 alone cannot recognize LPS; two other cell-surface proteins, CD14 and MD-2, are required. CD14 binds LPS and it is thought that the CD14:LPS complex is the actual ligand of TLR-4, although its direct binding to TLR-4 has yet to be demonstrated. MD-2 initially binds to TLR-4 within the cell and is necessary for the correct targeting of TLR-4 to the surface and its recognition of LPS. When the TLR-4:MD-2 complex interacts with LPS bound to CD14, this sends a signal to the cell nucleus that activates the transcription factor **NFκB** (Fig. 2.19). We will describe the signaling pathways used by TLRs in more detail in Chapter 6.

This NFκB signaling pathway was first discovered as the pathway used by the Toll receptor to determine dorso-ventral body pattern during embryogenesis in the fruitfly, and is often referred to as the Toll pathway. In the adult fly the same pathway leads to the formation of antimicrobial peptides in response to infection. Essentially the same signaling pathway is used by all the TLRs in their induction of innate immune responses in vertebrates, and a similar pathway is used by plants in their defense against viruses and other plant pathogens. Thus, the Toll pathway is an ancient signaling pathway that seems to be used in innate immunity in most, if not all, multicellular organisms.

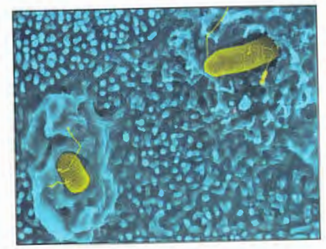


Fig. 2.18 Certain pathogens can invade directly through the intestinal or other internal epithelia. In the case of *Salmonella typhi*, which causes typhoid fever, shown here in the process of migrating through the intestinal epithelium, the flagellin proteins of the bacterial flagella are recognized by TLRs on macrophages and dendritic cells in the underlying tissues. This provokes an innate response that helps to control the infection. Photograph courtesy of J. Galan.



Interleukin Receptor-associated Kinase Deficiency

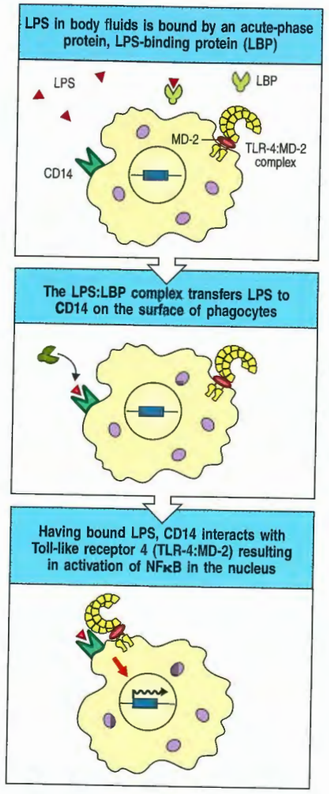


Fig. 2.19 Bacterial lipopolysaccharide signals through the Toll-like receptor TLR-4 to activate the transcription factor NFκB. In the plasma, LPS is bound by the soluble LPS-binding protein (LBP), which then loads its bound LPS onto the glycosylphosphatidylinositol (GPI)-tethered peripheral membrane protein CD14. This LPS:CD14 complex then triggers TLR-4, which is complexed with the protein MD-2, to signal to the nucleus, activating the transcription factor NFκB, which in turn activates genes encoding proteins involved in defense against infection.

2-9 The NOD proteins act as intracellular sensors of bacterial infection.

The TLRs are located in cellular membranes, either on the cell surface or in intracellular vesicles. Other proteins, sharing features of their ligand-binding domains with the TLRs, are present in the cytosol of the cell and are able to bind microbial products there and activate NFκB, thus initiating the same inflammatory processes as the TLRs (Fig. 2.20). These proteins are called **NOD1** and **NOD2**, because as well as a ligand-binding domain they contain a nucleotide-binding oligomerization (NOD) domain. They also contain protein domains that recruit caspases, a family of intracellular proteases, so the genes that encode the NOD proteins are referred to as members of the CARD family of genes—NOD1 is encoded by *CARD4* and NOD2 by *CARD15*.

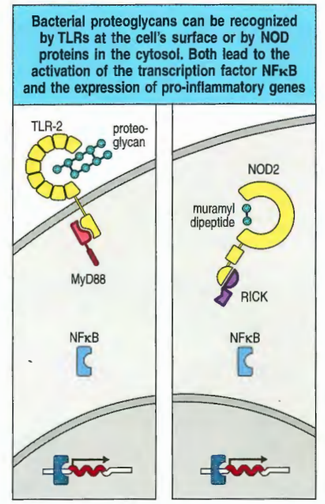
The NOD proteins recognize fragments of bacterial cell-wall proteoglycans—NOD1 binds γ-glutamyl diaminopimelic acid (iE-DAP), a breakdown product of proteoglycans from Gram-negative bacteria, whereas NOD2 binds muramyl dipeptide, which is present in the proteoglycans of both Gram-positive and Gram-negative bacteria. Thus, NOD2 is able to act as a general sensor of bacterial infection, whereas NOD1 is more restricted to sensing the presence of Gram-negative bacteria. In keeping with this role, the NOD proteins are expressed in cells that are routinely exposed to bacteria—in epithelial cells that form the barrier that bacteria must cross to establish an infection in the body, and in the macrophages and dendritic cells that ingest bacteria that have succeeded in entering the body. As macrophages and dendritic cells also express TLRs that can recognize bacterial proteoglycans, in these cells signals from NOD1 and NOD2 act in addition to the signals from the TLRs. In epithelial cells, however, the expression of TLRs is weak or absent, and in these cells NOD1 is an important activator of the innate immune response. NOD2 seems to have a more specialized role, being strongly expressed in the Paneth cells of the gut, where it induces the expression of potent antimicrobial peptides, the α-defensins.

2-10 Activation of Toll-like receptors and NOD proteins triggers the production of pro-inflammatory cytokines and chemokines, and the expression of co-stimulatory molecules.

In humans and all other vertebrates investigated, activation of NFκB by the Toll and NOD pathways leads to the production of several important mediators of innate immunity, such as cytokines (Fig. 2.21) and chemokines (see Fig. 2.46). (Appendices III and IV provide a detailed listing of these important mediators.) The pathway also leads to the cell-surface expression of **co-stimulatory molecules** that are essential for the induction of adaptive immune responses. These proteins, called **B7.1** (**CD80**) and **B7.2** (**CD86**), are produced by both macrophages and tissue dendritic cells in response to LPS signaling through TLR-4 (Fig. 2.22). It is these proteins, along with the antigenic microbial peptides presented by MHC class II proteins on dendritic cells and macrophages (see Section 1-18), that activate naive CD4 T cells (Fig. 2.23). These cells, in turn, are needed to initiate most adaptive immune responses. To encounter a naive CD4 T cell, the antigen-presenting dendritic cell must migrate to a nearby lymph node through which circulating naive T cells pass, and this migration is stimulated by cytokines such as TNF-α, which are also induced by signaling through TLR-4. Thus, the activation of adaptive immunity depends on molecules induced as a consequence of the innate immune recognition of pathogens.

Substances such as LPS that induce co-stimulatory activity have been used for years in mixtures that are co-injected with protein antigens to enhance their immunogenicity. These substances are known as **adjuvants** (see Appendix I, Section A-4), and it was found empirically that the best adjuvants contained microbial components. A range of microbial components (see Fig. 2.16) can induce macrophages and tissue dendritic cells to express co-stimulatory molecules and cytokines. The exact profile of cytokines produced by the macrophage or dendritic cell varies according to the receptors stimulated and, as we shall see in Chapters 8 and 10, the cytokines secreted will in turn influence the functional character of the adaptive immune response that develops. In this way the ability of the innate immune system to discriminate between different types of pathogen is used to ensure an appropriate type of adaptive immune response.

Fig. 2.20 Both membrane-bound and intracellular proteins act as bacterial sensors, recognizing bacterial proteoglycans and activating NFκB to induce the expression of pro-inflammatory genes. TLRs at the cell surface are able to bind microbial components—in the case of TLR-2, these are proteoglycans of the bacterial cell wall. Binding of bacterial proteoglycans to TLR-2 is signaled to the cell via the adaptor protein MyD88, leading to the activation of the transcription factor NFκB and its translocation into the nucleus to induce the expression of pro-inflammatory genes. Degradation of bacterial proteoglycans produces muramyl dipeptide, the ligand for the intracellular sensor of bacterial components, NOD2. By way of the adaptor protein kinase RICK (receptor-interacting serine–threonine kinase), NOD2 is able to activate NFκB, thus inducing the same pro-inflammatory genes as TLR-2.



Summary.

The innate immune system uses several different receptors to recognize and respond to pathogens. Those that recognize pathogen surfaces directly often bind to repeating patterns, for example of carbohydrate or lipid moieties, that are characteristic of microbial surfaces but are not found on host cells. Some of these receptors, such as the macrophage mannose receptor, directly

Cytokines secreted by macrophages and dendritic cells

Cytokine	Main producer	Acts upon	Effect
IL-1	Macrophages Keratinocytes	Lymphocytes	Enhances responses
		Liver	Induces acute-phase protein secretion
IL-6	Macrophages Dendritic cells	Lymphocytes	Enhances responses
		Liver	Induces acute-phase protein secretion
CXCL8 (IL-8)	Macrophages Dendritic cells	Phagocytes	Chemoattractant for neutrophils
IL-12	Macrophages Dendritic cells	Naive T cells	Diverts immune response to T_H1, pro-inflammatory, cytokine secretion
TNF-α	Macrophages Dendritic cells	Vascular endothelium	Induces changes in vascular endothelium (expression of cell-adhesion molecules (E- and P-selectin), changes in cell–cell junctions with increased fluid loss, local blood clotting)

Fig. 2.21 Important cytokines secreted by macrophages in response to bacterial products include IL-1β, IL-6, CXCL8, IL-12, and TNF-α. TNF-α is an inducer of a local inflammatory response that helps to contain infections; it also has systemic effects, many of which are harmful (see Section 2-27). The chemokine CXCL8 is also involved in the local inflammatory response, helping to attract neutrophils into the site of infection. IL-1β, IL-6, and TNF-α have a crucial role in inducing the acute-phase response in the liver (see Section 2-28) and induce fever, which favors effective host defense in several ways. IL-12 activates the natural killer (NK) cells of the innate immune response, and favors the differentiation of CD4 T cells into the T_H1 subset during an adaptive immune response.

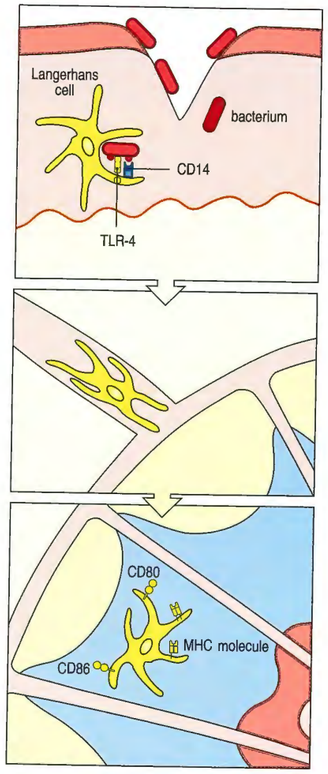


Fig. 2.22 Bacterial LPS induces changes in Langerhans cells, stimulating them to migrate and to initiate adaptive immunity by activating CD4 T cells. Langerhans cells are immature dendritic cells resident in the skin. During a bacterial infection they are activated by LPS through the TLR signaling pathway. This induces two types of changes in the cells. The first is a change in behavior and location. From being resting cells in the skin, the Langerhans cells become activated migrating cells in the afferent lymphatics, and eventually fully mature dendritic cells in the regional lymph nodes. The second is a drastic alteration in their cell-surface molecules. Resting Langerhans cells in the skin are highly phagocytic and macropinocytic but lack the ability to activate T lymphocytes. Mature dendritic cells in the lymph nodes have lost the ability to ingest antigen but have gained the ability to stimulate T cells. This is due to an increase in the number of MHC molecules on their surface and the expression of the co-stimulatory molecules CD80 (B7.1) and CD86 (B7.2).

stimulate phagocytosis, whereas others are produced as secreted molecules that promote the phagocytosis of pathogens by opsonization or by the activation of complement, as we shall see in the next part of this chapter. The innate immune system receptors that recognize pathogens also have an important role in signaling for the induced innate responses that are responsible for local inflammation, the recruitment of new effector cells, the containment of local infection, and the initiation of an adaptive immune response. Such signals can be transmitted through a family of signaling receptors, known as the Toll-like receptors (TLRs), that have been highly conserved across evolutionary time and serve to activate host defense through a signaling pathway that operates in most multicellular organisms. In vertebrates, TLRs also have a key role in enabling the initiation of adaptive immunity. TLR-4 detects the presence of Gram-negative bacteria through its association with the peripheral membrane protein CD14, which is a receptor for bacterial LPS. Other TLRs respond to other molecular patterns found on or in pathogens. TLRs activate the transcription factor NFκB, which then induces the transcription of a variety of genes, including those for the cytokines, chemokines, and co-stimulatory molecules that have essential roles in directing the course of the adaptive immune response later in infection. Whereas TLRs recognize the presence of bacteria and other microbes outside the cell, cytosolic proteins, the NOD proteins, detect similar bacterial products within the cytoplasm of the cell, activating the same NFκB pathway.

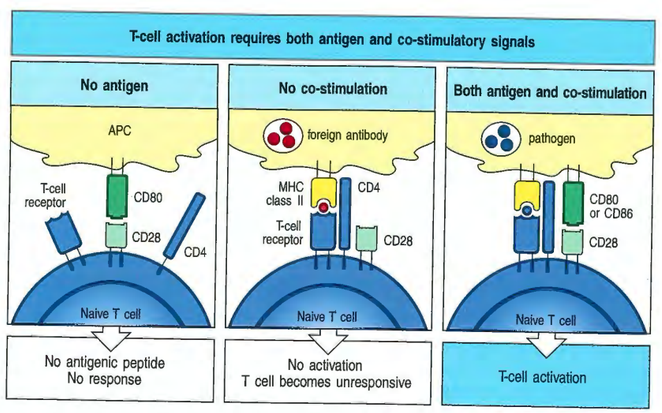


Fig. 2.23 For naive T cells to be activated by antigen, the antigen must be presented to them by an activated antigen-presenting cell that also expresses co-stimulatory molecules. Antigen is recognized by the T-cell receptor in the form of a peptide bound to an MHC molecule on an antigen-presenting cell (APC), such as a macrophage or dendritic cell. However, the T cell will be activated only if the antigen-presenting cell also expresses the co-stimulatory molecules CD80 or CD86.

The complement system and innate immunity.

Complement was discovered by **Jules Bordet** many years ago as a heat-labile component of normal plasma that augments the opsonization and killing of bacteria by antibodies. This activity was said to 'complement' the antibacterial activity of antibody; hence the name. Although first discovered as an effector arm of the antibody response, complement can also be activated early in infection in the absence of antibodies. Indeed, it now seems clear that complement first evolved as part of the innate immune system, where it still has an important role in coating pathogens and facilitating their destruction.

2-11 Complement is a system of plasma proteins that is activated by the presence of pathogens.

The **complement system** is made up of a large number of different plasma proteins that interact with one another both to opsonize pathogens and to induce a series of inflammatory responses that help to fight infection. A feature of the system is that several complement proteins are proteases that become activated only after cleavage, usually by another specific protease. In their inactive form, such enzymes are called pro-enzymes or **zymogens** and were first found in the gut. The digestive enzyme pepsin, for example, is stored inside cells and secreted as an inactive precursor, pepsinogen, which is only cleaved to pepsin in the acid environment of the stomach. The advantage to the host of not being autodigested is obvious.

The precursor zymogens of the complement system are widely distributed throughout body fluids and tissues. At sites of infection they are locally activated by the presence of the pathogen and trigger a series of powerful inflammatory events. The complement system becomes activated through a triggered-enzyme cascade in which an active complement protease generated by cleavage of its zymogen precursor then cleaves its substrate, another complement zymogen, to its active enzymatic form. This in turn cleaves and activates the next zymogen in the complement pathway. In this way, the activation of a small number of complement proteins at the start of the pathway is hugely amplified by each successive enzymatic reaction, resulting in the rapid generation of a disproportionately large complement response. The blood coagulation system mentioned earlier is another example of a triggered-enzyme cascade. In this case, a small injury to a blood vessel wall can lead to the development of a large blood clot.

A key site for the activation of the complement pathway is the surface of pathogens and there are three distinct pathways through which complement can be activated (Fig. 2.24). These pathways depend on different molecules for their initiation, but they converge to generate the same set of effector complement proteins. There are also three ways in which the complement system protects against infection (see Fig. 2.24). First, it generates large numbers of activated complement proteins that bind covalently to pathogens, opsonizing them for engulfment by phagocytes bearing receptors for complement. Second, the small fragments of some complement proteins act as chemoattractants to recruit more phagocytes to the site of complement activation, and also to activate these phagocytes. Third, the final components in the complement pathway damage certain bacteria by creating pores in the bacterial membrane.

As well as the direct effects of complement in eliminating infectious microorganisms, it also has an important role in activating the adaptive immune system. In part, this is a consequence of opsonization, because

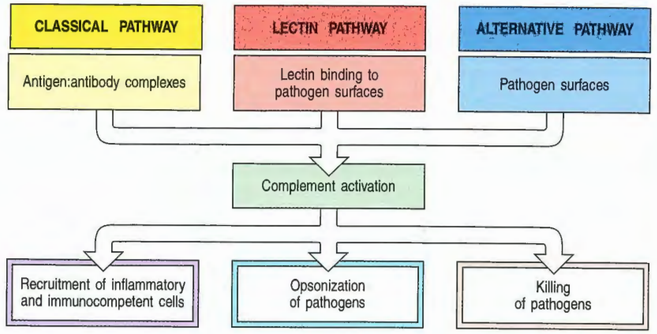


Fig. 2.24 Schematic overview of the complement cascade. There are three pathways of complement activation. One is the classical pathway, which is triggered by the binding of complement component C1q to antibody complexed with antigen by the direct binding of C1q to the pathogen surface, or by the binding of C1q to C-reactive protein bound to the pathogen. The second is the lectin pathway, which is triggered by mannose-binding lectin or the ficolin proteins, normal serum constituents that bind some encapsulated bacteria. The third is the alternative pathway, which is triggered directly on pathogen surfaces. All of these pathways generate a crucial enzymatic activity that, in turn, generates the effector molecules of complement. The three main consequences of complement activation are the opsonization of pathogens, the recruitment of inflammatory and immunocompetent cells, and the direct killing of pathogens.

antigen-presenting cells bear receptors for complement that enhance the uptake of complement-coated antigens and the presentation of these antigens to the adaptive immune system. In addition, as we will see in Chapter 9, B lymphocytes bear receptors for complement proteins that act as co-stimulators, enhancing the response of the B cell to complement-coated antigens.

Complement is not activated only by infectious organisms. Dying cells, such as those at sites of ischemic injury (injury to tissues caused by a lack of oxygen) can trigger complement activation. As complement-coated particles are more efficiently taken up by phagocytes, complement is important in the efficient disposal of dead, damaged, and apoptotic cells and, in doing so, protects against the development of autoimmunity—the attack by the immune system on the body's own antigens.

2-12 Complement interacts with pathogens to mark them for destruction by phagocytes.

In the early phases of an infection, the complement cascade can be activated on the surface of a pathogen through one or more of the three pathways shown in Fig. 2.25. The **classical pathway** is initiated by the binding of C1q, the first protein in the complement cascade, to the pathogen surface. C1q can bind to the surface of pathogens in one of three ways. It can bind directly to surface components of some bacteria, including certain proteins of bacterial cell walls and polyanionic surface structures such as lipoteichoic acid on Gram-positive bacteria. Second, C1q binds to C-reactive protein, an acute-phase protein of human plasma that binds to phosphocholine residues in bacterial polysaccharides, such as pneumococcal C polysaccharide; hence the name C-reactive protein. We shall discuss the acute-phase proteins of the early induced innate response later in the chapter. Third, C1q is a key link between the effector mechanisms of innate and adaptive immunity by binding to antibody:antigen complexes. The **lectin pathway** is initiated by the binding of carbohydrate-binding proteins to arrays of carbohydrates on the surface of pathogens. These carbohydrate-binding proteins include the lectin MBL, which binds to mannose-containing carbohydrates on bacteria or viruses, as described in Section 2-6, and the ficolins, which bind to the *N*-acetylglucosamine present on the surface of some pathogens. Finally, the **alternative pathway** of complement activation can be initiated by the binding of spontaneously activated complement component C3 in plasma to the surface of a pathogen.

In each pathway, a sequence of reactions generates a protease called a **C3 convertase**. These reactions are known as the 'early' events of complement

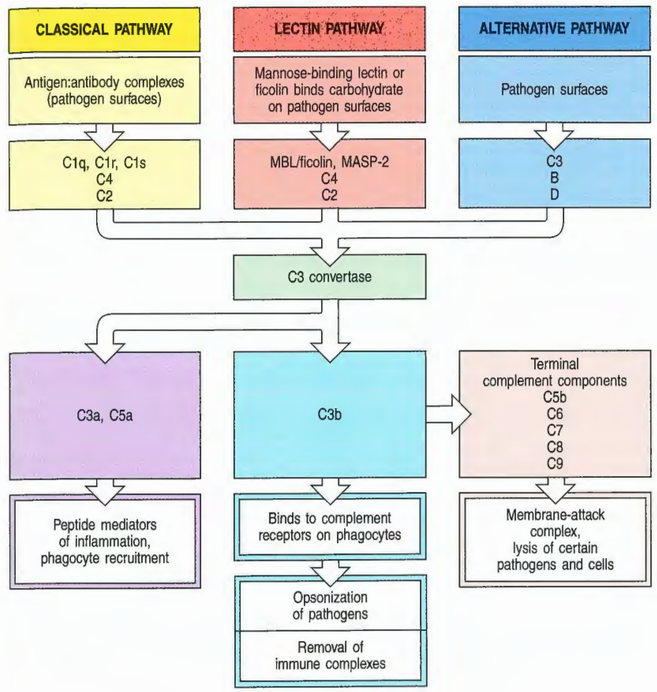


Fig. 2.25 Overview of the main components and effector actions of complement. Early on in all three pathways of complement activation a series of cleavage reactions culminates in the formation of an enzymatic activity called a C3 convertase, which cleaves complement component C3 into C3b and C3a. The production of the C3 convertase is the point at which the three pathways converge and the main effector functions of complement are generated. C3b binds covalently to the bacterial cell membrane and opsonizes the bacteria, enabling phagocytes to internalize them. C3a is a peptide mediator of local inflammation. C5a and C5b are generated by the cleavage of C5b by a C5 convertase formed by C3b bound to the C3 convertase (not shown in this simplified diagram). C5a is also a powerful peptide mediator of inflammation. C5b triggers the late events in which the terminal components of complement assemble into a membrane-attack complex that can damage the membrane of certain pathogens.

activation, and consist of triggered-enzyme cascades in which complement zymogens are successively cleaved to yield two fragments, the larger of which is an active serine protease. The active protease is retained at the pathogen surface; this ensures that the next complement zymogen in the pathway is also cleaved and activated at the pathogen surface. In contrast, the small peptide fragment is released from the site of the reaction and can act as a soluble mediator of inflammation.

The C3 convertases formed by these early events of complement activation are bound covalently to the pathogen surface. Here they cleave C3 to generate large amounts of **C3b**, the main effector molecule of the complement system, and C3a, a peptide mediator of inflammation. The C3b molecules act as opsonins; they bind covalently to the pathogen and thereby target it for destruction by phagocytes equipped with receptors for C3b. C3b also binds to the C3 convertase to form a **C5 convertase** that produces the most important and most potent inflammatory peptide, **C5a**, as well as a large active fragment, **C5b**, that initiates the 'late' events of complement activation. These comprise a sequence of polymerization reactions in which a set of complement proteins known as the terminal components interact to form a **membrane-attack complex**, which creates a pore in the cell membranes of certain pathogens that can lead to their death.

The nomenclature of complement proteins is an obstacle to understanding the system, and before discussing the complement cascade in more detail we will explain their naming. All components of the classical complement pathway and the membrane-attack complex are designated by the letter C

Functional protein classes in the complement system	
Binding to antigen:antibody complexes and pathogen surfaces	C1q
Binding to mannose on bacteria	MBL
Activating enzymes	C1r C1s C2a Bb D MASP-2
Membrane-binding proteins and opsonins	C4b C3b
Peptide mediators of inflammation	C5a C3a C4a
Membrane-attack proteins	C5b C6 C7 C8 C9
Complement receptors	CR1 CR2 CR3 CR4 C1qR
Complement-regulatory proteins	C1INH C4bp CR1 MCP DAF H I P CD59

Fig. 2.26 Functional protein classes in the complement system.

followed by a number. The native components have a simple number designation, for example, C1 and C2, but unfortunately the components were numbered in the order of their discovery rather than the sequence of reactions. The reaction sequence is C1, C4, C2, C3, C5, C6, C7, C8, and C9. The products of the cleavage reactions are designated by added lower-case letters, the larger fragment being designated b and the smaller a; thus, for example, C4 is cleaved to C4b, the large fragment of C4 that binds covalently to the surface of the pathogen, and C4a, a small fragment with weak pro-inflammatory properties. There is one exception to this rule of nomenclature. For C2, the larger fragment was originally named C2a, and it is this larger C2a component that contains the enzymatic activity. The components of the alternative pathway, instead of being numbered, are designated by different capital letters, for example factor B and factor D. As with the classical pathway, their cleavage products are designated by the addition of lower-case a and b: thus, the large fragment of B is called Bb and the small fragment Ba.

Finally, in the lectin pathway, the first enzymes to be activated are known as the *m*annose-binding-lectin-*a*ssociated *s*erine *p*roteases, **MASP-1** and **MASP-2**, after which the pathway is essentially the same as the classical pathway. Activated complement components are often designated by a horizontal line, for example, $\overline{C2a}$; however, we will not use this convention.

The formation of C3 convertase activity is pivotal in complement activation, leading to the production of the principal effector molecules, and initiating the late events. In the classical and lectin pathways the C3 convertase is formed from membrane-bound C4b complexed with C2a, designated C4b2a. In the alternative pathway, a homologous C3 convertase is formed from membrane-bound C3b complexed with Bb, C3bBb. The alternative pathway can act as an amplification loop for all three pathways, because it is initiated by the binding of C3b.

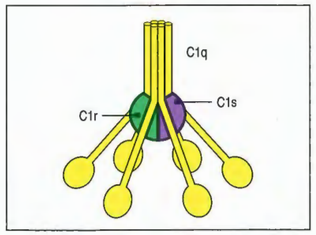
It is clear that a pathway leading to such potent inflammatory and destructive effects—and which, moreover, has a series of built-in amplification steps—is potentially dangerous and must be subject to tight regulation. One important safeguard is that key activated complement components are rapidly inactivated unless they bind to the pathogen surface on which their activation was initiated. There are also several points in the pathway at which regulatory proteins act on complement components to prevent the inadvertent activation of complement on host-cell surfaces, thereby protecting them from accidental damage. We will return to these regulatory mechanisms later.

We have now introduced all the relevant components of complement and are ready for a more detailed account of their functions. To help in distinguishing the different components according to their functions, we will use a color code in the tables in this part of the chapter. This is introduced in Fig. 2.26, where all the components of complement are grouped by function.

2-13 The classical pathway is initiated by activation of the C1 complex.

The classical pathway has a role in both innate and adaptive immunity. As we will see in Chapter 9, the first component of this pathway, C1q, links the adaptive humoral immune response to the complement system by binding to antibodies complexed with antigens. However, the classical pathway can be also be activated during innate immune responses. Antibodies called **natural antibodies** are produced by the immune system in the apparent absence of any infection. They have a broad specificity for self and microbial antigens, can react with many pathogens and, as in adaptive immunity, can activate complement through the binding of C1q. We will describe natural antibodies

Fig. 2.27 The first protein in the classical pathway of complement activation is C1, which is a complex of C1q, C1r, and C1s. C1q is composed of six identical subunits with globular heads and long collagen-like tails, and has been described as looking like 'a bunch of tulips'. The tails combine to bind to two molecules each of C1r and C1s, forming the C1 complex C1q:C1r_2:C1s_2. The heads can bind to the constant regions of immunoglobulin molecules or directly to the pathogen surface, causing a conformational change in C1r, which then cleaves and activates the C1s zymogen. Photograph (× 500,000) courtesy of K.B.M. Reid.





and the specialized subset of lymphocytes that produce them in more detail in Section 2-34. Here it is only important to note that most of the natural antibody that is produced is of the class known as IgM and that this is the class that is most efficient at binding C1q; thus, natural antibodies provide an effective means by which complement activation can be directed to pathogen surfaces immediately upon infection.

An additional function of C1q in innate immunity is that it can bind directly to the surface of certain pathogens and thus trigger complement activation in the absence of antibody. It can, for example, bind to C-reactive protein bound to phosphocholine on bacteria. Therefore, the activation of C1 by natural antibody and directly by pathogen surfaces represents an important humoral arm of innate immunity.

C1q is part of the **C1 complex**, which comprises a single C1q molecule bound to two molecules each of the zymogens C1r and C1s. C1q itself is a hexamer, each subunit of which is in turn a trimer, forming a globular domain with a triple-helical collagen-like tail. In the C1q hexamer the six globular heads are linked together by their collagen-like tails, which surround the $(C1r:C1s)_2$ complex (Fig. 2.27). Binding of more than one of the C1q heads to a pathogen surface or to the constant region of antibodies, known as the Fc region, in an immune complex of antigen and antibody causes a conformational change in the $(C1r:C1s)_2$ complex, which leads to the activation of an autocatalytic enzymatic activity in C1r; the active form of C1r then cleaves its associated C1s to generate an active serine protease.

Once activated, the C1s enzyme acts on the next two components of the classical pathway, cleaving C4 and then C2 to generate two large fragments, C4b and C2a, which together form the C3 convertase of the classical pathway. In the first step, C1s cleaves C4 to produce C4b, which may bind covalently to the surface of the pathogen. The covalently attached C4b then binds one molecule of C2, making it susceptible, in turn, to cleavage by C1s. C1s cleaves C2 to produce the large fragment C2a, which is itself a serine protease. C4b2a, the complex of C4b with the active serine protease C2a, remains covalently linked to the surface of the pathogen as the C3 convertase of the classical pathway. Its most important activity is to cleave large numbers of C3 molecules to produce C3b molecules that coat the pathogen surface. At the same time, the other cleavage product, C3a, initiates a local inflammatory response. These reactions, which comprise the classical pathway of complement activation, are shown in schematic form in Fig. 2.28; the proteins involved, and their active forms, are listed in Fig. 2.29.

2-14 The lectin pathway is homologous to the classical pathway.

The lectin pathway uses proteins very similar to C1q to trigger the complement cascade. One of these proteins is the mannose-binding lectin (MBL) introduced earlier. It binds specifically to mannose residues, and to certain other sugars, which are present on many pathogen surfaces in a pattern that allows binding, as shown schematically in Fig. 2.15. On vertebrate cells, however,

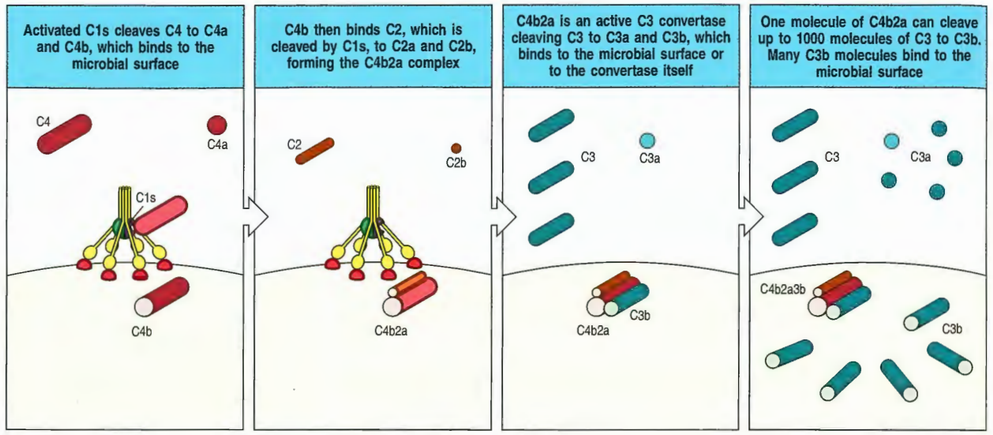


Fig. 2.28 The classical pathway of complement activation generates a C3 convertase that deposits large numbers of C3b molecules on the pathogen surface. The steps in the reaction are outlined here and detailed in the text. The cleavage of C4 by C1s exposes a reactive group on C4b that allows it to bind covalently to the pathogen surface. C4b then binds C2, making it susceptible to cleavage by C1s. The larger C2a fragment is the active protease component of the C3 convertase. It cleaves many molecules of C3 to produce C3b, which binds to the pathogen surface, and C3a, an inflammatory mediator.

mannose is covered by other sugar groups, especially by sialic acid. Thus, MBL is able to initiate complement activation by binding to pathogen surfaces while not becoming activated on host cells. It is present at low concentrations in the plasma of most individuals, and, as we will see in the last part of this chapter, its production by the liver is increased during the acute-phase reaction induced as part of the innate immune response.

MBL is a two- to six-headed molecule that, like C1q, forms a complex with two protease zymogens, which in the case of the MBL complex are the serine proteases MASP-1 and MASP-2 (Fig. 2.30). MASP-2 is closely related to C1r and C1s, and MASP-1 is a slightly more distantly related cousin; all four enzymes are likely to have evolved from the duplication of a gene for a common precursor. When the MBL complex binds to a pathogen surface, MASP-2 is activated to cleave C4 and C2. The role, if any, of MASP-1 in complement activation is uncertain; *in vitro* it is able to cleave C2 as efficiently as MASP-2, and so its role may be to enhance the activation of complement, even if it is unable to initiate it. Thus the lectin pathway initiates complement activation in a closely similar way to the classical pathway, forming a C3 convertase from C2a bound to C4b. People deficient in MBL or in MASP-2 experience a substantial increase in infections during early childhood, indicating the importance of the lectin pathway for host defense. The age window of susceptibility to infections associated with MBL deficiency illustrates the particular importance of innate host defense mechanisms in early childhood, which is a time before the child's adaptive immune responses are fully matured and after maternal antibodies transferred across the placenta and in colostrum have been lost.

Related in overall shape and function to MBL and Clq are the **ficolins**, which also bind carbohydrates on microbial surfaces and which, like the collectins, activate complement through the binding and activation of MASP-1 and MASP-2 (see Fig. 2.30). In humans, there are three ficolins: L-, M- and H-ficolin. Ficolins differ from the collectins in that instead of having a lectin domain attached to the collagen-like stalk, they have a fibrinogen-like domain; this fibrinogen-like domain binds carbohydrates and gives the ficolins their general specificity for oligosaccharides containing *N*-acetylglucosamine. In discussing the activation of complement by these innate activation molecules, we have used MBL as our prototype, but the ficolins

Proteins of the classical pathway of complement activation

Native component	Active form	Function of the active form
C1 ($C1q:C1r_2:C1s_2$)	C1q	Binds directly to pathogen surfaces or indirectly to antibody bound to pathogens, thus allowing autoactivation of C1r
	C1r	Cleaves C1s to active protease
	C1s	Cleaves C4 and C2
C4	C4b	Covalently binds to pathogen and opsonizes it. Binds C2 for cleavage by C1s
	C4a	Peptide mediator of inflammation (weak activity)
C2	C2a	Active enzyme of classical pathway C3/C5 convertase: cleaves C3 and C5
	C2b	Precursor of vasoactive C2 kinin
C3	C3b	Many molecules of C3b bind to pathogen surface and act as opsonins. Initiates amplification via the alternative pathway. Binds C5 for cleavage by C2b
	C3a	Peptide mediator of inflammation (intermediate activity)

Fig. 2.29 The proteins of the classical pathway of complement activation.

may be more important in practice, because their concentration in plasma is greater than that of MBL.

2-15 Complement activation is largely confined to the surface on which it is initiated.

We have seen that the classical and lectin pathways of complement activation are initiated by proteins that bind to pathogen surfaces. During the triggered-enzyme cascade that follows, it is important that activating events are confined to this same site, so that C3 activation also occurs on the surface of the pathogen and not in the plasma or on host-cell surfaces. This is achieved principally by the covalent binding of C4b to the pathogen surface. Cleavage

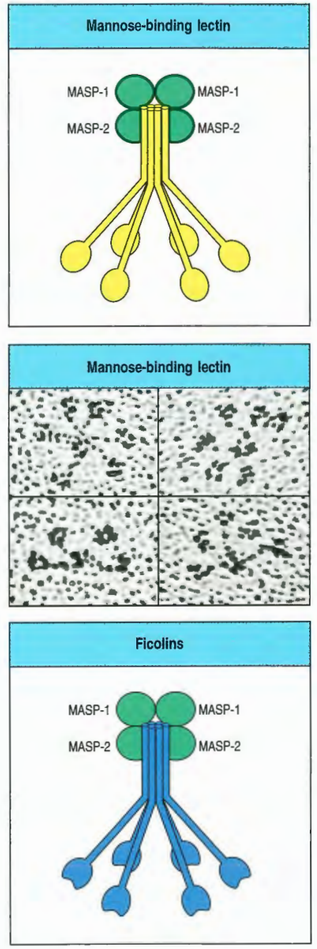


Fig. 2.30 The innate complement-activating molecules form a complex with serine proteases that resembles the complement C1 complex. Mannose-binding lectin (MBL) (top panel) forms clusters of two to six carbohydrate-binding heads around a central stalk formed from the collagen-like tails of the MBL monomers. This structure, easily discernible under the electron microscope (middle panels), closely resembles that of C1q. Associated with this complex are two serine proteases, MBL-associated serine protease 1 (MASP-1) and 2 (MASP-2). The structural disposition of the MASP proteins in the complex is not yet determined but it is likely that they interact with MBL in the same way in which C1r and C1s interact with C1q. On binding of MBL to bacterial surfaces, MASP-2 becomes activated and can then activate the complement system by cleaving and activating C4 and C2. The ficolins (bottom panel) resemble MBL in their overall structure, are associated with MASP-1 and MASP-2, and can activate C4 and C2 after binding to carbohydrate molecules present on microbial surfaces. The carbohydrate-binding domain of ficolins is a fibrinogen-like domain, rather than the lectin domain present in MBL. Photograph of MBL courtesy of K.B.M. Reid.

of C4 exposes a highly reactive thioester bond on the C4b molecule that allows it to bind covalently to molecules in the immediate vicinity of its site of activation. In innate immunity, C4 cleavage is catalyzed by a C1 or MBL complex bound to the pathogen surface, and C4b can bind adjacent proteins or carbohydrates on the pathogen surface. If C4b does not rapidly form this bond, the thioester bond is cleaved by reaction with water and this hydrolysis reaction irreversibly inactivates C4b (Fig. 2.31). This helps to prevent C4b from diffusing from its site of activation on the microbial surface and becoming coupled to host healthy cells.

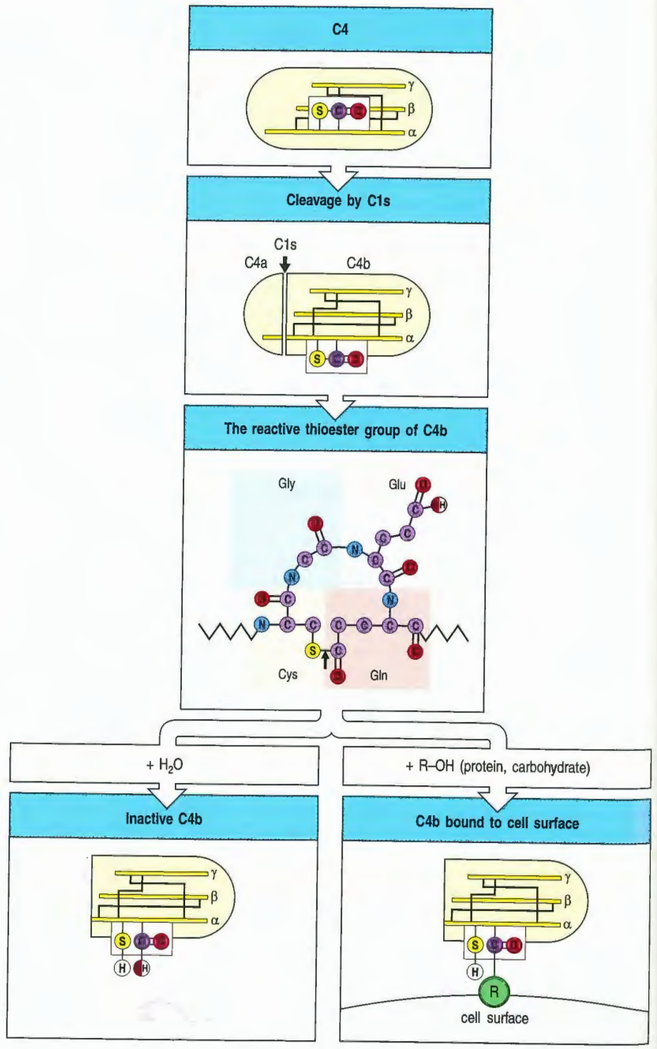


Fig. 2.31 Cleavage of C4 exposes an active thioester bond that causes the large fragment, C4b, to bind covalently to nearby molecules on the bacterial cell surface. Intact C4 consists of an α, a β, and a γ chain with a shielded thioester bond on the α chain. This is exposed when the α chain is cleaved by C1s to produce C4b. The thioester bond (marked by an arrow in the third panel) is rapidly hydrolyzed (that is, cleaved by water), inactivating C4b unless it reacts with hydroxyl or amino groups to form a covalent linkage with molecules on the pathogen surface. The homologous protein C3 has an identical reactive thioester bond that is also exposed on the C3b fragment when C3 is cleaved by C2a. The covalent attachment of C3b and C4b to the pathogen surface enables these molecules to act as opsonins and is important in confining complement activation to pathogen surfaces.

C2 becomes susceptible to cleavage by C1s only when it is bound by C4b, and the C2a serine protease is thereby also confined to the pathogen surface, where it remains associated with C4b, forming the C3 convertase C4b2a. The activation of C3 molecules thus also occurs at the surface of the pathogen. Furthermore, the C3b cleavage product is also rapidly inactivated unless it binds covalently by the same mechanism as C4b, and it therefore opsonizes only the surface on which complement activation has taken place. The opsonization of pathogens by C3b is more efficient when antibodies are bound to the pathogen surface, as phagocytes have receptors for both complement and antibody; these will be described in Chapter 9. Because the reactive forms of C3b and C4b are able to form a covalent bond with any adjacent protein or carbohydrate, when complement is activated by bound antibody a proportion of the reactive C3b or C4b will become linked to the antibody molecules themselves. This combination of antibody chemically cross-linked to complement is likely to be the most efficient trigger for phagocytosis.

2-16 Hydrolysis of C3 causes initiation of the alternative pathway of complement.

The alternative pathway is so named because it was discovered as a second, or 'alternative,' pathway for complement activation after the classical pathway had been defined. This pathway can proceed on many microbial surfaces in the absence of specific antibody, and it leads to the generation of a distinct C3 convertase designated C3bBb. In contrast to the classical and lectin pathways of complement activation, the alternative pathway does not depend on a pathogen-binding protein for its initiation; instead it is initiated through the spontaneous hydrolysis of C3, as shown in the top three panels of Fig. 2.32. The distinctive components of the pathway are listed in Fig. 2.33. Several mechanisms ensure that the activation pathway will proceed only on the surface of a pathogen or on damaged host cells, and not on normal host cells and tissues.

C3 is abundant in plasma, and C3b is produced at a significant rate by spontaneous cleavage (also known as 'tickover'). This occurs through the spontaneous hydrolysis of the thioester bond in C3 to form $C3(H_2O)$, which has an altered conformation, allowing binding of the plasma protein **factor B**. The binding of B by $C3(H_2O)$ then allows a plasma protease called **factor D** to cleave factor B to Ba and Bb, the latter remaining associated with $C3(H_2O)$ to form the $C3(H_2O)Bb$ complex. This complex is a fluid-phase C3 convertase, and although it is only formed in small amounts it can cleave many molecules of C3 to C3a and C3b. Much of this C3b is inactivated by hydrolysis, but some attaches covalently, through its reactive thioester group, to the surfaces of pathogens or of host cells. C3b bound in this way is able to bind factor B, allowing its cleavage by factor D to yield the small fragment Ba and the active protease Bb. This results in formation of the alternative pathway C3 convertase, C3bBb (Fig. 2.34).

2-17 Membrane and plasma proteins that regulate the formation and stability of C3 convertases determine the extent of complement activation under different circumstances.

The extent of complement activation is critically dependent on the stability of the C3bBb convertase. This stability is controlled by both positive and negative regulatory proteins. Normal host cells are protected from complement activation by several negative regulatory proteins, present in the plasma and in host-cell membranes, that protect normal host cells from the injurious effects of inappropriate complement activation. These proteins interact with

Fig. 2.32 Complement activated by the alternative pathway attacks pathogens while sparing host cells, which are protected by complement-regulatory proteins. The complement component C3 is cleaved spontaneously in plasma to give C3(H_2O), which binds factor B and enables the bound factor B to be cleaved by factor D (top panel). The resulting soluble C3 convertase cleaves C3 to give C3a and C3b, which can attach to host cells or pathogen surfaces (second panel). Covalently bound C3b binds factor B, which in turn is rapidly cleaved by factor D to Bb, which remains bound to C3b to form a C3 convertase, and Ba, which is released (third panel). If C3bBb forms on the surface of host cells (bottom left panels), it is rapidly inactivated by complement-regulatory proteins expressed by the host cell: complement receptor 1 (CR1), decay-accelerating factor (DAF), and membrane cofactor of proteolysis (MCP). Host cell surfaces also favor the binding of factor H from plasma. CR1, DAF, and factor H displace Bb from C3b, and CR1, MCP, and factor H catalyze the cleavage of bound C3b by the plasma protease factor I to produce inactive C3b (known as iC3b). Bacterial surfaces (bottom right panels) do not express complement-regulatory proteins and favor the binding of properdin (factor P), which stabilizes the C3bBb convertase activity. This convertase is the equivalent of C4b2a of the classical pathway (see Fig. 2.28).

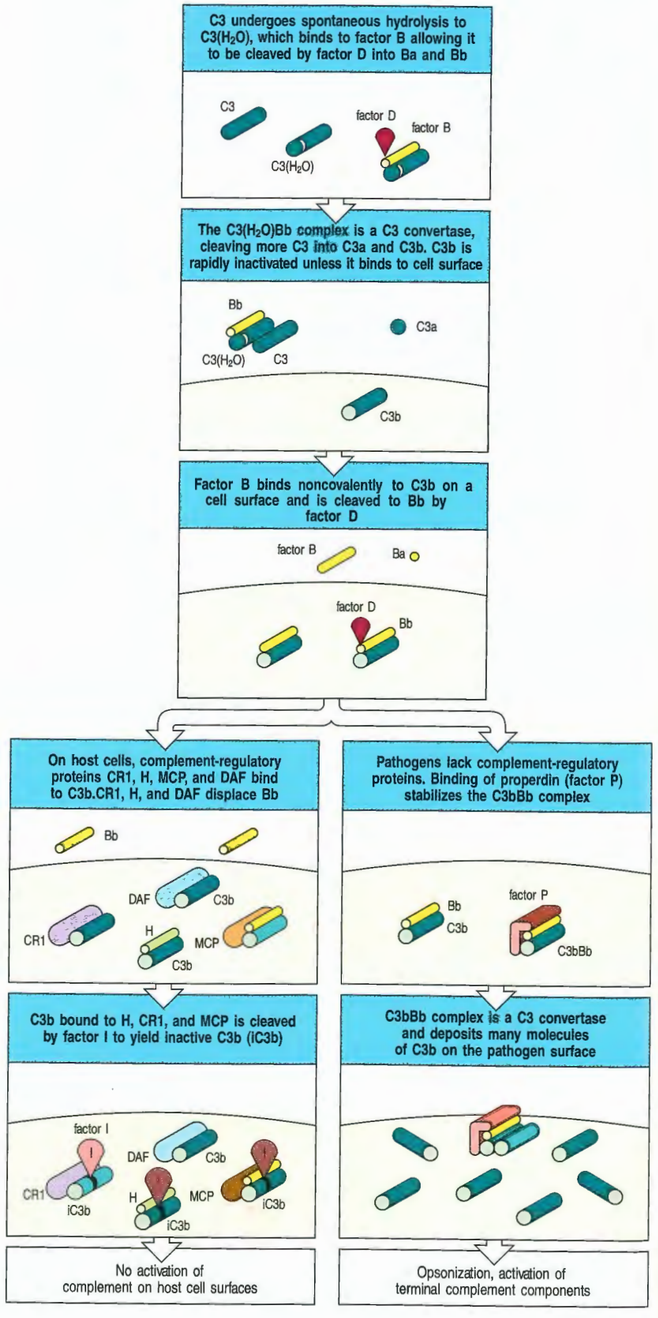


Fig 2.33 The proteins of the alternative pathway of complement activation.

Proteins of the alternative pathway of complement activation

Native component	Active fragments	Function
C3	C3b	Binds to pathogen surface, binds B for cleavage by D, C3bBb is C3 convertase and $C3b_2Bb$ is C5 convertase
Factor B (B)	Ba	Small fragment of B, unknown function
	Bb	Bb is active enzyme of the C3 convertase C3bBb and C5 convertase $C3b_2Bb$
Factor D (D)	D	Plasma serine protease, cleaves B when it is bound to C3b to Ba and Bb
Properdin (P)	P	Plasma protein that stabilizes the C3bBb convertase on bacterial cells



Factor I Deficiency

C3b and either prevent the convertase from forming or promote its rapid dissociation. Thus, a membrane-attached protein known as **decay-accelerating factor** (**DAF** or **CD55**) competes with factor B for binding to C3b on the cell surface, and can displace Bb from a convertase that has already formed. Convertase formation can also be prevented by cleaving C3b to its inactive derivative iC3b. This is achieved by a plasma protease, **factor I**, in conjunction with C3b-binding proteins that can act as cofactors, such as **membrane cofactor of proteolysis** (**MCP** or **CD46**), another host-cell membrane protein. Cell-surface complement receptor type 1 (CR1) has similar activities to DAF and MCP in the inhibition of C3 convertase formation and the promotion of the catabolism of C3b to inactive products, but has a more limited tissue distribution. **Factor H** is yet another complement-regulatory protein in plasma that binds C3b and, like CR1, it is able to compete with factor B to displace Bb from the convertase in addition to acting as a cofactor for factor I. Factor H binds preferentially to C3b bound to vertebrate cells because it has an affinity for the sialic acid residues present on the cell surface.

In contrast, when complement is activated on foreign surfaces, such as on bacterial surfaces, or indeed on host cells that have been damaged or modified by ischemia, viral infection, or antibody binding, C3 convertase enzymes are stabilized, which enables complement activation to proceed. On these cells a positive regulatory plasma protein, known as **properdin** or **factor P**, binds to the C3bBb convertase and enhances its stability, causing amplification of complement activation.

Fig. 2.34 The alternative pathway of complement activation can amplify the classical or the lectin pathway by forming an alternative C3 convertase and depositing more C3b molecules on the pathogen. C3b deposited by the classical or lectin pathway can bind factor B, making it susceptible to cleavage by factor D. The C3bBb complex is the C3 convertase of the alternative pathway of complement activation and its action, like that of C4b2a, results in the deposition of many molecules of C3b on the pathogen surface.

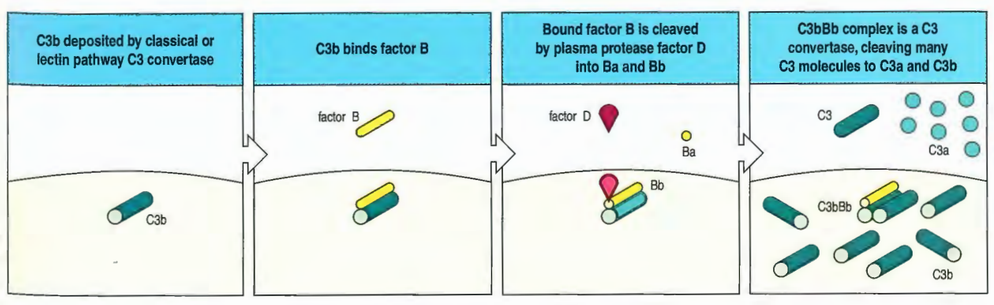


Fig. 2.35 There is a close relationship between the factors of the alternative, lectin, and classical pathways of complement activation. Most of the factors are either identical or the products of genes that have duplicated and then diverged in sequence. The proteins C4 and C3 are homologous and contain the unstable thioester bond by which their large fragments, C4b and C3b, bind covalently to membranes. The genes encoding proteins C2 and B are adjacent in the class III region of the MHC and arose by gene duplication. Factor H, CR1, and C4BP regulatory proteins share a repeat sequence common to many complement-regulatory proteins. The greatest divergence between the pathways is in their initiation: in the classical pathway the C1 complex binds either to certain pathogens or to bound antibody and in the latter circumstance it serves to convert antibody binding into enzyme activity on a specific surface; in the lectin pathway, mannose-binding lectin (MBL) associates with a serine protease, activating MBL-associated serine protease (MASP), to serve the same function as C1r:C1s; in the alternative pathway this enzyme activity is provided by factor D.

Step in pathway	Protein serving function in pathway			Relationship
	Alternative	Lectin	Classical	
Initiating serine protease	D	MASP	C1s	Homologous (C1s and MASP)
Covalent binding to cell surface	C3b	C4b		Homologous
C3/C5 convertase	Bb	C2b		Homologous
Control of activation	CR1 H	CR1 C4BP		Identical Homologous
Opsonization	C3b			Identical
Initiation of effector pathway	C5b			Identical
Local inflammation	C5a, C3a			Identical
Stabilization	P	None		Unique

Once formed on a surface that allows it to be stable, the C3bBb convertase rapidly cleaves yet more C3 to C3b, which can bind to the pathogen and either act as an opsonin or reinitiate the pathway to form another molecule of C3bBb convertase. Thus, the alternative pathway activates through an amplification loop that can proceed on the surface of a pathogen or on damaged host cells but not on normal host cells or tissues. This same amplification loop enables the alternative pathway to contribute to complement activation initially triggered through the classical or lectin pathways (see Fig. 2.25).

The C3 convertases resulting from activation of the classical and lectin pathways (C4b2b) and from the alternative pathway (C3bBb) are apparently distinct. However, understanding of the complement system is simplified somewhat by recognition of the close evolutionary relationships between the different complement proteins (Fig. 2.35). Thus the complement zymogens, factor B and C2, are closely related proteins encoded by homologous genes located in tandem within the major histocompatibility complex (MHC) on human chromosome 6. Furthermore, their respective binding partners, C3 and C4, both contain thioester bonds that provide the means of covalently attaching the C3 convertases to a pathogen surface. Only one component of the alternative pathway seems entirely unrelated to its functional equivalents in the classical and lectin pathways: the initiating serine protease, factor D. Factor D can also be singled out as the only activating protease of the complement system to circulate as an active enzyme rather than a zymogen. This is both necessary for the initiation of the alternative pathway through the cleavage of factor B bound to spontaneously activated C3, and safe for the host because factor D has no other substrate than factor B bound to C3b. This means that factor D finds its substrate only at a very low level in plasma, and at pathogen surfaces where the alternative pathway of complement activation is allowed to proceed.

Comparison of the different pathways of complement activation illustrates the general principle that most of the immune effector mechanisms that can be activated in a nonclonal fashion as part of the early nonadaptive response against infection have been harnessed during evolution to be used as effector mechanisms of adaptive immunity. It is almost certain that the adaptive response evolved by adding specific recognition to the original nonadaptive system. This is illustrated particularly clearly in the complement system, because here the components are defined and the functional homologs can be seen to be evolutionarily related.

2-18 Surface-bound C3 convertase deposits large numbers of C3b fragments on pathogen surfaces and generates C5 convertase activity.

The formation of C3 convertases is the point at which the three pathways of complement activation converge, because both the classical pathway and lectin pathway convertase C4b2a and the alternative pathway convertase C3bBb have the same activity, and they initiate the same subsequent events. They both cleave C3 to C3b and C3a. C3b binds covalently through its thioester bond to adjacent molecules on the pathogen surface; otherwise it is inactivated by hydrolysis. C3 is the most abundant complement protein in plasma, occurring at a concentration of 1.2 mg ml^{-1}, and up to 1000 molecules of C3b can bind in the vicinity of a single active C3 convertase (see Fig. 2.34). Thus, the main effect of complement activation is to deposit large quantities of C3b on the surface of the infecting pathogen, where it forms a covalently bonded coat that can signal the ultimate destruction of the pathogen by phagocytes.

The next step in the cascade is the generation of the C5 convertases. In the classical and the lectin pathways, a C5 convertase is formed by the binding of C3b to C4b2a to yield C4b2a3b. By the same token, the C5 convertase of the alternative pathway is formed by the binding of C3b to the C3 convertase to form $C3b_2Bb$. C5 is captured by these C5 convertase complexes through binding to an acceptor site on C3b, and is thus rendered susceptible to cleavage by the serine protease activity of C2a or Bb. This reaction, which generates C5b and C5a, is much more limited than cleavage of C3, because C5 can be cleaved only when it binds to C3b that is in turn bound to C4b2a or C3bBb to form the active C5 convertase complex. Thus, complement activated by all three pathways leads to the binding of large numbers of C3b molecules on the surface of the pathogen, the generation of a more limited number of C5b molecules, and the release of C3a and C5a (Fig. 2.36).

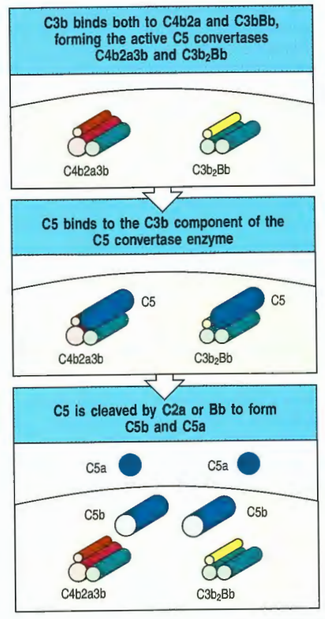


Fig. 2.36 Complement component C5 is cleaved when captured by a C3b molecule that is part of a C5 convertase complex. As shown in the top panel, C5 convertases are formed when C3b binds either the classical or lectin pathway C3 convertase C4b2a to form C4b2a3b, or the alternative pathway C3 convertase C3bBb to form C3b2Bb. C5 binds to C3b in these complexes (center panel). The bottom panel shows how C5 is cleaved by the active enzyme C2a or Bb to form C5b and the inflammatory mediator C5a. Unlike C3b and C4b, C5b is not covalently bound to the cell surface. The production of C5b initiates the assembly of the terminal complement components.

2-19 Ingestion of complement-tagged pathogens by phagocytes is mediated by receptors for the bound complement proteins.

The most important action of complement is to facilitate the uptake and destruction of pathogens by phagocytic cells. This occurs by the specific recognition of bound complement components by **complement receptors** (**CRs**) on phagocytes. These complement receptors bind pathogens opsonized with complement components: opsonization of pathogens is a major function of C3b and its proteolytic derivatives. C4b also acts as an opsonin but has a relatively minor role, largely because so much more C3b than C4b is generated.

The six known types of receptors for bound complement components are listed, with their functions and distributions, in Fig. 2.37. The best characterized is the C3b receptor **CR1** (**CD35**), which is expressed on both macrophages and neutrophils. Binding of C3b to CR1 cannot by itself stimulate phagocytosis, but it can lead to phagocytosis in the presence of other immune mediators that activate macrophages. For example, the small complement fragment C5a can activate macrophages to ingest bacteria bound to their CR1 receptors (Fig. 2.38). C5a binds to another receptor expressed by macrophages, the **C5a receptor**, which has seven membrane-spanning

Fig. 2.37 Distribution and function of cell-surface receptors for complement proteins. There are several different receptors specific for different bound complement components and their fragments. CR1 and CR3 are especially important in inducing phagocytosis of bacteria with complement components bound to their surface. CR2 is found mainly on B cells, where it is also part of the B-cell co-receptor complex and the receptor by which the Epstein–Barr virus selectively infects B cells, causing infectious mononucleosis. CR1 and CR2 share structural features with the complement-regulatory proteins that bind C3b and C4b. CR3 and CR4 are integrins; CR3 is known to be important for leukocyte adhesion and migration, whereas CR4 is only known to function in phagocytic responses. The C5a and C3a receptors are seven-span G-protein-coupled receptors. FDC, follicular dendritic cells; these are not involved in innate immunity and are discussed in later chapters.

Receptor	Specificity	Functions	Cell types
CR1 (CD35)	C3b, C4bi C3b	Promotes C3b and C4b decay Stimulates phagocytosis Erythrocyte transport of immune complexes	Erythrocytes, macrophages, monocytes, polymorphonuclear leukocytes, B cells, FDC
CR2 (CD21)	C3d, iC3b, C3dg Epstein–Barr virus	Part of B-cell co-receptor Epstein–Barr virus receptor	B cells, FDC
CR3 (Mac-1) (CD11b/ CD18)	iC3b	Stimulates phagocytosis	Macrophages, monocytes, polymorphonuclear leukocytes, FDC
CR4 (gp150, 95) (CD11c/ CD18)	iC3b	Stimulates phagocytosis	Macrophages, monocytes, polymorphonuclear leukocytes, dendritic cells
C5a receptor	C5a	Binding of C5a activates G protein	Endothelial cells, mast cells, phagocytes
C3a receptor	C3a	Binding of C3a activates G protein	Endothelial cells, mast cells, phagocytes

domains. Receptors of this type couple with intracellular guanine-nucleotide-binding proteins called G proteins, and the C5a receptor signals in this way. Proteins associated with the extracellular matrix, such as fibronectin, can also contribute to phagocyte activation; these are encountered when phagocytes are recruited to connective tissue and activated there.

Three other complement receptors—**CR2** (also known as **CD21**), **CR3** (**CD11b:CD18**), and **CR4** (**CD11c:CD18**)—bind to inactivated forms of C3b that remain attached to the pathogen surface. Like several other key components of complement, C3b is subject to regulatory mechanisms and can be cleaved into derivatives that cannot form an active convertase. One of the inactive derivatives of C3b, known as iC3b (see Section 2-17) acts as an opsonin in its own right when bound by the complement receptor CR3. Unlike the binding of iC3b to CR1, the binding of iC3b to CR3 is sufficient on its own to stimulate phagocytosis. A second breakdown product of C3b, called **C3dg**, binds only to CR2. CR2 is found on B cells as part of a co-receptor complex

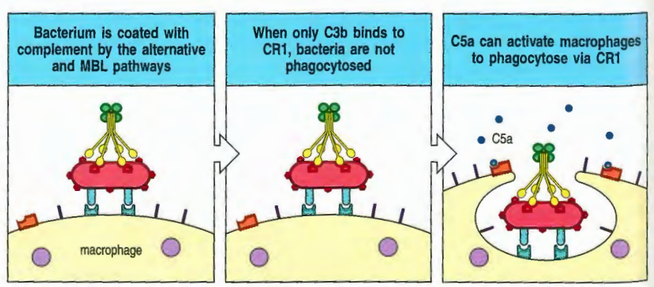


Fig. 2.38 The anaphylatoxin C5a can enhance the phagocytosis of opsonized microorganisms. Activation of complement, either by the alternative or by the mannose-binding lectin pathway, leads to the deposition of C3b on the surface of the microorganism (left panel). C3b can be bound by the complement receptor CR1 on the surface of phagocytes, but this on its own is insufficient to induce phagocytosis (center panel). Phagocytes also express receptors for the anaphylatoxin C5a, and binding of C5a will now activate the cell to phagocytose microorganisms bound through CR1 (right panel).

that can augment the signal received through the antigen-specific immunoglobulin receptor. Thus, a B cell whose antigen receptor is specific for a given pathogen will receive a strongly augmented signal on binding this pathogen if it is also coated with C3dg. The activation of complement can therefore contribute to producing a strong antibody response (see Chapters 6 and 9). This example of how an innate humoral immune response can contribute to activating adaptive humoral immunity parallels the contribution made by the innate cellular response of macrophages and dendritic cells to the initiation of a T-cell response, which we will discuss later in this chapter.

The central role of opsonization by C3b and its inactive fragments in the destruction of extracellular pathogens can be seen in the effects of various complement deficiency diseases. Whereas individuals deficient in any of the late components of complement are relatively unaffected, showing only an increase in susceptibility to *Neisseria* infections, individuals deficient in C3 or in molecules that catalyze C3b deposition show increased susceptibility to infection by a wide range of extracellular bacteria, as we will see in Chapter 12.

2-20 Small fragments of some complement proteins can initiate a local inflammatory response.

The small complement fragments C3a, C4a, and C5a act on specific receptors (see Fig. 2.37) to produce local inflammatory responses. When produced in large amounts or injected systemically, they induce a generalized circulatory collapse, producing a shock-like syndrome similar to that seen in a systemic allergic reaction involving antibodies of the IgE class (which is discussed in Chapter 13). Such a reaction is termed **anaphylactic shock** and these small fragments of complement are therefore often referred to as **anaphylatoxins**. Of the three, C5a has the highest specific biological activity. All three induce smooth muscle contraction and increase vascular permeability, but C5a and C3a also act on the endothelial cells lining blood vessels to induce adhesion molecules. In addition, C3a and C5a can activate the mast cells that populate submucosal tissues to release mediators such as histamine and TNF-α that cause similar effects. The changes induced by C5a and C3a recruit antibody, complement, and phagocytic cells to the site of an infection (Fig. 2.39), and the increased fluid in the tissues hastens the movement of pathogen-bearing antigen-presenting cells to the local lymph nodes, contributing to the prompt initiation of the adaptive immune response.

C5a also acts directly on neutrophils and monocytes to increase their adherence to vessel walls, their migration toward sites of antigen deposition, and their ability to ingest particles. C5a also increases the expression of CR1 and CR3 on the surfaces of these cells. In this way, C5a, and to a smaller extent C3a and C4a, act in concert with other complement components to hasten the destruction of pathogens by phagocytes. C5a and C3a signal through transmembrane receptors that activate G proteins; thus, the action of C5a in attracting neutrophils and monocytes is analogous to that of chemokines, which also act via G proteins to control cell migration.

2-21 The terminal complement proteins polymerize to form pores in membranes that can kill certain pathogens.

One of the important effects of complement activation is the assembly of the terminal components of complement (Fig. 2.40) to form a membrane-attack complex. The reactions leading to the formation of this complex are shown schematically and microscopically in Fig. 2.41. The end result is a pore in the lipid bilayer membrane that destroys membrane integrity. This is thought to

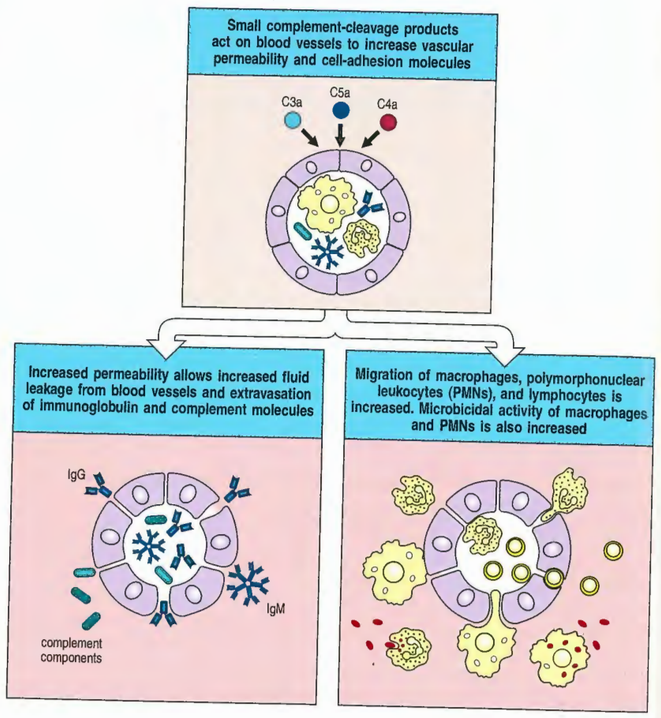


Fig. 2.39 Local inflammatory responses can be induced by small complement fragments, especially C5a. The small complement fragments are differentially active: C5a is more active than C3a, which is more active than C4a. They cause local inflammatory responses by acting directly on local blood vessels, stimulating an increase in blood flow, increased vascular permeability, and increased binding of phagocytes to endothelial cells. C5a also activates mast cells (not shown) to release mediators, such as histamine and TNF-α, that contribute to the inflammatory response. The increase in vessel diameter and permeability leads to the accumulation of fluid and protein. Fluid accumulation increases lymphatic drainage, bringing pathogens and their antigenic components to nearby lymph nodes. The antibodies, complement, and cells thus recruited participate in pathogen clearance by enhancing phagocytosis. The small complement fragments can also directly increase the activity of the phagocytes.

Fig. 2.40 The terminal complement components assemble to form the membrane-attack complex.

The terminal complement components that form the membrane-attack complex		
Native protein	**Active component**	**Function**
C5	C5a	Small peptide mediator of inflammation (high activity)
	C5b	Initiates assembly of the membrane-attack system
C6	C6	Binds C5b; forms acceptor for C7
C7	C7	Binds C5b6; amphiphilic complex inserts in lipid bilayer
C8	C8	Binds C5b67; initiates C9 polymerization
C9	$C9_n$	Polymerizes to C5b678 to form a membrane-spanning channel, lysing cell

kill the pathogen by destroying the proton gradient across the pathogen's cell membrane.

The first step in the formation of the membrane-attack complex is the cleavage of C5 by a C5 convertase to release C5b (see Fig. 2.36). In the next stages, shown in Fig. 2.41, C5b initiates the assembly of the later complement components and their insertion into the cell membrane. First, one molecule of C5b binds one molecule of C6, and the C5b6 complex then binds one molecule of C7. This reaction leads to a conformational change in the constituent molecules, with the exposure of a hydrophobic site on C7, which inserts into the lipid bilayer. Similar hydrophobic sites are exposed on the later components C8 and C9 when they are bound to the complex, allowing these proteins also to insert into the lipid bilayer. C8 is a complex of two proteins, C8β and C8α-γ. The C8β protein binds to C5b, and the binding of C8β to the membrane-associated C5b67 complex allows the hydrophobic domain of C8α-γ to insert into the lipid bilayer. Finally, C8α-γ induces the polymerization of 10–16 molecules of C9 into a pore-forming structure called the membrane-attack complex. The membrane-attack complex, shown schematically and by electron microscopy in Fig. 2.41, has a hydrophobic external face, allowing it to associate with the lipid bilayer, but a hydrophilic internal channel. The diameter of this channel is about 100 Å, allowing the free passage of solutes and water across the lipid bilayer. The disruption of the lipid bilayer leads to the loss of cellular homeostasis, the disruption of the proton gradient across the membrane, the penetration of enzymes such as lysozyme into the cell, and the eventual destruction of the pathogen.

Although the effect of the membrane-attack complex is very dramatic, particularly in experimental demonstrations in which antibodies against red blood cell membranes are used to trigger the complement cascade, the significance

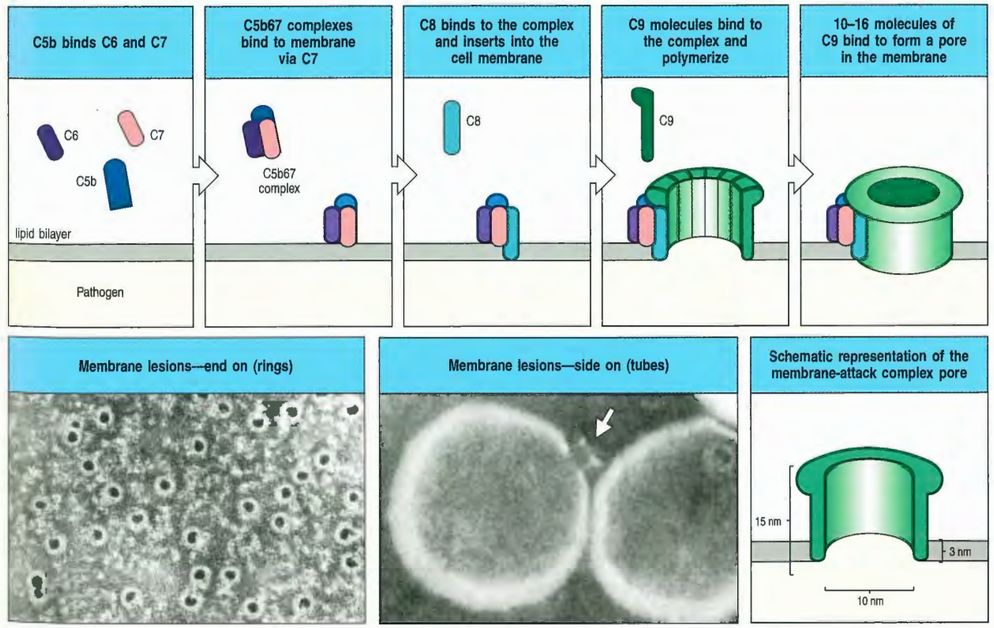


Fig. 2.41 Assembly of the membrane-attack complex generates a pore in the lipid bilayer membrane. The sequence of steps and their approximate appearance are shown here in schematic form. C5b triggers the assembly of a complex of one molecule each of C6, C7, and C8, in that order. C7 and C8 undergo conformational changes, exposing hydrophobic domains that insert into the membrane. This complex causes moderate membrane damage in its own right, and also serves to induce the polymerization of C9, again with the exposure of a hydrophobic site. Up to 16 molecules of C9 are then added to the assembly to generate a channel 100 Å in diameter in the membrane. This channel disrupts the bacterial cell membrane, killing the bacterium. The electron micrographs show erythrocyte membranes with membrane-attack complexes in two orientations, end on and side on. Photographs courtesy of S. Bhakdi and J. Tranum-Jensen.

Deficiency of the C8 Complement Component

of these components in host defense seems to be quite limited. So far, deficiencies in complement components C5–C9 have been associated with susceptibility only to *Neisseria* species, the bacteria that cause the sexually transmitted disease gonorrhea and a common form of bacterial meningitis. Thus, the opsonizing and inflammatory actions of the earlier components of the complement cascade are clearly more important for host defense against infection. Formation of the membrane-attack complex seems to be important only for the killing of a few pathogens, although, as we will see in Chapter 14, it might well have a major role in immunopathology.

2-22 Complement control proteins regulate all three pathways of complement activation and protect the host from its destructive effects.

Given the destructive effects of complement, and the way in which its activation is rapidly amplified through a triggered-enzyme cascade, it is not surprising that there are several mechanisms to prevent its uncontrolled activation. As we have seen, the effector molecules of complement are generated through the sequential activation of zymogens, which are present in plasma in an inactive form. The activation of these zymogens usually occurs on a pathogen surface, and the activated complement fragments produced in the ensuing cascade of reactions usually bind nearby or are rapidly inactivated by hydrolysis. These two features of complement activation act as safeguards against uncontrolled activation. Even so, all complement components are activated spontaneously at a low rate in plasma, and activated complement components will sometimes bind proteins on host cells. The potentially damaging consequences are prevented by a series of complement control proteins, summarized in Fig. 2.42, which regulate the complement cascade at different points. As we saw in discussing the alternative pathway of complement activation (see Section 2-16), many of these control proteins specifically protect normal host cells while allowing complement activation to proceed

Fig. 2.42 The proteins that regulate the activity of complement

Regulatory proteins of the classical and alternative pathways	
Name (symbol)	**Role in the regulation of complement activation**
C1 inhibitor (C1INH)	Binds to activated C1r, C1s, removing them from C1q, and to activated MASP-2, removing it from MBL
C4-binding protein (C4BP)	Binds C4b, displacing C2a; cofactor for C4b cleavage by I
Complement receptor 1 (CR1)	Binds C4b, displacing C2a, or C3b displacing Bb; cofactor for I
Factor H (H)	Binds C3b, displacing Bb; cofactor for I
Factor I (I)	Serine protease that cleaves C3b and C4b; aided by H, MCP, C4BP, or CR1
Decay-accelerating factor (DAF)	Membrane protein that displaces Bb from C3b and C2a from C4b
Membrane cofactor protein (MCP)	Membrane protein that promotes C3b and C4b inactivation by I
CD59 (protectin)	Prevents formation of membrane-attack complex on autologous or allogeneic cells. Widely expressed on membranes

on pathogen surfaces. The complement control proteins therefore allow complement to distinguish self from nonself.

The reactions that regulate the complement cascade are shown in Fig. 2.43. The top two panels show how the activation of C1 is controlled by a plasma serine protease inhibitor or **serpin**, the C1 inhibitor (C1INH). C1INH binds the active enzymes C1r:C1s and causes them to dissociate from C1q, which remains bound to the pathogen. In this way, C1INH limits the time during which active C1s is able to cleave C4 and C2. By the same means, C1INH limits the spontaneous activation of C1 in plasma. Its importance can be seen in the C1INH deficiency disease **hereditary angioneurotic edema**, in which chronic spontaneous complement activation leads to the production of excess cleaved fragments of C4 and C2. The small fragment of C2, C2b, is further cleaved into a peptide, the C2 kinin, which causes extensive swelling—the most dangerous being local swelling in the trachea, which can lead to suffocation. Bradykinin, which has similar actions to C2 kinin, is also produced in an uncontrolled fashion in this disease, as a result of the lack of inhibition of another plasma protease, kallikrein, a component of the kinin system discussed in Section 2-5, which is activated by tissue damage and is also regulated by C1INH. This disease is fully corrected by replacing C1INH. The large activated fragments of C4 and C2, which normally combine to form the C3 convertase, do not damage host cells in such patients because C4b is rapidly inactivated in plasma (see Fig. 2.31) and the convertase does not form. Furthermore, any convertase that accidentally forms on a host cell is inactivated by the mechanisms described below.

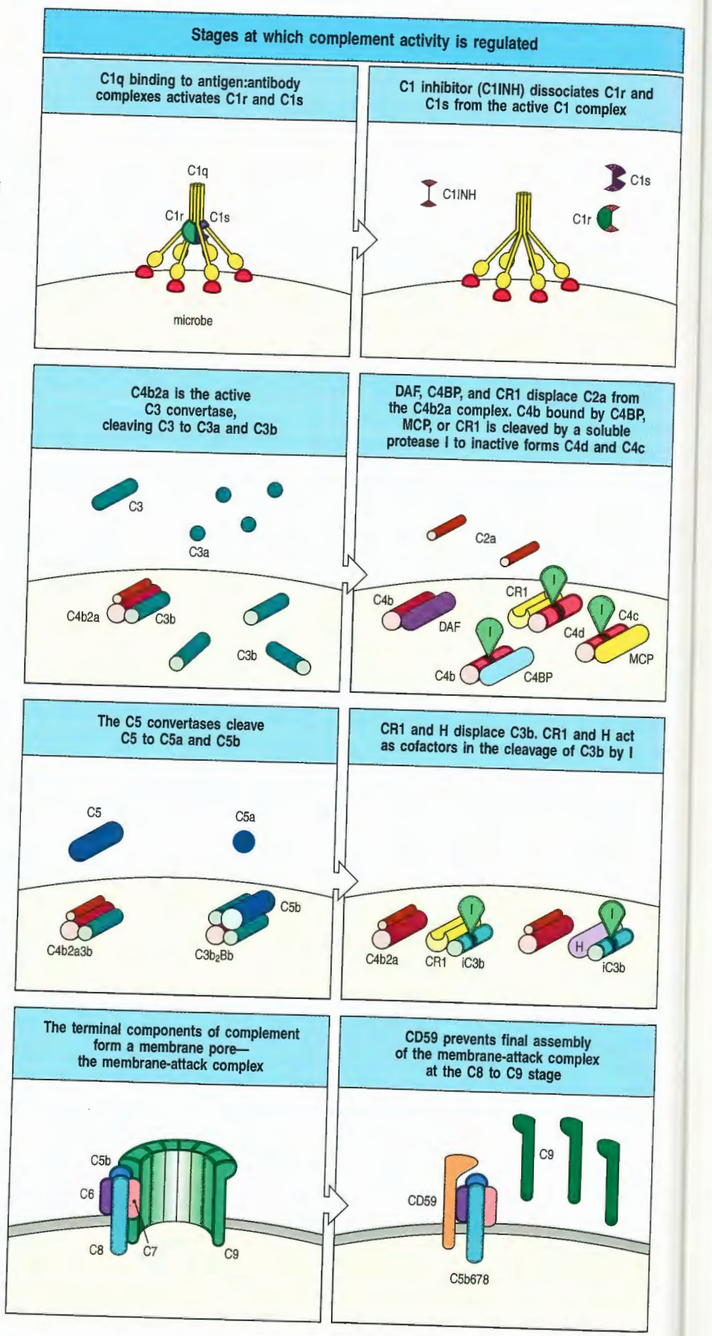
Hereditary Augioneurotic Edema

The thioester bond of activated C3 and C4 is extremely reactive and has no mechanism for distinguishing an acceptor hydroxyl or amine group on a host cell from a similar group on the surface of a pathogen. A series of protective mechanisms, mediated by other proteins, has evolved to ensure that the binding of a small number of C3 or C4 molecules to host cell membranes results in minimal formation of C3 convertase and little amplification of complement activation. We have already encountered most of these mechanisms in the description of the alternative pathway (see Fig. 2.32), but we will consider them again here because they are important regulators of the classical pathway convertase as well (see Fig. 2.43, second and third rows). The mechanisms can be divided into three categories. The first catalyze the cleavage of any C3b or C4b that does bind to host cells into inactive products. The complement-regulatory enzyme responsible is the plasma serine protease factor I; it circulates in active form but can cleave C3b and C4b only when they are bound to a membrane cofactor protein. In these circumstances, factor I cleaves C3b, first into iC3b and then further to C3dg, thus permanently inactivating it. C4b is similarly inactivated by cleavage into C4c and C4d. There are two cell-membrane proteins that bind C3b and C4b and possess cofactor activity for factor I; these are MCP and CR1 (see Section 2-17). Microbial cell walls lack these protective proteins and cannot promote the breakdown of C3b and C4b. Instead, these proteins act as binding sites for factor B and C2, promoting complement activation. The importance of factor I can be seen in people with genetically determined **factor I deficiency**. Because of uncontrolled complement activation, complement proteins rapidly become depleted and such people suffer repeated bacterial infections, especially with ubiquitous pyogenic bacteria.

Factor I Deficiency

There are also plasma proteins with cofactor activity for factor I. C4b is bound by a cofactor known as **C4b-binding protein** (**C4BP**), which acts mainly as a regulator of the classical pathway in the fluid phase. C3b is bound at cell membranes by cofactor proteins such as DAF and MCP. These regulatory molecules effectively compete with factor B for binding to C3b bound to cells. If factor B 'wins,' as typically happens on a pathogen surface, then more

Fig. 2.43 Complement activation is regulated by a series of proteins that serve to protect host cells from accidental damage. These act on different stages of the complement cascade, dissociating complexes or catalyzing the enzymatic degradation of covalently bound complement proteins. Stages in the complement cascade are shown schematically down the left side of the figure, with the regulatory reactions on the right. The alternative pathway C3 convertase is similarly regulated by DAF, CR1, MCP, and factor H.



C3bBb C3 convertase forms and complement activation is amplified. If DAF and MCP win, as occurs on cells of the host, then the bound C3b is catabolized by factor I to iC3b and C3dg and complement activation is inhibited.

The critical balance between the inhibition and the activation of complement on cell surfaces is illustrated in individuals heterozygous for mutations in the regulatory proteins MCP, factor I, or factor H. In such individuals the concentration of functional regulatory proteins is reduced, and the tipping of the balance towards complement activation leads to a predisposition to **hemolytic uremic syndrome**, a condition characterized by damage to platelets and red blood cells and by kidney inflammation, as a result of ineffectively controlled complement activation.

The competition between DAF or MCP and factor B for binding to surface-bound C3b is an example of the second mechanism for inhibiting complement activation on host cells. Several proteins competitively inhibit the binding of C2 to cell-bound C4b and of factor B to cell-bound C3b, thereby inhibiting convertase formation. These proteins bind to C3b and C4b on the cell surface, and also mediate protection against complement through a third mechanism, which is to augment the dissociation of C4b2a and C3bBb convertases that have already formed. Host-cell membrane molecules that regulate complement through both these mechanisms include DAF and CR1, which promote the dissociation of convertase in addition to their cofactor activity. All the proteins that bind the homologous C4b and C3b molecules share one or more copies of a structural element called the short consensus repeat (SCR), complement control protein (CCP) repeat, or (especially in Japan) the sushi domain.

In addition to the mechanisms for preventing C3 convertase formation and C4 and C3 deposition on cell membranes, there are also inhibitory mechanisms that prevent the inappropriate insertion of the membrane-attack complex into membranes. We saw in Section 2-21 that the membrane-attack complex polymerizes onto C5b molecules created by the action of C5 convertase. This complex mainly inserts into cell membranes adjacent to the site of the C5 convertase; that is, close to the site of complement activation on a pathogen. However, some newly formed membrane-attack complexes may diffuse from the site of complement activation and insert into adjacent host-cell membranes. Several plasma proteins including, notably, vitronectin, also known as S-protein, bind to the C5b67 complex and thereby inhibit its random insertion into cell membranes. Host-cell membranes also contain an intrinsic protein, **CD59** or **protectin**, which inhibits the binding of C9 to the C5b678 complex (see Fig. 2.43, bottom row). CD59 and DAF are both linked to the cell surface by a glycosylphosphatidylinositol (GPI) tail, like many other peripheral membrane proteins. One of the enzymes involved in the synthesis of GPI tails is encoded on the X chromosome. In people with a somatic mutation in this gene in a clone of hematopoietic cells, both CD59 and DAF fail to function. This causes the disease **paroxysmal nocturnal hemoglobinuria**, which is characterized by episodes of intravascular red blood cell lysis by complement. Red blood cells that lack CD59 only are also susceptible to destruction as a result of spontaneous activation of the complement cascade.

Summary.

The complement system is one of the major mechanisms by which pathogen recognition is converted into an effective host defense against initial infection. Complement is a system of plasma proteins that can be activated directly by pathogens or indirectly by pathogen-bound antibody, leading to a cascade of reactions that occurs on the surface of pathogens and generates

active components with various effector functions. There are three pathways of complement activation: the classical pathway, which is triggered directly by pathogen or indirectly by antibody binding to the pathogen surface; the lectin pathway; and the alternative pathway, which also provides an amplification loop for the other two pathways. All three pathways can be initiated independently of antibody as part of innate immunity. The early events in all pathways consist of a sequence of cleavage reactions in which the larger cleavage product binds covalently to the pathogen surface and contributes to the activation of the next component. The pathways converge with the formation of a C3 convertase enzyme, which cleaves C3 to produce the active complement component C3b. The binding of large numbers of C3b molecules to the pathogen is the central event in complement activation. Bound complement components, especially bound C3b and its inactive fragments, are recognized by specific complement receptors on phagocytic cells, which engulf pathogens opsonized by C3b and its inactive fragments. The small cleavage fragments of C3, C4, and especially C5 recruit phagocytes to sites of infection and activate them by binding to specific trimeric G-protein-coupled receptors. Together, these activities promote the uptake and destruction of pathogens by phagocytes. The molecules of C3b that bind the C3 convertase itself initiate the late events, binding C5 to make it susceptible to cleavage by C2a or Bb. The larger C5b fragment triggers the assembly of a membrane-attack complex, which can result in the lysis of certain pathogens. The activity of complement components is modulated by a system of regulatory proteins that prevent tissue damage as a result of inadvertent binding of activated complement components to host cells or of spontaneous activation of complement components in plasma.

Induced innate responses to infection.

In the final part of this chapter we will look at the induced responses of innate immunity. These depend on the cytokines and chemokines that are produced in response to pathogen recognition, and we will introduce these first. The chemokines are a large family of chemoattractant molecules with a central role in leukocyte migration. Adhesion molecules also have a central role in this process and we will also briefly consider them. We will then consider in more detail how macrophage-derived chemokines and cytokines promote the phagocytic response through the recruitment and production of fresh phagocytes, and the production of additional opsonizing molecules through the acute-phase reactions. We will also look at the role of the cytokines known as **interferons**, which are induced by viral infection, and at a class of lymphoid cells, known as **natural killer (NK) cells**, that are activated by interferons to contribute to innate host defense against viruses and other intracellular pathogens. We will also discuss the **innate-like lymphocytes (ILLs)**, which contribute to rapid responses to infection by acting early but use a limited set of antigen-receptor gene segments (see Section 1-11) to make immunoglobulins and T-cell receptors. The induced innate responses can either succeed in clearing the infection or contain it while an adaptive response develops. Adaptive immunity harnesses many of the same effector mechanisms that are used by the innate immune system, but adaptive immunity is able to target them with much greater precision. Thus, antigen-specific T cells activate the microbicidal and cytokine-secreting properties of macrophages harboring pathogens, antibodies activate complement, act as direct opsonins for phagocytes, and stimulate NK cells to kill infected cells. In addition, the adaptive immune response uses cytokines and chemokines in a manner similar to that

of innate immunity, to induce inflammatory responses that promote the influx of antibodies and effector lymphocytes to sites of infection. The effector mechanisms described here therefore serve as a primer for the later chapters that focus on adaptive immunity.

2-23 Activated macrophages secrete a range of cytokines that have a variety of local and distant effects.

Cytokines (see Appendix III) are small proteins (approximately 25 kDa) that are released by various cells in the body, usually in response to an activating stimulus, and they induce responses through binding to specific receptors. They can act in an autocrine manner, affecting the behavior of the cell that releases the cytokine, in a paracrine manner, affecting the behavior of adjacent cells, and some cytokines are stable enough to act in an endocrine manner, affecting the behavior of distant cells, although this depends on their ability to enter the circulation and on their half-life in the blood.

The cytokines secreted by macrophages in response to pathogens are a structurally diverse group of molecules that include interleukin-1β (IL-1β), IL-6, IL-12, TNF-α, and the chemokine CXCL8 (formerly known as IL-8). The name **interleukin (IL)** followed by a number (for example IL-1, IL-2, and so on) was coined in an attempt to develop a standardized nomenclature for molecules secreted by, and acting on, leukocytes. However, this became confusing when an ever-increasing number of cytokines with diverse origins, structures, and effects were discovered, and although the IL designation is still used, it is hoped that eventually a nomenclature based on cytokine structure will be developed. The cytokines are listed alphabetically, together with their receptors, in Appendix III. There are two major structural families of cytokines: the **hematopoietin family**, which includes growth hormones and also many interleukins with roles in both adaptive and innate immunity, and the **TNF family**, of which the prototype is TNF-α, which again functions in both innate and adaptive immunity and includes many members that are membrane-bound. Of the macrophage-derived interleukins, IL-6 belongs to the large family of hematopoietins, TNF-α is part of the TNF family, and IL-1 and IL-12 are structurally distinct. All have important local and systemic effects that contribute to both innate and adaptive immunity, and these are summarized in Fig. 2.44.

The recognition of different classes of pathogens by phagocytes and dendritic cells may involve signaling through different receptors, such as the different TLRs, and result in some variation in the cytokines induced. This is a way in which appropriate responses can be selectively activated as the released cytokines orchestrate the next phase of host defense. We will see how TNF-α, which is elicited by LPS-bearing pathogens, is particularly important in containing infection by these pathogens, and how the release of different chemokines can recruit and activate different types of effector cells.

2-24 Chemokines released by phagocytes and dendritic cells recruit cells to sites of infection.

Among the cytokines released by tissues in the earliest phases of infection are members of a family of chemoattractant cytokines known as **chemokines** (listed separately in Appendix IV). These small proteins induce directed chemotaxis in nearby responsive cells and were discovered only relatively recently. Because they were first detected in cytokine assays, they were initially named interleukins: interleukin-8 (now known as CXCL8) was the first chemokine to be cloned and characterized, and it remains typical of this family. All the chemokines are related in amino acid sequence, and their receptors

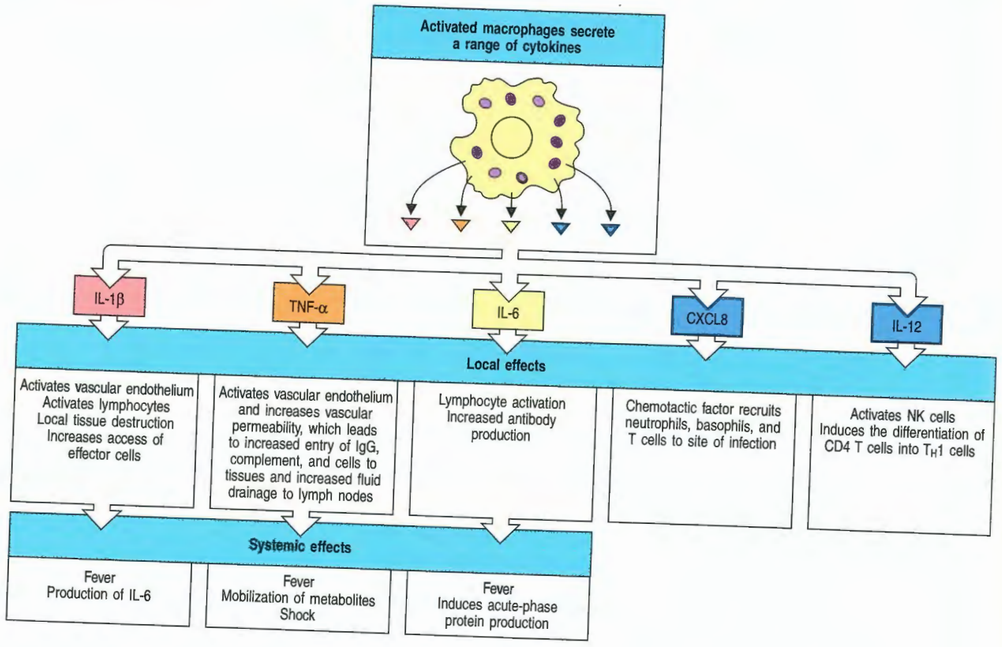


Fig. 2.44 Important cytokines secreted by macrophages in response to bacterial products include IL-1β, IL-6, CXCL8, IL-12, and TNF-α. TNF-α is an inducer of a local inflammatory response that helps to contain infections. It also has systemic effects, many of which are harmful (see Section 2-27). The chemokine CXCL8 is also involved in the local inflammatory response, helping to attract neutrophils to the site of infection. IL-1β, IL-6, and TNF-α have a critical role in inducing the acute-phase response in the liver (see Section 2-28) and induce fever, which favors effective host defense in various ways. IL-12 activates natural killer (NK) cells and favors the differentiation of CD4 T cells into the T_H1 subset in adaptive immunity.

are all seven-span transmembrane proteins that signal through coupled G proteins. No atomic structure of a chemokine receptor has yet been determined, but they are similar to other seven-span G-protein-coupled receptors such as rhodopsin (Fig. 2.45) and the muscarinic acetylcholine receptor. Chemokines function mainly as chemoattractants for leukocytes, recruiting monocytes, neutrophils, and other effector cells from the blood to sites of infection. They can be released by many different types of cells and they serve to guide cells involved in innate immunity to sites of infection. They also guide the lymphocytes in adaptive immunity, as we will learn in Chapters 8–10. Some chemokines also function in lymphocyte development and migration, and in angiogenesis (the growth of new blood vessels). The properties of a variety of chemokines are listed in Fig. 2.46 (see also Appendix IV). It is striking that there are so many chemokines; this may reflect their importance in delivering cells to their correct location, as seems to be the case for lymphocytes.

Members of the chemokine family fall mostly into two broad groups—CC chemokines with two adjacent cysteines near the amino terminus, and CXC chemokines, in which the equivalent two cysteine residues are separated by a single amino acid. The two groups of chemokines act on different sets of receptors. CC chemokines bind to CC chemokine receptors, of which there are nine, designated CCR1–9. CXC chemokines bind to CXC receptors; there are six of these, CXCR1–6. These receptors are expressed on different cell types, which are consequently attracted by different chemokines. In general, CXC chemokines with a Glu-Leu-Arg tripeptide motif immediately before the first cysteine promote the migration of neutrophils. CXCL8 is an example of this type of chemokine. Other CXC chemokines that lack this motif, such as the B-lymphocyte chemokine (CXCL13), guide lymphocytes to their proper

Hereditary Periodic Fever Syndromes

destination in the B-cell areas of the spleen, lymph nodes, and gut. The CC chemokines promote the migration of monocytes, lymphocytes, or other cell types. An example is macrophage chemoattractant protein-1 (CCL2). CXCL8 and CCL2 have similar, although complementary, functions: CXCL8 induces neutrophils to leave the bloodstream and migrate into the surrounding tissues; CCL2, by contrast, acts on monocytes, inducing their migration from the bloodstream to become tissue macrophages. Other CC chemokines such as CCL5 may promote the infiltration into tissues of a range of leukocytes, including effector T cells (see Section 10-6), with individual chemokines acting on different subsets of cells. The only known chemokine with only one cysteine (XCL1) was originally called lymphotactin; it is thought to attract T-cell precursors to the thymus by binding to XCR1. The chemokine fractalkine is unusual in several ways: it has three amino acid residues between the two cysteines, making it a CX_3CL chemokine. Most unusually, it exists in two forms, one tethered to the membrane of the endothelial and epithelial cells that express it, where it serves as an adhesion protein, and a soluble form released from the cell surface, which acts, like other chemokines, as a chemoattractant.

The role of chemokines such as CXCL8 and CCL2 in cell recruitment is twofold. First, they act on the leukocyte as it rolls along endothelial cells at sites of inflammation, converting this rolling into stable binding by triggering a change of conformation in the adhesion molecules known as leukocyte integrins. This allows the leukocyte to cross the blood vessel wall by squeezing between the endothelial cells, as we will see when we describe the process of extravasation. Second, the chemokine directs the migration of the leukocyte along a gradient of chemokine molecules bound to the extracellular matrix and the surfaces of endothelial cells. This gradient increases in concentration toward the site of infection.

Chemokines can be produced by a wide variety of cell types in response to bacterial products, viruses, and agents that cause physical damage, such as silica, alum, or the urate crystals that occur in gout. Thus, infection or physical damage to tissues sets in motion the production of chemokine gradients that can direct phagocytes to the sites where they are needed. In addition, peptides that act as chemoattractants for neutrophils are made by bacteria themselves. The fMLP peptide produced by bacteria is a potent chemotactic factor for inflammatory cells, especially neutrophils (see Section 2-6). The fMLP receptor is also a G-protein-coupled receptor, like the receptors for chemokines and for the complement fragments C5a, C3a, and C4a. Thus, there is a common mechanism for attracting neutrophils, whether by complement fragments, chemokines, or bacterial peptides. Neutrophils are the first cells to arrive in large numbers at a site of infection, with monocytes and immature dendritic cells being recruited later.

The complement peptide C5a and the chemokines CXCL8 and CCL2 also activate their respective target cells, so that not only are neutrophils and macrophages brought to potential sites of infection but, in the process, they are armed to deal with any pathogens they may encounter there. In particular, neutrophils exposed to CXCL8 and the cytokine TNF-α are activated to produce the respiratory burst that generates oxygen radicals and nitric oxide, and to release their stored lysosomal contents, thus contributing both to host defense and to the tissue destruction and pus formation seen in local sites, especially those infected with pyogenic bacteria.

Chemokines do not act alone in cell recruitment. They also require the action of vasoactive mediators to bring leukocytes close to the blood vessel endothelium (see Section 2-5) and cytokines such as TNF-α to induce the necessary adhesion molecules on the endothelial cells. We will return to the chemokines in later chapters, when they will be discussed in the context of



Fig. 2.45 Chemokines are a family of proteins of similar structures that bind to chemokine receptors, themselves part of a large family of G-protein-coupled receptors. The chemokines are represented here by CXCL8 (upper structure). The receptors for the chemokines are members of the family of seven-span receptors, which also includes the photoreceptor protein rhodopsin and many other receptors. They have seven transmembrane helices, and all the family interact with G proteins. The first solved structure of a seven-span membrane protein was of the bacterial protein bacteriorhodopsin; it is depicted here (lower structure), showing the orientation of the seven transmembrane helices (blue) with the bound ligand (in this case retinal) in red. Essentially all of this structure would be embedded within the cell membrane. Cylinders represent α helices and arrows β strands.

the adaptive immune response. We will now turn to the molecules that mediate leukocyte adhesion to the endothelium, and then describe the process of leukocyte extravasation step-by-step as it has been shown for both neutrophils and monocytes.

Class	Chemokine	Produced by	Receptors	Cells attracted	Major effects
CXC	CXCL8 (IL-8)	Monocytes Macrophages Fibroblasts Keratinocytes Endothelial cells	CXCR1 CXCR2	Neutrophils Naive T cells	Mobilizes, activates and degranulates neutrophils Angiogenesis
	CXCL7 (PBP, β-TG NAP-2)	Platelets	CXCR2	Neutrophils	Activates neutrophils Clot resorption Angiogenesis
	CXCL1 (GROα) CXCL2 (GROβ) CXCL3 (GROγ)	Monocytes Fibroblasts Endothelium	CXCR2	Neutrophils Naive T cells Fibroblasts	Activates neutrophils Fibroplasia Angiogenesis
	CXCL10 (IP-10)	Keratinocytes Monocytes T cells Fibroblasts Endothelium	CXCR3	Resting T cells NK cells Monocytes	Immunostimulant Antiangiogenic Promotes T_H1 immunity
	CXCL12 (SDF-1)	Stromal cells	CXCR4	Naive T cells Progenitor ($CD34^+$) B cells	B-cell development Lymphocyte homing Competes with HIV-1
	CXCL13 (BLC)	Stromal cells	CXCR5	B cells	Lymphocyte homing
CC	CCL3 (MIP-1α)	Monocytes T cells Mast cells Fibroblasts	CCR1, 3, 5	Monocytes NK and T cells Basophils Dendritic cells	Competes with HIV-1 Antiviral defense Promotes T_H1 immunity
	CCL4 (MIP-1β)	Monocytes Macrophages Neutrophils Endothelium	CCR1, 3, 5	Monocytes NK and T cells Dendritic cells	Competes with HIV-1
	CCL2 (MCP-1)	Monocytes Macrophages Fibroblasts Keratinocytes	CCR2B	Monocytes NK and T cells Basophils Dendritic cells	Activates macrophages Basophil histamine release Promotes T_H2 immunity
	CCL5 (RANTES)	T cells Endothelium Platelets	CCR1, 3, 5	Monocytes NK and T cells Basophils Eosinophils Dendritic cells	Degranulates basophils Activates T cells Chronic inflammation
	CCL11 (Eotaxin)	Endothelium Monocytes Epithelium T cells	CCR3	Eosinophils Monocytes T cells	Role in allergy
	CCL18 (DC-CK)	Dendritic cells	?	Naive T cells	Role in activating naive T cells
C	XCL1 (Lymphotactin)	CD8>CD4 T cells	CXCR1	Thymocytes Dendritic cells NK cells	Lymphocyte trafficking and development
CXXXC (CX_3C)	CX3CL1 (Fractalkine)	Monocytes Endothelium Microglial cells	CX_3CR1	Monocytes T cells	Leukocyte–endothelial adhesion Brain inflammation

Fig. 2.46 Properties of selected chemokines. Chemokines fall mainly into two related but distinct groups. The CXC chemokines, the genes for which are found mainly in a cluster on chromosome 17 in humans, have an amino acid residue (X) between two invariant cysteines (C) in the amino-terminal region. CC chemokines, which in humans are mostly encoded in one region of chromosome 4, have these two invariant cysteines adjacent. These classes can be divided further by the presence or absence of an amino acid triplet (ELR: glutamic acid–leucine–arginine) preceding the first of these invariant cysteines. All the chemokines that attract neutrophils have this motif, whereas most of the other CXC chemokines, including those that interact with receptors CXCR3, 4, and 5, lack it. A C chemokine with only one cysteine at this location, and fractalkine, a CX_3C chemokine, are encoded elsewhere in the genome. Each chemokine interacts with one or more receptors, and affects one or more types of cells. A comprehensive list of chemokines and their receptors is given in Appendix IV.

2-25 Cell-adhesion molecules control interactions between leukocytes and endothelial cells during an inflammatory response.

The recruitment of activated phagocytes to sites of infection is one of the most important functions of innate immunity. Recruitment occurs as part of the inflammatory response and is mediated by cell-adhesion molecules that are induced on the surface of the local blood vessel endothelium. Before we consider the process of inflammatory cell recruitment, we will first describe some of the cell-adhesion molecules involved.

As with the complement components, a significant barrier to understanding cell-adhesion molecules is their nomenclature. Most adhesion molecules, especially those on leukocytes, which are relatively easy to analyze functionally, were named after the effects of specific monoclonal antibodies directed against them; only later were these characterized by gene cloning. Their names therefore bear no relation to their structure. For instance, the **leukocyte functional antigens LFA-1**, **LFA-2**, and **LFA-3** are actually members of two different protein families. In Fig. 2.47, the adhesion molecules are grouped according to their molecular structure, which is shown in schematic form, alongside their different names, sites of expression, and ligands. Three families of adhesion molecules are important for leukocyte recruitment. The **selectins** are membrane glycoproteins with a distal lectin-like domain that binds specific carbohydrate groups. Members of this family are induced on activated endothelium and initiate endothelium–leukocyte interactions by binding to fucosylated oligosaccharide ligands on passing leukocytes (see Fig. 2.47).

The next step in leukocyte recruitment depends on tighter adhesion, which is due to the binding of **intercellular adhesion molecules** (**ICAMs**) on the endothelium to heterodimeric proteins of the **integrin** family on leukocytes.

		Name	Tissue distribution	Ligand
Selectins Bind carbohydrates. Initiate leukocyte–endothelial interaction	P-selectin	P-selectin (PADGEM, CD62P)	Activated endothelium and platelets	PSGL-1, sialyl-Lewis^x
		E-selectin (ELAM-1, CD62E)	Activated endothelium	Sialyl-Lewis^x
Integrins Bind to cell-adhesion molecules and extracellular matrix. Strong adhesion	LFA-1	α_L:β_2 (LFA-1, CD11a:CD18)	Monocytes, T cells, macrophages, neutrophils, dendritic cells	ICAMs
		α_M:β_2 (CR3, Mac-1, CD11b:CD18)	Neutrophils, monocytes, macrophages	ICAM-1, iC3b, fibrinogen
		α_X:β_2 (CR4, p150.95, CD11c:CD18)	Dendritic cells, macrophages, neutrophils	iC3b
		α_5:β_1 (VLA-5, CD49d:CD29)	Monocytes, macrophages	Fibronectin
Immunoglobulin superfamily Various roles in cell adhesion. Ligand for integrins	ICAM-1	ICAM-1 (CD54)	Activated endothelium	LFA-1, Mac1
		ICAM-2 (CD102)	Resting endothelium, dendritic cells	LFA-1
		VCAM-1 (CD106)	Activated endothelium	VLA-4
		PECAM (CD31)	Activated leukocytes, endothelial cell–cell junctions	CD31

Fig. 2.47 Adhesion molecules involved in leukocyte interactions. Several structural families of adhesion molecules have a role in leukocyte migration, homing, and cell–cell interactions: the selectins, the integrins, and proteins of the immunoglobulin superfamily. The figure shows schematic representations of an example from each family, a list of other family members that participate in leukocyte interactions, their cellular distribution, and their ligand in adhesive interactions. The family members shown here are limited to those that participate in inflammation and other innate immune mechanisms. The same molecules and others participate in adaptive immunity and will be considered in Chapters 8 and 10. The nomenclature of the different molecules in these families is confusing because it often reflects the way in which the molecules were first identified rather than their related structural characteristics. Alternative names for each of the adhesion molecules are given in parentheses. Sulfated sialyl-Lewis^x, which is recognized by P- and E-selectin, is an oligosaccharide present on the cell-surface glycoproteins of circulating leukocytes. Sulfation can occur at either the sixth carbon atom of the galactose or the *N*-acetylglucosamine, but not both.

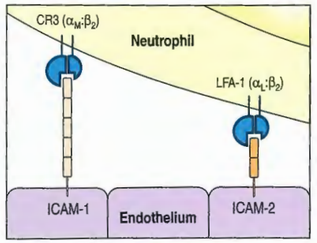


Fig. 2.48 Phagocyte adhesion to vascular endothelium is mediated by integrins. When vascular endothelium is activated by inflammatory mediators it expresses two adhesion molecules, namely ICAM-1 and ICAM-2. These are ligands for integrins expressed by phagocytes—α_M:β_2 (also called CR3, Mac-1, or CD11b:CD18) and $\alpha_L\beta_2$ (also called LFA-1 or CD11a:CD18).

The leukocyte integrins important for extravasation are **LFA-1** (α_L:β_2, also known as CD11a:CD18) and CR3 (α_M:β_2, complement receptor type 3, also known as CD11b:CD18 or Mac-1; we met CR3 in Section 2-19 as a receptor for iC3b, but that is just one of the ligands for this integrin) and they both bind to **ICAM-1** and to **ICAM-2** (Fig. 2.48). Strong adhesion between leukocytes and endothelial cells is promoted by the induction of ICAM-1 on inflamed endothelium and the activation of a conformational change in LFA-1 and CR3 that occurs in response to chemokine binding by the leukocyte. The importance of the leukocyte integrins in inflammatory cell recruitment is illustrated by the disease **leukocyte adhesion deficiency**, which stems from a defect in the β_2 chain common to both LFA-1 and CR3. People with this disease suffer from recurrent bacterial infections and impaired healing of wounds.

The activation of endothelium is driven by interactions with macrophage cytokines, particularly TNF-α, which induce the rapid externalization of granules called **Weibel–Palade bodies** in the endothelial cells. These granules contain preformed **P-selectin**, which is thus expressed within minutes on the surface of local endothelial cells after the production of TNF-α by macrophages. Shortly after the appearance of P-selectin on the cell surface, mRNA encoding **E-selectin** is synthesized, and within 2 hours the endothelial cells are expressing mainly E-selectin. Both these proteins interact with the sulfated sialyl-Lewis^x that is present on the surface of neutrophils.

Resting endothelium carries low levels of ICAM-2, apparently in all vascular beds. This may be used by circulating monocytes to navigate out of the vessels and into their tissue sites. This monocyte migration happens continuously and essentially ubiquitously. However, upon exposure to TNF-α, local expression of ICAM-1 is strongly induced on the endothelium of small vessels near or within the infectious focus. ICAM-1 in turn binds to LFA-1 or CR3 on circulating monocytes and polymorphonuclear leukocytes, in particular neutrophils, as shown in Fig. 2.48. Cell-adhesion molecules have many other roles in the body, directing many aspects of tissue and organ development. In this brief description we have considered only those that participate in the recruitment of inflammatory cells in the hours to days after the establishment of an infection.

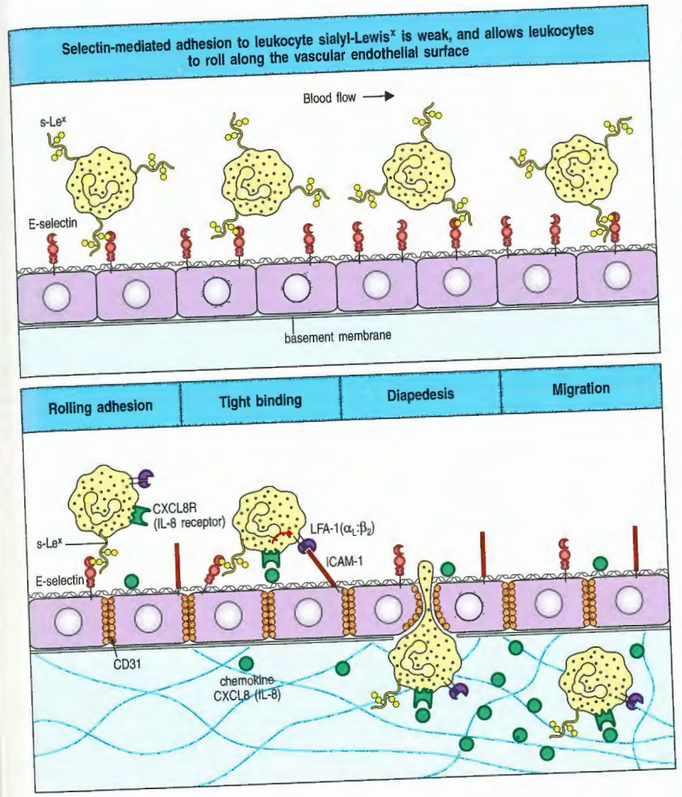
Leukocyte Adhesion Deficiency

2-26 Neutrophils make up the first wave of cells that cross the blood vessel wall to enter inflammatory sites.

The physical changes that accompany the initiation of the inflammatory response have been described in Section 2-5; here we give a step-by-step account of how the required effector cells are recruited into sites of infection. Under normal conditions, leukocytes flow in the center of small blood vessels, where blood flow is fastest. In inflammatory sites, the vessels are dilated and the slower blood flow allows the leukocytes to move out of the center of the blood vessel and interact with the vascular endothelium. Even in the absence of infection, monocytes migrate continuously into the tissues, where they differentiate into macrophages. During an inflammatory response, the induction of adhesion molecules on the endothelial cells by the infection focus, as well as induced changes in the adhesion molecules expressed on leukocytes, recruit large numbers of circulating leukocytes, initially neutrophils and later monocytes, into the site of an infection. The migration of leukocytes out of blood vessels, a process known as extravasation, is thought to occur in four steps. We will describe this process as it is known to occur for monocytes and neutrophils (Fig. 2.49).

The first step involves selectins. P-selectin appears on endothelial cell surfaces within a few minutes of exposure to leukotriene B4, the complement fragment C5a, or histamine, which is released from mast cells in response to C5a. The appearance of P-selectin can also be induced by exposure to TNF-α or LPS, and both of these have the additional effect of inducing the synthesis of a second selectin, E-selectin, which appears on the endothelial cell surface a few hours later. These selectins recognize the sulfated sialyl-Lewis^x moiety of certain leukocyte glycoproteins that are exposed on the tips of leukocyte microvilli. The interaction of P-selectin and E-selectin with these glycoproteins allows monocytes and neutrophils to adhere reversibly to the vessel wall, so that circulating leukocytes can be seen to 'roll' along endothelium that has been treated with inflammatory cytokines (see Fig. 2.49, top panel). This adhesive interaction permits the stronger interactions of the next step in leukocyte migration.

This second step depends on interactions between the leukocyte integrins LFA-1 and CR3 with molecules on endothelium such as ICAM-1, which can also be induced on endothelial cells by TNF-α, and ICAM-2 (see Fig. 2.49, bottom panel). LFA-1 and CR3 normally adhere only weakly, but CXCL8 or other chemokines, bound to proteoglycans on the surface of endothelial cells, bind to specific chemokine receptors on the leukocyte and signal the cell to trigger a conformational change in LFA-1 and CR3 on the rolling leukocyte, which greatly increases the adhesive properties of the neutrophil. As a result of this greatly increased adhesion, the leukocyte attaches firmly to the endothelium and its rolling is arrested.



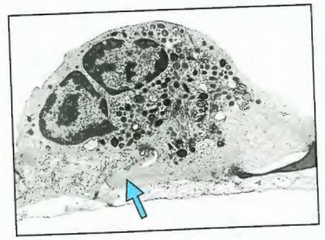


Fig. 2.49 Neutrophils leave the blood and migrate to sites of infection in a multi-step process involving adhesive interactions that are regulated by macrophage-derived cytokines and chemokines. The first step (top panel) involves the reversible binding of a neutrophil to vascular endothelium through interactions between selectins induced on the endothelium and their carbohydrate ligands on the neutrophil, shown here for E-selectin and its ligand the sialyl-Lewisx moiety (s-Lex). This interaction cannot anchor the cells against the shearing force of the flow of blood, and they roll along the endothelium, continually making and breaking contact. The binding does, however, allow stronger interactions, which only result when binding of a chemokine such as CXCL8 to its specific receptor on the neutrophil triggers the activation of the integrins LFA-1 and CR3 (Mac-1) (not shown). Inflammatory cytokines such as TNF-α are also necessary to induce the expression of adhesion molecules such as ICAM-1 and ICAM-2, the ligands for these integrins, on the vascular endothelium. Tight binding between ICAM-1 and the integrins arrests the rolling and allows the neutrophil to squeeze between the endothelial cells forming the wall of the blood vessel (i.e., to extravasate). The leukocyte integrins LFA-1 and CR3 are required for extravasation, and for migration toward chemoattractants. Adhesion between molecules of CD31, expressed on both the neutrophil and the junction of the endothelial cells, is also thought to contribute to extravasation. The neutrophil also needs to traverse the basement membrane; it penetrates this with the aid of a matrix metalloproteinase enzyme that it expresses at the cell surface. Finally, the neutrophil migrates along a concentration gradient of chemokines (shown here as CXCL8) secreted by cells at the site of infection. The electron micrograph shows a neutrophil extravasating between endothelial cells. The blue arrow indicates the pseudopod that the neutrophil is inserting between the endothelial cells. Photograph (× 5500) courtesy of I. Bird and J. Spragg.

In the third step the leukocyte extravasates, or crosses the endothelial wall. This step also involves LFA-1 and CR3, as well as a further adhesive interaction involving an immunoglobulin-related molecule called **PECAM** or **CD31**, which is expressed both on the leukocyte and at the intercellular junctions of endothelial cells. These interactions enable the phagocyte to squeeze between the endothelial cells. It then penetrates the basement membrane with the aid of enzymes that break down the extracellular matrix proteins of the basement membrane. The movement through the basement membrane is known as **diapedesis**, and it enables phagocytes to enter the subendothelial tissues.

The fourth and final step in extravasation is the migration of leukocytes through the tissues under the influence of chemokines. Chemokines such as CXCL8 and CCL2 (see Section 2-24) are produced at the site of infection and bind to proteoglycans in the extracellular matrix and on similar molecules on endothelial cell surfaces. This forms a matrix-associated concentration gradient of chemokine on a solid surface along which the leukocyte can migrate to the focus of infection (see Fig. 2.49). CXCL8 is released by the macrophages that first encounter pathogens and recruits neutrophils, which enter the infected tissue in large numbers in the early part of the induced response. Their influx usually peaks within the first 6 hours of an inflammatory response, whereas monocytes can be recruited later, through the action of chemokines such as CCL2. Once in an inflammatory site, neutrophils are able to eliminate many pathogens by phagocytosis. They act as phagocytic effectors in an innate immune response through receptors that opsonize or capture infectious agents and their derived components through innate immune recognition as well as by recognizing pathogens directly. In addition, as we will see in Chapter 9, they act as phagocytic effectors in humoral adaptive immunity. The importance of neutrophils is dramatically illustrated by diseases or treatments that severely reduce neutrophil numbers. Such patients are said to have **neutropenia**, and they are very susceptible to deadly infection with a wide range of pathogens and commensal organisms. Restoring neutrophil levels in such patients by transfusion of neutrophil-rich blood fractions or by stimulating their production with specific growth factors largely corrects this susceptibility.

2-27 TNF-α is an important cytokine that triggers local containment of infection but induces shock when released systemically.

Inflammatory mediators also stimulate endothelial cells to express proteins that trigger blood clotting in the local small vessels, occluding them and thus cutting off blood flow. This can be important in preventing the pathogen from entering the bloodstream and spreading through the blood to organs all over the body. Instead, the fluid that has leaked into the tissue in the early phases of an infection carries the pathogen, usually enclosed in dendritic cells, via the lymph to the regional lymph nodes, where an adaptive immune response can be initiated. The importance of TNF-α in the containment of local infection is illustrated by experiments in which rabbits were infected locally with a bacterium. Normally, the infection would be contained at the site of the inoculation; if, however, an injection of anti-TNF-α antibody was also given to block the action of TNF-α, the infection spread via the blood to other organs.

Once an infection spreads to the bloodstream, however, the same mechanisms by which TNF-α so effectively contains local infection instead become catastrophic (Fig. 2.50). The presence of infection in the bloodstream, or sepsis, is accompanied by the release of TNF-α by macrophages in the liver, spleen, and other systemic sites. The systemic release of TNF-α causes vasodilation, which leads to a loss of blood pressure and increased vascular permeability, leading

to a loss of plasma volume and eventually to shock. In septic shock, disseminated intravascular coagulation (blood clotting) is also triggered by TNF-α, leading to the generation of clots in many small vessels and the massive consumption of clotting proteins, thus causing the patient's ability to clot blood appropriately to be lost. This condition frequently leads to the failure of vital organs such as the kidneys, liver, heart, and lungs, which are quickly compromised by the failure of normal perfusion; consequently, septic shock has a very high mortality rate.

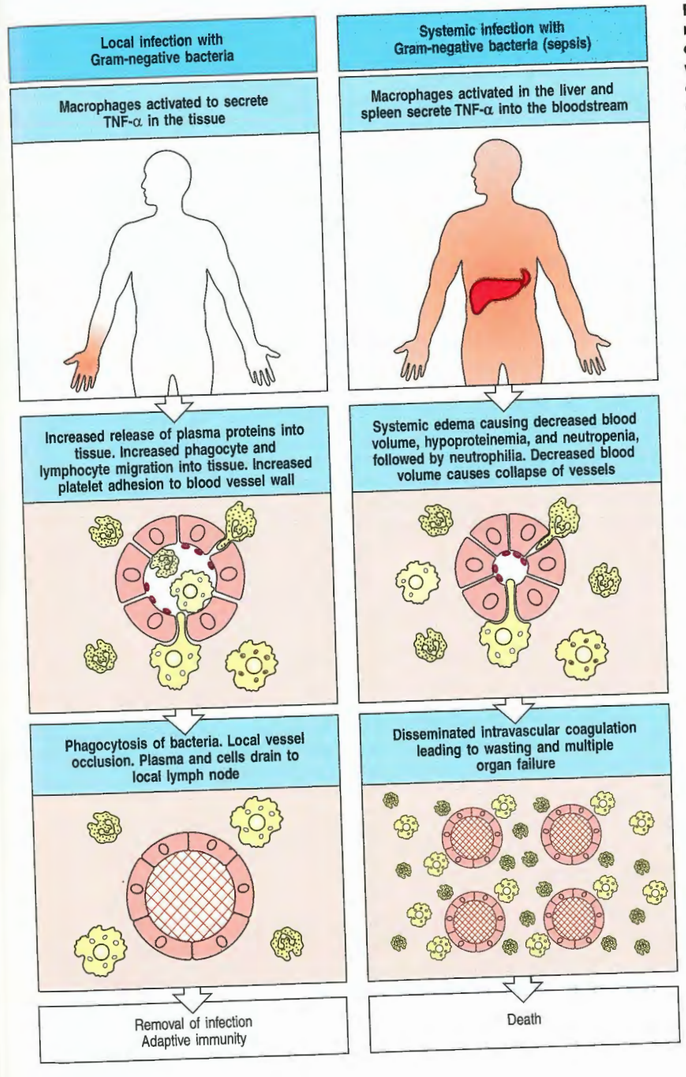


Fig. 2.50 The release of TNF-α by macrophages induces local protective effects, but TNF-α can be damaging when released systemically. The panels on the left show the causes and consequences of local release of TNF-α, and the panels on the right show the causes and consequences of systemic release. In both cases TNF-α acts on blood vessels, especially venules, to increase blood flow and vascular permeability to fluid, proteins, and cells, and to increase endothelial adhesiveness for leukocytes and platelets (center panels). Local release thus allows an influx of fluid, cells, and proteins into the infected tissue, where they participate in host defense. Later, blood clots form in the small vessels (lower left panel), preventing spread of infection via the blood, and the accumulated fluid and cells drain to regional lymph nodes, where an adaptive immune response is initiated. When there is a systemic infection, or sepsis, with bacteria that elicit TNF-α production, TNF-α is released into the blood by macrophages in the liver and spleen and acts in a similar way on all small blood vessels (lower right panel). The result is shock, disseminated intravascular coagulation with depletion of clotting factors, and consequent bleeding, multiple organ failure, and frequently death.

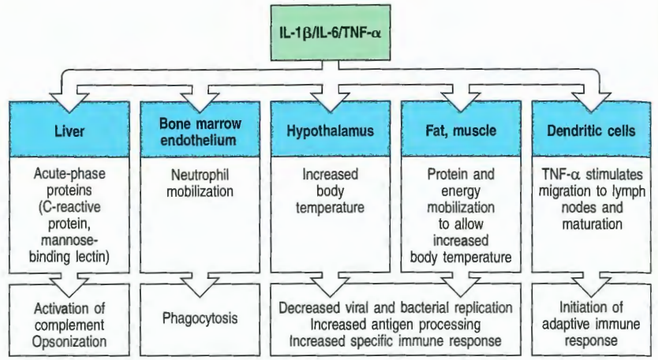


Fig. 2.51 The cytokines TNF-α, IL-1β, and IL-6 have a wide spectrum of biological activities that help to coordinate the body's responses to infection. IL-1β, IL-6, and TNF-α activate hepatocytes to synthesize acute-phase proteins, and bone marrow endothelium to release neutrophils. The acute-phase proteins act as opsonins, whereas the disposal of opsonized pathogens is augmented by the enhanced recruitment of neutrophils from the bone marrow. IL-1β, IL-6, and TNF-α are also endogenous pyrogens, raising body temperature, which is believed to help in eliminating infections. A major effect of these cytokines is to act on the hypothalamus, altering the body's temperature regulation, and on muscle and fat cells, altering energy mobilization to increase the body temperature. At higher temperatures, bacterial and viral replication is less efficient, whereas the adaptive immune response operates more efficiently.

Mice with a mutant TNF-α receptor gene are resistant to septic shock; however, such mice are also unable to control local infection. The features of TNF-α that make it so valuable in containing local infection are precisely those that give it a central role in the pathogenesis of septic shock. It is clear from the evolutionary conservation of TNF-α that its benefits in the former area far outweigh the devastating consequences of its systemic release.

2-28 Cytokines released by phagocytes activate the acute-phase response.

As well as their important local effects, the cytokines produced by macrophages have long-range effects that contribute to host defense. One of these is the elevation of body temperature, which is caused mainly by TNF-α, IL-1β, and IL-6. These are termed **endogenous pyrogens** because they cause fever and derive from an endogenous source rather than from bacterial components such as LPS, which is an **exogenous pyrogen**. Endogenous pyrogens cause fever by inducing the synthesis of prostaglandin E2 by the enzyme cyclooxygenase-2, the expression of which is induced by these cytokines. Prostaglandin E2 then acts on the hypothalamus, resulting in an increase in heat production by brown fat and increased vasoconstriction, decreasing the loss of excess heat through the skin. Exogenous pyrogens are able to induce fever both by inducing the production of the endogenous pyrogens and also by directly inducing cyclooxygenase-2 as a consequence of signaling through TLR-4, leading to the production of prostaglandin E2. Fever is generally beneficial to host defense; most pathogens grow better at lower temperatures and adaptive immune responses are more intense at elevated temperatures. Host cells are also protected from the deleterious effects of TNF-α at raised temperatures.

Hereditary Periodic Fever Syndromes

The effects of TNF-α, IL-1, and IL-6 are summarized in Fig. 2.51. One of the most important of these is the initiation of a response known as the **acute-phase response** (Fig. 2.52). This involves a shift in the proteins synthesized and secreted by the liver into the plasma and results from the action of IL-1, IL-6, and TNF-α on hepatocytes. In the acute-phase response, levels of some plasma proteins go down, while levels of others increase markedly. The proteins whose synthesis is induced by TNF-α, IL-1, and IL-6 are called **acute-phase proteins**. Several of these are of particular interest because they mimic the action of antibodies, but, unlike antibodies, these proteins have broad specificity for PAMPs and depend only on the presence of cytokines for their production.

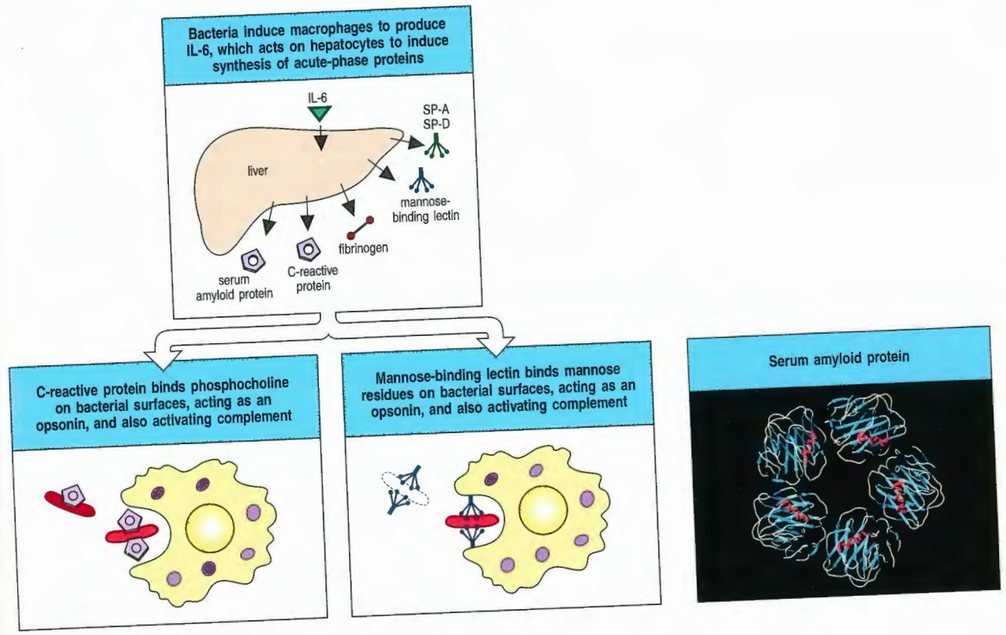


Fig. 2.52 The acute-phase response produces molecules that bind pathogens but not host cells. Acute-phase proteins are produced by liver cells in response to cytokines released by macrophages in the presence of bacteria. They include serum amyloid protein (SAP) (in mice but not humans), C-reactive protein (CRP), fibrinogen, and mannose-binding lectin (MBL). SAP and CRP are homologous in structure; both are pentraxins, forming five-membered discs, as shown for SAP (photograph on the right). CRP binds phosphocholine on certain bacterial and fungal surfaces but does not recognize it in the form in which it is found in host cell membranes. It both acts as an opsonin in its own right and activates the classical complement pathway by binding C1q to augment opsonization. MBL is a member of the collectin family, which includes the lung surfactant proteins SP-A and SP-D. It also resembles C1q in its structure. Like CRP, MBL can act as an opsonin in its own right, as can SP-A and SP-D. Model structure courtesy of J. Emsley.

One of these proteins, the **C-reactive protein**, is a member of the **pentraxin** protein family, so called because they are formed from five identical subunits. C-reactive protein is another example of a multipronged pathogen-recognition molecule, and binds to the phosphocholine portion of certain bacterial and fungal cell-wall lipopolysaccharides. Phosphocholine is also found in mammalian cell membrane phospholipids, but it cannot be bound by C-reactive protein. When C-reactive protein binds to a bacterium, it is not only able to opsonize it but can also activate the complement cascade by binding to C1q, the first component of the classical pathway of complement activation, as we learnt in Section 2-13. The interaction with C1q involves the collagen-like parts of C1q rather than the globular heads contacted by pathogen surfaces, but the same cascade of reactions is initiated.

The second acute-phase protein of interest is MBL, which we have already met as a pathogen-binding molecule (see Fig. 2.15) and as a trigger for the complement cascade (see Section 2-14). MBL is found in normal serum at low levels but is produced in increased amounts during the acute-phase response. It acts as an opsonin for monocytes, which, unlike tissue macrophages, do not express the macrophage mannose receptor. Two other proteins with opsonizing properties that are produced by the liver in increased quantities during the acute-phase response are the pulmonary surfactant proteins SP-A and SP-D (see Section 2-6). They are found along with macrophages in the alveolar fluid of the lung and are important in promoting the phagocytosis of respiratory pathogens such as *Pneumocystis carinii*, one of the main causes of pneumonia in patients with AIDS.

Thus, within a day or two, the acute-phase response provides the host with several proteins with the functional properties of antibodies but able to bind a broad range of pathogens. However, unlike the antibodies we shall learn

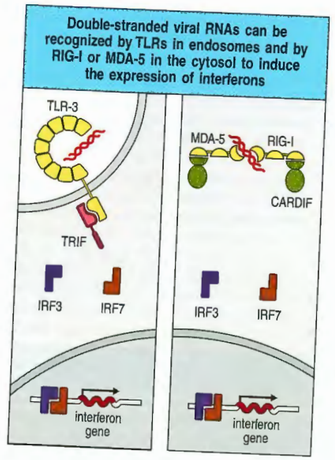


Fig. 2.53 Double-stranded RNAs induce the expression of interferons by activating the interferon regulatory factors IRF3 and IRF7. Long double-stranded RNAs can be recognized by the Toll-like receptor TLR-3, which is present in endosomes (left panel). TLR-3 signals through an adaptor molecule, TRIF, to activate the transcription factors IRF3 and IRF7. Similarly, the intracellular receptors RIG-I and MDA-5 also bind long double-stranded RNAs (right panel) and activate IRF3 and IRF7, but in this case through the adaptor protein CARDIF (CARD adaptor inducing IFN-β). Activated IRF3 and IRF7 can form both homodimers (not shown) and IRF3:IRF7 heterodimers that enter the nucleus and activate the transcription of a number of genes, principally those for IFN-α and IFN-β.

about in Chapters 3 and 9 they have no structural diversity and are made in response to any stimulus that triggers the release of TNF-α, IL-1, and IL-6. Therefore, unlike antibodies, their synthesis is not specifically induced and targeted.

A final distant effect of the cytokines produced by phagocytes is to induce a **leukocytosis**, an increase in circulating neutrophils. The neutrophils come from two sources: the bone marrow, from which mature leukocytes are released in increased numbers; and sites in blood vessels where they are attached loosely to endothelial cells. Thus, the effects of these cytokines contribute to the control of infection while the adaptive immune response is being developed. As shown in Fig. 2.51, TNF-α also has a role in stimulating the migration of dendritic cells from their sites in peripheral tissues to the lymph node and in their maturation into nonphagocytic but highly co-stimulatory antigen-presenting cells.

2-29 Interferons induced by viral infection make several contributions to host defense.

Infection of cells with viruses induces the production of proteins that are known as interferons, because they were found to interfere with viral replication in previously uninfected tissue culture cells. They are believed to have a similar role *in vivo*, blocking the spread of viruses to uninfected cells. These antiviral effector molecules, called **IFN-α** and **IFN-β**, are quite distinct from **IFN-γ**. This cytokine is not directly induced by viral infection but is produced later and does have an important role in the adaptive immune response to intracellular pathogens, as we will see in later chapters. IFN-α, actually a family of several closely related proteins, and IFN-β, the product of a single gene, are synthesized by many cell types after their infection by diverse viruses. Interferon synthesis is thought to occur in response to the presence of double-stranded RNA, because synthetic double-stranded RNA is a potent inducer of IFN-α and IFN-β. Double-stranded RNA forms the genome of some viruses and might be made as part of the infectious cycle of all viruses. Although double-stranded RNA molecules are found in mammalian cells, they are present only as relatively short molecules, usually less than 100 nucleotides long, whereas double-stranded RNA genomes are thousands of nucleotides long. Therefore, long double-stranded RNA molecules might be the common element in interferon induction; such long molecules are recognized as a distinct molecular pattern by the Toll-like receptor TLR-3 (Fig. 2.53), which induces the synthesis of IFN-α and IFN-β.

Long double-stranded viral RNA can also induce the expression of interferons by activating the cytoplasmic proteins **RIG-I** and **MDA-5** (see Fig. 2.53). These proteins contain RNA helicase-like domains that bind double-stranded RNAs, and two CARD domains (see Section 2-9) that allow them to interact with adaptor proteins within the cell to deliver a signal that viral RNAs are present. For both RIG-I and MDA-5, the adaptors link the binding of double-stranded RNAs to the activation of the interferon-regulatory factors IRF3 and IRF7, transcription factors that induce the production of IFN-α and IFN-β.

Interferons make several contributions to defense against viral infection (Fig. 2.54). An obvious and important effect is the induction of a state of resistance to viral replication in all cells. IFN-α and IFN-β secreted by the infected cell bind to a common cell-surface receptor, known as the **interferon receptor**, on both the infected cell and nearby uninfected cells. The interferon receptor, like many other cytokine receptors, is coupled to a **Janus-family tyrosine kinase**, through which it signals. This signaling pathway, which we will describe in detail in Chapter 6, rapidly induces new gene transcription as the

Janus-family kinases directly phosphorylate signal-transducing activators of transcription known as **STAT proteins**. The phosphorylated STAT proteins enter the nucleus, where they activate the transcription of several different genes, including those encoding proteins that help to inhibit viral replication.

One such protein is the enzyme **oligoadenylate synthetase**, which polymerizes ATP into 2′–5′-linked oligomers (nucleotides in nucleic acids are normally linked 3′–5′). These activate an endoribonuclease that then degrades viral RNA. A second protein activated by IFN-α and IFN-β is a serine–threonine kinase called **PKR kinase**. This phosphorylates the eukaryotic protein synthesis initiation factor eIF-2, inhibiting translation and thus further contributing to the inhibition of viral replication. Another interferon-inducible protein, called **Mx**, is known to be required for cellular resistance to influenza virus replication. Mice that lack the gene for Mx are highly susceptible to infection with the influenza virus, whereas mice that can make Mx are not. Another way in which interferons act in innate immunity is to activate NK cells, which can kill virus-infected cells, as described in more detail in the next section.

Last, interferons have a more general role in the process by which recognition of pathogens by the innate immune system underlies and augments activation of the adaptive immune response. We have already discussed how recognition of double-stranded RNAs by TLR-3 can lead to the induction of IFN-α and IFN-β. Other TLRs, notably TLR-4, can also induce these interferons in response to the recognition of bacterial cell-wall components. Interferons in turn induce the expression of co-stimulatory molecules on macrophages and dendritic cells, which enables them to act as antigen-presenting cells that can fully activate T cells (see Section 1-7). Thus, a macrophage or dendritic cell that becomes activated when its Toll-like receptors bind pathogens is in turn able to signal to other macrophages and dendritic cells and recruit them to initiate an adaptive immune response. IFN-α and IFN-β also stimulate the increased expression of MHC class I molecules on all types of cells. The cytotoxic T lymphocytes of the adaptive immune system recognize virus-infected cells by the complexes of viral antigens and MHC class I molecules they display on their surface (see Fig. 1.30), and so, indirectly, interferons help promote the killing of virus-infected cells by CD8 cytotoxic T cells.

Almost all types of cells can produce IFN-α and IFN-β if necessary, but some cells seem to be specialized for the task. The **plasmacytoid dendritic cells** (also known as interferon-producing cells or natural interferon-producing cells) are circulating dendritic cells that accumulate in peripheral lymphoid tissues during an infection and can secrete up to 1000 times more interferon than other cell types. They are described in more detail in Chapter 8.

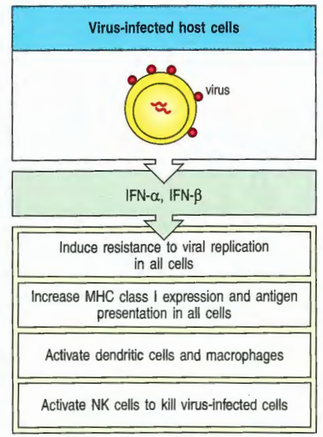


Fig. 2.54 Interferons are antiviral proteins produced by cells in response to viral infection. The interferons IFN-α and IFN-β have three major functions. First, they induce resistance to viral replication in uninfected cells by activating genes that cause the destruction of mRNA and inhibit the translation of viral and some host proteins. Second, they can induce MHC class I expression in most cell types in the body, thus enhancing their resistance to NK cells; they may also induce increased synthesis of MHC class I molecules in cells that are newly infected by virus, thus making them more susceptible to killing by CD8 cytotoxic T cells (see Chapter 8). Third, they activate NK cells, which then selectively kill virus-infected cells.

2-30 NK cells are activated by interferons and macrophage-derived cytokines to serve as an early defense against certain intracellular infections.

Natural killer cells (**NK cells**) develop in the bone marrow from the common lymphoid progenitor cell and circulate in the blood. They are larger than T and B lymphocytes, have distinctive cytoplasmic granules, and are functionally identified by their ability to kill certain lymphoid tumor cell lines *in vitro* without the need for prior immunization or activation. The mechanism of killing by NK cells is the same as that used by the cytotoxic T cells generated in an adaptive immune response; cytotoxic granules are released onto the surface of the bound target cell, and the effector proteins they contain penetrate the cell membrane and induce programmed cell death. However, NK cell killing is triggered by invariant receptors that recognize components of infected cell surfaces, and their known function in host defense is in the early

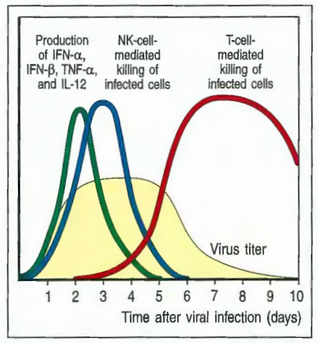


Fig. 2.55 Natural killer cells (NK cells) are an early component of the host response to virus infection. Experiments in mice have shown that IFN-α, IFN-β, and the cytokines TNF-α and IL-12 appear first, followed by a wave of NK cells, which together control virus replication but do not eliminate the virus. Virus elimination is accomplished when virus-specific CD8 T cells are produced. Without NK cells, the levels of some viruses are much higher in the early days of the infection, and can be lethal unless treated vigorously with antiviral compounds.

phases of infection with several intracellular pathogens, particularly herpes viruses and the protozoan parasite *Leishmania*. NK cells are classed as part of the innate immune system because of their invariant receptors.

NK cells are activated in response to interferons or macrophage-derived cytokines. Although NK cells that can kill sensitive targets can be isolated from uninfected individuals, this activity is increased 20–100-fold when NK cells are exposed to IFN-α and IFN-β or to the NK-cell-activating factor IL-12, which is one of the cytokines produced early in many infections. Activated NK cells serve to contain virus infections while the adaptive immune response is generating antigen-specific cytotoxic T cells that can clear the infection (Fig. 2.55). At present the only clue to the physiological function of NK cells in humans comes from rare patients deficient in these cells. Such patients prove to be highly susceptible to the early phases of herpes virus infection. A similar finding has recently been made in mice infected with murine cytomegalovirus, a herpes virus.

IL-12, acting in synergy with TNF-α, can also stimulate NK cells to secrete large amounts of the cytokine IFN-γ, and this is crucial in controlling some infections before the IFN-γ produced by activated CD8 cytotoxic T cells becomes available. This early production of IFN-γ by NK cells may also influence the CD4 T-cell response to infectious agents, inducing activated CD4 T cells to differentiate into inflammatory T_H1 cells able to activate macrophages (see Section 8-19).

2-31 NK cells possess receptors for self molecules that prevent their activation by uninfected cells.

If NK cells are to defend the body against infection with viruses and other pathogens, they must have some mechanism for distinguishing infected from uninfected cells. Exactly how this is achieved has not yet been worked out in every case, but it is thought that an NK cell is activated by a combination of direct recognition of changes in cell-surface glycoproteins induced by metabolic stress, such as malignant transformation or viral or bacterial infection, together with a recognition of 'altered self', which involves changes in the expression of MHC molecules. Altered expression of MHC class I molecules may be a common feature of cells infected by intracellular pathogens, because many of these pathogens have developed strategies to interfere with the ability of MHC class I molecules to capture and display peptides to T cells. Thus, one mechanism by which NK cells distinguish infected from uninfected cells is by recognizing alterations in MHC class I expression (Fig. 2.56).

NK cells are able to sense changes in the expression of MHC class I molecules by integrating the signals from two types of surface receptors, which together control their cytotoxic activity. One type is an activating receptor that triggers killing by the NK cell. Several classes of receptor provide this activation signal, including members of the immunoglobulin-like and the C-type lectin families of proteins. Stimulation of these receptors activates the NK cells, causing the release of cytokines such as IFN-γ and the directed killing of the stimulating cell through the release of cytotoxic granules containing granzymes and perforin. This killing mechanism is the same as that used by cytotoxic T cells and will be described in detail when we discuss the functions of that effector T-cell population in Chapter 8. NK cells also carry receptors for immunoglobulin, and binding of antibodies to these receptors activates NK cells to release their cytotoxic granules; this is known as antibody-dependent cellular cytotoxicity, or ADCC, and will be described in Chapter 9. A second set of receptors inhibits activation and prevents NK cells from killing normal host cells. These inhibitory receptors are specific for various MHC class I molecules, which helps to explain why NK cells selectively kill cells bearing low levels of

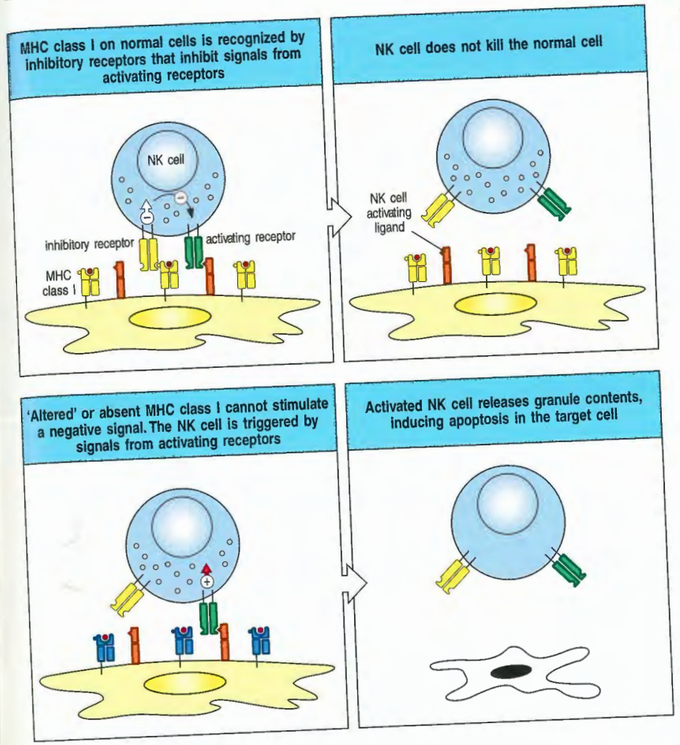


Fig. 2.56 NK cell killing depends on the balance between activating and inhibitory signals. NK cells have several different activating receptors that recognize common carbohydrate ligands on the surfaces of the cells of the body and signal the NK to kill the bound cell. However, NK cells are prevented from a wholesale attack by another set of receptors that recognize MHC class I molecules (which are present on almost all cell types) and inhibit killing by overruling the actions of the activating receptors. This inhibitory signal is lost when the target cells do not express MHC class I and perhaps also in cells infected with virus, many of which specifically inhibit MHC class I expression or alter its conformation so as to avoid recognition by CD8 T cells. Another possibility is that normal uninfected cells respond to IFN-α and IFN-β by increasing the expression of MHC class I molecules, making them resistant to killing by activated NK cells. In contrast, infected cells can fail to increase MHC class I expression, making them targets for activated NK cells.

MHC class I molecules but are prevented from killing cells with normal numbers. The higher the level of expression of MHC class I on a cell surface, the better protected it is against destruction by NK cells. This is why interferons, which induce the expression of MHC class I molecules, can protect uninfected host cells from NK cells. At the same time the interferon activates the NK cell to kill virus-infected cells.

The receptors that regulate the activity of NK cells fall into two large families (Fig. 2.57) that contain a number of other cell-surface receptors in addition to the NK receptors. One is composed of receptors homologous to C-type lectins; these are called **killer lectin-like receptors** (**KLRs**). The genes for KLRs are found within a gene cluster called the NK receptor complex, or NKC.

The other family of receptors is composed of receptors with immunoglobulin-like domains; hence their name **killer cell immunoglobulin-like receptors** (**KIRs**). The KIR genes form part of a larger cluster of immunoglobulin-like receptors known as the leukocyte receptor complex, or LRC. Both the NKC and LRC clusters are present in mice and in humans, but mice lack the KIR genes and therefore rely on the C-type lectin-like receptors of the NKC to control their NK-cell activity.

One complication in understanding the regulation of NK cell activity is that the same structural families of NK receptors contain both activating and inhibitory receptors. In humans and mice, NK cells express a heterodimer of two C-type lectins, called CD94 and NKG2, which interacts with nonpolymorphic MHC

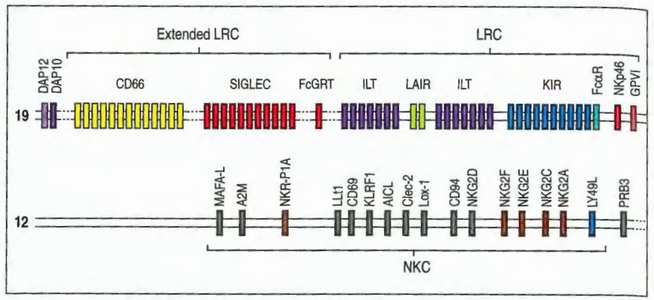


Fig. 2.57 The genes that encode NK receptors fall into two large families. The first, the leukocyte receptor complex (LRC), comprises a large cluster of genes encoding a family of proteins composed of immunoglobulin-like domains. These include the killer immunoglobulin-like receptors (KIRs) expressed by NK cells, the ILT (immunoglobulin-like transcript) class, and the leukocyte-associated immunoglobulin-like receptor (LAIR) gene families. The signaling lectins (SIGLECs) and members of the CD66 family are located nearby. In humans, this complex is located on chromosome 19. The second gene cluster is called the NK receptor complex (NKC), and encodes killer lectin-like receptors, a receptor family that includes the NKG2 proteins and CD94, with which an NKG2 molecule pairs to form a functional receptor. This complex is located on human chromosome 12. Some NK receptor genes are found outside of these two major gene clusters; for example, the genes for the natural cytotoxicity receptors NKp30 and NKp44 are located within the major histocompatibility complex on chromosome 6. Figure based on data courtesy of J. Trowsdale, University of Cambridge.

class I-like molecules, including HLA-E in humans and Qa-1 in mice, that bind leader peptide fragments from other MHC class I molecules. Thus, CD94:NKG2 could be sensitive to the presence of several different MHC class I variants. In humans, the NKG2 family has six members, NKG2A, B, C, D, E, and F. Of these, for example, NKG2A and B are inhibitory, whereas NKG2C is activating (Fig. 2.58). NKG2D is also activating but is distinct from the other NKG2 family members and will be discussed separately in the next section. In mice, Ly49H, a member of the Ly49 C-type lectin family, seems to be distinct, because binding of this molecule is an activating event that triggers a cytotoxic response, whereas binding to the other Ly49 members is inhibitory.

In the KIR receptor family, too, some members are activating whereas others are inhibitory. Different KIR genes also encode proteins with differing numbers of immunoglobulin domains; some, called KIR-2D, have two immunoglobulin domains while others, called KIR-3D, have three. Whether a KIR protein is activating or inhibitory depends on the presence or absence of specific signaling motifs in its cytoplasmic domain. Sequence motifs that are recognized by intracellular inhibitory adaptor proteins are found in KIR proteins that have long cytoplasmic tails; these proteins are designated KIR-2DL and KIR-3DL. KIR proteins with short cytoplasmic tails lack these inhibitory motifs, and instead associate with the activating adaptor protein DAP12 (also known as KARAP). Activating KIR receptors are therefore designated as KIR-2DS and KIR-3DS (see Fig. 2.58). Other inhibitory NK receptors specific for the products of the MHC class I loci are rapidly being defined, and all are members of either the immunoglobulin-like KIR family or the Ly49-like C-type lectins. Clearly, the regulation of NK-cell activity is complex, and whether any individual NK cell is activated by another cell will depend on the overall balance of activating and inhibitory receptors that the NK cell is expressing.

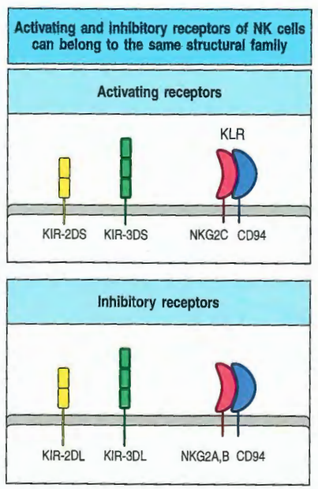


Fig. 2.58 The structural families of NK receptors encode both activating and inhibitory receptors. The families of killer immunoglobulin-like receptors (KIR) and killer lectin-like receptors (KLR) have members that send activating signals to the NK cell, as shown in the upper panel, and those that send inhibitory signals, as shown in the lower panel. KIR family members are designated according to the number of immunoglobulin-like domains they possess and by the length of their cytoplasmic tails. Activating KIR receptors have short cytoplasmic tails and bear the designation 'S'. These associate with a signaling adaptor protein, DAP12. The activating KLR receptor is a heterodimer of NKG2C with another C-type lectin family member, CD94. The inhibitory KIR receptors have longer cytoplasmic tails and are designated 'L'; these do not associate constitutively with adaptor proteins but contain signaling motifs that when phosphorylated are recognized by inhibitory phosphatases. Like the activating KLR, the inhibitory KLRs (NKG2A and NKG2B) form heterodimers with CD94.

The overall response of NK cells to differences in MHC expression is further complicated by the polymorphism of KIR genes; for example, for one of the KIR genes there are two alleles, one of which is activating and the other inhibitory. Moreover, the KIR gene cluster seems to be a very dynamic part of the human genome, because different numbers of activating and inhibitory KIR genes are found in different people. What advantage this diversity might have is not clear. As noted earlier, the KIR locus is not present in mice, and they use only the KLR molecules to regulate NK cell activity. So whatever the driving force is for the evolution of the KIR locus and its diversity, it may have arisen relatively recently in evolutionary terms.

Signaling by the inhibitory NK receptors suppresses the killing activity of NK cells. This means that NK cells will not kill healthy, genetically identical cells with normal expression of MHC class I molecules, such as the other cells of the body. Virus-infected cells, however, can become susceptible to being killed by NK cells by a variety of mechanisms. First, some viruses inhibit all protein synthesis in their host cells, so that synthesis of MHC class I proteins would be blocked in infected cells, even while their production in uninfected cells is being stimulated by the actions of interferon. The reduced level of MHC class I expression in infected cells would make them correspondingly less able to inhibit NK cells through their MHC-specific receptors, and they would therefore be more susceptible to being killed. Second, some viruses can selectively prevent the export of MHC class I molecules to the cell surface. This might allow the infected cell to evade recognition by cytotoxic T cells, but would make them susceptible to being killed by NK cells. Virus infection also alters the glycosylation of cellular proteins, perhaps allowing recognition by NK cell-activating receptors to dominate, or removing the normal ligand for the inhibitory receptors. This could allow infected cells to be detected even when the level of MHC class I expression has not changed.

Clearly, much remains to be learned about this innate mechanism of cytotoxic attack and its physiological relevance. The role of MHC class I molecules in allowing NK cells to detect intracellular infections is of particular interest because these same proteins govern the response of T cells to intracellular pathogens. It is possible that NK cells, which use a diverse set of nonclonal receptors to detect altered MHC, represent the modern remnants of the evolutionary forebears of T cells. Those T-cell ancestors went on to evolve rearranging genes that encode a vast repertoire of antigen-specific T-cell receptors geared to recognizing MHC molecules 'altered' by binding peptide antigens.

2-32 NK cells bear receptors that activate their killer function in response to ligands expressed on infected cells or tumor cells.

In addition to the KIR and KLR receptors, which have a role in sensing the level of MHC class I expression on other cells, NK cells also express receptors that more directly sense the presence of infection or other perturbations in a cell. The most important activating receptors for the recognition of infected cells are the **natural cytotoxicity receptors (NCRs)** NKp30, NKp44, and NKp46, which are immunoglobulin-like receptors, and the C-type lectin family member NKG2D (Fig. 2.59). The ligands recognized by the natural cytotoxicity receptors are not well defined, although NKp46 is known to recognize heparan sulfate proteoglycans as well as some viral proteins.

NKG2D seems to have a specialized role in activating NK cells. Other NKG2 family members (NKG2A, C, and E) form heterodimers with CD94 and bind the MHC class I molecule HLA-E. NKG2D does neither; instead, the ligands for the NKG2D receptor are families of proteins that are distantly related to the MHC class I molecules but have a completely different function, being

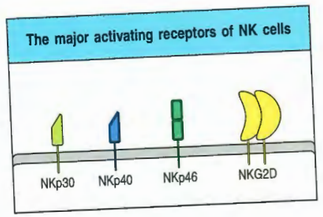


Fig. 2.59 The major activating receptors of NK cells are the natural cytotoxicity receptors and NKG2D. The natural cytotoxicity receptors are immunoglobulin-like proteins. NKp30 and NKp40 have an extracellular domain that resembles a single variable domain of an immunoglobulin molecule. They activate the NK cell through their association with homodimers of the CD3ζ chain or the Fc receptor γ chain (these are signaling proteins that also associate with other types of receptors and will be described in more detail in Chapter 6). NKp46 resembles the KIR-2D molecules in having two domains that resemble the constant domains of an immunoglobulin molecule. NKG2D is a member of the C-type lectin family and forms a homodimer.

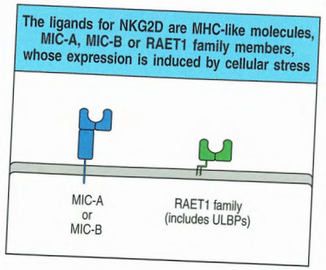


Fig. 2.60 The ligands for the activating NK receptor NKG2D are proteins that are expressed in conditions of cellular stress. The MIC proteins MIC-A and MIC-B are MHC-like molecules induced on epithelial and other cells by stresses, such as heat shock, metabolic stress, or infection. RAET1 family members, including the subset designated as UL16-binding proteins (ULBPs), also resemble a portion of an MHC class I molecule, the α_1 and α_2 domains, and are attached to the cell via a glycophosphatidylinositol linkage.

produced in response to stress. The ligands in humans for NKG2D, as shown in Fig. 2.60, are the MHC class I-like MIC molecules, MIC-A and MIC-B, and the RAET1 protein family, which are homologous to the α_1 and α_2 domains of MHC class I molecules (which will be described when we discuss MHC molecule structure in Chapter 3). The RAET1 family has 10 members, 3 of which were initially characterized as ligands for the cytomegalovirus UL16 protein and are therefore also called UL16-binding proteins, or ULBPs. Mice do not express an equivalent of the MIC molecules, and the ligands for mouse NKG2D have a very similar structure to the RAET1 proteins, and are probably homologs of them. In fact, these ligands were first identified in mice as the retinoic acid early inducible 1 (Rae1) protein family.

The ligands for NKG2D are expressed in response to cellular or metabolic stress, and so are upregulated on cells that are infected with intracellular bacteria or with some viruses, such as cytomegalovirus, as well as on incipient tumor cells that have become malignantly transformed. Thus, recognition by NKG2D acts as a generalized 'danger' signal to the immune system. NKG2D is also expressed on activated macrophages and activated CD8 cytotoxic T cells, and recognition of NKG2D ligands by these cells provides a potent co-stimulatory signal that enhances their effector functions.

2-33 The NKG2D receptor activates a different signaling pathway from that of the other activating NK receptors.

As well as the ligands it recognizes, NKG2D also differs from other activating receptors of NK cells in the signaling pathway it engages within the cell. The other activating receptors, both the natural cytotoxicity receptors and activating KIRs, bind adaptor molecules such as the CD3ζ chain, the Fc receptor γ chain, and DAP12, which all contain specific signaling motifs called **immunoreceptor tyrosine-based activation motifs** (**ITAMs**). When the NK receptor binds its ligand, the ITAMS become phosphorylated, leading to the binding and activation of the intracellular tyrosine kinase Syk and further signaling events in the cell (see Section 6-17). In contrast, NKG2D binds a different adaptor protein, DAP10, which does not contain an ITAM sequence and instead activates the intracellular lipid kinase phosphatidylinositol-3-kinase (PI 3-kinase), initiating a different series of intracellular signaling events. Nevertheless, both signaling pathways result in the activation of the NK cell. In mice, the workings of NKG2D are even more complicated, because mouse NKG2D is produced in two alternatively spliced forms, one of which binds DAP12 while the other binds DAP10. Mouse NKG2D can thus activate both signaling pathways, whereas human NKG2D signals only through DAP10 to activate the PI 3-kinase pathway.

2-34 Several lymphocyte subpopulations behave as innate-like lymphocytes.

Receptor gene rearrangements are a defining characteristic of the lymphocytes of the adaptive immune system, and they allow the generation of an infinite variety of antigen receptors, each expressed by a different individual T cell or B cell (see Section 1-11). There are, however, several minor lymphocyte subsets that produce antigen receptors of this type but with only very limited diversity, encoded by a few common gene rearrangements. Because their receptors are relatively invariant and because they occur only in specific locations within the body, these lymphocytes do not need to undergo clonal expansion before responding effectively to the antigens they recognize; they are therefore known as **innate-like lymphocytes** (**ILLs**) (Fig. 2.61). To produce antigen receptors in these cells requires the recombinases RAG-1 and RAG-2;

Innate-like lymphocytes		
B-1 cells	**Epithelial γ:δ cells**	**NK T cells**
Make natural antibody, protect against infection with *Streptococcus pneumoniae*	Produce cytokines rapidly	Produce cytokines rapidly
Ligands not MHC associated	Ligands are MHC class IB associated	Ligands are lipids bound to CD1d
Cannot be boosted	Cannot be boosted	Cannot be boosted

Fig. 2.61 The three main classes of innate-like lymphocytes and their properties.

these proteins and their role in gene rearrangement in lymphocytes are described in Chapter 4. Because they express RAG-1 and RAG-2 and undergo the process of antigen receptor gene rearrangement, ILLs are, by definition, cells of the adaptive immune system. They behave, however, more like a part of the innate immune system, and so we will discuss them here.

One type of ILL is the subset of **γ:δ T cells** that resides within epithelia, such as the skin. γ:δ T cells are themselves a minor subset of the T cells introduced in Chapter 1. Their antigen receptors are composed of a γ chain and a δ chain, rather than the α and β chains that make up the antigen receptors on the majority subset of T cells involved in adaptive immunity. γ:δ T cells were discovered purely as a consequence of their having immunoglobulin-related receptors encoded by rearranged genes, and their function is still being clarified.

One of the most striking features of γ:δ T cells is their division into two highly distinct subsets. One is found in the lymphoid tissues of all vertebrates and, like B cells and the α:β T cells, has highly diversified T-cell receptors. In contrast, intraepithelial γ:δ T cells occur variably in different vertebrates, and commonly display receptors of very limited diversity, particularly in the skin and the female reproductive tract of mice, where the γ:δ T cells are essentially homogeneous in any one site. On the basis of this limited diversity and lack of recirculation, it has been proposed that intraepithelial γ:δ T cells may recognize ligands that are derived from the epithelium in which they reside, but which are expressed only when a cell has become infected. Candidate ligands are heat-shock proteins, MHC class Ib molecules (described in Chapter 5), and unorthodox nucleotides and phospholipids; there is evidence of recognition of all these ligands by γ:δ T cells.

Unlike α:β T cells, γ:δ T cells do not generally recognize antigen as peptides presented by MHC molecules; instead, they seem to recognize their target antigens directly, and thus could recognize and respond rapidly to molecules expressed by many different cell types. Recognition of molecules expressed as a consequence of infection, rather than recognition of pathogen-specific antigens themselves, would distinguish intraepithelial γ:δ T cells from other lymphocytes and would place them in the innate-like class.

Another subset of lymphocytes with antigen receptors of limited diversity is the **B-1** subset of B cells. B-1 cells are distinguished from other B cells by the cell-surface protein CD5 and have properties quite distinct from those of the conventional B cells that mediate adaptive humoral immunity. B-1 cells are in many ways analogous to intraepithelial γ:δ T cells: they arise early in embryonic development, they use a distinctive and limited set of gene rearrangements to make their receptors, they are self-renewing in tissues outside the central lymphoid organs, and they are the predominant lymphocyte in a distinctive

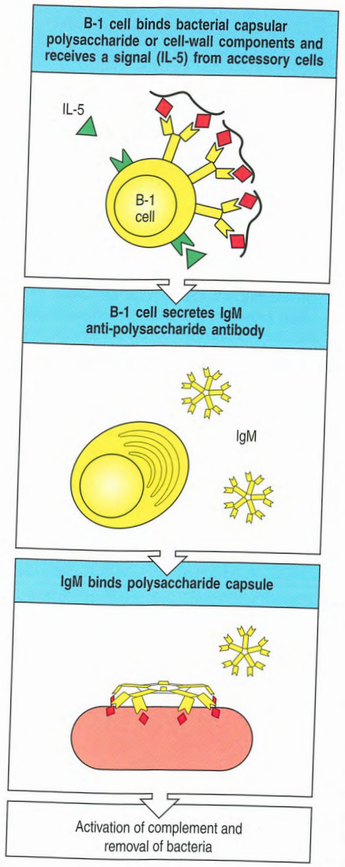


Fig. 2.62 B-1 cells might be important in the response to carbohydrate antigens such as bacterial polysaccharides. These responses occur rapidly, with antibody appearing within 48 hours after infection, presumably because there is a high frequency of precursors of the responding lymphocytes so that little clonal expansion is required. Unlike the responses to many other antigens, this response does not need the 'help' of T cells. In the absence of such help, only IgM is made (for reasons that will be explained in Chapter 9) and, in mice, these responses therefore clear bacteria mainly through the activation of complement, which is most efficient when the antibody is of the IgM isotype.

microenvironment—the peritoneal and pleural cavities. B-1 cells seem to make antibody responses mainly to polysaccharide antigens and can produce antibodies of the IgM class without needing help from T cells (Fig. 2.62). Although these responses can be augmented by T cells, they first appear within 48 hours of exposure to antigen, and so T cells cannot be involved. Thus, B-1 cells are not active in an antigen-specific adaptive immune response. The lack of an antigen-specific interaction with helper T cells might explain why immunological memory is not generated as a result of B-1 cell responses: repeated exposures to the same antigen elicit similar, or decreased, responses with each exposure. These responses, although generated by lymphocytes with rearranging receptors, therefore resemble innate rather than adaptive immune responses.

As with γ:δ T cells, the precise role of B-1 cells in host defense is uncertain. Mice that are deficient in B-1 cells are more susceptible to infection with *Streptococcus pneumoniae* because they fail to produce an anti-phosphocholine antibody that provides protection against this bacterium. A significant fraction of the B-1 cells can make antibodies of this specificity, and because no antigen-specific T-cell help is required, a potent response can be produced early in infection with this pathogen. Whether human B-1 cells have the same role is not certain.

A third subset of ILLs, known as **NK T cells**, exists both in the thymus and in peripheral lymphoid organs. These cells express an invariant T-cell receptor α chain, paired with one of three different β chains, and are able to recognize glycolipid antigens. The main response of NK T cells seems to be the rapid secretion of cytokines, including IL-4, IL-10, and IFN-γ, and it is thought that these cells may have a primarily regulatory function. We shall encounter these unusual cells again in Chapter 10.

From an evolutionary perspective, it is interesting to note that γ:δ T cells seem to defend the body surfaces, whereas B-1 cells defend the body cavity. Both cell types are relatively limited in their range of specificities and in the efficiency of their responses. It is possible that these two cell types represent a transitional phase in the evolution of the adaptive immune response, guarding the two main compartments of primitive organisms—the epithelial surfaces and the body cavity. It is not yet clear whether they are still critical to host defense or whether they represent an evolutionary relic. Nevertheless, because each cell type is prominent in certain sites in the body and contributes to responses against certain pathogens, they must be incorporated into our thinking about host defense.

A final component of the body's innate immune defenses are the IgM antibodies that are known as **natural antibodies**. This natural IgM is encoded by rearranged antibody genes that have not undergone further diversification by the process known as somatic hypermutation (which is described in Chapter 4). It makes up a considerable amount of the IgM circulating in humans and does not seem to be the consequence of an antigen-specific adaptive immune response to infection. It has a low affinity for many microbial pathogens and is highly cross-reactive, even binding to some self molecules. Furthermore, it is not known whether it is produced in response to the normal flora of the epithelial surfaces of the body or in response to self. However, it can have a role in host defense against *Streptococcus pneumoniae* by binding to phosphocholine in the bacterial cell envelope, leading to clearance of the bacteria before they become dangerous.

Summary.

Innate immunity uses a variety of induced effector mechanisms to clear an infection or, failing that, to hold it in check until the pathogen can be recognized by the adaptive immune system. These effector mechanisms are all regulated by germline-encoded receptor systems that are able to discriminate between normal self molecules on uninfected cells and infectious nonself ligands. Thus, the phagocyte's ability to discriminate between self and pathogen controls its release of pro-inflammatory chemokines and cytokines that act together to recruit more phagocytic cells to the site of infection. Especially prominent is the early recruitment of neutrophils that can recognize pathogens directly. Furthermore, cytokines released by tissue phagocytic cells induce fever, the production of acute-phase response proteins, including the pathogen-binding mannose-binding lectin and C-reactive protein, and the mobilization of antigen-presenting cells that induce the adaptive immune response. Viral pathogens are recognized by the cells in which they replicate, leading to the production of interferons that serve to inhibit viral replication and to activate NK cells, which in turn can distinguish infected from uninfected cells. As we will see later in this book, cytokines, chemokines, phagocytic cells, and NK cells are all effector mechanisms that are also employed in the adaptive immune response, which uses variable receptors to target specific pathogen antigens.

Summary to Chapter 2.

The innate system of host defense against infection is made up of several distinct components. The first of these are the barrier functions of the body's epithelia, which can simply prevent an infection from becoming established. Next, there are cells and molecules available to control or destroy the pathogen once it has breached the epithelial defenses. The most important of these are the tissue macrophages, which mediate cellular defense of the borders. The understanding of how the innate immune system recognizes pathogens is rapidly growing, and structural studies, such as those of mannose-binding lectin, have begun to reveal in detail how innate immune receptors can distinguish pathogen surfaces from those of host cells. Furthermore, the identification of the receptor for bacterial LPS and its link to human TLR-4 has opened a window onto innate immune recognition of microbe-associated molecular patterns. Recognition by the innate immune system leads to the elimination of invading pathogens through various effector mechanisms. Most of these have been known for a long time; indeed, the elimination of microorganisms by phagocytosis was the first immune response to be observed. However, more is being learned all the time; the chemokines, for example, have only been known of for about 15 years, and over 50 chemokine proteins have now been discovered. The complement system of proteins mediates humoral innate immunity of the tissue spaces and the blood. The induction of powerful effector mechanisms on the basis of innate immune recognition through germline-encoded receptors clearly has some dangers. Indeed, the double-edged sword embodied by the effects of the cytokine TNF-α—beneficial when it is released locally but disastrous when it is released systemically—illustrates the evolutionary knife-edge along which all innate mechanisms of host defense travel. The innate immune system can be viewed as a defense system that mainly frustrates the establishment of a focus of infection; however, even when it is inadequate to this function, it can still set the scene for the adaptive immune response, which forms an essential part of host defense in humans. Thus, having introduced the study of immunology with a consideration of innate immune function, we will now turn our attention to the adaptive immune response. This has been the focus of nearly all studies in immunology, because it is much easier to follow and experiment with by using reagents and responses that are specific for defined antigens.

Questions.

2.1 The innate immune system uses two different strategies to identify pathogens: recognition of nonself and recognition of self. (a) Give examples of each and discuss how each example contributes to the ability of the organism to protect itself from infection. (b) What are the disadvantages of these different strategies?

2.2 The complement system gives rise to inflammatory signals, opsonins and molecules that lyse bacteria directly. (a) Describe the general properties of each class and discuss their utility in host defense. (b) Say which you think is most important in host defense, and why.

2.3 "The Toll receptors represent the most ancient pathways of host defense." Is this statement justified? Give an explanation for your answer.

2.4 Elie Metchnikoff discovered the protective role of macrophages by observing what happened in a starfish injured by a sea urchin spine. Describe the sequence of events that would follow were you to be speared by a sea urchin spine.

2.5 The complement system is a cascade of enzymes capable of producing powerful deleterious effects. (a) How is complement harnessed to protect us rather than creating harm? (b) What happens when things go wrong?

2.6 During their development and in order to perform their various functions efficiently, cells of the immune system must find their way to the correct part of the body. How do they manage to do this?

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PART II THE RECOGNITION OF ANTIGEN

Chapter 3 Antigen Recognition by B-cell and T-cell Receptors

The structure of a typical antibody molecule.

The interaction of the antibody molecule with specific antigen.

Antigen recognition by T cells.

Chapter 4 The Generation of Lymphocyte Antigen Receptors

Primary immunoglobulin gene rearrangement.

T-cell receptor gene rearrangement.

Structural variation in immunoglobulin constant regions.

Secondary diversification of the antibody repertoire.

Chapter 5 Antigen Presentation to T Lymphocytes

The generation of T-cell receptor ligands.

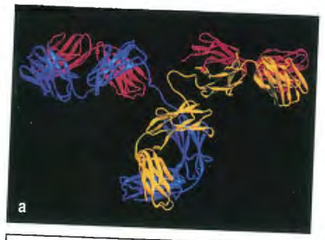
The major histocompatibility complex and its functions.

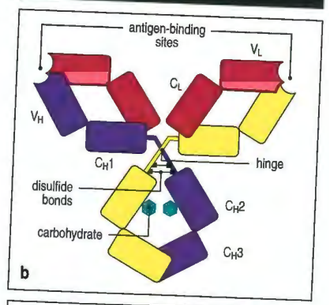
3 Antigen Recognition by B-cell and T-cell Receptors

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 secreted yet nonspecific defenses, as we have learned in Chapter 2. 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. The antigen-recognition molecules of B cells are the **immunoglobulins** (**Ig**). These proteins are produced by B 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**). Immuno-globulin 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, is the main effector function of B cells in adaptive immunity.

Antibodies were the first molecules 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 molecules from the pathogen that elicited the immune response; the other is to recruit other cells and molecules to destroy the pathogen once the antibody is bound to it. For example, binding by antibody 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 various elimination mechanisms. The antigen-binding region varies extensively between antibody molecules and is thus 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 thus 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. Its function is to recognize and bind antigen by the V regions exposed on the surface of the cell, thus transmitting a signal that causes B-cell activation, leading to clonal expansion and specific antibody production.

The antigen-recognition molecules of T cells are made solely as membrane-bound proteins and function only to signal T cells for activation. These **T-cell receptors** (**TCRs**) are related to immunoglobulins both in their protein





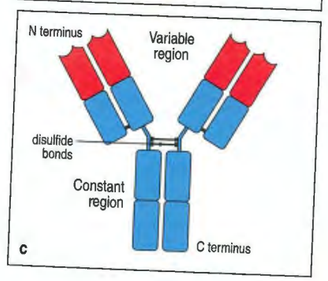


Fig. 3.1 Structure of an antibody molecule. Panel a illustrates a ribbon diagram based on the X-ray crystallographic structure of an IgG antibody, showing the course of the backbones of the polypeptide chains. Three globular regions form a Y shape. The two antigen-binding sites are at the tips of the arms, which are tethered to the trunk of the Y by a flexible hinge region. A schematic representation of the structure in 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. Panel a courtesy of A. McPherson and L. Harris.

structure—having both V and C regions—and in the genetic mechanism that produces their great variability, which will be discussed in Chapter 4. The T-cell receptor differs from the B-cell receptor in an important way, however: it does not recognize and bind antigen directly, but instead recognizes short peptide fragments of protein antigens, which are bound to proteins known as **MHC molecules** on the surfaces of cells.

The MHC molecules are glycoproteins encoded in the large cluster of genes known as the **major histocompatibility complex** (**MHC**). Their most striking structural feature is a cleft running across their outermost surface, in which a variety of peptides can be bound. MHC molecules are highly **polymorphic**: that is, each type of MHC molecule occurs in many different versions within the population. Most people are therefore heterozygous for the MHC molecules: 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 not simply for a foreign peptide antigen but for a unique combination of a 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 5 and 7, respectively.

In this chapter we focus on the structure and antigen-binding properties of immunoglobulins and T-cell receptors. Both receptors are also associated with intracellular signaling complexes, which transmit the antigen-binding signal onwards into the cell; these are described in Chapter 6. Although B cells and T cells recognize 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. As they are soluble and secreted 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, which has been determined by X-ray crystallography, are shown in Fig. 3.1. The aim of this part of the chapter is to 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 hand to a limited number of effector molecules and cells. As we will see, each of these tasks is performed by separable parts of the molecule. The two arms of the Y end in regions that vary between different antibody molecules—the V regions. These are involved in antigen binding, whereas the stem of the Y, or 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. Within this general category, however, five different **classes** of immunoglobulins—**IgM**, **IgD**, **IgG**, **IgA**, and **IgE**—can be distinguished by their C region. 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.

3-1 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 chain. One, of approximately 50 kDa, is termed the **heavy** or **H chain**, and the other, of 25 kDa, is termed the **light** or **L chain** (Fig. 3.2). 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. 3.1) and thus the ability to bind simultaneously to two identical structures.

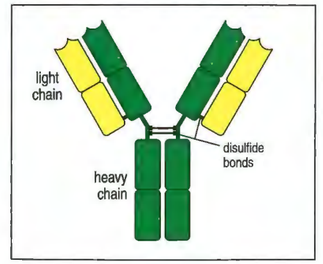


Fig. 3.2 Immunoglobulin molecules are composed of two types of protein chains: heavy chains and light chains. Each immunoglobulin molecule is made up of two heavy chains (green) and two light chains (yellow) joined by disulfide bonds so that each heavy chain is linked to a light chain and the two heavy chains are linked together.

Two types of light chain, termed **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 would 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 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). Their distinctive functional properties 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 4. 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 the B-cell receptor structure 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 sequence that anchors the molecule in the membrane, and in the antibody it is a hydrophilic sequence that allows secretion.

3-2 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. 3.1a). This suggests that the immunoglobulin chains have evolved by repeated duplication of an ancestral gene corresponding to a single domain.

The second important feature revealed by comparisons of amino acid sequences is that the amino-terminal sequences of both the heavy and light chains vary greatly between different antibodies. The variability in sequence 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. 3.1b 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.

3-3 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. 3.1b). 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 sites are formed by the paired V_H and V_L domains at the ends of the two arms of the Y (see Fig. 3.1b).

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. 3.3). Two fragments are identical and contain the antigen-binding activity. These are termed the **Fab fragments**, for **F**ragment **a**ntigen **b**inding. The Fab fragments correspond to the two identical arms of the antibody molecule, each of which consists of a complete light chain paired with the V_H and C_H1 domains of a heavy chain. 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. This fragment 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.

The protein fragments obtained after proteolysis are determined by where the protease cuts the antibody molecule in relation to the disulfide bonds that link the two heavy chains. These lie in the hinge region between the C_H1 and C_H2 domains and, as illustrated in Fig. 3.3, papain cleaves the antibody molecule on the amino-terminal side of the disulfide bonds. This releases the two arms of the antibody as separate Fab fragments, whereas in the Fc fragment the carboxy-terminal halves of the heavy chains remain linked.

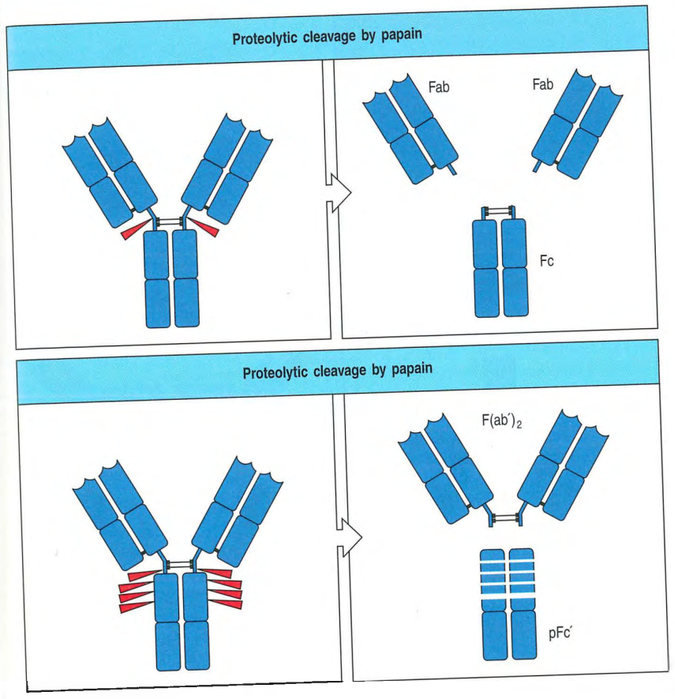


Fig. 3.3 The Y-shaped immunoglobulin molecule can be dissected by partial digestion with proteases. Upper panels: papain cleaves the immunoglobulin molecule into three pieces, two Fab fragments and one Fc fragment. The Fab fragment contains the V regions and binds antigen. The Fc fragment is crystallizable 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.

Another protease, pepsin, cuts in the same general region of the antibody molecule as papain but on the carboxy-terminal side of the disulfide bonds (see Fig. 3.3). This produces a fragment, the **F(ab′)$_2$ fragment**, in which the two antigen-binding arms of the antibody molecule remain linked. In this case the remaining part of the heavy chain is cut 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 is thus of potential value in the therapeutic applications of antibodies as well as in research into the functional role of the Fc portion.

Genetic engineering techniques also now permit the construction of many different antibody-related molecules. One important type 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 variable. Fv molecules may become valuable therapeutic agents because of their small size, which allows them to penetrate tissues readily. For example, Fv molecules specific for tumor antigens and coupled to protein toxins have potential applications in tumor therapy, as discussed in Chapter 15.

3-4 The immunoglobulin molecule is flexible, especially at the hinge region.

The region that links the Fc and Fab portions of the antibody molecule is in reality not a rigid hinge but a flexible tether, allowing independent movement

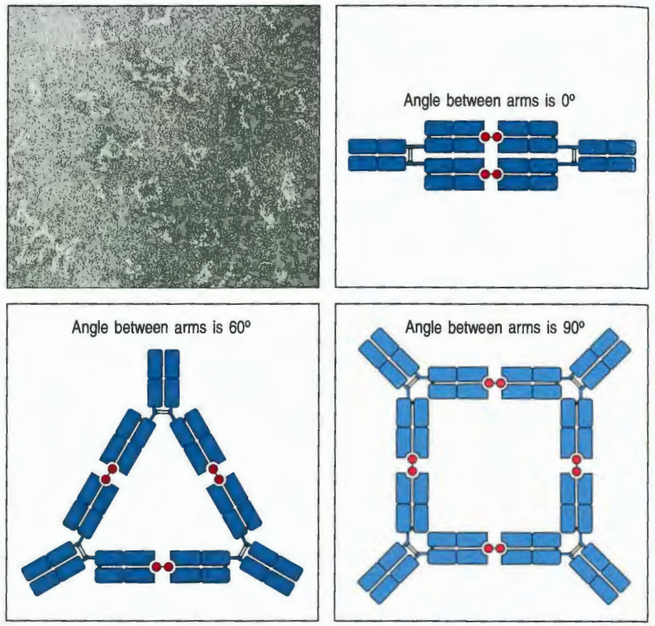


Fig. 3.4 Antibody arms are joined by a flexible hinge. An antigen consisting of two hapten molecules (red balls in diagrams) that can cross-link two antigen-binding sites is used to create antigen:antibody complexes, which can be seen in the electron micrograph. Linear, triangular, and square forms are 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 the complexes is shown in the diagrams. The angle between the arms of the antibody molecules varies, from 0° in the antibody dimers, through 60° in the triangular forms, to 90° in the square forms, showing that the connections between the arms are flexible. Photograph (× 300,000) courtesy of N.M. Green.

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 can be 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. 3.4). 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 and rotation of the V domain relative to the C domain. For example, in the antibody molecule shown in Fig. 3.1a, 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.

3-5 The domains of an immunoglobulin molecule have similar structures.

As we saw in Section 3-2, 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 three-dimensional structure there are distinct differences between V and C domains. The structural similarities and

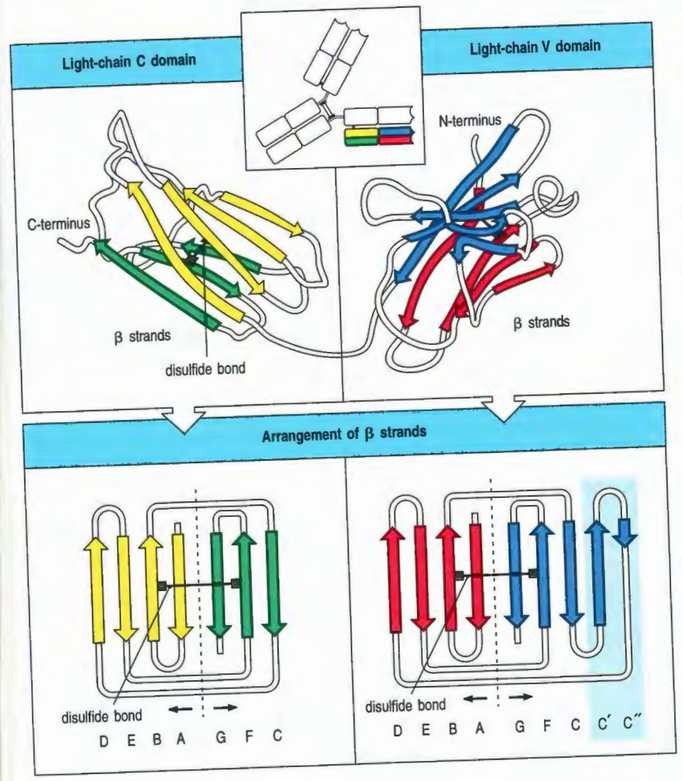


Fig. 3.5 The structure of immunoglobulin constant and variable domains. The upper panels show schematically the folding pattern of the constant (C) and variable (V) domains of an immunoglobulin light chain. Each domain is a barrel-shaped structure in which strands of polypeptide chain (β strands) running 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 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.

differences can be seen in the diagram of a light chain in Fig. 3.5. 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 difference between them are most clearly seen in the bottom panels of Fig. 3.5, where 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 C and V domains of immunoglobulin chains lie in the core of the immunoglobulin fold and are essential for its stability. For that reason, other proteins with sequences similar to those of immunoglobulins are believed to form domains of similar structure, and in many cases this has been demonstrated by crystallography. These **immunoglobulin-like domains** are present in many other 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**.

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. 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.

The interaction of the antibody molecule with specific antigen.

We have described the structure of the antibody molecule and how the V regions of the heavy and light chains fold and pair to form the antigen-binding site. In this part of the chapter we look at the antigen-binding site 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.

3-6 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 can be clearly seen in what is termed a **variability plot** (Fig. 3.6), 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

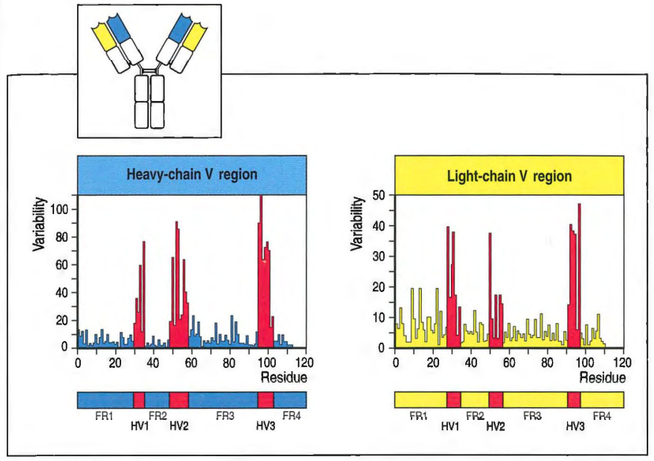


Fig. 3.6 There are discrete regions of hypervariability in V domains. A variability plot derived from comparison of the amino acid sequences of several dozen heavy-chain and light-chain V domains is shown. At each amino acid position the degree of variability is the ratio of the number of different amino 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 and are also known as the complementarity-determining regions, CDR1, CDR2, and CDR3. They are flanked by less variable framework regions (FR1, FR2, FR3, and FR4, shown in blue or yellow).

three loops at the outer edge of the β barrel, which are juxtaposed in the folded domain (Fig. 3.7). Thus, not only is diversity concentrated in particular parts of the V domain sequence, but it is localized to a particular region on the surface of the molecule. When the V_H and V_L domains are paired in the antibody molecule, the hypervariable loops from each domain are brought together, creating a single hypervariable site at the tip of each arm of the molecule. This is the binding site for antigen, the **antigen-binding site** or **antibody combining site**. The six hypervariable loops determine antigen specificity by forming a surface complementary to the antigen, and are more commonly termed the **complementarity-determining regions**, or **CDRs** (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. Thus, one way in which the immune system is able to generate antibodies of different specificities is by generating different combinations of heavy- and light-chain V regions. This means of producing variability is known as **combinatorial diversity**; we will encounter a second form of combinatorial diversity when we consider in Chapter 4 how the genes encoding the heavy- and light-chain V regions are created from smaller segments of DNA.

3-7 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 3-4) 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

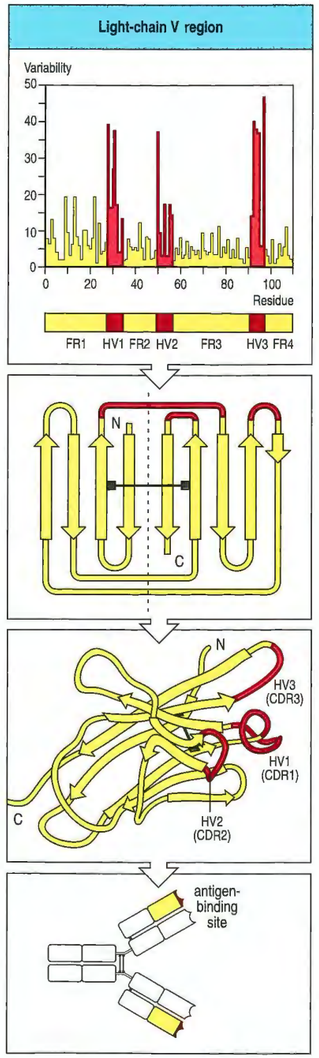


Fig. 3.7 The hypervariable regions lie in discrete loops of the folded structure. When the hypervariable regions (CDRs) are positioned on the structure of a V domain it can be seen that they lie in loops that are brought together in the folded structure. In the antibody molecule, the pairing of a heavy 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. C, carboxy terminus; N, amino terminus.

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 are the shapes 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- and light-chain V domains (Fig. 3.8a 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 (Fig. 3.8c). 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 Fig. 3.8d, where an antibody binding to the HIV gp120 antigen projects a long loop against its target.

3-8 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 from 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 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.

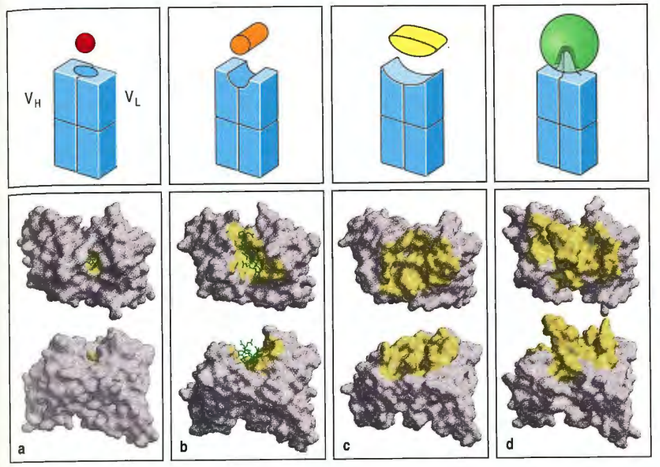


Fig. 3.8 Antigens can bind in pockets, or grooves, or on extended surfaces in the binding sites of antibodies. The panels in the top row show schematic representations of the different types of binding sites in a Fab fragment of an antibody: first panel, pocket; second panel, groove; third panel, extended 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- 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 elongated CDR3 loops that protrude into a recess in the surface of the antigen. The structure of the complex between this antibody and gp120 has not been solved, and so the yellow area on the images in the lower panels represents the extent of the CDR regions rather than the actual region of contact between antibody and antigen. Photographs courtesy of I.A. Wilson and R.L. Stanfield.

3-9 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 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. 3.9.

Electrostatic interactions occur between charged amino acid side chains, as in salt bridges. 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

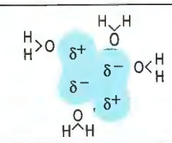
Noncovalent forces	Origin	
Electrostatic forces	Attraction between opposite charges	$-\overset{\oplus}{N}H_3 \quad \overset{\ominus}{O}OC-$
Hydrogen bonds	Hydrogen shared between electronegative atoms (N,O)	$>N-H\cdots O=C<$ (δ^- δ^+ δ^-)
Van der Waals forces	Fluctuations in electron clouds around molecules oppositely polarize neighboring atoms	δ^+ ⇌ δ^- ⇌ δ^- ⇌ δ^+
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. 3.9 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.

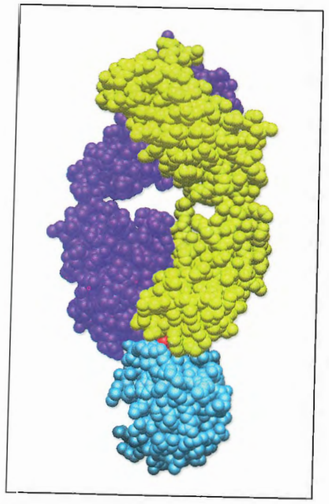


Fig. 3.10 The complex of lysozyme with the antibody D1.3. The interaction of the Fab fragment of D1.3 with hen egg-white lysozyme is shown, with the lysozyme in blue, the heavy chain in purple and the light chain in yellow. A glutamine residue of lysozyme, shown in red, protrudes between the two V domains of the antigen-binding site and makes hydrogen bonds that are important to the antigen–antibody binding. Courtesy of R. J. Poljak.

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. 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 participate in both hydrogen bonding and in 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. Amino acids that possess charged side chains, such as arginine, are also over-represented at antigen-binding sites.

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 (Fig. 3.10), 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. 3.8c), 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, genetic engineering by site-directed mutagenesis can tailor an antibody to bind even more strongly to its epitope.

Summary.

X-ray crystallographic analysis of antigen:antibody complexes has shown that the hypervariable loops (complementarity-determining regions, CDRs) of immunoglobulin V regions determine the binding specificity of an antibody. With protein antigens, an antibody molecule contacts the antigen over a broad area of its 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 play little part in the direct contact with the antigen but 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 the cleft 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 only recognize foreign antigens that 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 cells have 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 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 5.

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.

3-10 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. 3.11), and they account for antigen recognition by most T cells. A minority of T cells bear an alternative,

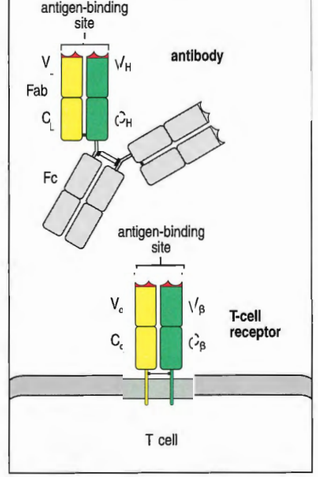


Fig. 3.11 The T-cell receptor resembles a membrane-bound Fab fragment. The Fab fragment of an antibody molecule is a disulfide-linked heterodimer, each chain of which contains one immunoglobulin C domain and one V domain; the juxtaposition of the V domains forms the antigen-binding site (see Section 3-6). 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.

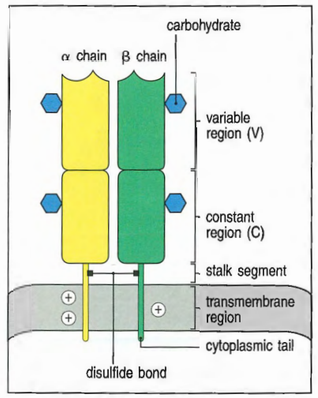


Fig. 3.12 Structure of the T-cell receptor. The T-cell receptor heterodimer is composed of two transmembrane glycoprotein chains, α and β. The extracellular portion of each chain consists of two domains, resembling immunoglobulin V and C domains, respectively. Both chains have carbohydrate side chains attached to each domain. A short stalk segment, analogous to an immunoglobulin hinge region, connects the immunoglobulin-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.

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 2-34). In the rest of this chapter we shall use the term T-cell receptor to mean the α:β receptor, except where specified otherwise. Both types of T-cell receptor 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.

The first 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 clearly 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. 3.12). 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 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 structure is indeed similar to that of a Fab fragment. The T-cell receptor chains fold in much the same way as those of a Fab fragment (Fig. 3.13a), 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 immunoglobulin-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 immunoglobulin-like domains, but the other half of the domain is formed of loosely packed strands and a short segment of α helix (Fig. 3.13b). In a C_α domain the intramolecular disulfide bond, which in immunoglobulin-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 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. 3.13b). 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. 3.13c). 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.

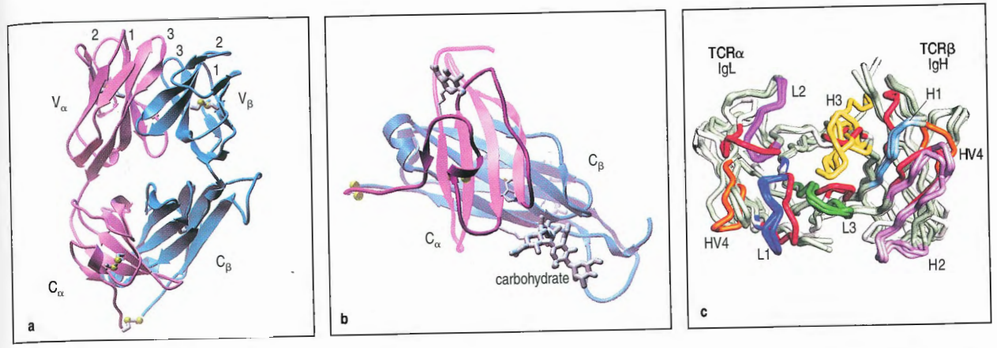


Fig. 3.13 The crystal structure of an α:β T-cell receptor resolved at 2.5 Å. In panels a and b the α chain is shown in pink and the β chain in blue. Disulfide bonds are shown in green. In panel a, the T-cell receptor is viewed from the side as it would sit on a cell surface, with the CDR loops that form 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 immunoglobulin-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.

3-11 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. Antigen recognition by B cells involves the direct binding of immunoglobulin to the intact antigen and, as discussed in Section 3-8, 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

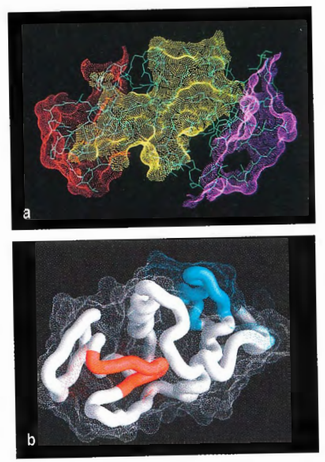


Fig. 3.14 Differences in the recognition of hen egg-white lysozyme by immunoglobulins and T-cell receptors. Antibodies can be shown by X-ray crystallography to bind epitopes on the surface of proteins, as shown in panel a, where the epitopes for three antibodies are shown in different colors on the surface of hen egg-white lysozyme (see also Fig. 3.10). 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.

contiguous amino acid sequences in proteins. 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. 3.14). We shall see in Chapter 5 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 recently 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.

3-12 There are two classes of MHC molecules with distinct subunit composition 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 expression pattern on the tissues of the body. As shown in Figs 3.15 and 3.16, MHC class I and MHC class II molecules are closely related in overall structure but differ in their subunit composition.

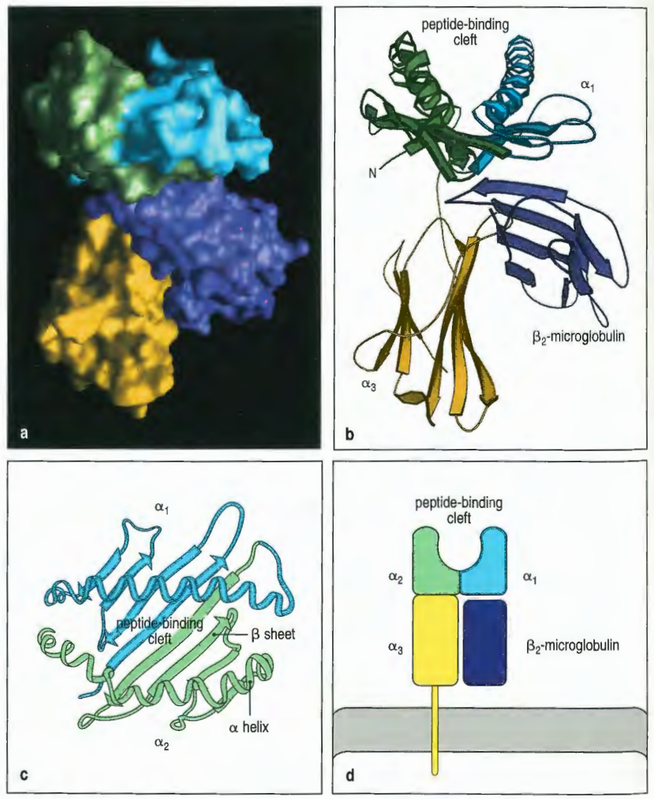


Fig. 3.15 The structure of an MHC class I molecule determined by X-ray crystallography. Panel a shows a computer graphic representation of a human MHC class I molecule, HLA-A2, which has been cleaved from the cell surface by the enzyme papain. The surface of the molecule is shown, colored according to 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.

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. 3.15) 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 immunoglobulin-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.

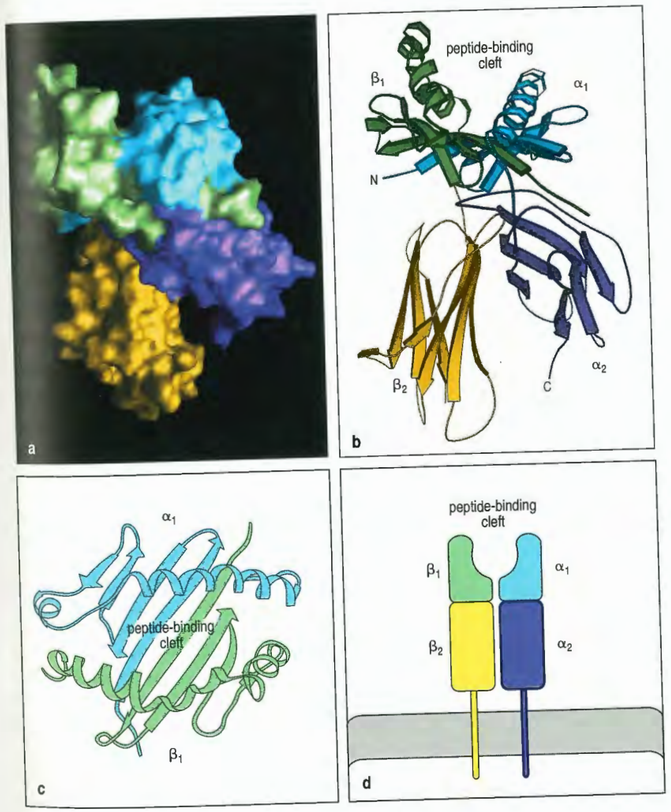


Fig. 3.16 MHC class II molecules resemble MHC class I molecules in overall structure. The MHC class II molecule is composed of two transmembrane glycoprotein chains, α (34 kDa) and β (29 kDa), as shown schematically in panel d. Each chain has two domains, and the two chains together form a compact four-domain structure similar to that of the MHC class I molecule (compare with panel d of Fig. 3.15). 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. 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.

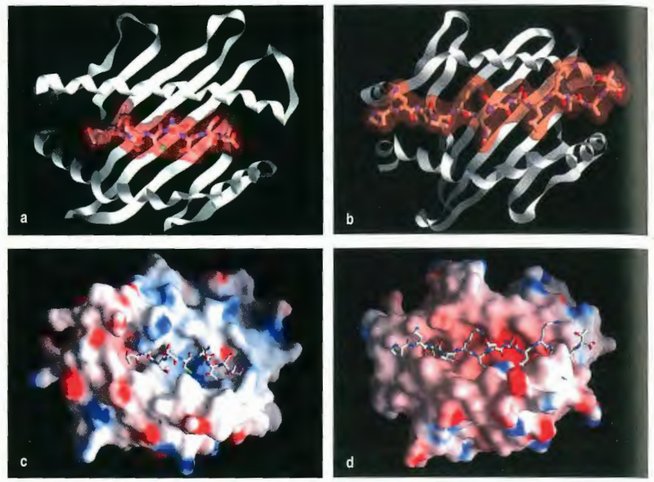


Fig. 3.17 MHC molecules bind peptides tightly within the cleft. When MHC molecules are crystallized with a single synthetic peptide antigen, the details of peptide binding are revealed. In MHC class I molecules (panels a and c) the peptide is bound in an elongated conformation with both ends tightly 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.

An MHC class II molecule consists of a noncovalent complex of two chains, α and β, both of which span the membrane (see Fig. 3.16). 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 in MHC class II molecules 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. 3.17). The T-cell receptor interacts with this compound ligand, making contacts both with the MHC molecule and with the peptide antigen. The sites of major polymorphism in MHC class II molecules are again located in the peptide-binding cleft.

3-13 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, the proteins of which 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 analyzed. Peptides are released from the MHC molecules by denaturing the complex in acid; they can then be purified and sequenced. Pure synthetic peptides can also be incorporated into previously 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.

3-14 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. 3.18). 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 be able to bind, particularly if they can bind at their carboxy terminus, but 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.

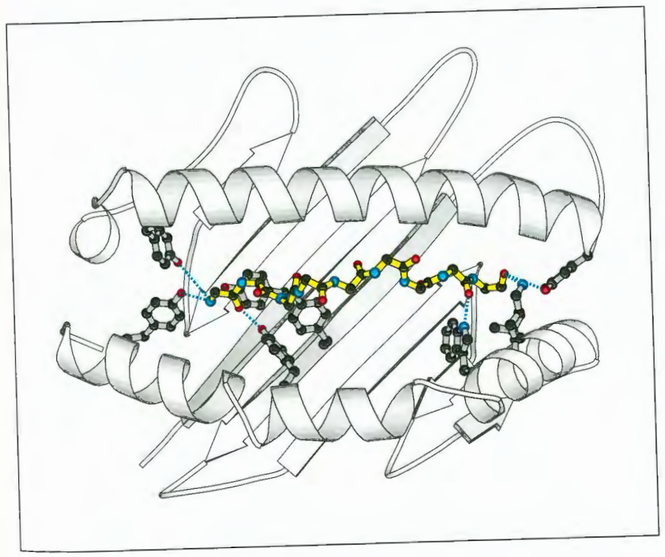


Fig. 3.18 Peptides are bound to MHC class I molecules by their ends. MHC class I molecules interact with the backbone of a bound peptide (shown in yellow) through a series of hydrogen bonds and ionic interactions (shown as dotted blue lines) at each end of the peptide. The amino terminus of the peptide 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.

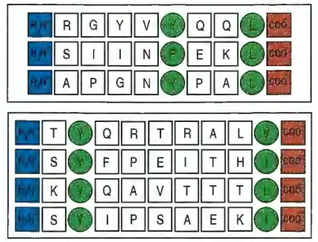


Fig. 3.19 Peptides bind to MHC molecules through structurally related anchor residues. Peptides eluted from two different MHC class I molecules are shown in the upper and lower panels, respectively. The anchor residues (green) differ for peptides that bind different alleles of MHC class I molecules but 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.

These interactions give all MHC class I molecules their 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 as a whole, and each individual carries only a small selection. The main differences between the allelic MHC variants are found at certain sites in the peptide-binding cleft, resulting in different amino acids in key peptide-interaction sites in the different MHC variants. The consequence of this is that 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 the binding of these side chains anchors the peptide to the MHC molecule, the peptide residues involved have been called **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. 3.19). 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.

3-15 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. 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 MHC class II 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. 3.20). Although there are fewer crystal structures of MHC class II-bound peptides than of peptides bound to MHC class I, the available 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 molecule (Fig. 3.21). Nevertheless, by comparing the sequences of known binding peptides it is usually possible to detect a pattern of permissive amino acids for each of the different alleles of MHC class II molecules, and to model how the amino acids of this peptide sequence motif will interact with the amino acids that make up 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

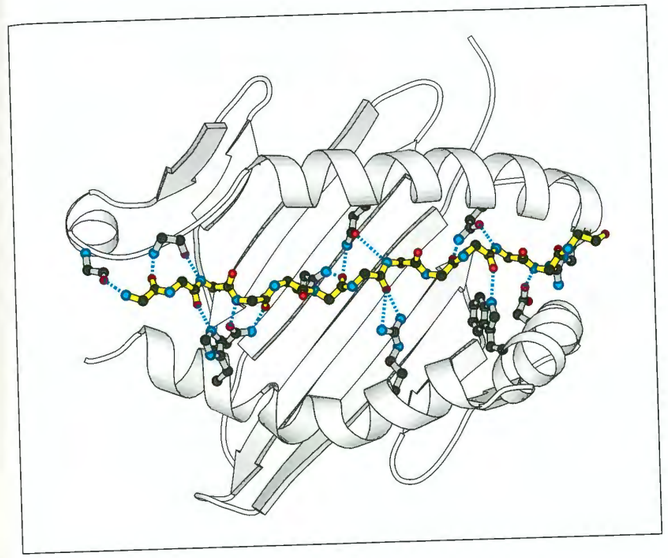


Fig. 3.20 Peptides bind to MHC class II molecules by interactions along the length of the binding groove. A peptide (yellow; shown as the peptide backbone only, with the amino terminus to the left and the carboxy terminus to the right) is bound by an MHC class II molecule through a series of hydrogen 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.

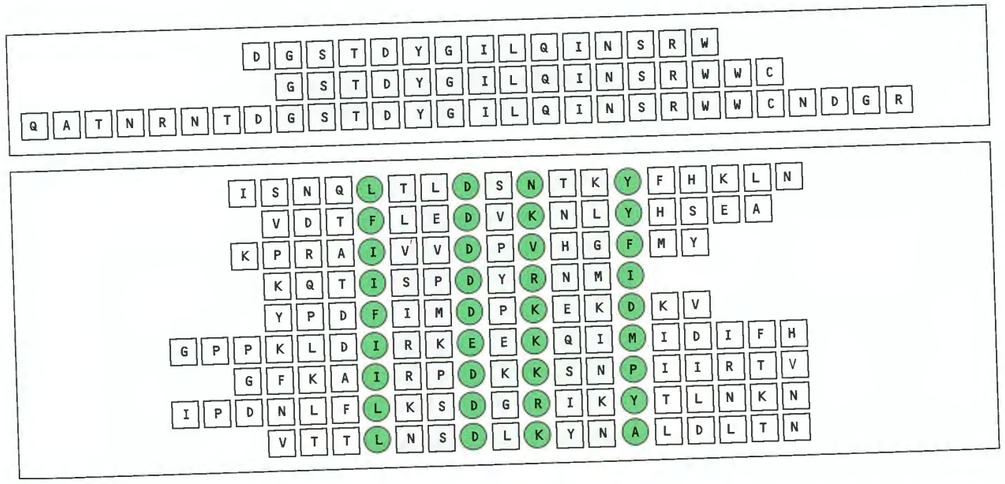


Fig. 3.21 Peptides that bind MHC class II molecules are variable in length and their anchor residues lie at various distances from the ends of the peptide. The sequences of a set of peptides that bind to the mouse MHC class II A^k allele are shown in the upper panel. All contain the same core sequence (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

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 13–17 amino acids in most cases. Like MHC class I molecules, MHC class II molecules that lack bound peptide are unstable, but the critical stabilizing interactions that the peptide makes with the MHC class II molecule are not yet known.

3-16 The crystal structures of several peptide:MHC:T-cell receptor complexes show a similar T-cell receptor orientation 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. 3.22), which had been forecast 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. 3.23) 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 terminus of the bound peptide, whereas the V_β domain contacts primarily the carboxy terminus of the bound peptide. Both chains also interact with the α helices of the MHC class I molecule (see Fig. 3.22). 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

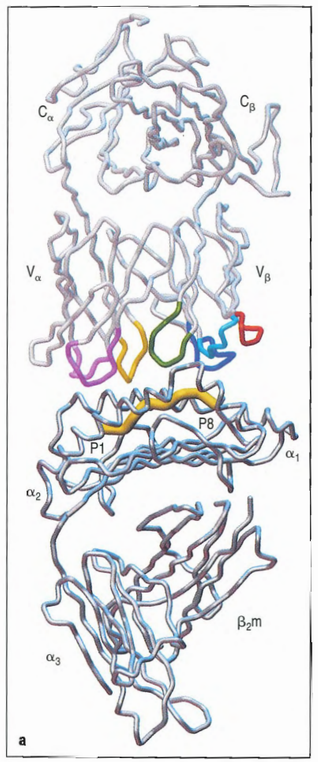


Fig. 3.22 The T-cell receptor binds to the peptide:MHC complex. Panel a: the T-cell receptor binds to the top of the peptide:MHC complex, straddling, in the case of the MHC class I molecule shown here, both the α_1 and α_2 domain helices. The CDRs of the T-cell receptor are indicated in color: the CDR1 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 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.

flexibility in the CDR3 loop demonstrated by these two structures helps to explain how the T-cell receptor can adopt conformations that can 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. 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 may provide the essential contacts that determine the specificity and strength of binding. It is known that alterations as simple as changing a leucine to isoleucine in the peptide are sufficient to alter a T-cell response from strong killing to absolutely no response at all. Studies show that 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 5. 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. We discuss the evidence for this in Chapter 7.

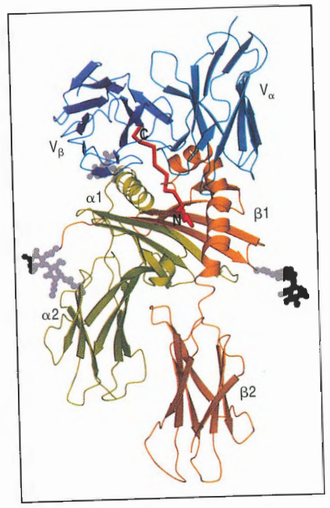


Fig. 3.23 The T-cell receptor interacts with MHC class I and MHC class II molecules in a similar fashion. The structure of a T-cell receptor binding to an MHC class II molecule has been determined, and shows the T-cell receptor binding to an equivalent site, and in an equivalent orientation, to the way in which T-cell receptors bind to MHC class I molecules (see Fig. 3.22). 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.

3-17 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 with its antigen receptor, T cells make additional interactions with the MHC molecule that stabilize the interaction and are required for the T cell to respond effectively to antigen. T cells fall into two major classes that 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 it is to activate other cells (see Section 1-19). CD4 and CD8 were known as markers for these different functional sets of T cells for some time before it became clear that this distinction was based on their ability to recognize the different classes of MHC molecules: CD8 recognizes MHC class I molecules and CD4 recognizes MHC class II molecules. During antigen recognition, CD4 or CD8 molecules (depending on the type of T cell) associate on the T-cell surface with the T-cell receptor and bind 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 molecule composed of four immunoglobulin-like domains (Fig. 3.24). The first two domains (D1 and D2) of the CD4 molecule 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). There is some evidence that CD4 forms homodimers on the T cell suface that are functional in recognition of MHC class II, although the structural basis of their formation is still uncertain. CD4 binds MHC class II mole-

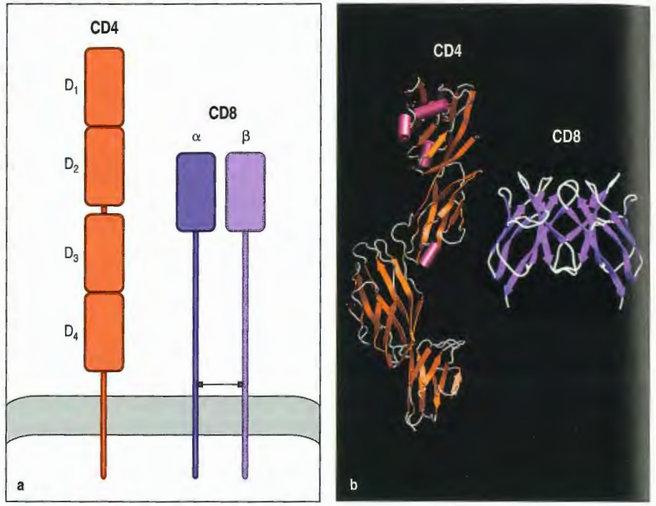


Fig. 3.24 The structures of the CD4 and CD8 co-receptor molecules. The CD4 molecule contains four immunoglobulin-like domains, shown in schematic form in panel a, and as a ribbon diagram of the crystal structure in panel b. The amino-terminal domain, D_1, is similar in structure to an immunoglobulin 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 rodlike 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.

cules through a region that is located mainly on a lateral face of the first domain, D1. 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. 3.25a), 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 6. When CD4 and the T-cell receptor simultaneously bind 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.

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 immunoglobulin-like domain linked to the membrane by a segment of extended polypeptide (see Fig. 3.24). 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 5.

CD8 binds weakly to an invariant site in the α_3 domain of an MHC class I molecule (see Fig. 3.25b). Although only the interaction of the CD8α homodimer with MHC class I is known in detail, this shows that the MHC class I binding site of the CD8 α:β heterodimer is formed by the interaction of the CD8α and β chains. In addition, CD8 (most probably through the α chain) interacts 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 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.

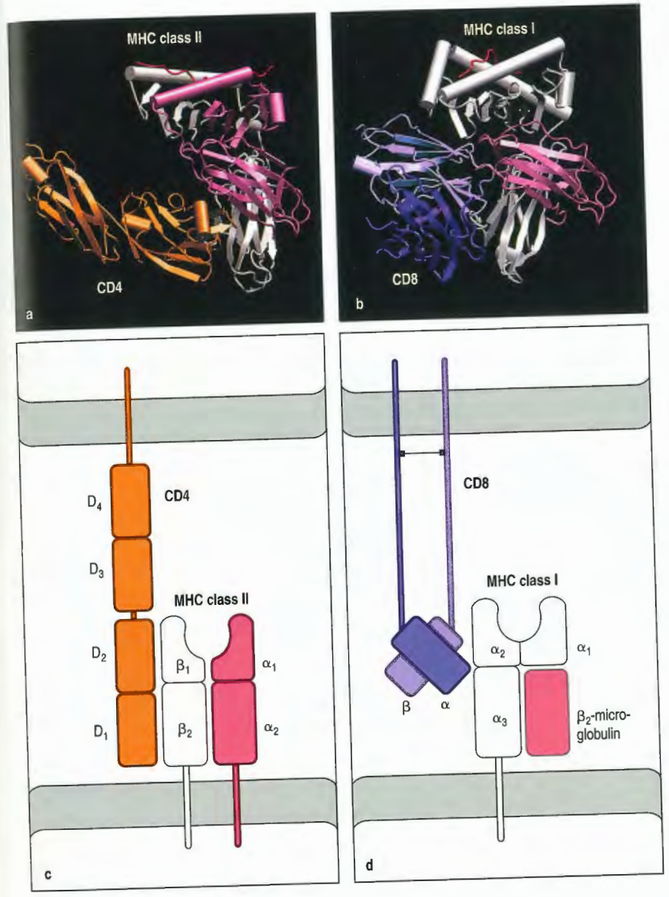


Fig. 3.25 The binding sites for CD4 and CD8 on MHC class II and class I molecules lie in the immunoglobulin-like domains. The binding sites for CD4 and CD8 on the MHC class II and class I molecules, respectively, lie in the immunoglobulin-like domains nearest to the membrane and distant from the peptide-binding 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 the same 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.

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. 3.26. 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. And 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.

3-18 The two classes of MHC molecules are expressed differentially on cells.

MHC class I and MHC class II molecules have distinct distributions among cells that reflect the different effector functions of the T cells that recognize them (Fig. 3.27). MHC class I molecules present peptides from pathogens,



Fig. 3.26 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 CD8α subunit in red and the CD8β subunit in blue. Courtesy of G. Gao.

commonly viruses, to CD8 cytotoxic T cells, which are specialized 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. 3.27). Non-nucleated 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

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	+	–†
Non-nucleated cells		
Red blood cells	–	–

Fig. 3.27 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.

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. 3.27). 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 8 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-γ (IFN-γ), for example, increases the expression of 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.

3-19 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 7-11 and 7-12.

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. 3.28). 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 5. 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 is covered in Sections 4-11 and 7-12.

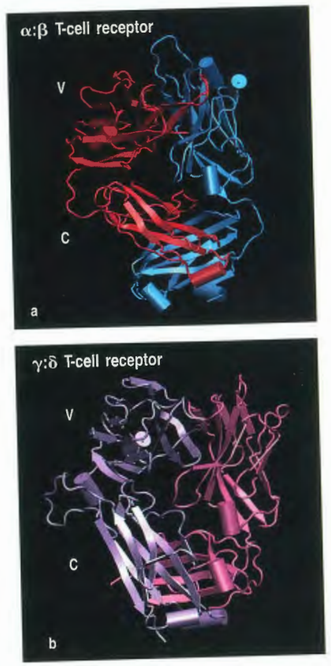


Fig. 3.28 Structures of α:β and γ:δ T-cell receptors. The structures of the α:β and the γ:δ T-cell receptors have both been determined by X-ray crystallography. The α:β T-cell receptor is shown in panel a, with the α chain colored red and the β chain blue. Panel b shows the γ:δ receptor, with the γ chain colored purple and the δ chain pink. Both 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, as do 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 bound stably in a

peptide-binding cleft on the surface of the MHC molecule. There are two classes of MHC molecule, 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 class I peptide:MHC 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 class II peptide:MHC 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 seems to bind different ligands, including nonpeptide ligands. It is thought not to be MHC restricted. It is found on a minority population of T cells, the γ:δ T cells.

Summary to Chapter 3.

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 9, 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 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. They thus have no requirement for 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.

3.1 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 contributes to its complementarity determining regions (CDRs) and how do the V-type and C-type immunoglobulin domains differ in those regions.

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

3.3 T cells must deliver effector functions appropriate for the cellular localization of pathogens, whereas B cells are not so constrained. (a) How does this account for the different recognition properties of B- and T-cell antigen receptors? (b) Describe the similarities and differences between B- and T-cell antigen receptors. (c) Given these differences, what is the essential difference in the function of B cells and T cells.

3.4 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) If MHC class I and class II peptide-binding regions are so similar, how can T cells functionally distinguish between antigens presented by MHC class I and class II molecules?

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Immunologists' Toolbox

APPENDIX I

Immunization.

Natural adaptive immune responses are normally directed at antigens borne by pathogenic microorganisms. The immune system can also be induced to respond to simple nonliving antigens, and experimental immunologists have focused on the responses to these simple antigens in developing our understanding of the immune response. The deliberate induction of an immune response is known as **immunization**. Experimental immunizations are routinely carried out by injecting the test antigen into the animal or human subject. The route, dose, and form in which antigen is administered can profoundly affect whether a response occurs and the type of response that is produced, and are considered in Sections A-1–A-4. The induction of protective immune responses against common microbial pathogens in humans is often called vaccination, although this term is correctly only applied to the induction of immune responses against smallpox by immunizing with the cross-reactive cowpox virus, vaccinia.

To determine whether an immune response has occurred and to follow its course, the immunized individual is monitored for the appearance of immune reactants directed at the specific antigen. Immune responses to most antigens elicit the production of both specific antibodies and specific effector T cells. Monitoring the antibody response usually involves the analysis of relatively crude preparations of **antiserum** (plural: **antisera**). The **serum** is the fluid phase of clotted blood, which, if taken from an immunized individual, is called antiserum because it contains specific antibodies against the immunizing antigen as well as other soluble serum proteins. To study immune responses mediated by T cells, blood lymphocytes or cells from lymphoid organs such as the spleen are tested; T-cell responses are more commonly studied in experimental animals than in humans.

Any substance that can elicit an immune response is said to be **immunogenic** and is called an **immunogen**. There is a clear operational distinction between an immunogen and an antigen. An antigen is defined as any substance that can bind to a specific antibody. All antigens therefore have the potential to elicit specific antibodies, but some need to be attached to an immunogen in order to do so. This means that although all immunogens are antigens, not all antigens are immunogenic. The antigens used most frequently in experimental immunology are proteins, and antibodies to proteins are of enormous utility in experimental biology and medicine. Purified proteins are, however, not always highly immunogenic and to provoke an immune response have to be administered with an adjuvant (see Section A-4). Carbohydrates, nucleic acids, and other types of molecules are all potential antigens, but will often only induce an immune response if attached to a protein carrier. Thus, the immunogenicity of protein antigens determines the outcome of virtually every immune response.

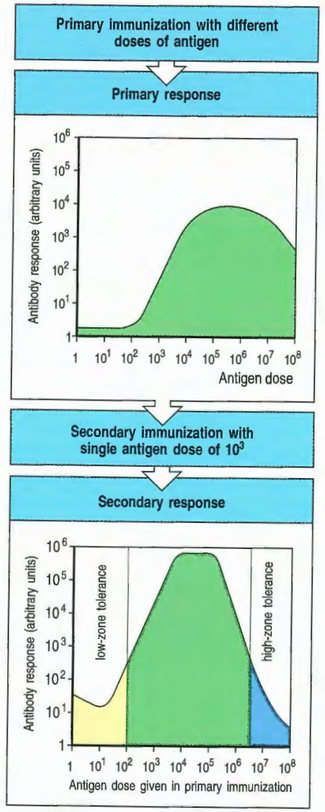


Fig. A.1 The dose of antigen used in an initial immunization affects the primary and secondary antibody response. The typical antigen dose–response curve shown here illustrates the influence of dose on both a primary antibody response (amounts of antibody produced expressed in arbitrary units) and the effect of the dose used for priming on a secondary antibody response elicited by a dose of antigen of 10^3 arbitrary mass units. Very low doses of antigen do not cause an immune response at all. Slightly higher doses appear to inhibit specific antibody production, an effect known as low-zone tolerance. Above these doses there is a steady increase in the response with antigen dose to reach a broad optimum. Very high doses of antigen also inhibit immune responsiveness to a subsequent challenge, a phenomenon known as high-zone tolerance.

Antisera generated by immunization with even the simplest antigen will contain many different antibody molecules that bind to the immunogen in slightly different ways. Some of the antibodies in an antiserum are cross-reactive. A **cross-reaction** is defined as the binding of an antibody to an antigen other than the immunogen; most antibodies cross-react with closely related antigens but, on occasion, some bind antigens having no clear relationship to the immunogen. These cross-reacting antibodies can create problems when the antiserum is used to detect a specific antigen. They can be removed from an antiserum by **absorption** with the cross-reactive antigen, leaving behind the antibodies that bind only to the immunogen. Absorption can be performed by affinity chromatography using immobilized antigen, a technique that is also used for purification of antibodies or antigens (see Section A-5). Most problems of cross-reactivity can be avoided, however, by making monoclonal antibodies (see Section A-12).

Although almost any structure can be recognized by antibody as an antigen, usually only proteins elicit fully developed adaptive immune responses. This is because proteins have the ability to engage T cells, which contribute to inducing most antibody responses and are required for immunological memory. Proteins engage T cells because the T cells recognize antigens as peptide fragments of proteins bound to major histocompatibility complex (MHC) molecules. An adaptive immune response that includes immunological memory can be induced by nonpeptide antigens only when they are attached to a protein carrier that can engage the necessary T cells (see Section 9-3 and Fig. 9.4).

Immunological memory is produced as a result of the initial or **primary immunization**, which evokes the **primary immune response**. This is also known as **priming**, as the animal or person is now 'primed' like a pump to mount a more potent response to subsequent challenges with the same antigen. The response to each immunization is increasingly intense, so that **secondary**, **tertiary**, and subsequent responses are of increasing magnitude (Fig. A.1). Repetitive challenge with antigen to achieve a heightened state of immunity is known as **hyperimmunization**.

Certain properties of a protein that favor the priming of an adaptive immune response have been defined by studying antibody responses to simple natural proteins like hen egg-white lysozyme and to synthetic polypeptide antigens (Fig. A.2). The larger and more complex a protein, and the more distant its relationship to self proteins, the more likely it is to elicit a response. This is because such responses depend on the proteins being degraded into peptides that can bind to MHC molecules, and on the subsequent recognition of these peptide:MHC complexes by T cells. The larger and more distinct the protein antigen, the more likely it is to contain such peptides. Particulate or aggregated antigens are more immunogenic because they are taken up more efficiently by the specialized antigen-presenting cells responsible for initiating a response. Indeed small soluble proteins are unable to induce a response unless they are made to aggregate in some way. Many vaccines, for example, use aggregated protein antigens to potentiate the immune response.

A-1 Haptens.

Small organic molecules of simple structure, such as phenyl arsonates and nitrophenyls, do not provoke antibodies when injected by themselves. However, antibodies can be raised against them if the molecule is attached covalently, by simple chemical reactions, to a protein carrier. Such small molecules were termed **haptens** (from the Greek *haptein*, to fasten) by the immunologist Karl Landsteiner, who first studied them in the early 1900s. He found that animals immunized with a hapten–carrier conjugate produced three distinct sets of antibodies (Fig. A.3). One set comprised hapten-specific

Fig. A.2 Intrinsic properties and extrinsic factors that affect the immunogenicity of proteins.

Factors that influence the immunogenicity of proteins		
Parameter	**Increased immunogenicity**	**Decreased immunogenicity**
Size	Large	Small (MW<2500)
Dose	Intermediate	High or low
Route	Subcutaneous > intraperitoneal > intravenous or intragastric	
Composition	Complex	Simple
Form	Particulate	Soluble
	Denatured	Native
Similarity to self protein	Multiple differences	Few differences
Adjuvants	Slow release	Rapid release
	Bacteria	No bacteria
Interaction with host MHC	Effective	Ineffective

antibodies that reacted with the same hapten on any carrier, as well as with free hapten. The second set of antibodies was specific for the carrier protein, as shown by their ability to bind both the hapten-modified and unmodified carrier protein. Finally, some antibodies reacted only with the specific conjugate of hapten and carrier used for immunization. Landsteiner studied mainly the antibody response to the hapten, as these small molecules could be synthesized in many closely related forms. He observed that antibodies raised against a particular hapten bind that hapten but, in general, fail to bind even very closely related chemical structures. The binding of haptens by anti-hapten antibodies has played an important part in defining the precision of antigen binding by antibody molecules. Anti-hapten antibodies are also important medically as they mediate allergic reactions to penicillin and other compounds that elicit antibody responses when they attach to self proteins (see Section 13-11).

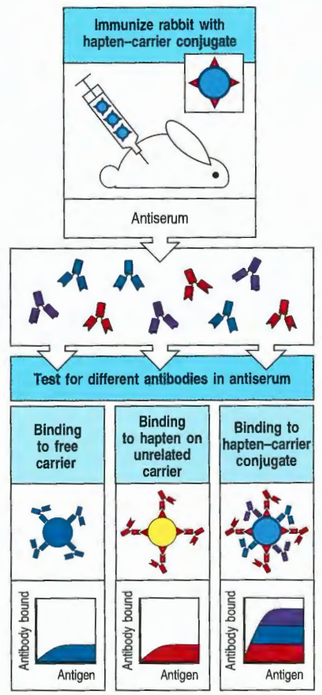


Fig. A.3 Antibodies can be elicited by small chemical groups called haptens only when the hapten is linked to an immunogenic protein carrier. Three types of antibodies are produced. One set (blue) binds the carrier protein alone and is called carrier-specific. One set (red) binds to the hapten on any carrier or to free hapten in solution and is called hapten-specific. One set (purple) only binds the specific conjugate of hapten and carrier used for immunization, apparently binding to sites at which the hapten joins the carrier, and is called conjugate-specific. The amount of antibody of each type in this serum is shown schematically in the graphs at the bottom; note that the original antigen binds more antibody than the sum of anti-hapten and anti-carrier antibodies owing to the additional binding of conjugate-specific antibody.

A-2 Routes of immunization.

The route by which antigen is administered affects both the magnitude and the type of response obtained. The most common routes by which antigen is introduced experimentally or as a vaccine into the body are injection into tissue by **subcutaneous (s.c.)** injection between the epidermis and dermal layers, or by **intradermal (i.d.)** injection, or **intramuscular (i.m.)** injection; by **intravenous (i.v.)** injection or transfusion directly into the bloodstream; into the gastrointestinal tract by oral administration; into the respiratory tract by **intranasal (i.n.)** administration or inhalation.

Antigens injected subcutaneously generally elicit the strongest responses, most probably because the antigen is taken up by Langerhans' cells and efficiently presented in local lymph nodes, and so this is the method most commonly used when the object of the experiment is to elicit specific antibodies or T cells against a given antigen. Antigens injected or transfused directly into the bloodstream tend to induce immune unresponsiveness or tolerance unless they bind to host cells or are in the form of aggregates that are readily taken up by antigen-presenting cells.

Antigen administration via the gastrointestinal tract is used mostly in the study of allergy. It has distinctive effects, frequently eliciting a local antibody response in the intestinal lamina propria, while producing a systemic state of tolerance that manifests as a diminished response to the same antigen if subsequently administered in immunogenic form elsewhere in the body. This 'split tolerance' may be important in avoiding allergy to antigens in food, as the local response prevents food antigens from entering the body, while the inhibition of systemic immunity helps to prevent the formation of IgE antibodies, which are the cause of such allergies (see Chapter 13).

Introduction of antigen into the respiratory tract is also used mainly in the study of allergy. Protein antigens that enter the body through the respiratory epithelium tend to elicit allergic responses, for reasons that are not clear.

A-3 Effects of antigen dose.

The magnitude of the immune response depends on the dose of immunogen administered. Below a certain threshold dose, most proteins do not elicit any immune response. Above the threshold dose, there is a gradual increase in the response as the dose of antigen is increased, until a broad plateau level is reached, followed by a decline at very high antigen doses (see Fig. A.1). As most infectious agents enter the body in small numbers, immune responses are generally elicited only by pathogens that multiply to a level sufficient to exceed the antigen dose threshold. The broad response optimum allows the system to respond to infectious agents across a wide range of doses. At very high antigen doses the immune response is inhibited, which may be important in maintaining tolerance to abundant self proteins such as plasma proteins. In general, secondary and subsequent immune responses occur at lower antigen doses and achieve higher plateau values, which is a sign of immunological memory. However, under some conditions, very low or very high doses of antigen may induce specific unresponsive states, known respectively as acquired **low-zone** or **high-zone tolerance**.

A-4 Adjuvants.

Most proteins are poorly immunogenic or nonimmunogenic when administered by themselves. Strong adaptive immune responses to protein antigens almost always require that the antigen be injected in a mixture known as an

adjuvant. An adjuvant is any substance that enhances the immunogenicity of substances mixed with it. Adjuvants differ from protein carriers in that they do not form stable linkages with the immunogen. Furthermore, adjuvants are needed primarily for initial immunizations, whereas carriers are required to elicit not only primary but also subsequent responses to haptens. Commonly used adjuvants are listed in Fig. A.4.

Adjuvants can enhance immunogenicity in two different ways. First, adjuvants convert soluble protein antigens into particulate material, which is more readily ingested by antigen-presenting cells such as macrophages. For example, the antigen can be adsorbed on particles of the adjuvant (such as alum), made particulate by emulsification in mineral oils, or incorporated into the colloidal particles of ISCOMs. This enhances immunogenicity somewhat, but such adjuvants are relatively weak unless they also contain bacteria or bacterial products. Such microbial constituents are the second means by which adjuvants enhance immunogenicity, and although their exact contribution to enhancing immunogenicity is unknown, they are clearly the more important component of an adjuvant. Microbial products may signal macrophages or dendritic cells to become more effective antigen-presenting cells (see Chapter 2). One of their effects is to induce the production of inflammatory cytokines and potent local inflammatory responses; this effect is probably intrinsic to their activity in enhancing responses, but precludes their use in humans.

Nevertheless, some human vaccines contain microbial antigens that can also act as effective adjuvants. For example, purified constituents of the bacterium *Bordetella pertussis*, which is the causal agent of whooping cough, are used as both antigen and adjuvant in the triplex DPT (diphtheria, pertussis, tetanus) vaccine against these diseases.

Adjuvants that enhance immune responses

Adjuvant name	Composition	Mechanism of action
Incomplete Freund's adjuvant	Oil-in-water emulsion	Delayed release of antigen; enhanced uptake by macrophages
Complete Freund's adjuvant	Oil-in-water emulsion with dead mycobacteria	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators in macrophages
Freund's adjuvant with MDP	Oil-in-water emulsion with muramyldipeptide (MDP), a constituent of mycobacteria	Similar to complete Freund's adjuvant
Alum (aluminum hydroxide)	Aluminum hydroxide gel	Delayed release of antigen; enhanced macrophage uptake
Alum plus *Bordetella pertussis*	Aluminum hydroxide gel with killed *B. pertussis*	Delayed release of antigen; enhanced uptake by macrophages; induction of co-stimulators
Immune stimulatory complexes (ISCOMs)	Matrix of Quil A containing viral proteins	Delivers antigen to cytosol; allows induction of cytotoxic T cells

Fig. A.4 Common adjuvants and their use. Adjuvants are mixed with the antigen and usually render it particulate, which helps to retain the antigen in the body and promotes uptake by macrophages. Most adjuvants include bacteria or bacterial components that stimulate macrophages, aiding in the induction of the immune response. ISCOMs (immune stimulatory complexes) are small micelles of the detergent Quil A; when viral proteins are placed in these micelles, they apparently fuse with the antigen-presenting cell, allowing the antigen to enter the cytosol. Thus, the antigen-presenting cell can stimulate a response to the viral protein, much as a virus infecting these cells would stimulate an anti-viral response.

The detection, measurement, and characterization of antibodies and their use as research and diagnostic tools.

B cells contribute to adaptive immunity by secreting antibodies, and the response of B cells to an injected immunogen is usually measured by analyzing the specific antibody produced in a **humoral immune response**. This is most conveniently achieved by assaying the antibody that accumulates in the fluid phase of the blood or **plasma**; such antibodies are known as circulating antibodies. Circulating antibody is usually measured by collecting blood, allowing it to clot, and then isolating the serum from the clotted blood. The amount and characteristics of the antibody in the resulting antiserum are then determined using the assays we will describe in Sections A-5–A-11.

The most important characteristics of an antibody response are the specificity, amount, isotype or class, and affinity of the antibodies produced. The **specificity** determines the ability of the antibody to distinguish the immunogen from other antigens. The amount of antibody can be determined in many different ways and is a function of the number of responding B cells, their rate of antibody synthesis, and the persistence of the antibody after production. The persistence of an antibody in the plasma and extracellular fluid bathing the tissues is determined mainly by its isotype or class (see Sections 4-12 and 9-14); each isotype has a different half-life *in vivo*. The isotypic composition of an antibody response also determines the biological functions these antibodies can perform and the sites in which antibody will be found. Finally, the strength of binding of the antibody to its antigen in terms of a single antigen-binding site binding to a monovalent antigen is termed its **affinity** (the total binding strength of a molecule with more than one binding site is called its **avidity**). Binding strength is important, since the higher the affinity of the antibody for its antigen, the less antibody is required to eliminate the antigen, as antibodies with higher affinity will bind at lower antigen concentrations. All these parameters of the humoral immune response help to determine the capacity of that response to protect the host from infection.

Antibody molecules are highly specific for their corresponding antigen, being able to detect one molecule of a protein antigen out of more than 10^8 similar molecules. This makes antibodies both easy to isolate and study, and invaluable as probes of biological processes. Whereas standard chemistry would have great difficulty in distinguishing between two such closely related proteins as human and pig insulin, or two such closely related structures as *ortho-* and *para*-nitrophenyl, antibodies can be made that discriminate between these two structures absolutely. The value of antibodies as molecular probes has stimulated the development of many sensitive and highly specific techniques to measure their presence, to determine their specificity and affinity for a range of antigens, and to ascertain their functional capabilities. Many standard techniques used throughout biology exploit the specificity and stability of antigen binding by antibodies. Comprehensive guides to the conduct of these antibody assays are available in many books on immunological methodology; we will illustrate here only the most important techniques, especially those used in studying the immune response itself.

Some assays for antibody measure the direct binding of the antibody to its antigen. Such assays are based on **primary interactions**. Others determine the amount of antibody present by the changes it induces in the physical

state of the antigen, such as the precipitation of soluble antigen or the clumping of antigenic particles; these are called secondary interactions. Both types of assay can be used to measure the amount and specificity of the antibodies produced after immunization, and both can be applied to a wide range of other biological questions.

As assays for antibody were originally conducted with antisera from immune individuals, they are commonly referred to as **serological assays**, and the use of antibodies is often called **serology**. The amount of antibody is usually determined by antigen-binding assays after titration of the antiserum by serial dilution, and the point at which binding falls to 50% of the maximum is usually referred to as the **titer** of an antiserum.

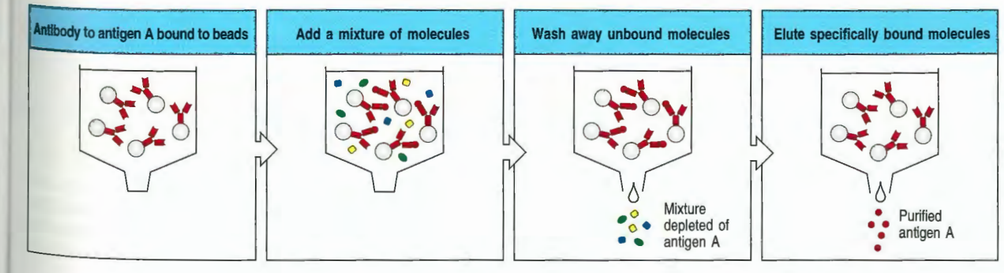
A-5 Affinity chromatography.

Specific antibody can be isolated from an antiserum by **affinity chromatography**, which exploits the specific binding of antibody to antigen held on a solid matrix (Fig. A.5). Antigen is bound covalently to small, chemically reactive beads, which are loaded into a column, and the antiserum is allowed to pass over the beads. The specific antibodies bind, while all the other proteins in the serum, including antibodies to other substances, can be washed away. The specific antibodies are then eluted, typically by lowering the pH to 2.5 or raising it to greater than 11. Antibodies bind stably under physiological conditions of salt concentration, temperature, and pH, but the binding is reversible as the bonds are noncovalent. Affinity chromatography can also be used to purify antigens from complex mixtures by using beads coated with specific antibody. The technique is known as affinity chromatography because it separates molecules on the basis of their affinity for one another.

A-6 Radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and competitive inhibition assay.

Radioimmunoassay (RIA) and **enzyme-linked immunosorbent assay (ELISA)** are direct binding assays for antibody (or antigen) and both work on the same principle, but the means of detecting specific binding is different. Radioimmunoassays are commonly used to measure the levels of hormones in blood and tissue fluids, while ELISA assays are frequently used in viral

Fig. A.5 Affinity chromatography uses antigen–antibody binding to purify antigens or antibodies. To purify a specific antigen from a complex mixture of molecules, a monoclonal antibody is attached to an insoluble matrix, such as chromatography beads, and the mixture of molecules is passed over the matrix. The specific antibody binds the antigen of interest; other molecules are washed away. Specific antigen is then eluted by altering the pH, which can usually disrupt antibody–antigen bonds. Antibodies can be purified in the same way on beads coupled to antigen (not shown).



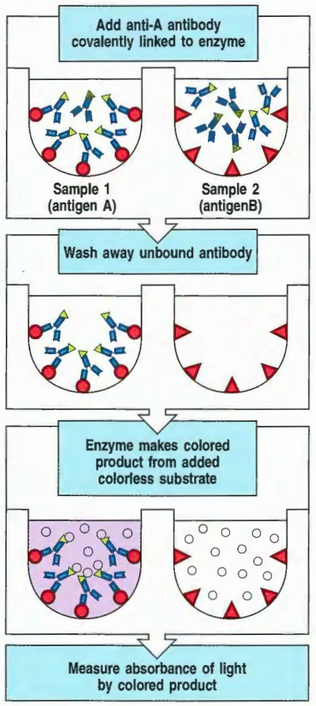


Fig. A.6 The principle of the enzyme-linked immunosorbent assay (ELISA). To detect antigen A, purified antibody specific for antigen A is linked chemically to an enzyme. The samples to be tested are coated onto the surface of plastic wells to which they bind nonspecifically; residual sticky sites on the plastic are blocked by adding irrelevant proteins (not shown). The labeled antibody is then added to the wells under conditions where nonspecific binding is prevented, so that only binding to antigen A causes the labeled antibody to be retained on the surface. Unbound labeled antibody is removed from all wells by washing, and bound antibody is detected by an enzyme-dependent color-change reaction. This assay allows arrays of wells known as microtiter plates to be read in fiberoptic multichannel spectrometers, greatly speeding the assay. Modifications of this basic assay allow antibody or antigen in unknown samples to be measured as shown in Figs A.7 and A.30 (see also Section A-10).

diagnostics, for example in detecting cases of HIV infection. For both these methods one needs a pure preparation of a known antigen or antibody, or both, in order to standardize the assay. We will describe the assay with a sample of pure antibody, which is the more usual case, but the principle is similar if pure antigen is used instead. In RIA for an antigen, pure antibody against that antigen is radioactively labeled, usually with ^{125}I; for the ELISA, an enzyme is linked chemically to the antibody. The unlabeled component, which in this case would be antigen, is attached to a solid support, such as the wells of a plastic multiwell plate, which will adsorb a certain amount of any protein.

The labeled antibody is allowed to bind to the unlabeled antigen, under conditions where nonspecific adsorption is blocked, and any unbound antibody and other proteins are washed away. Antibody binding in RIA is measured directly in terms of the amount of radioactivity retained by the coated wells, whereas in ELISA, binding is detected by a reaction that converts a colorless substrate into a colored reaction product (Fig. A.6). The color change can be read directly in the reaction tray, making data collection very easy, and ELISA also avoids the hazards of radioactivity. This makes ELISA the preferred method for most direct-binding assays. Labeled anti-immunoglobulin antibodies (see Section A-10) can also be used in RIA or ELISA to detect binding of unlabeled antibody to unlabeled antigen-coated plates. In this case, the labeled anti-immunoglobulin antibody is used in what is termed a 'second layer.' The use of such a second layer also amplifies the signal, as at least two molecules of the labeled anti-immunoglobulin antibody are able to bind to each unlabeled antibody. RIA and ELISA can also be carried out with unlabeled antibody stuck to the plates and labeled antigen added.

A modification of ELISA known as a **capture** or **sandwich ELISA** (or more generally as an **antigen-capture assay**) can be used to detect secreted products such as cytokines. Rather than the antigen being directly attached to a plastic plate, antigen-specific antibodies are bound to the plate. These are able to bind antigen with high affinity, and thus concentrate it on the surface of the plate, even with antigens that are present in very low concentrations in the initial mixture. A separate labeled antibody that recognizes a different epitope to the immobilized first antibody is then used to detect the bound antigen.

These assays illustrate two crucial aspects of all serological assays. First, at least one of the reagents must be available in a pure, detectable form in order to obtain quantitative information. Second, there must be a means of separating the bound fraction of the labeled reagent from the unbound, free fraction so that the percentage of specific binding can be determined. Normally, this separation is achieved by having the unlabeled partner trapped on a solid support. Labeled molecules that do not bind can then be washed away, leaving just the labeled partner that has bound. In Fig. A.6, the unlabeled antigen is attached to the well and the labeled antibody is trapped by binding to it. The separation of bound from free is an essential step in every assay that uses antibodies.

RIA and ELISA do not allow one to measure directly the amount of antigen or antibody in a sample of unknown composition, as both depend on the binding of a pure labeled antigen or antibody. There are various ways around this problem, one of which is to use a **competitive inhibition assay**, as shown in Fig. A.7. In this type of assay, the presence and amount of a particular antigen in an unknown sample is determined by its ability to compete with a labeled reference antigen for binding to an antibody attached to a plastic well. A standard curve is first constructed by adding varying amounts of a known, unlabeled standard preparation; the assay can then measure the amount of antigen in unknown samples by comparison with the standard.

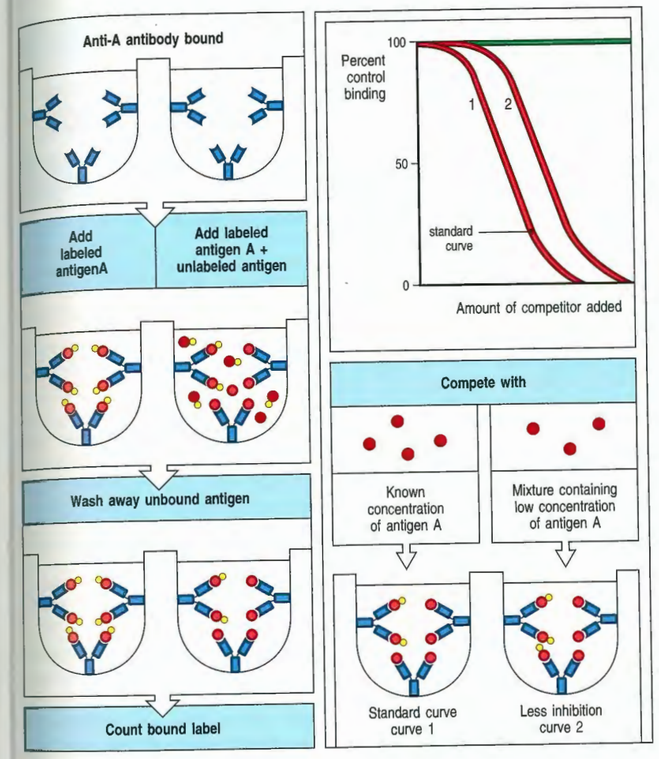


Fig. A.7 Competitive inhibition assay for antigen in unknown samples. A fixed amount of unlabeled antibody is attached to a set of wells, and a standard reference preparation of a labeled antigen is bound to it. Unlabeled standard or test samples are then added in various amounts and the displacement of labeled antigen is measured, generating characteristic inhibition curves. A standard curve is obtained by using known amounts of unlabeled antigen identical to that used as the labeled species, and comparison with this curve allows the amount of antigen in unknown samples to be calculated. The green line on the graph represents a sample lacking any substance that reacts with anti-A antibodies.

The competitive binding assay can also be used for measuring antibody in a sample of unknown composition by attaching the appropriate antigen to the plate and measuring the ability of the test sample to inhibit the binding of a labeled specific antibody.

A-7 Hemagglutination and blood typing.

The direct measurement of antibody binding to antigen is used in most quantitative serological assays. However, some important assays are based on the ability of antibody binding to alter the physical state of the antigen it binds to. These secondary interactions can be detected in a variety of ways. For instance, when the antigen is displayed on the surface of a large particle such as a bacterium, antibodies can cause the bacteria to clump or **agglutinate**. The same principle applies to the reactions used in blood typing, only here the target antigens are on the surface of red blood cells and the clumping reaction caused by antibodies against them is called **hemagglutination** (from the Greek *haima*, blood).

Hemagglutination is used to determine the **ABO blood group** of blood donors and transfusion recipients. Clumping or agglutination is induced by antibodies or agglutinins called anti-A or anti-B that bind to the A or B blood-group substances, respectively (Fig. A.8). These blood-group antigens are arrayed in

Fig. A.8 Hemagglutination is used to type blood groups and match compatible donors and recipients for blood transfusion. Common gut bacteria bear antigens that are similar or identical to blood-group antigens, and these stimulate the formation of antibodies to these antigens in individuals who do not bear the corresponding antigen on their own red blood cells (left column); thus, type O individuals, who lack A and B, have both anti-A and anti-B antibodies, while type AB individuals have neither. The pattern of agglutination of the red blood cells of a transfusion donor or recipient with anti-A and anti-B antibodies reveals the individual's ABO blood group. Before transfusion, the serum of the recipient is also tested for antibodies that agglutinate the red blood cells of the donor, and vice versa, a procedure called a cross-match, which may detect potentially harmful antibodies to other blood groups that are not part of the ABO system.

Serum from individuals of type	Red blood cells from individuals of type: O	A	B	AB
	Express the carbohydrate structures: R–GlcNAc–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc)	R–GlcNAc–Gal–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc) and R–GlcNAc–Gal–Gal (Fuc)
O — Anti-A and anti-B antibodies	no agglutination	agglutination	agglutination	agglutination
A — Anti-B antibodies	no agglutination	no agglutination	agglutination	agglutination
B — Anti-A antibodies	no agglutination	agglutination	no agglutination	agglutination
AB — No antibodies to A or B	no agglutination	no agglutination	no agglutination	no agglutination

many copies on the surface of the red blood cell, allowing the cells to agglutinate when cross-linked by antibodies. Because hemagglutination involves the cross-linking of blood cells by the simultaneous binding of antibody molecules to identical antigens on different cells, this reaction demonstrates that each antibody molecule has at least two identical antigen-binding sites.

A-8 Precipitin reaction.

When sufficient quantities of antibody are mixed with soluble macromolecular antigens, a visible precipitate consisting of large aggregates of antigen cross-linked by antibody molecules can form. The amount of precipitate depends on the amounts of antigen and antibody, and on the ratio between them (Fig. A.9). This **precipitin reaction** provided the first quantitative assay for antibody, but is now seldom used in immunology. However, it is important to understand the interaction of antigen with antibody that leads to this reaction, as the production of **antigen:antibody complexes**, also known as **immune complexes**, *in vivo* occurs in almost all immune responses and occasionally can cause significant pathology (see Chapters 13 and 14).

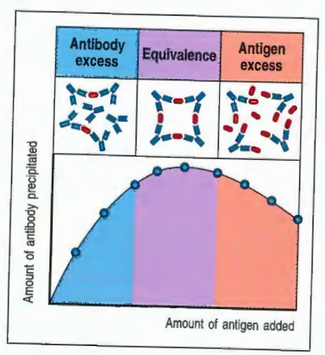


Fig. A.9 Antibody can precipitate soluble antigen. Analysis of the precipitate can generate a precipitin curve. Different amounts of antigen are added to a fixed amount of antibody, and precipitates form by antibody cross-linking of antigen molecules. The precipitate is recovered and the amount of precipitated antibody measured; the supernatant is tested for residual antigen or antibody. This defines zones of antibody excess, equivalence, and antigen excess. At equivalence, the largest antigen:antibody complexes form. In the zone of antigen excess, some of the immune complexes are too small to precipitate. These soluble immune complexes can cause pathological damage to small blood vessels when they form *in vivo* (see Chapter 14).

In the precipitin reaction, various amounts of soluble antigen are added to a fixed amount of serum containing antibody. As the amount of antigen added increases, the amount of precipitate generated also increases up to a maximum and then declines (see Fig. A.9). When small amounts of antigen are added, antigen:antibody complexes are formed under conditions of antibody excess so that each molecule of antigen is bound by antibody and cross-linked to other molecules of antigen. When large amounts of antigen are added, only small antigen:antibody complexes can form and these are often soluble in this zone of antigen excess. Between these two zones, all of the antigen and antibody is found in the precipitate, generating a zone of equivalence. At equivalence, very large lattices of antigen and antibody are formed by cross-linking. While all antigen:antibody complexes can potentially produce disease, the small, soluble immune complexes formed in the zone of antigen excess may persist and cause pathology *in vivo*.

The precipitin reaction is affected by the number of binding sites that each antibody has for antigen, and by the maximum number of antibodies that can be bound by an antigen molecule or particle at any one time. These quantities are defined as the **valence** of the antibody and the valence of the antigen: the valence of both the antibodies and the antigen must be two or greater before any precipitation can occur. The valence of an antibody depends on its structural class (see Section 4-16).

Antigen will be precipitated only if it has several antibody-binding sites. This condition is usually satisfied in macromolecular antigens, which have a complex surface with binding sites for several different antibodies. The site on an antigen to which each distinct antibody molecule binds is called an **antigenic determinant** or an **epitope**. Steric considerations limit the number of distinct antibody molecules that can bind to a single antigen molecule at any one time however, because antibody molecules binding to epitopes that partially overlap will compete for binding. For this reason, the valence of an antigen is almost always less than the number of epitopes on the antigen (Fig. A.10).

A-9 Equilibrium dialysis: measurement of antibody affinity and avidity.

The affinity of an antibody is the strength of binding of a monovalent ligand to a single antigen-binding site on the antibody. The affinity of an antibody that binds small antigens, such as haptens, that can diffuse freely across a dialysis membrane can be determined directly by the technique of **equilibrium dialysis**. A known amount of antibody, whose molecules are too large to cross a dialysis membrane, is placed in a dialysis bag and offered various amounts of antigen. Molecules of antigen that bind to the antibody are no longer free to diffuse across the dialysis membrane, so only the unbound molecules of antigen equilibrate across it. By measuring the concentration of antigen inside the bag and in the surrounding fluid, one can determine the amount of the antigen that is bound as well as the amount that is free when equilibrium has been achieved. Given that the amount of antibody present is known, the affinity of the antibody and the number of specific binding sites for the antigen per molecule of antibody can be determined from this information. The data are usually analyzed using **Scatchard analysis** (Fig. A.11); such analyses were used to demonstrate that a molecule of IgG antibody has two identical antigen-binding sites.

Whereas affinity measures the strength of binding of an antigenic determinant to a single antigen-binding site, an antibody reacting with an antigen that has multiple identical epitopes or with the surface of a pathogen will often bind the same molecule or particle with both of its antigen-binding sites. This increases the apparent strength of binding, since both binding sites must release at the same time in order for the two molecules to dissociate.

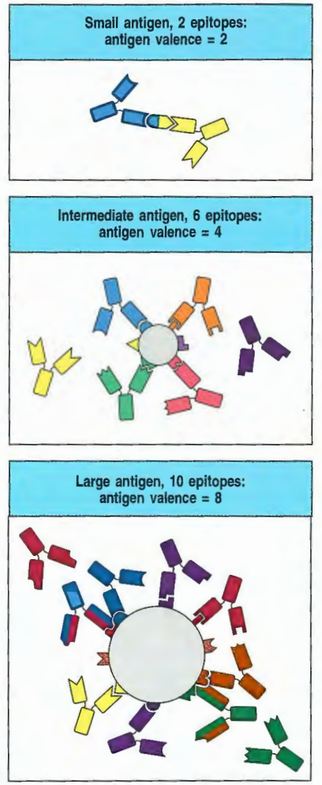


Fig. A.10 Different antibodies bind to distinct epitopes on an antigen molecule. The surface of an antigen possesses many potential antigenic determinants or epitopes, distinct sites to which an antibody can bind. The number of antibody molecules that can bind to a molecule of antigen at one time defines the antigen's valence. Steric considerations can limit the number of different antibodies that bind to the surface of an antigen at any one time (center and bottom panels) so that the number of epitopes on an antigen is always greater than or equal to its valence.

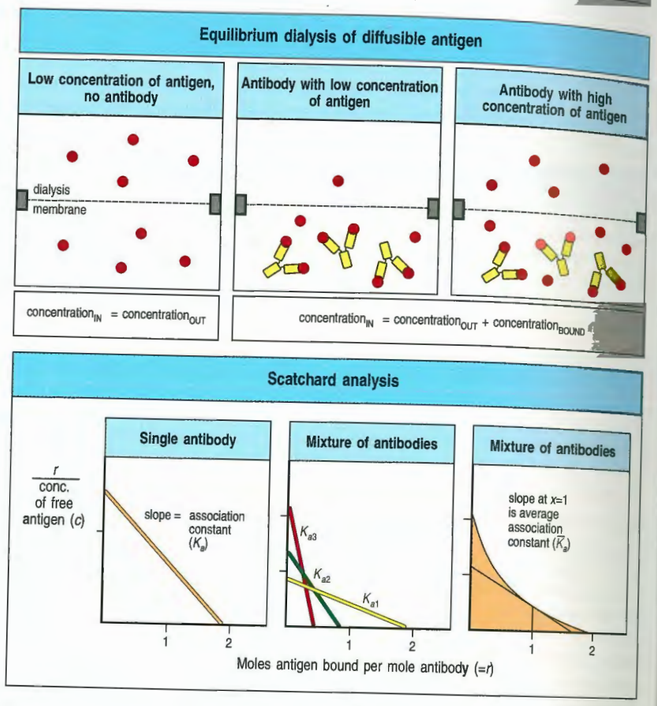


Fig. A.11 The affinity and valence of an antibody can be determined by equilibrium dialysis. A known amount of antibody is placed in the bottom half of a dialysis chamber and exposed to different amounts of a diffusible monovalent antigen, such as a hapten. At equilibrium, the concentration of free antigen will be the same on each side of the membrane, so that at each concentration of antigen added, the fraction of the antigen bound is determined from the difference in concentration of total antigen in the top and bottom chambers. This information can be transformed into a Scatchard plot as shown here. In Scatchard analysis, the ratio *r*/*c* (where *r* = moles of antigen bound per mole of antibody and *c* = molar concentration of free antigen) is plotted against r. The number of binding sites per antibody molecule can be determined from the value of r at infinite free-antigen concentration, where *r*/free = 0, in other words at the x-axis intercept. The analysis of a monoclonal IgG antibody molecule, in which there are two identical antigen-binding sites per molecule, is shown in the left panel. The slope of the line is determined by the affinity of the antibody molecule for its antigen; if all the antibody molecules in a preparation are identical, as for this monoclonal antibody, then a straight line is obtained whose slope is equal to $-K_a$, where K_a is the association (or affinity) constant and the dissociation constant $K_d = 1/K_a$. However, antiserum raised even against a simple antigenic determinant such as a hapten contains a heterogeneous population of antibody molecules (see Section A-1). Each antibody molecule would, if isolated, make up part of the total and give a straight line whose x-axis intercept is less than two, as this antibody molecule contains only a fraction of the total binding sites in the population (middle panel). As a mixture, they give curved lines with an x-axis intercept of two for which an average affinity ($\bar{K}_a$) can be determined from the slope of this line at a concentration of antigen where 50% of the sites are bound, or at x = 1 (right panel). The association constant determines the equilibrium state of the reaction Ag + Ab = Ag:Ab, where antigen = Ag and antibody = Ab, and K_a = [Ag:Ab]/[Ag][Ab]. This constant reflects the 'on' and 'off' rates for antigen binding to the antibody; with small antigens such as haptens, binding is usually as rapid as diffusion allows, whereas differences in 'off' rates determine the affinity constant. However, with larger antigens the 'on' rate may also vary as the interaction becomes more complex.

This is often referred to as **cooperativity** in binding, but it should not be confused with the cooperative binding found in a protein such as hemoglobin, in which binding of ligand at one site enhances the affinity of a second binding site for its ligand. The overall strength of binding of an antibody molecule to an antigen or particle is called its avidity (Fig. A.12). For IgG antibodies, bivalent binding can significantly increase avidity; in IgM antibodies, which have ten identical antigen-binding sites, the affinity of each site for a monovalent antigen is usually quite low, but the avidity of binding of the whole antibody to a surface such as a bacterium that displays multiple identical epitopes can be very high.

A-10 Anti-immunoglobulin antibodies.

A general approach to the detection of bound antibody that avoids the need to label each preparation of antibody molecules is to detect bound, unlabeled antibody with a labeled antibody specific for immunoglobulins themselves. Immunoglobulins, like other proteins, are immunogenic when used to immunize individuals of another species. The majority of **anti-immunoglobulin antibodies** raised in this way recognize conserved features shared by all immunoglobulin molecules of the immunizing species. Others can be specific for immunoglobulin chains, heavy or light chains, for example, or for individual isotypes. Antibodies raised by immunization of goats with mouse IgG are commonly used in experimental immunology. Such goat anti-mouse IgG antibodies can be purified using affinity chromatography, then labeled and used as a general probe for bound IgG antibodies. Anti-immunoglobulin

antisera have found many uses in clinical medicine and biological research since their introduction. Fluorescently labeled anti-immunoglobulin antibodies are now widely used both in immunology and other areas of biology as secondary reagents for detecting specific antibodies bound, for example, to cell structures (see Sections A-14 and A-16). Labeled anti-immunoglobulin antibodies can also be used in radioimmunoassay or ELISA (see Section A-6) to detect binding of unlabeled antibody to antigen-coated plates.

When an immunoglobulin is used as an antigen to immunize a different species of animal, it will be treated like any other foreign protein and will elicit an antibody response. Anti-immunoglobulin antibodies can be made that recognize the amino acids that characterize the isotype of the injected antibody. Such **anti-isotypic antibodies** recognize all immunoglobulins of the same isotype in all members of the species from which the injected antibody came.

It is also possible to raise antibodies that recognize differences in immunoglobulins from members of the same species that are due to the presence of multiple alleles of the individual C genes in the population (genetic polymorphism). Such allelic variants are called **allotypes**. In contrast to anti-isotypic antibodies, anti-allotypic antibodies will recognize immunoglobulin of a particular isotype only in some members of a species. Finally, as individual antibodies differ in their variable regions, one can raise antibodies against unique features of the antigen-binding site, which are called **idiotypes**.

A schematic picture of the differences between idiotypes, allotypes, and isotypes is given in Fig. A.13. Historically, the main features of immunoglobulins were defined by using isotypic and allotypic genetic markers identified by antisera raised in different species or in genetically distinct members of the same species (see Section A-10). The independent segregation of allotypic and isotypic markers revealed the existence of separate heavy-chain, κ, and λ genes. Such anti-idiotypic, allotypic, and isotypic antibodies are still enormously useful in detecting antibodies and B cells in scientific experimentation and medical diagnostics.

Antibodies specific for individual immunoglobulin isotypes can be produced by immunizing an animal of a different species with a pure preparation of one isotype and then removing those antibodies that cross-react with immunoglobulins of other isotypes by using affinity chromatography (see Section A-5). Anti-isotype antibodies can be used to measure how much antibody of a particular isotype in an antiserum reacts with a given antigen. This reaction is particularly important for detecting small amounts of specific antibodies of the IgE isotype, which are responsible for most allergies. The presence in an individual's serum of IgE binding to an antigen correlates with allergic reactions to that antigen.

An alternative approach to detecting bound antibodies exploits bacterial proteins that bind to immunoglobulins with high affinity and specificity. One of these, **Protein A** from the bacterium *Staphylococcus aureus*, has been exploited widely in immunology for the affinity purification of immuno-globulin and for the detection of bound antibody. The use of standard second reagents such as labeled anti-immunoglobulin antibodies or Protein A to detect antibody bound specifically to its antigen allows great savings in reagent labeling costs, and also provides a standard detection system so that results in different assays can be compared directly.

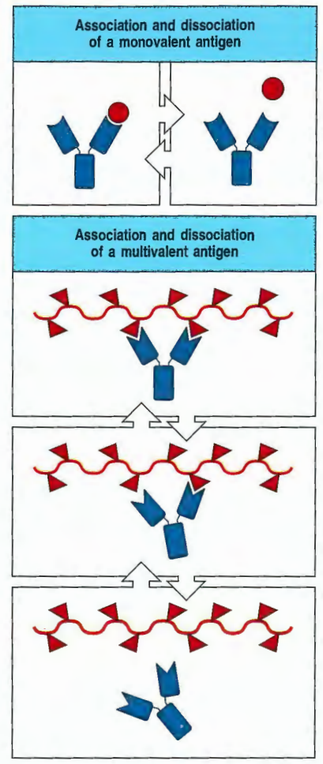


Fig. A.12 The avidity of an antibody is its strength of binding to intact antigen. When an IgG antibody binds a ligand with multiple identical epitopes, both binding sites can bind the same molecule or particle. The overall strength of binding, called avidity, is greater than the affinity, the strength of binding of a single site, since both binding sites must dissociate at the same time for the antibody to release the antigen. This property is very important in the binding of antibody to bacteria, which usually have multiple identical epitopes on their surfaces.

A-11 Coombs tests and the detection of Rhesus incompatibility.

These tests use anti-immunoglobulin antibodies (see Section A-10) to detect the antibodies that cause **hemolytic disease of the newborn**, or **erythroblastosis**

Event	Process	Nature of change	Process occurs in:	
			B cells	T cells
V-region assembly	Somatic recombination of DNA	Irreversible	Yes	Yes
Junctional diversity	Imprecise joining, N-sequence insertion in DNA	Irreversible	Yes	Yes
Transcriptional activation	Activation of promoter by proximity to the enhancer	Irreversible but regulated	Yes	Yes
Switch recombination	Somatic recombination of DNA	Irreversible	Yes	No
Somatic hypermutation	DNA point mutation	Irreversible	Yes	No
IgM, IgD expression on surface	Differential splicing of RNA	Reversible, regulated	Yes	No
Membrane vs secreted form	Differential splicing of RNA	Reversible, regulated	Yes	No

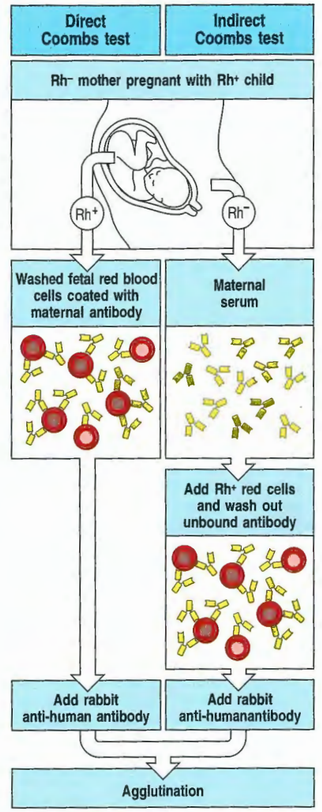
Fig. A.13 Different types of variation between immunoglobulins. Differences between constant regions due to usage of different C-region genes are called isotypes; differences due to different alleles of the same C gene are called allotypes; differences due to particular rearranged V_H and V_L genes are called idiotypes.

fetalis. Anti-immunoglobulin antibodies were first developed by Robin Coombs and the test for this disease is still called the Coombs test. Hemolytic disease of the newborn occurs when a mother makes IgG antibodies specific for the **Rhesus** or **Rh blood group antigen** expressed on the red blood cells of her fetus. Rh-negative mothers make these antibodies when they are exposed to Rh-positive fetal red blood cells bearing the paternally inherited Rh antigen. Maternal IgG antibodies are normally transported across the placenta to the fetus, where they protect the newborn infant against infection. However, IgG anti-Rh antibodies coat the fetal red blood cells, which are then destroyed by phagocytic cells in the liver, causing a hemolytic anemia in the fetus and newborn infant.

Since the Rh antigens are widely spaced on the red blood cell surface, the IgG anti-Rh antibodies cannot fix complement and cause lysis of red blood cells *in vitro*. Furthermore, for reasons that are not fully understood, antibodies to Rh blood group antigens do not agglutinate red blood cells as do antibodies to the ABO blood group antigens. Thus, detecting these antibodies was difficult until anti-human immunoglobulin antibodies were developed. With these, maternal IgG antibodies bound to the fetal red blood cells can be detected after washing the cells to remove unbound immunoglobulin that is present in the fetal serum. Adding anti-human immunoglobulin antibodies to the washed fetal red blood cells agglutinates any cells to which maternal antibodies are bound. This is the **direct Coombs test** (Fig. A.14), so called because it directly detects antibody bound to the surface of the fetal red blood cells. An **indirect Coombs test** is used to detect nonagglutinating anti-Rh antibody in maternal serum; the serum is first incubated with Rh-positive red blood cells, which bind the anti-Rh antibody, after which the antibody-coated cells are washed to remove unbound immunoglobulin and are then agglutinated with anti-immunoglobulin antibody (see Fig. A.14). The indirect Coombs test allows Rh incompatibilities that might lead to hemolytic disease of the newborn to be detected, and this knowledge allows the disease to be prevented (see Section 10-19). The Coombs test is also commonly used to detect antibodies to drugs that bind to red blood cells and cause hemolytic anemia.

Fig. A.14 The Coombs direct and indirect anti-globulin tests for antibody to red blood cell antigens. A Rh^- mother of a Rh^+ fetus can become immunized to fetal red blood cells that enter the maternal circulation at the time of delivery. In a subsequent pregnancy with a Rh^+ fetus, IgG anti-Rh antibodies can cross the placenta and damage the fetal red blood cells. In contrast to anti-Rh antibodies, maternal anti-ABO antibodies are of the IgM isotype and cannot cross the placenta, and so do not cause harm.

Anti-Rh antibodies do not agglutinate red blood cells but their presence on the fetal red blood cell surface can be shown by washing away unbound immunoglobulin and then adding antibody to human immunoglobulin, which agglutinates the antibody-coated cells. Anti-Rh antibodies can be detected in the mother's serum in an indirect Coombs test; the serum is incubated with Rh^+ red blood cells, and once the antibody binds, the red blood cells are treated as in the direct Coombs test.



A-12 Monoclonal antibodies.

The antibodies generated in a natural immune response or after immunization in the laboratory are a mixture of molecules of different specificities and affinities. Some of this heterogeneity results from the production of antibodies that bind to different epitopes on the immunizing antigen, but even antibodies directed at a single antigenic determinant such as a hapten can be markedly heterogeneous, as shown by **isoelectric focusing**. In this technique, proteins are separated on the basis of their isoelectric point, the pH at which their net charge is zero. By electrophoresing proteins in a pH gradient for long enough, each molecule migrates along the pH gradient until it reaches the pH at which it is neutral and is thus concentrated (focused) at that point. When antiserum containing anti-hapten antibodies is treated in this way and then transferred to a solid support such as nitrocellulose paper, the anti-hapten antibodies can be detected by their ability to bind labeled hapten. The binding of antibodies of various isoelectric points to the hapten shows that even antibodies that bind the same antigenic determinant can be heterogeneous.

Antisera are valuable for many biological purposes but they have certain inherent disadvantages that relate to the heterogeneity of the antibodies they contain. First, each antiserum is different from all other antisera, even if raised in a genetically identical animal by using the identical preparation of antigen and the same immunization protocol. Second, antisera can be produced in only limited volumes, and thus it is impossible to use the identical serological reagent in a long or complex series of experiments or clinical tests. Finally, even antibodies purified by affinity chromatography (see Section A-5) may include minor populations of antibodies that give unexpected cross-reactions, which confound the analysis of experiments. To avoid these problems, and to harness the full potential of antibodies, it was necessary to develop a way of making an unlimited supply of antibody molecules of homogeneous structure and known specificity. This has been achieved through the production of monoclonal antibodies from cultures of hybrid antibody-forming cells or, more recently, by genetic engineering.

Biochemists in search of a homogeneous preparation of antibody that they could subject to detailed chemical analysis turned early to proteins produced by patients with multiple myeloma, a common tumor of plasma cells. It was known that antibodies are normally produced by plasma cells and since this disease is associated with the presence of large amounts of a homogeneous gamma globulin called a **myeloma protein** in the patient's serum, it seemed likely that myeloma proteins would serve as models for normal antibody molecules. Thus, much of the early knowledge of antibody structure came from studies on myeloma proteins. These studies showed that monoclonal antibodies could be obtained from immortalized plasma cells. However, the

antigen specificity of most myeloma proteins was unknown, which limited their usefulness as objects of study or as immunological tools.

This problem was solved by Georges Köhler and Cesar Milstein, who devised a technique for producing a homogeneous population of antibodies of known antigenic specificity. They did this by fusing spleen cells from an immunized mouse to cells of a mouse myeloma to produce hybrid cells that both proliferated indefinitely and secreted antibody specific for the antigen used to immunize the spleen cell donor. The spleen cell provides the ability to make specific antibody, while the myeloma cell provides the ability to grow indefinitely in culture and secrete immunoglobulin continuously. By using a myeloma cell partner that produces no antibody proteins itself, the antibody produced by the hybrid cells comes only from the immune spleen cell partner. After fusion, the hybrid cells are selected using drugs that kill the myeloma parental cell, while the unfused parental spleen cells have a limited life-span and soon die, so that only hybrid myeloma cell lines or **hybridomas** survive. Those hybridomas producing antibody of the desired specificity are then identified and cloned by regrowing the cultures from single cells (Fig. A.15). Since each hybridoma is a **clone** derived from fusion with a single B cell, all the antibody molecules it produces are identical in structure, including their antigen-binding site and isotype. Such antibodies are called **monoclonal antibodies**. This technology has revolutionized the use of antibodies by providing a limitless supply of antibody of a single and known specificity. Monoclonal antibodies are now used in most serological assays, as diagnostic probes, and as therapeutic agents. So far, however, only mouse monoclonals are routinely produced and efforts to use this same approach to make human monoclonal antibodies have met with very limited success.

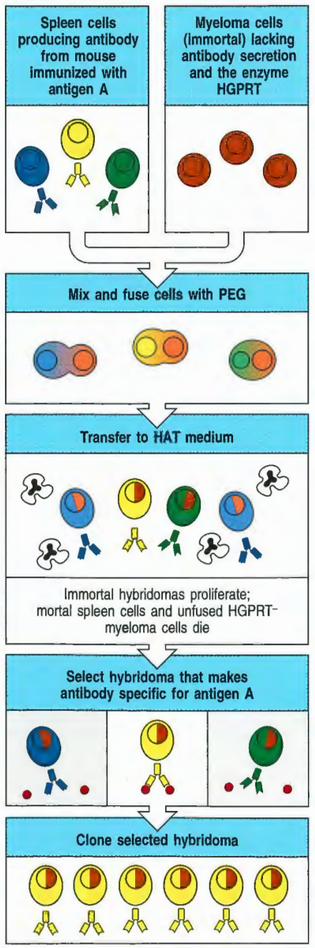


Fig. A.15 The production of monoclonal antibodies. Mice are immunized with antigen A and given an intravenous booster immunization three days before they are killed, in order to produce a large population of spleen cells secreting specific antibody. Spleen cells die after a few days in culture. In order to produce a continuous source of antibody they are fused with immortal myeloma cells by using polyethylene glycol (PEG) to produce a hybrid cell line called a hybridoma. The myeloma cells are selected beforehand to ensure that they are not secreting antibody themselves and that they are sensitive to the hypoxanthine-aminopterin-thymidine (HAT) medium that is used to select hybrid cells because they lack the enzyme hypoxanthine:guanine phosphoribosyl transferase (HGPRT). The HGPRT gene contributed by the spleen cell allows hybrid cells to survive in the HAT medium, and only hybrid cells can grow continuously in culture because of the malignant potential contributed by the myeloma cells. Therefore, unfused myeloma cells and unfused spleen cells die in the HAT medium, as shown here by cells with dark, irregular nuclei. Individual hybridomas are then screened for antibody production, and cells that make antibody of the desired specificity are cloned by growing them up from a single antibody-producing cell. The cloned hybridoma cells are grown in bulk culture to produce large amounts of antibody. As each hybridoma is descended from a single cell, all the cells of a hybridoma cell line make the same antibody molecule, which is thus called a monoclonal antibody.

A-13 Phage display libraries for antibody V-region production.

This is a technique for producing antibody-like molecules. Gene segments encoding the antigen-binding variable or V domains of antibodies are fused to genes encoding the coat protein of a bacteriophage. Bacteriophage containing such gene fusions are used to infect bacteria, and the resulting phage particles have coats that express the antibody-like fusion protein, with the antigen-binding domain displayed on the outside of the bacteriophage. A collection of recombinant phage, each displaying a different antigen-binding domain on its surface, is known as a **phage display library**. In much the same

way that antibodies specific for a particular antigen can be isolated from a complex mixture by affinity chromatography (see Section A-5), phage expressing antigen-binding domains specific for a particular antigen can be isolated by selecting the phage in the library for binding to that antigen. The phage particles that bind are recovered and used to infect fresh bacteria. Each phage isolated in this way will produce a monoclonal antigen-binding particle analogous to a monoclonal antibody (Fig. A.16). The genes encoding the antigen-binding site, which are unique to each phage, can then be recovered from the phage DNA and used to construct genes for a complete antibody molecule by joining them to parts of immunoglobulin genes that encode the invariant parts of an antibody. When these reconstructed antibody genes are introduced into a suitable host cell line, such as the nonantibody-producing myeloma cells used for hybridomas, the transfected cells can secrete antibodies with all the desirable characteristics of monoclonal antibodies produced from hybridomas.

In much the same way that a collection of phage can display a wide variety of potential antigen-binding sites, the phage can also be engineered to display a wide variety of antigens; such a library is known as an **antigen display library**. In such cases, the antigens displayed are often short peptides encoded by chemically synthesized DNA sequences that have mixtures of all four nucleotides in some positions, so that all possible amino acids are incorporated. It is not usual for every position in a peptide to be allowed to vary in this way, since the number of different peptide sequences increases dramatically with the number of variable positions; there are over 2×10^{10} possible sequences of eight amino acids.

A-14 Immunofluorescence microscopy.

Since antibodies bind stably and specifically to their corresponding antigen, they are invaluable as probes for identifying a particular molecule in cells, tissues, or biological fluids. Antibody molecules can be used to locate their target molecules accurately in single cells or in tissue sections by a variety of different labeling techniques. When the antibody itself, or the anti-immunoglobulin antibody used to detect it, is labeled with a fluorescent dye the technique is known as **immunofluorescence microscopy**. As in all serological techniques, the antibody binds stably to its antigen, allowing unbound antibody to be removed by thorough washing. As antibodies to proteins recognize the surface features of the native, folded protein, the native structure of the protein being sought usually needs to be preserved, either by using only the most gentle chemical fixation techniques or by using frozen tissue sections that are fixed only after the antibody reaction has been performed. Some

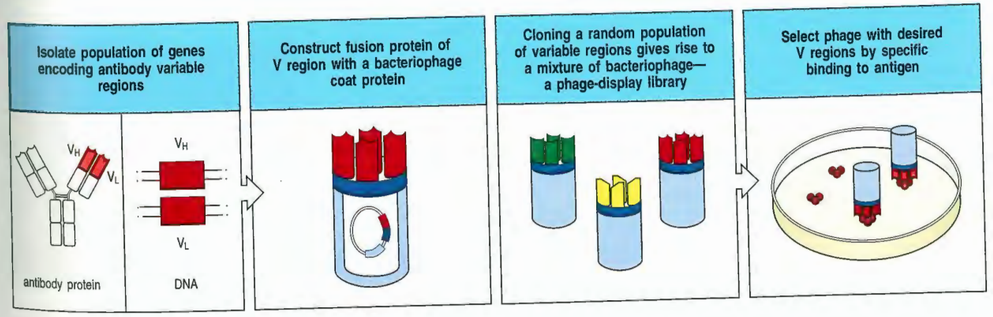


Fig. A.16 The production of antibodies by genetic engineering. Short primers to consensus sequences in heavy- and light-chain variable (V) regions of immunoglobulin genes are used to generate a library of heavy- and light-chain V-region DNAs by the polymerase chain reaction, with spleen DNA as the starting material. These heavy- and light+chain V-region genes are cloned randomly into a filamentous phage such that each phage expresses one heavy-chain and one light-chain V region as a surface fusion protein with antibody-like properties. The resulting phage display library is multiplied in bacteria, and the phage are then bound to a surface coated with antigen. The unbound phage are washed away; the bound phage are recovered, multiplied in bacteria, and again bound to antigen. After a few cycles, only specific high-affinity antigenbinding phage are left. These can be used like antibody molecules, or their V genes can be recovered and engineered into anti-body genes to produce genetically engineered antibody molecules (not shown). This technology may replace the hybridoma technology for producing monoclonal antibodies and has the advantage that humans can be used as the source of DNA.

Fig. A.17 Excitation and emission wavelengths for common fluorochromes.

Excitation and emission wavelengths of some commonly used fluorochromes		
Probe	**Excitation (nm)**	**Emission (nm)**
R-phycoerythrin (PE)	480; 565	578
Fluorescein	495	519
PerCP	490	675
Texas Red	589	615
Rhodamine	550	573

antibodies, however, bind proteins even if they are denatured, and such antibodies will bind specifically even to protein in fixed tissue sections.

The fluorescent dye can be covalently attached directly to the specific antibody, but more commonly, the bound antibody is detected by fluorescent anti-immunoglobulin, a technique known as **indirect immunofluorescence**. The dyes chosen for immunofluorescence are excited by light of one wavelength, usually blue or green, and emit light of a different wavelength in the visible spectrum. The most common fluorescent dyes are fluorescein, which emits green light, Texas Red and Peridinin chlorophyll protein (PerCP), which emit red light, and rhodamine and phycoerythrin (PE) which emit orange/red light (Fig. A.17). By using selective filters, only the light coming from the dye or fluorochrome used is detected in the fluorescence microscope (Fig. A.18). Although Albert Coons first devised this technique to identify the plasma cell as the source of antibody, it can be used to detect the distribution of any protein. By attaching different dyes to different antibodies, the distribution of two or more molecules can be determined in the same cell or tissue section (see Fig. A.18).

The recent development of the **confocal fluorescent microscope**, which uses computer-aided techniques to produce an ultrathin optical section of a cell or tissue, gives very high resolution immunofluorescence microscopy without the need for elaborate sample preparation. The resolution of the confocal microscope can be further increased using low-intensity illumination so that two photons are required to excite the fluorochrome. A pulsed laser beam is used, and only when it is focused into the focal plane of the microscope is the intensity sufficient to excite fluorescence. In this way the fluorescence emission itself can be restricted to the optical section.

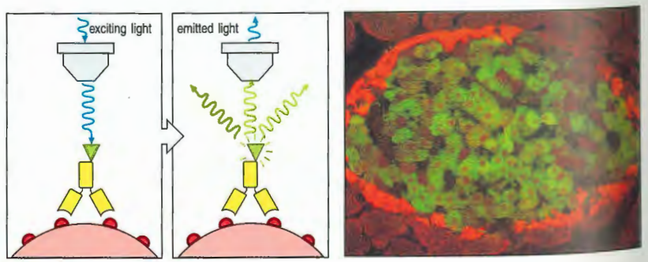


Fig. A.18 Immunofluorescence microscopy. Antibodies labeled with a fluorescent dye such as fluorescein (green triangle) are used to reveal the presence of their corresponding antigens in cells or tissues. The stained cells are examined in a microscope that exposes them to blue or green light to excite the fluorescent dye. The excited dye emits light at a characteristic wavelength, which is captured by viewing the sample through a selective filter. This technique is applied widely in biology to determine the location of molecules in cells and tissues. Different antigens can be detected in tissue sections by labeling antibodies with dyes of distinctive color. Here, antibodies to the protein glutamic acid decarboxylase (GAD) coupled to a green dye are shown to stain the β cells of pancreatic islets of Langerhans. The α cells do not make this enzyme and are labeled with antibodies to the hormone glucagon coupled with an orange fluorescent dye. GAD is an important antigen in diabetes, a disease in which the insulin-secreting β cells of the islets of Langerhans are destroyed by an immune attack on self tissues (see Chapter 14). Photograph courtesy of M. Solimena and P. De Camilli.

One important development in the area of microscopy has been the use of **time-lapse video microscopy**, in which sensitive digital video cameras record the movement of fluorescently labeled molecules in cell membranes and their redistribution when cells come into contact with each other. Cell-surface molecules can be fluorescently labeled in two main ways. One is by the binding of fluorochrome-labeled Fab fragments of antibodies specific for the protein of interest; the other is by generating a fusion protein, in which the protein of interest has been attached to one of a family of fluorescent proteins obtained from jellyfish. The first of these fluorescent proteins to come into wide use was green fluorescent protein (GFP), isolated from the jellyfish, *Aequorea victoria*. Other variants of this protein with different properties are now available, and the list of available fluorescent labels now includes red, blue, cyan, or yellow fluorescent proteins. Using cells transfected with the genes encoding such fusion proteins, it is possible to show the redistribution of T-cell receptors, co-receptors, adhesion molecules, and other signaling molecules, such as CD45, that takes place when a T cell makes contact with a target cell. It is clear from these observations that the interface between the T cell and its target does not represent a simple apposition of two cell membranes, but is an actively organized and dynamic structure, often now referred to as the 'immunological synapse.'

A-15 Immunoelectron microscopy.

Antibodies can be used to detect the intracellular location of structures or particular proteins at high resolution by electron microscopy, a technique known as **immunoelectron microscopy**. Antibodies against the required antigen are labeled with gold particles and then applied to ultrathin sections, which are then examined in the transmission electron microscope. Antibodies labeled with gold particles of different diameters enable two or more proteins to be studied simultaneously (see Fig. 5.10). The difficulty with this technique is in staining the ultrathin section adequately, as few molecules of antigen are present in each section.

A-16 Immunohistochemistry.

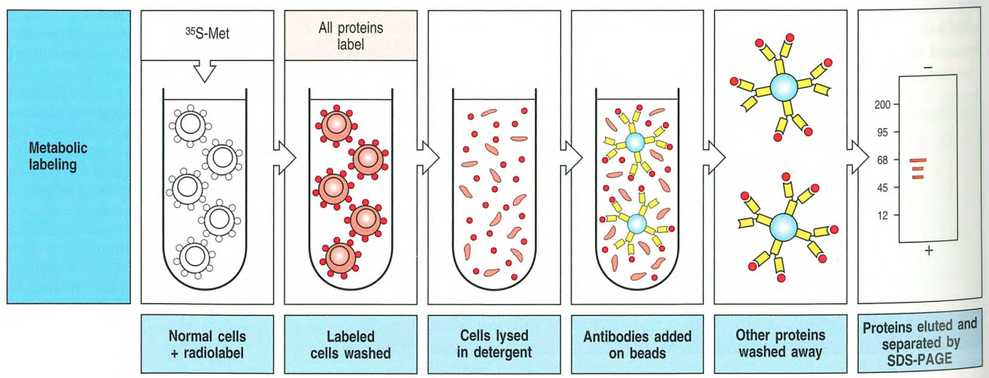
An alternative to immunofluorescence (see Section A-14) for detecting a protein in tissue sections is **immunohistochemistry**, in which the specific antibody is chemically coupled to an enzyme that converts a colorless substrate into a colored reaction product *in situ*. The localized deposition of the colored product where antibody has bound can be directly observed under a light microscope. The antibody binds stably to its antigen, allowing unbound antibody to be removed by thorough washing. This method of detecting bound antibody is analogous to ELISA (see Section A-6) and frequently uses the same coupled enzymes, the difference in detection being primarily that in immunohistochemistry the colored products are insoluble and precipitate at the site where they are formed. Horseradish peroxidase and alkaline phosphatase are the two enzymes most commonly used in these applications. Horseradish peroxidase oxidises the substrate diaminobenzidine to produce a brown precipitate, while alkaline phosphatase can produce red or blue dyes depending on the substrates used; a common substrate is 5-bromo-4-chloro-3-indolyl phosphate plus nitroblue tetrazolium (BCIP/NBT), which gives rise to a dark blue or purple stain. As with immunofluorescence, the native structure of the protein being sought usually needs to be preserved, so that it will be recognized by the antibody. Tissues are fixed by the most gentle chemical fixation techniques or frozen tissue sections are used that are fixed only after the antibody reaction has been performed.

A-17 Immunoprecipitation and co-immunoprecipitation.

In order to raise antibodies to membrane proteins and other cellular structures that are difficult to purify, mice are often immunized with whole cells or crude cell extracts. Antibodies to the individual molecules are then obtained by using these immunized mice to produce hybridomas making monoclonal antibodies (see Section A-12) that bind to the cell type used for immunization. To characterize the molecules identified by the antibodies, cells of the same type are labeled with radioisotopes and dissolved in nonionic detergents that disrupt cell membranes but do not interfere with antigen–antibody interactions. This allows the labeled protein to be isolated by binding to the antibody in a reaction known as **immunoprecipitation**. The antibody is usually attached to a solid support, such as the beads that are used in affinity chromatography (see Section A-5), or to Protein A. Cells can be labeled in two main ways for immunoprecipitation analysis. All the proteins in a cell can be labeled metabolically by growing the cell in radioactive amino acids that are incorporated into cellular protein (Fig. A.19). Alternatively, one can label only the cell-surface proteins by radioiodination under conditions that prevent iodine from crossing the plasma membrane and labeling proteins inside the cell, or by a reaction that labels only membrane proteins with biotin, a small molecule that is detected readily by labeled avidin, a protein found in egg whites that binds biotin with very high affinity.

Once the labeled proteins have been isolated by the antibody, they can be characterized in several ways. The most common is polyacrylamide gel electrophoresis (PAGE) of the proteins after dissociating them from antibody in the strong ionic detergent sodium dodecyl sulfate (SDS), a technique generally

Fig. A.19 Cellular proteins reacting with an antibody can be characterized by immunoprecipitation of labeled cell lysates. All actively synthesized cellular proteins can be labeled metabolically by incubating cells with radioactive amino acids (shown here for methionine) or one can label just the cell-surface proteins by using radioactive iodine in a form that cannot cross the cell membrane or by a reaction with the small molecule biotin, detected by its reaction with labeled avidin (not shown). Cells are lysed with detergent and individual labeled cell-associated proteins can be precipitated with a monoclonal antibody attached to beads. After unbound proteins have been washed away, the bound protein is eluted in the detergent sodium dodecyl sulfate (SDS), which dissociates it from the antibody and also coats the protein with a strong negative charge, allowing it to migrate according to its size in polyacrylamide gel electrophoresis (PAGE). The positions of the labeled proteins are determined by autoradiography using X-ray film. This technique of SDS-PAGE can be used to determine the molecular weight and subunit composition of a protein. Patterns of protein bands observed with metabolic labeling are usually more complex than those revealed by radioiodination, owing to the presence of precursor forms of the protein (right panel). The mature form of a surface protein can be identified as being the same size as that detected by surface iodination or biotinylation (not shown).



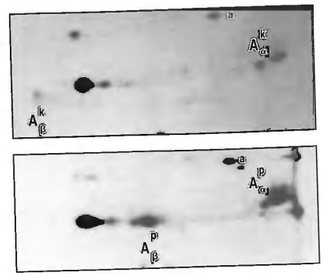


Fig. A.20 Two-dimensional gel electrophoresis of MHC class II molecules. Proteins in mouse spleen cells have been labeled metabolically (see Fig. A.19), precipitated with a monoclonal antibody against the mouse MHC class II molecule H2-A, and separated by isoelectric focusing in one direction and SDS-PAGE in a second direction at right angles to the first; hence the term two-dimensional gel electrophoresis. This allows one to distinguish molecules of the same molecular weight on the basis of their charge. The separated proteins are detected by autoradiography. The MHC class II molecules are composed of two chains, α and β, and in the different MHC class II molecules these have different isoelectric points (compare upper and lower panels). The MHC genotype of mice is indicated by lower case superscripts (k,p). Actin, a common contaminant, is marked a. Photographs courtesy of J.F. Babich.

abbreviated as **SDS-PAGE**. SDS binds relatively homogeneously to proteins, conferring a charge that allows the electrophoretic field to drive protein migration through the gel. The rate of migration is controlled mainly by protein size (see Fig. A.19). Proteins of differing charges can be separated using isoelectric focusing (see Section A-12). This technique can be combined with SDS-PAGE in a procedure known as **two-dimensional gel electrophoresis**. For this, the immunoprecipitated protein is eluted in urea, a nonionic solubilizing agent, and run on an isoelectric focusing gel in a narrow tube of polyacrylamide. This first-dimensional isoelectric focusing gel is then placed across the top of an SDS-PAGE slab gel, which is then run vertically to separate the proteins by molecular weight (Fig. A.20). Two-dimensional gel electro-phoresis is a powerful technique that allows many hundreds of proteins in a complex mixture to be distinguished from one another.

Immunoprecipitation and the related technique of immunoblotting (see Section A-18) are useful for determining the molecular weight and isoelectric point of a protein as well as its abundance, distribution, and whether, for example, it undergoes changes in molecular weight and isoelectric point as a result of processing within the cell.

A-18 Immunoblotting (Western blotting).

Like immunoprecipitation (see Section A-17), **immunoblotting** is used for identifying the presence of a given protein in a cell lysate, but it avoids the problem of having to label large quantities of cells with radioisotopes. Unlabeled cells are placed in detergent to solubilize all cell proteins and the lysate is run on SDS-PAGE to separate the proteins (see Section A-17). The size-separated proteins are then transferred from the gel to a stable support such as a nitrocellulose membrane. Specific proteins are detected by treatment with antibodies able to react with SDS-solubilized proteins (mainly those that react with denatured sequences); the bound antibodies are revealed by anti-immunoglobulin antibodies labeled with radioisotopes or an enzyme. The term **Western blotting** as a synonym for immunoblotting arose because the comparable technique for detecting specific DNA sequences is known as Southern blotting, after Ed Southern who devised it, which in turn provoked the name Northern for blots of size-separated RNA, and Western for blots of size-separated proteins. Western blots have many applications in basic research and clinical diagnosis. They are often used to test sera for the presence of antibodies to specific proteins, for example to detect antibodies to different constituents of the human immunodeficiency virus, HIV (Fig. A.21).

Co-immunoprecipitation is an extension of the immunoprecipitation technique which is used to determine whether a given protein interacts physically with another given protein. Cell extracts containing the presumed interaction

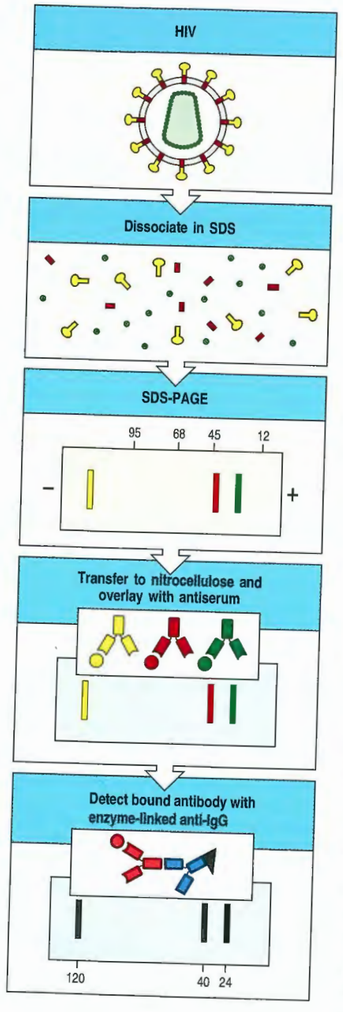


Fig. A.21 Western blotting is used to identify antibodies to the human immunodeficiency virus (HIV) in serum from infected individuals. The virus is dissociated into its constituent proteins by treatment with the detergent SDS, and its proteins are separated using SDS-PAGE. The separated proteins are transferred to a nitro-cellulose sheet and reacted with the test serum. Anti-HIV antibodies in the serum bind to the various HIV proteins and are detected with enzyme-linked anti-human immunoglobulin, which deposits colored material from a colorless substrate. This general methodology will detect any combination of antibody and antigen and is used widely, although the denaturing effect of SDS means that the technique works most reliably with antibodies that recognize the antigen when it is denatured.

complex are first immunoprecipitated with antibody against one of the proteins. The material identified by this means is then tested for the presence of the other protein by immunoblotting with a specific antibody.

A-19 Use of antibodies in the isolation and identification of genes and their products.

As a first step in isolating the gene that codes for a particular protein, antibodies specific for the protein can be used to isolate the purified protein from cells using affinity chromatography (see Section A-5). Small amounts of amino acid sequence can then be obtained from the protein's amino-terminal end or from peptide fragments generated by proteolysis. The information in these amino acid sequences is used to construct a set of synthetic oligonucleotides corresponding to the possible DNA sequences, which are then used as probes to isolate the gene encoding the protein from either a library of DNA sequences complementary to mRNA (a cDNA library) or a genomic DNA library (a library of chromosomal DNA fragments).

An alternative approach to gene identification uses antibodies to identify the protein product of a gene that has been introduced into a cell that does not normally express it. This technique is most commonly applied to the identification of genes encoding cell-surface proteins. A set of cDNA-containing expression vectors is first made from a cDNA library prepared from the total mRNA from a cell type that does express the protein of interest. The vectors are used to transfect a cell type that does not normally express the protein of interest, and the vector drives expression of the cDNA it contains without integrating into the host cell DNA. Cells expressing the required protein after transfection are then isolated by binding to specific antibodies that detect the presence of the protein on the cell surface. The vector containing the required gene can then be recovered from these cells (Fig. A.22).

The recovered vectors are then introduced into bacterial cells where they replicate rapidly, and these amplified vectors are used in a second round of transfection in mammalian cells. After several cycles of transfection, isolation, and amplification in bacteria, single colonies of bacteria are picked and the vectors prepared from cultures of each colony are used in a final transfection to identify a cloned vector carrying the cDNA of interest, which is then isolated and characterized. This methodology has been used to isolate many genes encoding cell-surface molecules.

The full amino acid sequence of the protein can be deduced from the nucleotide sequence of its cDNA, and this often gives clues to the nature of the protein and its biological properties. The nucleotide sequence of the gene and its regulatory regions can be determined from genomic DNA clones. The gene can be manipulated and introduced into cells by transfection for larger-scale production and functional studies. This approach has been used to

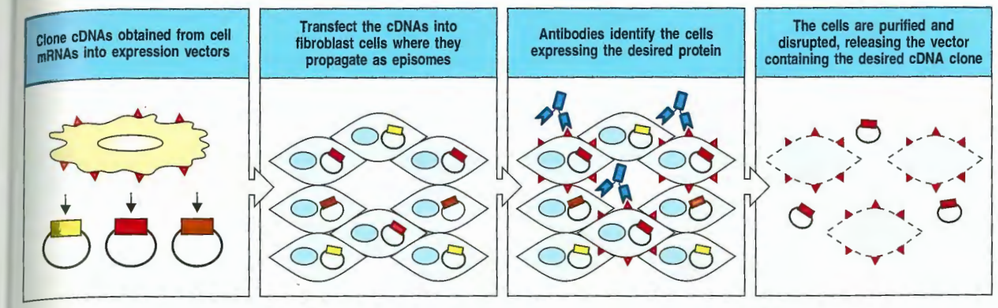


Fig. A.22 The gene encoding a cell-surface molecule can be isolated by expressing it in fibroblasts and detecting its protein product with monoclonal antibodies. Total mRNA from a cell line or tissue expressing the protein is isolated, converted into cDNA, and cloned as cDNAs in a vector designed to direct expression of the cDNA in fibroblasts. The entire cDNA library is used to transfect cultured fibroblasts. Fibroblasts that have taken up cDNA encoding a cell-surface protein express the protein on their surface; they can be isolated by binding a monoclonal antibody against that protein. The vector containing the gene is isolated from the cells that express the antigen and used for more rounds of transfection and reisolation until uniform positive expression is obtained, ensuring that the correct gene has been isolated. The cDNA insert can then be sequenced to determine the sequence of the protein it encodes and can also be used as the source of material for large-scale expression of the protein for analysis of its structure and function. The method illustrated is limited to cloning genes for single-chain proteins (that is, those encoded by only one gene) that can be expressed in fibroblasts. It has been used to clone many genes of immunological interest such as that for CD4.

characterize many immunologically important proteins, such as the MHC glycoproteins.

The converse approach is taken to identify the unknown protein product of a cloned gene. The gene sequence is used to construct synthetic peptides of 10–20 amino acids that are identical to part of the deduced protein sequence, and antibodies are then raised against these peptides by coupling them to carrier proteins; the peptides behave as haptens. These anti-peptide antibodies often bind the native protein and so can be used to identify its distribution in cells and tissues and to try to ascertain its function (Fig. A.23). This approach to identifying the function of a gene is often called 'reverse genetics' as it works from gene to phenotype rather than from phenotype to gene, which is the classical genetic approach. The great advantage of reverse genetics over the classical approach is that it does not require a detectable phenotypic genetic trait in order to identify a gene.

Antibodies can also be used in the determination of the function of gene products. Some antibodies are able to act as agonists, when the binding of the antibody to the molecule mimics the binding of the natural ligand and activates the function of the gene product. For example, antibodies to the CD3 molecule

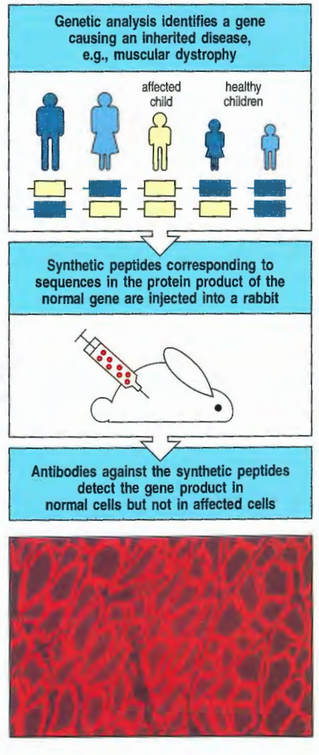


Fig. A.23 The use of antibodies to detect the unknown protein product of a known gene is called reverse genetics. When the gene responsible for a genetic disorder such as Duchenne muscular dystrophy is isolated, the amino acid sequence of the unknown protein product of the gene can be deduced from the nucleotide sequence of the gene, and synthetic peptides representing parts of this sequence can be made. Antibodies are raised against these peptides and purified from the antiserum by affinity chromatography on a peptide column (see Fig. A.5). Labeled antibody is used to stain tissue from individuals with the disease and from unaffected individuals to determine differences in the presence, amount, and distribution of the normal gene product. The product of the dystrophin gene is present in normal mouse skeletal muscle cells, as shown in the bottom panel (red fluorescence), but is missing from the cells of mice bearing the mutation mdx, the mouse equivalent of Duchenne muscular dystrophy (not shown). Photograph (× 15) courtesy of H.G.W. Lidov and L. Kunkel.

have been used to stimulate T cells, replacing the interaction of the T-cell receptor with MHC:peptide antigens in cases where the specific peptide antigen is not known. Conversely, antibodies can function as antagonists, inhibiting the binding of the natural ligand and thus blocking the function.

Isolation of lymphocytes

A-20 Isolation of peripheral blood lymphocytes by Ficoll-Hypaque™ gradient.

The first step in studying lymphocytes is to isolate them so that their behavior can be analyzed *in vitro*. Human lymphocytes can be isolated most readily from peripheral blood by density centrifugation over a step gradient consisting of a mixture of the carbohydrate polymer Ficoll™ and the dense iodine-containing compound metrizamide. This yields a population of mononuclear cells at the interface that has been depleted of red blood cells and most polymorphonuclear leukocytes or granulocytes (Fig. A.24). The resulting population, called **peripheral blood mononuclear cells (PBMCs)**, consists mainly of lymphocytes and monocytes. Although this population is readily accessible, it is not necessarily representative of the lymphoid system, as only recirculating lymphocytes can be isolated from blood.

A particular cell population can be isolated from a sample or culture by binding to antibody-coated plastic surfaces, a technique known as **panning**, or by removing unwanted cells by treatment with specific antibody and complement to kill them. Cells can also be passed over columns of antibody-coated, nylon-coated steel wool and different populations differentially eluted. This technique extends affinity chromatography to cells, and is now a very popular way to separate cells. All these techniques can also be used as a pre-purification step prior to sorting out highly purified populations by FACS (see Section A-22).

A-21 Isolation of lymphocytes from tissues other than blood.

In experimental animals, and occasionally in humans, lymphocytes are isolated from lymphoid organs, such as spleen, thymus, bone marrow, lymph nodes, or mucosal-associated lymphoid tissues, most commonly the palatine tonsils in humans (see Fig. 11.5). A specialized population of lymphocytes resides in surface epithelia; these cells are isolated by fractionating the epithelial layer after its detachment from the basement membrane. Finally, in

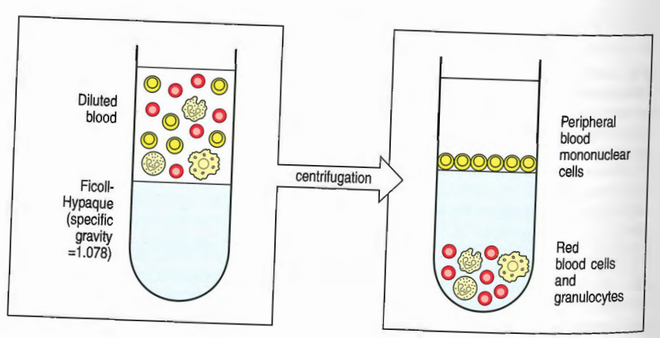


Fig. A.24 Peripheral blood mononuclear cells can be isolated from whole blood by Ficoll-Hypaque™ centrifugation. Diluted anticoagulated blood (left panel) is layered over Ficoll-Hypaque™ and centrifuged. Red blood cells and polymorphonuclear leukocytes or granulocytes are more dense and centrifuge through the Ficoll-Hypaque™, while mononuclear cells consisting of lymphocytes together with some monocytes band over it and can be recovered at the interface (right panel).

situations where local immune responses are prominent, lymphocytes can be isolated from the site of the response itself. For example, in order to study the autoimmune reaction that is thought to be responsible for rheumatoid arthritis, an inflammatory response in joints, lymphocytes are isolated from the fluid aspirated from the inflamed joint space.

A-22 Flow cytometry and FACS analysis.

Resting lymphocytes present a deceptively uniform appearance, all being small round cells with a dense nucleus and little cytoplasm (see Fig. 1.6). However, these cells comprise many functional subpopulations, which are usually identified and distinguished from each other on the basis of their differential expression of cell-surface proteins, which can be detected using specific antibodies (Fig. A.25). B and T lymphocytes, for example, are identified unambiguously and separated from each other by antibodies to the constant regions of the B- and T-cell antigen receptors. T cells are further subdivided on the basis of expression of the co-receptor proteins CD4 and CD8.

An immensely powerful tool for defining and enumerating lymphocytes is the flow cytometer, which detects and counts individual cells passing in a stream through a laser beam. A flow cytometer equipped to separate the identified cells is called a **fluorescence-activated cell sorter (FACS)**. These instruments are used to study the properties of cell subsets identified using monoclonal antibodies to cell-surface proteins. Individual cells within a mixed population are first tagged by treatment with specific monoclonal antibodies labeled with fluorescent dyes, or by specific antibodies followed by labeled anti-immunoglobulin antibodies. The mixture of labeled cells is then forced with a much larger volume of saline through a nozzle, creating a fine stream of liquid containing cells spaced singly at intervals. As each cell passes through a laser beam it scatters the laser light, and any dye molecules bound to the cell will be excited and will fluoresce. Sensitive photomultiplier tubes detect both the scattered light, which gives information on the size and granularity of the cell, and the fluorescence emissions, which give information on the binding of the labeled monoclonal antibodies and hence on the expression of cell-surface proteins by each cell (Fig. A.26).

In the cell sorter, the signals passed back to the computer are used to generate an electric charge, which is passed from the nozzle through the liquid stream at the precise time that the stream breaks up into droplets, each containing no more than a single cell; droplets containing a charge can then be deflected from the main stream of droplets as they pass between plates of opposite charge, so that positively charged droplets are attracted to a negatively charged plate, and vice versa. In this way, specific subpopulations of cells, distinguished by the binding of the labeled antibody, can be purified from a mixed population of cells. Alternatively, to deplete a population of cells, the same fluorochrome can be used to label different antibodies directed at marker proteins expressed by the various undesired cell types. The cell sorter can be used to direct labeled cells to a waste channel, retaining only the unlabeled cells.

When cells are labeled with a single fluorescent antibody, the data from a flow cytometer are usually displayed in the form of a histogram of fluorescence intensity versus cell numbers. If two or more antibodies are used, each coupled to different fluorescent dyes, then the data are more usually displayed in the form of a two-dimensional scatter diagram or as a contour diagram, where the fluorescence of one dye-labeled antibody is plotted against that of a second, with the result that a population of cells labeling with one antibody can be further subdivided by its labeling with the second antibody (see Fig. A.26). By examining large numbers of cells, flow cytometry can give quantitative data on the percentage of cells bearing different molecules, such as surface

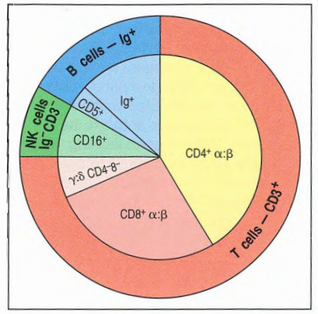


Fig. A.25 The distribution of lymphocyte subpopulations in human peripheral blood. As shown on the outside of the circle, lymphocytes can be divided into T cells bearing T-cell receptors (detected with anti-CD3 antibodies), B cells bearing immuno-globulin receptors (detected with anti-immunoglobulin antibodies), and null cells including natural killer (NK) cells, that label with neither. Further divisions of the T-cell and B-cell populations are shown inside. Using anti-CD4 and anti-CD8 antibodies, α:β T cells can be subdivided into two populations, whereas γ:δ T cells are identified with antibodies against the γ:δ T-cell receptor and mainly lack CD4 and CD8. A minority population of B cells express CD5 on their surface (see Section 7-28).

immunoglobulin, which characterizes B cells, the T-cell receptor-associated molecules known as CD3, and the CD4 and CD8 co-receptor proteins that distinguish the major T-cell subsets. Likewise, FACS analysis has been instrumental in defining stages in the early development of B and T cells. As the power of

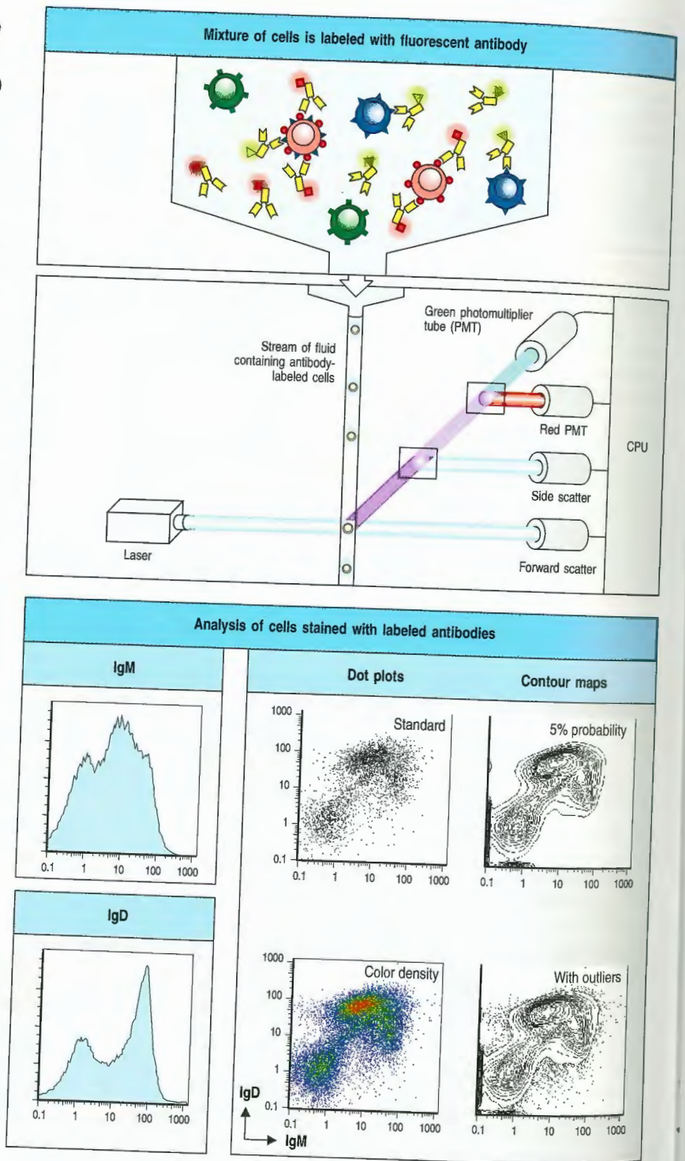


Fig. A.26 The FACS™ allows individual cells to be identified by their cell-surface antigens and to be sorted. Cells to be analyzed by flow cytometry are first labeled with fluorescent dyes (top panel). Direct labeling uses dye-coupled antibodies specific for cell-surface antigens (as shown here), while indirect labeling uses a dye-coupled immunoglobulin to detect unlabeled cell-bound antibody. The cells are forced through a nozzle in a single-cell stream that passes through a laser beam (second panel). Photomultiplier tubes (PMTs) detect the scattering of light, which is a sign of cell size and granularity, and emissions from the different fluorescent dyes. This information is analyzed by computer (CPU). By examining many cells in this way, the number of cells with a specific set of characteristics can be counted and levels of expression of various molecules on these cells can be measured. The lower part of the figure shows how this data can be represented, using the expression of two surface immuno-globulins, IgM and IgD, on a sample of B cells from a mouse spleen. The two immunoglobulins have been labeled with different colored dyes. When the expression of just one type of molecule is to be analyzed (IgM or IgD), the data is usually displayed as a histogram, as in the left-hand panels. Histograms display the distribution of cells expressing a single meas-ured parameter (for example, size, granularity, fluorescence intensity). When two or more parameters are measured for each cell (IgM and IgD), various types of two-color plots can be used to display the data, as shown in the right-hand panel. All four plots represent the same data. The horizontal axis represents intensity of IgM fluorescence and the vertical axis the intensity of IgD fluorescence. Two-color plots provide more information than histo-grams; they allow recognition, for example, of cells that are 'bright' for both colors, 'dull' for one and bright for the other, dull for both, negative for both, and so on. For example, the cluster of dots in the extreme lower left portions of the plots represents cells that do not express either immunoglobulin, and are mostly T cells. The standard dot plot (upper left) places a single dot for each cell whose fluorescence is measured. It is good for picking up cells that lie outside the main groups but tends to saturate in areas containing a large number of cells of the same type. A second means of presenting these data is the color dot plot (lower left), which uses color density to indicate high-density areas. A contour plot (upper right) draws 5% 'probability' contours, with 5% of the cells lying between each contour providing the best monochrome visualization of regions of high and low density. The lower right plot is a 5% probability contour map which also shows outlying cells as dots.

the FACS technology has grown, progressively more antibodies labeled with distinct fluorescent dyes can be used at the same time. Three-, four-, and even five-color analyses can now be handled by very powerful machines. FACS analysis has been applied to a broad range of problems in immunology; indeed, it played a vital role in the early identification of AIDS as a disease in which T cells bearing CD4 are depleted selectively (see Chapter 12).

A-23 Lymphocyte isolation using antibody-coated magnetic beads.

Although the FACS is superb for isolating small numbers of cells in pure form, when large numbers of lymphocytes must be prepared quickly, mechanical means of separating cells are preferable. A powerful and efficient way of isolating lymphocyte populations is to couple paramagnetic beads to monoclonal antibodies that recognize distinguishing cell-surface molecules. These antibody-coated beads are mixed with the cells to be separated, and run through a column containing material that attracts the paramagnetic beads when the column is placed in a strong magnetic field. Cells binding the magnetically labeled antibodies are retained; cells lacking the appropriate surface molecule can be washed away (Fig. A.27). The bound cells are positively selected for expression of the particular cell-surface molecule, while the unbound cells are negatively selected for its absence.

A-24 Isolation of homogeneous T-cell lines.

The analysis of specificity and effector function in T cells depends heavily on the study of monoclonal populations of T lymphocytes. These can be obtained in four distinct ways. First, as for B-cell hybridomas (see Section A-12), normal T cells proliferating in response to specific antigen can be fused to malignant T-cell lymphoma lines to generate **T-cell hybrids**. The hybrids express the receptor of the normal T cell, but proliferate indefinitely owing to the cancerous state of the lymphoma parent. T-cell hybrids can be cloned to yield a population of cells all having the same T-cell receptor. When stimulated by their specific antigen these cells release cytokines such as the T-cell growth factor interleukin-2 (IL-2), and the production of cytokines is used as an assay to assess the antigen specificity of the T-cell hybrid.

T-cell hybrids are excellent tools for the analysis of T-cell specificity, as they grow readily in suspension culture. However, they cannot be used to analyze the regulation of specific T-cell proliferation in response to antigen because they are continually dividing. T-cell hybrids also cannot be transferred into an animal to test for function *in vivo* because they would give rise to tumors. Functional analysis of T-cell hybrids is also confounded by the fact that the malignant partner cell affects their behavior in functional assays. Therefore, the regulation of T-cell growth and the effector functions of T cells must be studied using **T-cell clones**. These are clonal cell lines of a single T-cell type and antigen specificity, which are derived from cultures of heterogeneous T cells,

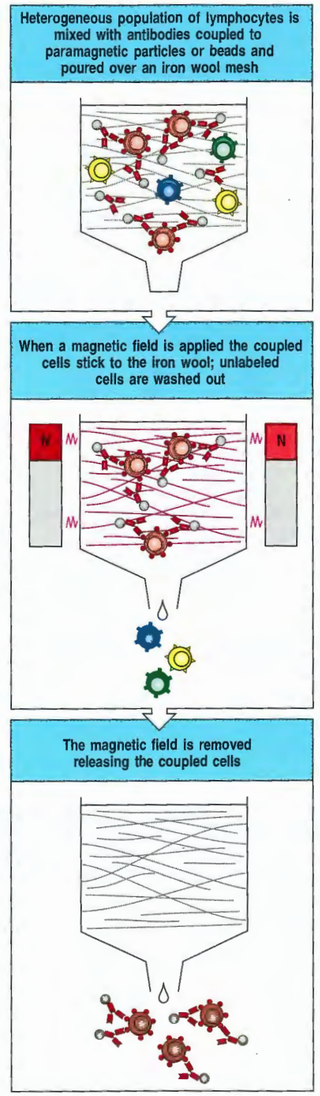


Fig. A.27 Lymphocyte subpopulations can be separated physically by using antibodies coupled to paramagnetic particles or beads. A mouse monoclonal antibody specific for a particular cell-surface molecule is coupled to paramagnetic particles or beads. It is mixed with a heterogeneous population of lymphocytes and poured over an iron wool mesh in a column. A magnetic field is applied so that the antibody-bound cells stick to the iron wool while cells which have not bound antibody are washed out; these cells are said to be negatively selected for lack of the molecule in question. The bound cells are released by removing the magnetic field; they are said to be positively selected for presence of the antigen recognized by the antibody.

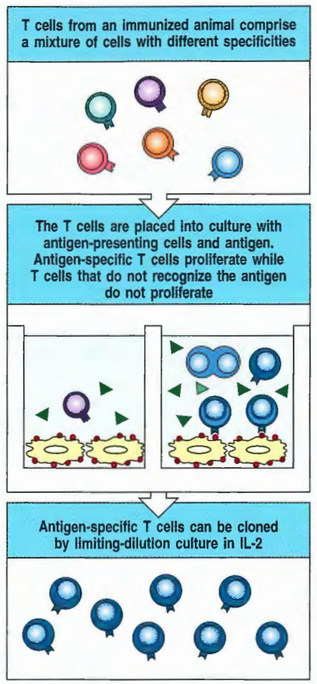


Fig. A.28 Production of cloned T-cell lines. T cells from an immune donor, comprising a mixture of cells with different specificities, are activated with antigen and antigen-presenting cells. Single responding cells are cultured by limiting dilution in the T-cell growth factor IL-2, which selectively stimulates the responding cells to proliferate. From these single cells, cloned lines specific for antigen are identified and can be propagated by culture with antigen, antigen-presenting cells, and IL-2.

called **T-cell lines**, whose growth is dependent on periodic restimulation with specific antigen and, frequently, on the addition of T-cell growth factors (Fig. A.28). T-cell clones also require periodic restimulation with antigen and are more tedious to grow than T-cell hybrids but, because their growth depends on specific antigen recognition, they maintain antigen specificity, which is often lost in T-cell hybrids. Cloned T-cell lines can be used for studies of effector function both *in vitro* and *in vivo*. In addition, the proliferation of T cells, a critical aspect of clonal selection, can be characterized only in cloned T-cell lines, where such growth is dependent on antigen recognition. Thus, both types of monoclonal T-cell line have valuable applications in experimental studies.

Studies of human T cells have relied largely on T-cell clones because a suitable fusion partner for making T-cell hybrids has not been identified. However, a human T-cell lymphoma line, called Jurkat, has been characterized extensively because it secretes IL-2 when its antigen receptor is cross-linked with anti-receptor monoclonal antibodies. This simple assay system has yielded much information about signal transduction in T cells. One of the Jurkat cell line's most interesting features, shared with T-cell hybrids, is that it stops growing when its antigen receptor is cross-linked. This has allowed mutants lacking the receptor or having defects in signal transduction pathways to be selected simply by culturing the cells with anti-receptor antibody and selecting those that continue to grow. Thus, T-cell tumors, T-cell hybrids, and cloned T-cell lines all have valuable applications in experimental immunology.

Finally, primary T cells from any source can be isolated as single, antigen-specific cells by limiting dilution rather than by first establishing a mixed population of T cells in culture as a T-cell line and then deriving clonal subpopulations. During the growth of T-cell lines, particular T-cell clones can come to dominate the cultures and give a false picture of the number and specificities in the original sample. Direct cloning of primary T cells avoids this artifact.

Characterization of lymphocyte specificity, frequency, and function.

B cells are relatively easy to characterize as they have only one function—antibody production. T cells are more difficult to characterize as there are several different classes with different functions. It is also technically more difficult to study the membrane-bound T-cell receptors than the antibodies secreted in large amounts by B cells. All the methods in this part of the appendix can be used for T cells. Some are also used to detect and count B cells.

On many occasions it is important to know the frequency of antigen-specific lymphocytes, especially T cells, in order to measure the efficiency with which an individual responds to a particular antigen, for example, or the degree to which specific immunological memory has been established. There are a number of methods for doing this, either by detecting the cells directly by the specificity of their receptor, or by detecting activation of the cells to provide some particular function, such as cytokine secretion or cytotoxicity.

The first technique of this type to be established was the limiting-dilution culture (see Section A-25), in which the frequency of specific T or B cells responding to a particular antigen could be estimated by plating the cells into 96-well plates at increasing dilutions and measuring the number of wells in which there was no response. However, in this type of assay it became laborious to ask detailed questions about the phenotype of the responding cells, and to compare responses from different cell subpopulations.

A simpler assay for measuring the responses of T-cell populations has been developed from a variant of the antigen-capture ELISA method (see Section A-6), called the ELISPOT assay (see Section A-26). It assays T cells on the basis of cytokine production. In the ELISPOT assay, cytokine secreted by individual activated T cells is immobilized as discrete spots on a plastic plate, which are counted to give the number of activated T cells. The ELISPOT assay suffers from many of the same problems as the limiting-dilution assay in giving information about the nature of the activated cells, and it can be difficult to determine whether individual cells are capable of secreting mixtures of cytokines. It was therefore important to develop assays that could make these measurements on single cells. Measurements based on flow cytometry (see Section A-22) proved the answer, with the development of methods for detecting fluorescently labeled cytokines within activated T cells. The drawback of intracellular cytokine staining (see Section A-27) was that the T cells have to be killed and permeabilized by detergents to enable the cytokines to be detected. This led to the more sophisticated technique of capturing secreted labeled cytokines on the surfaces of the living T cells (see Section A-27).

Finally, methods for directly detecting T cells on the basis of the specificity of their receptor, using fluorochrome-tagged tetramers of specific MHC:peptide complexes (see Section A-28), have revolutionized the study of T-cell responses in a similar way to the use of monoclonal antibodies.

A-25 Limiting-dilution culture.

The response of a lymphocyte population is a measure of the overall response, but the frequency of lymphocytes able to respond to a given antigen can be determined only by **limiting-dilution culture**. This assay makes use of the Poisson distribution, a statistical function that describes how objects are distributed at random. For instance, when a sample of heterogeneous T cells is distributed equally into a series of culture wells, some wells will receive no T cells specific for a given antigen, some will receive one specific T cell, some two, and so on. The T cells in the wells are activated with specific antigen, antigen-presenting cells, and growth factors. After allowing several days for their growth and differentiation, the cells in each well are tested for a response to antigen, such as cytokine release or the ability to kill specific target cells (Fig. A.29). The assay is replicated with different numbers of T cells in the samples. The logarithm of the proportion of wells in which there is no response is plotted against the number of cells initially added to each well. If cells of one type, typically antigen-specific T cells because of their rarity, are the only limiting factor for obtaining a response, then a straight line is obtained. From the Poisson distribution, it is known that there is, on average, one antigen-specific cell per well when the proportion of negative wells is 37%. Thus, the frequency of antigen-specific cells in the population equals the reciprocal of the number of cells added to each well when 37% of the wells are negative. After priming, the frequency of specific cells goes up substantially, reflecting the antigen-driven proliferation of antigen-specific cells. The limiting-dilution assay can also be used to measure the frequency of B cells that can make antibody to a given antigen.

A-26 ELISPOT assays.

A modification of the ELISA antigen-capture assay (see Section A-6), called the **ELISPOT assay**, has provided a powerful tool for measuring the frequency of T-cell responses. Populations of T cells are stimulated with the antigen of interest, and are then allowed to settle onto a plastic plate coated with antibodies to the cytokine that is to be assayed (Fig. A.30). If an activated T cell is

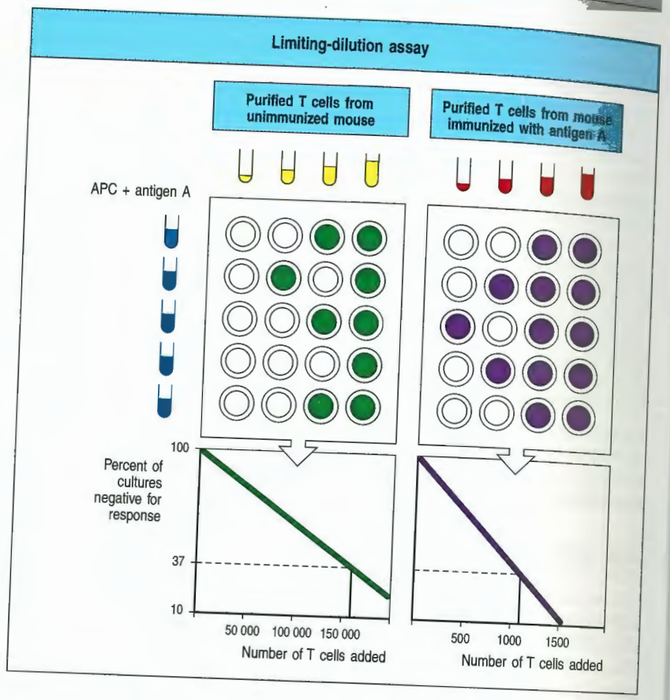


Fig. A.29 The frequency of specific lymphocytes can be determined using limiting-dilution assay. Varying numbers of lymphoid cells from normal or immunized mice are added to individual culture wells and stimulated with antigen and antigen-presenting cells (APCs) or polyclonal mitogen and added growth factors. After several days, the wells are tested for a specific response to antigen, such as cytotoxic killing of target cells. Each well that initially contained a specific T cell will make a response to its target, and from the Poisson distribution one can determine that when 37% of the wells are negative, each well contained, on average, one specific T cell at the beginning of the culture. In the example shown, for the unimmunized mouse 37% of the wells are negative when 160,000 T cells have been added to each well; thus the frequency of antigen-specific T cells is 1 in 160,000. When the mouse is immunized, 37% of the wells are negative when only 1100 T cells have been added; hence the frequency of specific T cells after immunization is 1 in 1100, an increase in responsive cells of 150-fold.

secreting that cytokine, it is captured by the antibody on the plastic plate. After a period the cells are removed, and a second antibody to the cytokine is added to the plate to reveal a circle of bound cytokine surrounding the position of each activated T cell; counting each spot, and knowing the number of T cells originally added to the plate allows a simple calculation of the frequency of T cells secreting that particular cytokine, giving the ELISPOT assay its name. ELISPOT can also be used to detect specific antibody secretion by B cells, in this case by using antigen-coated surfaces to trap specific antibody and labeled anti-immunoglobulin to detect the bound antibody.

A-27 Identification of functional subsets of T cells by staining for cytokines.

One problem with the detection of cytokine production on a single-cell level is that the cytokines are secreted by the T cells into the surrounding medium, and any association with the originating cell is lost. Two methods have been devised that allow the cytokine profile produced by individual cells to be determined. The first, that of **intracellular cytokine staining** (Fig. A.31), relies on the use of metabolic poisons that inhibit protein export from the cell. The cytokine thus accumulates within the endoplasmic reticulum and vesicular network of the cell. If the cells are subsequently fixed and rendered permeable by the use of mild detergents, antibodies can gain access to these intracellular compartments and detect the cytokine. The T cells can be stained for other markers simultaneously, and thus the frequency, for example, of IL-10-producing $CD25^+$ $CD4$ T cells can be easily obtained.

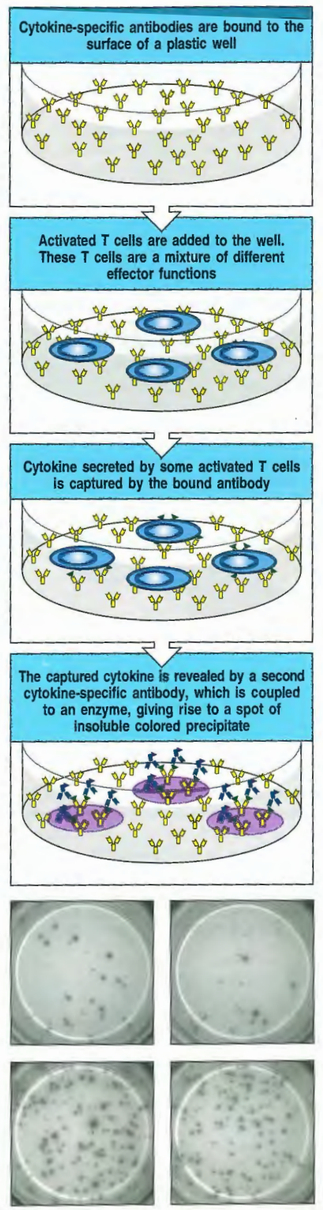


Fig. A.30 The frequency of cytokine-secreting T cells can be determined by the ELISPOT assay. The ELISPOT assay is a variant of the ELISA assay in which antibodies bound to a plastic surface are used to capture cytokines secreted by individual T cells. Usually, cytokine specific antibodies are bound to the surface of a plastic tissue-culture well and the unbound antibodies are removed (top panel). Activated T cells are then added to the well and settle onto the antibody-coated surface (second panel). If a T cell is secreting the appropriate cytokine, this will then be captured by the antibody molecules on the plate surrounding the T cell (third panel). After a period of time the T cells are removed, and the presence of the specific cytokine is detected using an enzyme-labeled second antibody specific for the same cytokine. Where this binds, a colored reaction product can be formed (fourth panel). Each T cell that originally secreted cytokine gives rise to a single spot of color, hence the name of the assay. The results of such an ELISPOT assay for T cells secreting IFN-γ in response to different stimuli are shown in the last panel. In this example, T cells from a stem cell transplant recipient were treated with a control peptide (top two panels) or a peptide from cytomegalovirus (bottom two panels). You can see a greater number of spots in the bottom two panels, indicating clearly that the patient's T cells are able to respond to the viral peptide and produce IFNγ. Photographs courtesy of S. Nowack.

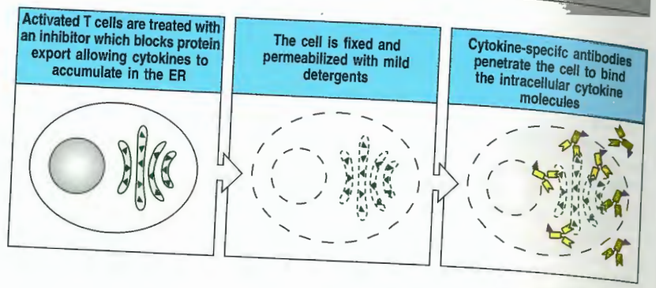
A second method, which has the advantage that the cells being analyzed are not killed in the process, is called **cytokine capture**. This technique uses hybrid antibodies, in which the two separate heavy- and light-chain pairs from different antibodies are combined to give a mixed antibody molecule in which the two antigen-binding sites recognize different ligands (Fig. A.32). In the bispecific antibodies used to detect cytokine production, one of the antigen-binding sites is specific for a T-cell surface marker, while the other is specific for the cytokine in question. The bispecific antibody binds to the T cells through the binding site for the cell-surface marker, leaving the cytokine-binding site free. If that T cell is secreting the particular cytokine, it is captured by the bound antibody before it diffuses away from the surface of the cell. It can then be detected by adding a fluorochrome-labeled second antibody specific for the cytokine to the cells.

A-28 Identification of T-cell receptor specificity using MHC:peptide tetramers.

For many years, the ability to identify antigen-specific T cells directly through their receptor specificity eluded immunologists. Foreign antigen could not be used directly to identify T cells, since, unlike B cells, they do not recognize antigen alone but rather the complexes of peptide fragments of antigen bound to self MHC molecules. Moreover, the affinity of interaction between the T-cell receptor and the MHC:peptide complex was in practice so low that attempts to label T cells with their specific MHC:peptide complexes routinely failed. The breakthrough in labeling antigen-specific T cells came with the idea of making multimers of the MHC:peptide complex, so as to increase the avidity of the interaction.

Peptides can be biotinylated using the bacterial enzyme BirA, which recognizes a specific amino acid sequence. Recombinant MHC molecules containing this target sequence are used to make MHC:peptide complexes which are then biotinylated. Avidin, or the bacterial counterpart streptavidin, contains four sites that bind biotin with extremely high affinity. Mixing the biotinylated MHC:peptide complex with avidin or streptavidin results in the formation of an **MHC:peptide tetramer**—four specific MHC:peptide complexes bound to a single molecule of streptavidin (Fig. A.33). Routinely, the streptavidin moiety is labeled with a fluorochrome to allow detection of those T cells capable of binding the MHC:peptide tetramer.

Fig. A.31 Cytokine-secreting cells can be identified by intracellular cytokine staining. The cytokines secreted by activated T cells can be determined by using fluorochrome-labeled antibodies to detect cytokine molecules that have been allowed to accumulate inside the cell. The accumulation of cytokine molecules, to allow them to reach a high enough concentration for efficient detection, is achieved by treating the activated T cells with inhibitors of protein export. In such treated cells, proteins destined to be secreted are instead retained within the endoplasmic reticulum (first panel). These treated cells are then fixed, to cross-link the proteins inside the cell and in the cell membranes, so that they are not lost when the cell is permeabilized by dissolving the cell membrane in a mild detergent (center panel). Fluorochrome-labeled antibodies can now enter the permeabilized cell and bind to the cytokines inside the cell (last panel). Cells labeled in this way can also be labeled with antibodies that bind to cell-surface proteins to determine which subsets of T cells are secreting particular cytokines.



MHC:peptide tetramers have been used to identify populations of antigen-specific T cells in, for example, patients with acute Epstein–Barr virus infections (infectious mononucleosis), showing that up to 80% of the peripheral T cells in infected individuals can be specific for a single MHC:peptide complex. They have also been used to follow responses over longer timescales in individuals with HIV or, in the example we show, cytomegalovirus infections. These reagents have also been important in identifying the cells responding, for example, to nonclassical class I molecules such as HLA-E or HLA-G, in both cases showing that these nonclassical molecules are recognized by subsets of NK receptors.

A-29 Assessing the diversity of the T-cell repertoire by 'spectratyping.'

The extent of the diversity of the T-cell repertoire, either generally or during specific immune responses, is often of interest. In particular, as T cells do not undergo somatic hypermutation and affinity maturation in the same way that B cells do, the relationship between the repertoire of T cells making a primary response to antigen and the repertoire of T cells involved in secondary

Fig. A.32 Hybrid antibodies containing cell-specific and cytokine-specific binding sites can be used to assay cytokine secretion by living cells and to purify cells secreting particular cytokines. Hybrid antibodies can be made by mixing together heavy- and light-chain pairs from antibodies of different specificities, for example, an antibody to an MHC class I molecule and an antibody specific for a cytokine such as IL-4 (first panel). The hybrid antibodies are then added to a population of activated T cells, and bind to each cell via the MHC class I binding arm (second panel). If some of the cells in the population are secreting the appropriate cytokine, IL-4, this is captured by the cytokine specific arm of the hybrid antibody (third panel). The presence of the cytokine can then be revealed, for example using a fluorochrome-labeled second antibody specific for the same cytokine, but binding to a different site to the one used for the hybrid antibody (last panel). Such labeled cells may be analyzed by flow cytometry, or can be isolated using a fluorescence activated cell sorter. Alternatively, the second cytokine specific antibody may be coupled to magnetic beads, and the cytokine producing cells isolated magnetically.

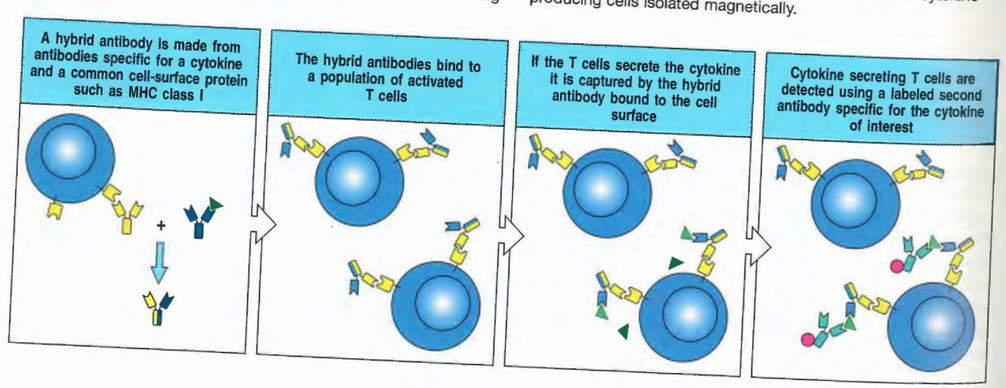
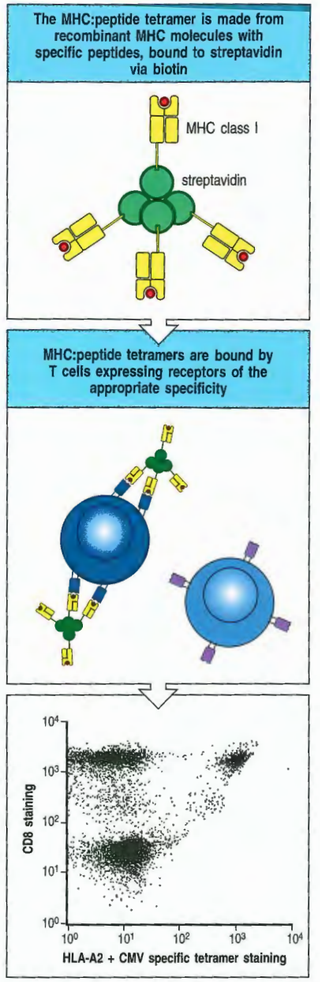


Fig. A.33 MHC:peptide complexes coupled to streptavidin to form tetramers are able to stain antigen-specific T cells. MHC:peptide tetramers are formed from recombinant refolded MHC:peptide complexes containing a single defined peptide epitope. The MHC molecules can be chemically derivatized to contain biotin, but more usually, the recombinant MHC heavy chain is linked to a bacterial biotinylation sequence, a target for the *E. coli* enzyme BirA, which is used to add a single biotin group to the MHC molecule. Streptavidin is a tetramer, each subunit having a single binding site for biotin, hence the streptavidin/MHC:peptide complex creates a tetramer of MHC:peptide complexes (top panel). While the affinity between the T-cell receptor and its MHC:peptide ligand is too low for a single complex to bind stably to a T cell, the tetramer, by being able to make a more avid interaction with multiple MHC:peptide complexes binding simultaneously, is able to bind to T cells whose receptors are specific for the particular MHC:peptide complex (center panel). Routinely, the streptavidin molecules are coupled to a fluoro-chrome, so that the binding to T cells can be monitored by flow cytometry. In the example shown in the bottom panel, T cells have been stained simultaneously with antibodies specific for CD3 and CD8, and with a tetramer of HLA-A2 molecules containing a cytomegalovirus peptide. Only the $CD3^+$ cells are shown, with the staining of CD8 displayed on the vertical axis and the tetramer staining displayed along the horizontal axis. The $CD8^-$ cells (mostly $CD4^+$) on the bottom left of the figure show no specific tetramer staining, while the bulk of the $CD8^+$ cells, on the top left, likewise show no tetramer staining. However, a discrete population of tetramer positive $CD8^+$ cells, at the top right of the panel, comprising some 5% of the total $CD8^+$ cells, can clearly be demonstrated. Data courtesy of G. Aubert.



and subsequent responses to antigen has been difficult to determine. This information has usually been obtained through the laborious process of cloning the T cells involved in specific responses (see Section A-24), and the cloning and sequencing of their T-cell receptors.

It is possible, however, to estimate the diversity of T-cell responses by making use of the junctional diversity generated when T-cell receptors are created by somatic recombination, a technique known as **spectratyping**. Variability in the length of the CDR3 segments is created during the recombination process, both by variation in the exact positions at which the junctions between gene segments occur, and by variation in the number of N-nucleotides added. Both these processes result in the length of the V_β CDR3 varying by up to nine amino acids. The problem in detecting this variability is that there are 24 families of V_β gene segments in humans and it is not possible to design a single oligonucleotide primer that will anneal to all of these families. Specific oligonucleotide primers can, however, be designed for each family, and these can be used in the polymerase chain reaction (PCR), together with a primer specific for the C_β region, to amplify, for each individual family, a segment of the mRNA for the T-cell receptor β chain that spans the CDR3 region. A population of TCR V_β genes will therefore show a distribution, or 'spectrum,' of CD3 lengths, and will give rise to PCR products of different lengths that can be resolved, usually by polyacrylamide gel electrophoresis (Fig. A.34). The deletion and addition of nucleotides during the generation of T-cell receptors by rearrangement is random, and so in a normal individual the CDR3 lengths follow a Gaussian distribution. Deviations from this Gaussian distribution, such as an excess of one particular CDR3 length, indicate the presence of clonal expansions of T cells, such as occurs during a T-cell response.

A-30 Biosensor assays for measuring the rates of association and disassociation of antigen receptors for their ligands.

Two of the important questions that are always asked of any receptor–ligand interaction is: what is the strength of binding, or affinity, of the interaction,

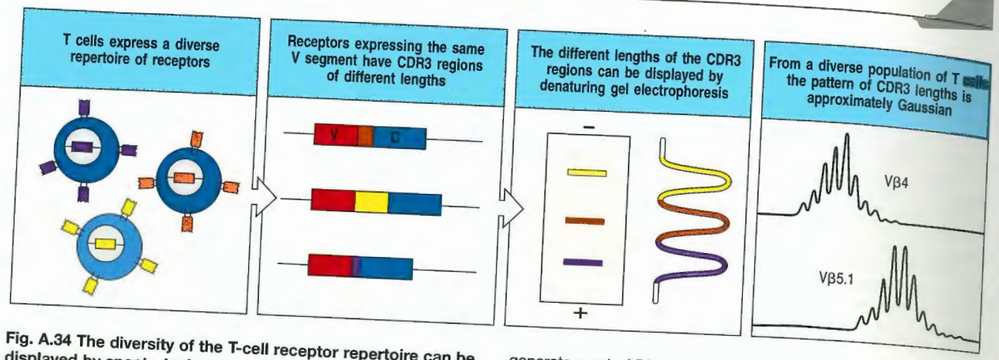


Fig. A.34 The diversity of the T-cell receptor repertoire can be displayed by spectratyping, a PCR-based technique that separates different receptors on the basis of their CDR3 length. The process of generation of T-cell receptors is stochastic, giving rise to a population of mature T cells whose receptors are clonally distributed (first panel). In each of the cells expressing a particular V_β gene segment, all of the differences between the unique receptors are restricted to the CDR3 region, where there will be differences in length as well as sequence as a consequence of the imprecision of the rearrangement process (second panel). Using sets of primers for the PCR reaction that are specific for individual V_β gene segments at one end and for a conserved part of the C region at the other, it is possible to generate a set of DNA fragments that span the CDR3 region. If these are separated by denaturing acrylamide gel electrophoresis then a series of bands are formed or, since these fragments can be labeled with fluorochromes and analyzed by automated gel readers, a series of peaks corresponding to the different length fragments (third panel). The pattern of peaks obtained in this way is known as a spectratype. From a diverse population of cells, the distribution of fragment lengths is Gaussian, as shown in the last panel, where the spectratypes of two different V_β regions from the same individual are shown. In this case, both of the patterns are approximately Gaussian; deviations from a Gaussian distribution may indicate expansion of particular clones of T cells, perhaps in response to antigenic challenge. Data courtesy of L. McGreavey.

and what are the rates of association and disassociation? Traditionally, measurements of affinity have been made by equilibrium binding measurements (see Section A-9), and measurements of rates of binding have been difficult to obtain. Equilibrium binding assays also cannot be performed on T-cell receptors, which have large macromolecular ligands and which cannot be isolated and purified in large quantity.

It is now possible to measure binding rates directly, by following the binding of ligands to receptors immobilized on gold-plated glass slides, using a phenomenon known as **surface plasmon resonance (SPS)** to detect the binding (Fig. A.35). A full explanation of surface plasmon resonance is beyond the scope of this textbook, as it is based on advanced physical and quantum mechanical principles. Briefly, it relies on the total internal reflection of a beam of light from the surface of a gold-coated glass slide. As the light is reflected, some of its energy excites electrons in the gold coating and these excited electrons are in turn affected by the electric field of any molecules binding to the surface of the glass coating. The more molecules that bind to the surface, the greater the effect on the excited electrons, and this in turn affects the reflected light beam. The reflected light thus becomes a sensitive measure of the number of atoms bound to the gold surface of the slide.

If a purified receptor is immobilized on the surface of the gold-coated glass slide, to make a biosensor 'chip,' and a solution containing the ligand is flowed over that surface, the binding of ligand to the receptor can be followed until it reaches equilibrium (see Fig. A.35). If the ligand is then washed out, dissociation of ligand from the receptor can easily be followed and the dissociation rate calculated. A new solution of the ligand at a different concentration can then be flowed over the chip and the binding once again measured. The affinity of binding can be calculated in a number of ways in this type of assay. Most simply, the ratio of the rates of association and dissociation will give an estimate of the affinity, but more accurate estimates can be obtained from the measurements of the binding at different concentrations of ligand. From measurements of binding at equilibrium, a Scatchard plot (see Fig. A.11) will give a measurement of the affinity of the receptor–ligand interaction.

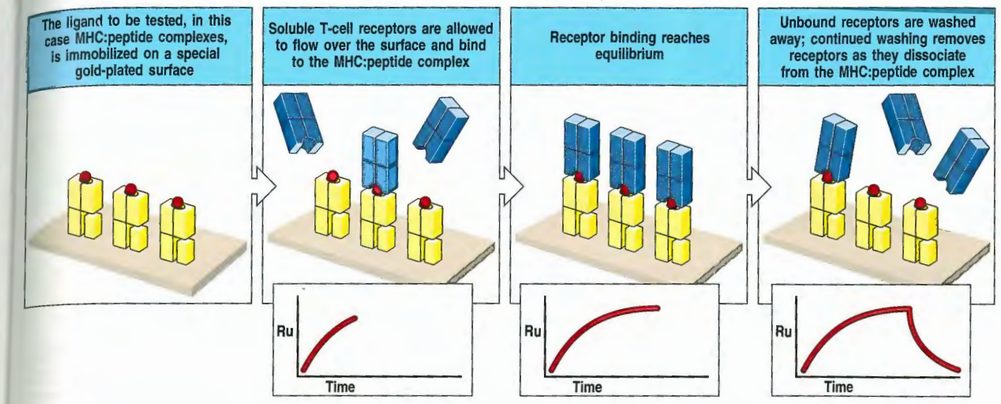


Fig. A.35 Measurement of receptor–ligand interactions can be made in real time using a biosensor. Biosensors are able to measure the binding of molecules on the surface of gold-plated glass chips through the indirect effects of the binding on the total internal reflection of a beam of polarized light at the surface of the chip. Changes in the angle and intensity of the reflected beam are measured in 'resonance units' (Ru) and plotted against time in what is termed a 'sensorgram.' Depending on the exact nature of the receptor–ligand pair to be analyzed, either the receptor or the ligand can be immobilized on the surface of the chip. In the example shown, MHC:peptide complexes are immobilized on such a surface (first panel). T-cell receptors in solution are now allowed to flow over the surface, and to bind to the immobilized MHC:peptide complexes (second panel). As the T-cell receptors bind, the sensorgram (inset panel below the main panel) reflects the increasing amount of protein bound. As the binding reaches either saturation or equilibrium (third panel), the sensorgram shows a plateau, as no more protein binds. At this point, unbound receptors can be washed away. With continued washing, bound receptors now start to dissociate and are removed in the flow of the washing solution (last panel). The sensorgram now shows a declining curve, reflecting the rate at which the receptor and ligand dissociation occurs.

A-31 Stimulation of lymphocyte proliferation by treatment with polyclonal mitogens or specific antigen.

To function in adaptive immunity, rare antigen-specific lymphocytes must proliferate extensively before they differentiate into functional effector cells in order to generate sufficient numbers of effector cells of a particular specificity. Thus, the analysis of induced lymphocyte proliferation is a central issue in their study. It is, however, difficult to detect the proliferation of normal lymphocytes in response to specific antigen because only a minute proportion of cells will be stimulated to divide. Great impetus was given to the field of lymphocyte culture by the finding that certain substances induce many or all lymphocytes of a given type to proliferate. These substances are referred to collectively as **polyclonal mitogens** because they induce mitosis in lymphocytes of many different specificities or clonal origins. T and B lymphocytes are stimulated by different polyclonal mitogens (Fig. A.36). Polyclonal mitogens seem to trigger essentially the same growth response mechanisms as antigen. Lymphocytes normally exist as resting cells in the G_0 phase of the cell cycle. When stimulated with polyclonal mitogens, they rapidly enter the G_1 phase and progress through the cell cycle. In most studies, lymphocyte proliferation is most simply measured by the incorporation of ^{3}H-thymidine into DNA.

Mitogen	Responding cells
Phytohemagglutinin (PHA) (red kidney bean)	T cells
Concanavalin (ConA) (Jack bean)	T cells
Pokeweed mitogen (PWM) (Pokeweed)	T and B cells
Lipopolysaccharide (LPS) (*Escherichia coli*)	B cells (mouse)

Fig. A.36 Polyclonal mitogens, many of plant origin, stimulate lymphocyte proliferation in tissue culture. Many of these mitogens are used to test the ability of lymphocytes in human peripheral blood to proliferate.

This assay is used clinically for assessing the ability of lymphocytes from patients with suspected immunodeficiencies to proliferate in response to a nonspecific stimulus.

Once lymphocyte culture had been optimized using the proliferative response to polyclonal mitogens as an assay, it became possible to detect antigen-specific T-cell proliferation in culture by measuring ^{3}H-thymidine uptake in response to an antigen to which the T-cell donor had been previously immunized (Fig. A.37). This is the assay most commonly used for assessing T-cell responses after immunization, but it reveals little about the functional capabilities of the responding T cells. These must be ascertained by functional assays, as outlined in Sections A-33 and A-34.

A-32 Measurements of apoptosis by the TUNEL assay.

Apoptotic cells can be detected by a procedure known as **TUNEL staining**. In this technique, the 3′ ends of the DNA fragments generated in apoptotic cells are labeled with biotin-coupled uridine by using the enzyme terminal deoxynucleotidyl transferase (TdT). The biotin label is then detected with enzyme tagged streptavidin, which binds to biotin. When the colorless substrate of the enzyme is added to a tissue section or cell culture, it is reacted upon to produce a colored precipitate only in cells that have undergone apoptosis (Fig. A.38). This technique has revolutionized the detection of apoptotic cells.

A-33 Assays for cytotoxic T cells.

Activated CD8 T cells generally kill any cells that display the specific peptide:MHC class I complex they recognize. Thus CD8 T-cell function can be determined using the simplest and most rapid T-cell bioassay—the killing of a target cell by a cytotoxic T cell. This is usually detected in a ^{51}Cr-release assay. Live cells will take up, but do not spontaneously release, radioactively labeled sodium chromate, $Na_2{}^{51}CrO_4$. When these labeled cells are killed, the radioactive chromate is released and its presence in the supernatant of mixtures of target cells and cytotoxic T cells can be measured (Fig. A.39). In a similar assay, proliferating target cells such as tumor cells can be labeled with ^{3}H-thymidine, which is incorporated into the replicating DNA. On attack by a cytotoxic T cell, the DNA of the target cells is rapidly fragmented and retained in the filtrate, while large, unfragmented DNA is collected on a filter, and one can measure either the release of these fragments or the retention of ^{3}H-thymidine in chromosomal DNA. These assays provide a rapid, sensitive, and specific measure of the activity of cytotoxic T cells.

A-34 Assays for CD4 T cells.

CD4 T-cell functions usually involve the activation rather than the killing of cells bearing specific antigen. The activating effects of CD4 T cells on B cells or macrophages are mediated in large part by nonspecific mediator proteins

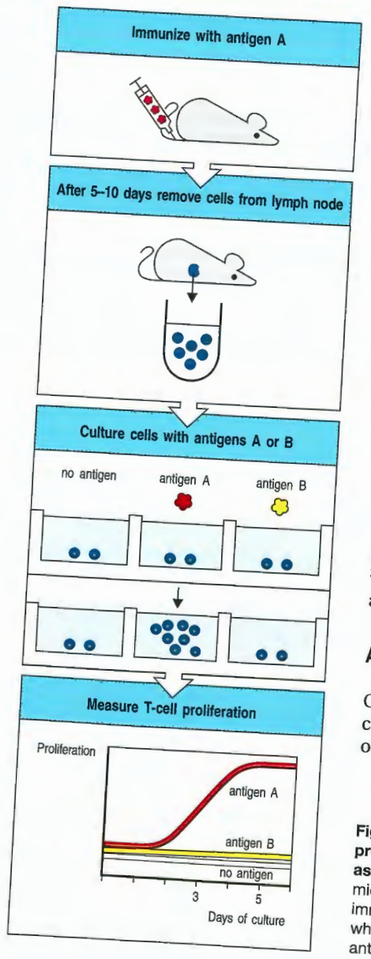


Fig. A.37 Antigen-specific T-cell proliferation is used frequently as an assay for T-cell responses. T cells from mice or humans that have been immunized with an antigen (A) proliferate when they are exposed to antigen A and antigen-presenting cells but not when cultured with unrelated antigens to which they have not been immunized (antigen B). Proliferation can be measured by incorporation of ^{3}H-thymidine into the DNA of actively dividing cells. Antigen-specific proliferation is a hallmark of specific CD4 T-cell immunity.

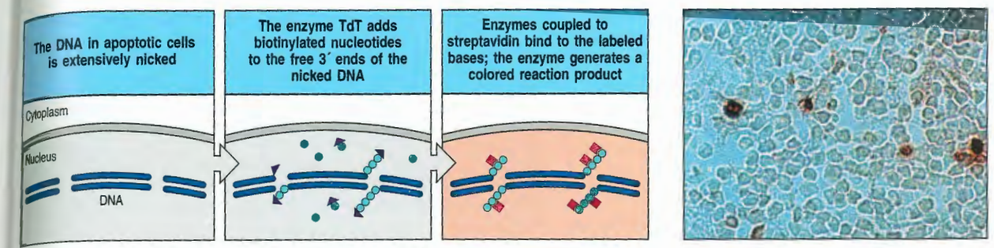


Fig. A.38 Fragmented DNA can be labeled by terminal deoxynucleotidyl transferase (TdT) to reveal apoptotic cells. When cells undergo programmed cell death, or apoptosis, their DNA becomes fragmented (left panel). The enzyme TdT is able to add nucleotides to the ends of DNA fragments; most commonly in this assay, biotin-labeled nucleotides (usually dUTP) are added (second panel). The biotinylated DNA can be detected by using streptavidin, which binds to biotin, coupled to enzymes that convert a colorless substrate into a colored insoluble product (third panel). Cells stained in this way can be detected by light microscopy, as shown in the photograph of apoptotic cells (stained red) in the thymic cortex. Photograph courtesy of R. Budd and J. Russell.

called cytokines, which are released by the T cell when it recognizes antigen. Thus, CD4 T-cell function is usually studied by measuring the type and amount of these released proteins. As different effector T cells release different amounts and types of cytokines, one can learn about the effector potential of that T cell by measuring the proteins it produces.

Cytokines can be detected by their activity in biological assays of cell growth, where they serve either as growth factors or growth inhibitors. A more specific assay is a modification of ELISA known as a capture or sandwich ELISA (see Section A-6). In this assay, the cytokine is characterized by its ability to bridge between two monoclonal antibodies reacting with different epitopes on the cytokine molecule. Cytokine-secreting cells can also be detected by ELISPOT (see Section A-26).

Sandwich ELISA and ELISPOT avoid a major problem of cytokine bioassays, the ability of different cytokines to stimulate the same response in a bioassay. Bioassays must always be confirmed by inhibition of the response with neutralizing monoclonal antibodies specific for the cytokine. Another way of identifying cells actively producing a given cytokine is to stain them with a fluorescently tagged anti-cytokine monoclonal antibody and identify and count them by FACS (see Section A-22).

A quite different approach to detecting cytokine production is to determine the presence and amount of the relevant cytokine mRNA in stimulated T cells. This can be done for single cells by *in situ* hybridization and for cell populations by **reverse transcriptase–polymerase chain reaction (RT–PCR)**. Reverse transcriptase is an enzyme used by certain RNA viruses,

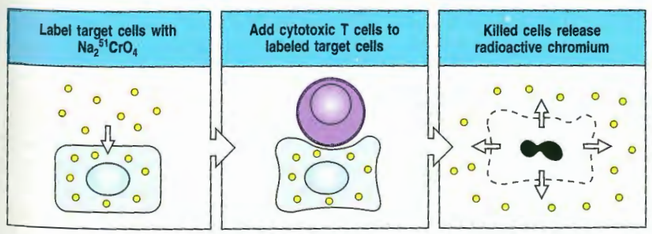


Fig. A.39 Cytotoxic T-cell activity is often assessed by chromium release from labeled target cells. Target cells are labeled with radioactive chromium as $Na_2{}^{51}CrO_4$, washed to remove excess radioactivity and exposed to cytotoxic T cells. Cell destruction is measured by the release of radioactive chromium into the medium, detectable within 4 hours of mixing target cells with T cells.

such as the human immunodeficiency virus (HIV-1) that causes AIDS, to convert an RNA genome into a DNA copy, or cDNA. In RT–PCR, mRNA is isolated from cells and cDNA copies made using reverse transcriptase. The desired cDNA is then selectively amplified by PCR using sequence-specific primers. When the products of the reaction are subjected to electrophoresis on an agarose gel, the amplified DNA can be visualized as a band of a specific size. The amount of amplified cDNA sequence will be proportional to its representation in the mRNA; stimulated T cells actively producing a particular cytokine will produce large amounts of that particular mRNA and thus give correspondingly large amounts of the selected cDNA on RT–PCR. The level of cytokine mRNA in the original tissue is usually determined by comparison with the outcome of RT–PCR on the mRNA produced by a so-called 'housekeeping gene' expressed by all cells.

A-35 DNA microarrays.

Any cell expresses, at any one time, many hundreds or even thousands of genes. Some of the products are expressed at high levels, the actin that forms the cytoskeleton of the cell is one example, while others may only be expressed in a few copies per cell. Different cell types, or cells at different stages of maturation, or even tumor cells compared to their normal counterparts, will express different sets of genes, and trying to identify these differences is an important field of research, in immunology as well as in other areas of biology. One important new technique in analyzing these differences makes use of arrays of hundreds of DNA sequences attached to a glass surface—a so-called **DNA microarray** or 'DNA chip.' The array contains a range of DNA sequences from known genes, arranged in a fixed pattern, and the differential expression of those genes in a particular cell type or tissue is tested by exposing the array to labeled mRNA (or cDNA made from it) from the tissue. Hybridization of labeled mRNAs to their corresponding DNA sequences in the array is detected by standard techniques and the whole technique is readily automated. Many different samples can be examined in parallel, which makes this a powerful analytical technique, as can be seen from the example we illustrate in Fig. A.40. Here the DNA microarray has been constructed with almost 18,000 cDNA clones known to be expressed in either B or T cells and B-cell tumors. This array was then probed with fluorochrome-labeled cDNAs from 96 normal and malignant cells, and the level of expression of approximately 18,000 genes in each of the cell lines was measured simultaneously. In this particular case the patterns of expression of the different genes revealed that the malignant B cells formed discrete subtypes, which were then found to have different clinical prognoses.

Detection of immunity *in vivo*.

A-36 Assessment of protective immunity.

An adaptive immune response against a pathogen often confers long-lasting immunity against infection with that pathogen; successful vaccination achieves the same end. The very first experiment in immunology, Jenner's successful vaccination against smallpox, is still the model for assessing the presence of such protective immunity. The assessment of protective immunity conferred by vaccination has three essential steps. First, an immune response is elicited by immunization with a candidate vaccine. Second, the immunized individuals, along with unimmunized controls, are challenged with the infectious agent (Fig. A.41). Finally, the prevalence and severity of infection in the immunized individual is compared with the course of the disease in the unimmunized controls. For obvious reasons, such experiments are usually carried out first in animals, if a suitable animal model for the infection exists. However, eventually a trial must be carried out in humans. In this case, the infectious challenge is usually provided naturally by carrying out the trial in a region where the disease is prevalent. The efficacy of the vaccine is determined by assessing the prevalence and severity of new infections in the immunized and control populations. Such studies necessarily give less precise results than a direct experiment but, for most diseases, they are the only way of assessing a vaccine's ability to induce protective immunity in humans.

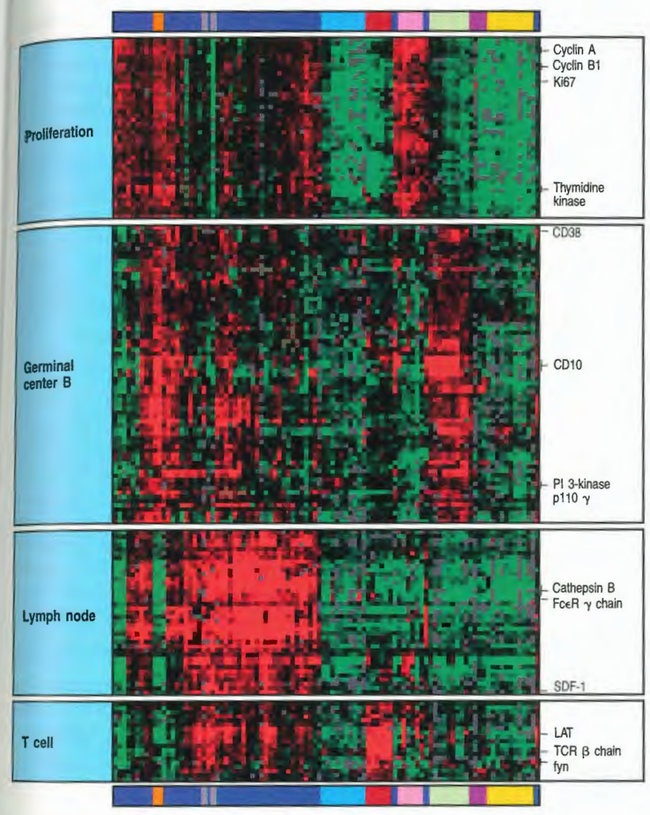


Fig. A.40 DNA microarrays allow a rapid, simultaneous screening of a great many genes for changes in expression between different cells. In the experiment whose results are shown here, nearly 18,000 cDNA clones made from lymphoid cells and lymphoid tumors were arrayed horizontally across the chip. Some of these cDNAs represent the products of known genes, and examples of these are indicated on the right hand edge of the diagram. To these cDNAs mRNA was hybridized from 96 normal cells, cell lines and lymphoid tumor cells, with the mRNA from each individual cell arrayed vertically. The types of cells used are indicated by the colored bars at the top, with the colors explained in the key. The malignant cells used were from diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) or mantle cell lymphoma, and chronic lymphocytic leukemia (CLL). A number of established lymphoid cell lines were included. Normal cells are represented by resting B cells from peripheral blood (Resting Blood B), B cells activated by cross-linking cell-surface IgM with or without additional cytokines and co-stimulation (Activated blood B) germinal center B cells from tonsil (Germinal Center B), and normal, noninflamed tonsil and lymph node (NI. Node/Tonsil) were used as representatives of different stages in B-cell maturation. Normal T cells, CD4 T cells, either resting or stimulated with PMA and ionomycin (Resting/Activated T) were also used. Each point on the array represents, therefore, the hybridization of the mRNA from one of these cell lines to one of the cDNAs, and is displayed in color to represent the level of expression of the mRNA in question, green being those expressed at lower levels than in a control cell, while red represents those expressed at a higher level. The data shown has been clustered by patterns of expression of the various genes, to give clusters of genes upregulated in proliferating cells, germinal center B cells, lymph node B cells, and in T cells. Courtesy of L. M. Staudt.

A-37 Transfer of protective immunity.

The tests described in Section A-36 show that protective immunity has been established, but cannot show whether it involves humoral immunity, cell-mediated immunity, or both. When these studies are carried out in inbred mice, the nature of protective immunity can be determined by transferring

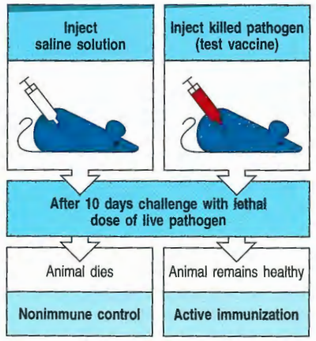


Fig. A.41 *In vivo* assay for the presence of protective immunity after vaccination in animals. Mice are injected with the test vaccine or a control such as saline solution. Different groups are then challenged with lethal or pathogenic doses of the test pathogen or with an unrelated pathogen as a specificity control (not shown). Unimmunized animals die or become severely infected. Successful vaccination is seen as specific protection of immunized mice against infection with the test pathogen. This is called active immunity and the process is called active immunization.

serum or lymphoid cells from an immunized donor animal to an unimmunized syngeneic recipient (that is, a genetically identical animal of the same inbred strain) (Fig. A.42). If protection against infection can be conferred by the transfer of serum, the immunity is provided by circulating antibodies and is called **humoral immunity**. Transfer of immunity by antiserum or purified antibodies provides immediate protection against many pathogens and against toxins such as those of tetanus and snake venom. However, although protection is immediate, it is temporary, lasting only so long as the transferred antibodies remain active in the recipient's body. This type of transfer is therefore called **passive immunization**. Only **active immunization** with antigen can provide lasting immunity. Moreover, the recipient may become immunized to the antiserum used to transfer immunity. Horse or sheep sera are the usual sources of anti-snake venoms used in humans, and repeated administration can lead either to serum sickness (see Section 13-18) or, if the recipient becomes allergic to the foreign serum, to anaphylaxis (see Section 13-11).

Protection against many diseases cannot be transferred with serum but can be transferred by lymphoid cells from immunized donors. The transfer of lymphoid cells from an immune donor to a normal syngeneic recipient is called **adoptive transfer** or **adoptive immunization**, and the immunity transferred is called **adoptive immunity**. Immunity that can be transferred only with lymphoid cells is called **cell-mediated immunity**. Such cell transfers must be between genetically identical donors and recipients, such as members of the same inbred strain of mouse, so that the donor lymphocytes are not rejected by the recipient and do not attack the recipient's tissues. Adoptive transfer of immunity is used clinically in humans in experimental approaches to cancer therapy or as an adjunct to bone marrow transplantation; in these cases, the patient's own T cells, or the T cells of the bone marrow donor, are given.

A-38 The tuberculin test.

Local responses to antigen can indicate the presence of active immunity. Active immunity is often studied *in vivo*, especially in humans, by injecting antigens locally in the skin. If a reaction appears, this indicates the presence of antibodies or immune lymphocytes that are specific for that antigen; the **tuberculin test** is an example of this. When people have had tuberculosis they develop cell-mediated immunity that can be detected as a local response when their skin is injected with a small amount of tuberculin, an extract of *Mycobacterium tuberculosis*, the pathogen that causes tuberculosis. The response typically appears a day or two after the injection and consists of a raised, red, and hard (or indurated) area in the skin, which then disappears as the antigen is degraded.

A-39 Testing for allergic responses.

Local **intracutaneous** injections of minute doses of the antigens that cause allergies are used to determine which antigen triggers a patient's allergic reactions. Local responses that happen in the first few minutes after antigen injection in immune recipients are called **immediate hypersensitivity reactions**, and they can be of several forms, one of which is the wheal-and-flare response (see Fig. 13.14). Immediate hypersensitivity reactions are mediated by specific antibodies of the IgE class formed as a result of earlier exposures to the antigen. Responses that take hours to days to develop, such as the tuberculin test, are referred to as **delayed-type hypersensitivity responses** and are caused by preexisting immune T cells. This latter type of response was observed by Jenner when he tested vaccinated individuals with a local injection of vaccinia virus.

These tests work because the local deposit of antigen remains concentrated in the initial site of injection, eliciting responses in local tissues. They do not cause generalized reactions if sufficiently small doses of antigen are used. However, local tests carry a risk of systemic allergic reactions, and they should be used with caution in people with a history of hypersensitivity.

A-40 Assessment of immune responses and immunological competence in humans.

The methods used for testing immune function in humans are necessarily more limited than those used in experimental animals, but many different tests are available. They fall into several groups depending on the reason the patient is being studied.

Assessment of protective immunity in humans generally relies on tests conducted *in vitro*. To assess humoral immunity, specific antibody levels in the patient's serum are assayed by RIA or, more commonly, ELISA (see Section A-6), using the test microorganism or a purified microbial product as antigen. To test for humoral immunity against viruses, antibody production is often measured by the ability of serum to neutralize the infectivity of live virus for tissue culture cells. In addition to providing information about protective immunity, the presence of antibody to a particular pathogen indicates that the patient has been exposed to it, making such tests of crucial importance in epidemiology. At present, testing for antibody to HIV is the main screening test for infection with this virus, critical both for the patient and in blood banking, where blood from infected donors must be excluded from the supply. Essentially similar tests are used in investigating allergy, where allergens are used as the antigens in tests for specific IgE antibody by ELISA or RIA (see Section A-6), which may be used to confirm the results of skin tests.

Cell-mediated immunity, that is immunity mediated by T cells, is technically more difficult to measure than humoral immunity. This is principally because T cells do not make a secreted antigen-binding product, so there is no simple binding assay for their antigen-specific responses. T-cell activity can be divided into an induction phase, in which T cells are activated to divide and differentiate, and an effector phase, in which their function is expressed. Both phases require that the T cell interacts with another cell and that it recognizes specific antigen displayed in the form of peptide:MHC complexes on the surface of this interacting cell. In the induction phase, the interaction must be with an antigen-presenting cell able to deliver co-stimulatory signals, whereas, in the effector phase, the nature of the target cell depends on the type of effector T cell that has been activated. Most commonly, the presence of T cells that have responded to a specific antigen is detected by their subsequent *in vitro* proliferation when reexposed to the same antigen (see Section A-31).

T-cell proliferation indicates only that cells able to recognize that antigen have been activated previously; it does not reveal what effector function they mediate. The effector function of a T cell is assayed by its effect on an appropriate target cell. Assays for cytotoxic CD8 T cells (see Section A-33) and for cytokine production by CD4 T cells (see Sections A-26, A-27, and A-34) are used to characterize the immune response. Cell-mediated immunity to infectious agents can also be tested by skin test with extracts of the pathogen, as in the tuberculin test (see Section A-36). These tests provide information about the exposure of the patient to the disease and also about their ability to mount an adaptive immune response to it.

Patients with immune deficiency (see Chapter 12) are usually detected clinically by a history of recurrent infection. To determine the competence of the

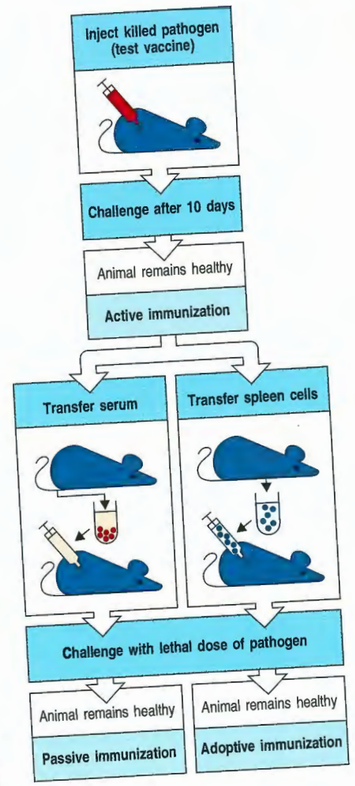


Fig. A.42 Immunity can be transferred by antibodies or by lymphocytes. Successful vaccination leads to a long-lived state of protection against the specific immunizing pathogen. If this immune protection can be transferred to a normal syngeneic recipient with serum from an immune donor, then immunity is mediated by antibodies; such immunity is called humoral immunity and the process is called passive immunization. If immunity can only be transferred by infusing lymphoid cells from the immune donor into a normal syngeneic recipient, then the immunity is called cell-mediated immunity and the transfer process is called adoptive transfer or adoptive immunization. Passive immunity is short-lived, as antibody is eventually catabolized, but adoptively transferred immunity is mediated by immune cells, which can survive and provide longer-lasting immunity.

immune system in such patients, a battery of tests are usually conducted (see Appendix V); these focus with increasing precision as the nature of the defect is narrowed down to a single element. The presence of the various cell types in blood is determined by routine hematology, often followed by FACS analysis of lymphocyte subsets (see Section A-22), and the measurement of serum immunoglobulins. The phagocytic competence of freshly isolated polymorphonuclear leukocytes and monocytes is tested, and the efficiency of the complement system (see Chapters 2 and 9) is determined by testing the dilution of serum required for lysis of 50% of antibody-coated red blood cells (this is denoted the CH_{50}).

In general, if such tests reveal a defect in one of the broad compartments of immune function, more specialized testing is then needed to determine the precise nature of the defect. Tests of lymphocyte function are often valuable, starting with the ability of polyclonal mitogens to induce T-cell proliferation and B-cell secretion of immunoglobulin in tissue culture (see Section A-31). These tests can eventually pinpoint the cellular defect in immunodeficiency.

In patients with autoimmune diseases (see Chapter 14), the same parameters are usually analyzed to determine whether there is a gross abnormality in the immune system. However, most patients with such diseases show few abnormalities in general immune function. To determine whether a patient is producing antibody against their own cellular antigens, the most informative test is to react their serum with tissue sections, which are then examined for bound antibody by indirect immunofluorescence using anti-human immunoglobulin labeled with fluorescent dye (see Section A-14). Most autoimmune diseases are associated with the production of broadly characteristic patterns of autoantibodies directed at self tissues. These patterns aid in the diagnosis of the disease and help to distinguish autoimmunity from tissue inflammation due to infectious causes.

It is also possible to investigate allergies by administration of possible allergens by routes other than intracutaneous administration. Allergen may be given by inhalation to test for asthmatic allergic responses (see Fig. 13.14); this is mainly done for experimental purposes in studies of the mechanisms and treatment of asthma. Similarly, food allergens may be given by mouth. The administration of allergens is potentially very dangerous because of the risk of causing anaphylaxis, and must only be carried out by trained and experienced investigators in an environment in which full resuscitation facilities are available.

A-41 The Arthus reaction.

This is an experimental method using only animal models for studying the formation of immune complexes in tissues and how immune complexes cause inflammation (see Section 13-18). The original reaction described by Maurice Arthus was induced by the repeated injection of horse serum into rabbits. Initial injections of horse serum into the skin induced no reaction, but later injections, following the production of antibodies to the proteins in horse serum, induced an inflammatory reaction at the site of injection after several hours, characterized by the presence of edema, hemorrhage, and neutrophil infiltration, which frequently progressed to tissue necrosis. Most investigators now use passive models of the Arthus reaction in which either antibody is infused systemically and antigen given locally (passive Arthus reaction) or antigen is infused systemically and antibody injected locally (reverse passive Arthus reaction).

Manipulation of the immune system.

A-42 Adoptive transfer of lymphocytes.

Ionizing radiation from X-ray or γ-ray sources kills lymphoid cells at doses that spare the other tissues of the body. This makes it possible to eliminate immune function in a recipient animal before attempting to restore immune function by adoptive transfer, and allows the effect of the adoptively transferred cells to be studied in the absence of other lymphoid cells. James Gowans originally used this technique to prove the role of the lymphocyte in immune responses. He showed that all active immune responses could be transferred to irradiated recipients by small lymphocytes from immunized donors. This technique can be refined by transferring only certain lymphocyte subpopulations, such as B cells, CD4 T cells, and so on. Even cloned T-cell lines have been tested for their ability to transfer immune function, and have been shown to confer adoptive immunity to their specific antigen. Such adoptive transfer studies are a cornerstone in the study of the intact immune system, as they can be carried out rapidly, simply, and in any strain of mouse.

A-43 Hematopoietic stem-cell transfers.

All cells of hematopoietic origin can be eliminated by treatment with high doses of X rays, allowing replacement of the entire hematopoietic system, including lymphocytes, by transfusion of donor bone marrow or purified hematopoietic stem cells from another animal. The resulting animals are called **radiation bone marrow chimeras** from the Greek word *chimera*, a mythical animal that had the head of a lion, the tail of a serpent, and the body of a goat. This technique is used experimentally to examine the development of lymphocytes, as opposed to their effector functions, and has been particularly important in studying T-cell development. Essentially the same technique is used in humans to replace the hematopoietic system when it fails, as in aplastic anemia or after nuclear accidents, or to eradicate the bone marrow and replace it with normal marrow in the treatment of certain cancers. In man, bone marrow is the main source of hematopoietic stem cells, but increasingly they are being obtained from peripheral blood after the donor has been treated with hematopoietic growth factors such as GM-CSF, or from umbilical cord blood, which is rich in such stem cells.

A-44 *In vivo* depletion of T cells.

The importance of T-cell function *in vivo* can be ascertained in mice with no T cells of their own. Under these conditions, the effect of a lack of T cells can be studied, and T-cell subpopulations can be restored selectively to analyze their specialized functions. T lymphocytes originate in the thymus, and neonatal **thymectomy**, the surgical removal of the thymus of a mouse at birth, prevents T-cell development from occurring because the export of most functionally mature T cells only occurs after birth in the mouse. Alternatively, adult mice can be thymectomized and then irradiated and reconstituted with bone marrow; such mice will develop all hematopoietic cell types except mature T cells.

The recessive *nude* mutation in mice is caused by a mutation in the gene for the transcription factor Wnt and in homozygous form causes hairlessness

and absence of the thymus. Consequently, these animals fail to develop T cells from bone marrow progenitors. Grafting thymectomized or *nude/nude* mice with thymic epithelial elements depleted of lymphocytes allows the graft recipients to develop normal mature T cells. This procedure allows the role of the nonlymphoid thymic stroma to be examined; it has been crucial in determining the role of thymic stromal cells in T-cell development (see Chapter 7).

A-45 *In vivo* depletion of B cells.

There is no single site of B-cell development in mice, so techniques such as thymectomy cannot be applied to the study of B-cell function and development in rodents. However, **bursectomy**, the surgical removal of the Bursa of Fabricius in birds, can inhibit the development of B cells in these species. In fact, it was the effect of thymectomy versus bursectomy that led to the naming of T cells for thymus-derived lymphocytes and B cells for bursal-derived lymphocytes. There are no known spontaneous mutations (analogous to the *nude* mutation) in mice that produce animals with T cells but no B cells. However, such mutations exist in humans, leading to a failure to mount humoral immune responses or make antibody. The diseases produced by such mutations are called agammaglobulinemias because they were originally detected as the absence of gamma globulins. The genetic basis for one form of this disease in humans has now been established (see Chapter 12), and some features of the disease can be reproduced in mice by targeted disruption of the corresponding gene (see Section A-47). Several different mutations in crucial regions of immunoglobulin genes have already been produced by gene targeting and have provided mice lacking B cells.

A-46 Transgenic mice.

The function of genes has traditionally been studied by observing the effects of spontaneous mutations in whole organisms and, more recently, by analyzing the effects of targeted mutations in cultured cells. The advent of gene cloning and *in vitro* mutagenesis now make it possible to produce specific mutations in whole animals. Mice with extra copies or altered copies of a gene in their genome can be generated by **transgenesis**, which is now a well established procedure. To produce **transgenic mice**, a cloned gene is introduced into the mouse genome by microinjection into the male pronucleus of a fertilized egg, which is then implanted into the uterus of a pseudopregnant female mouse. In some of the eggs, the injected DNA becomes integrated randomly into the genome, giving rise to a mouse that has an extra genetic element of known structure, the transgene (Fig. A.43).

The transgene, to be studied in detail, needs to be introduced onto a stable, well-characterized genetic background. However, it is difficult to prepare transgenic embryos successfully in inbred strains of mice, and transgenic

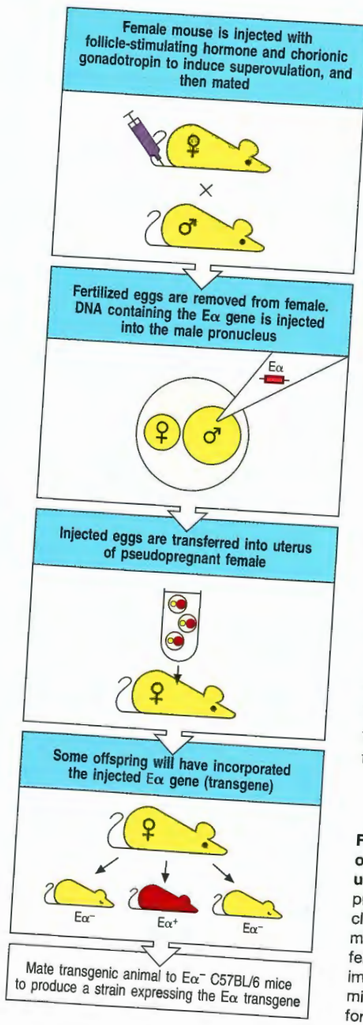


Fig. A.43 The function and expression of genes can be studied in vivo by using transgenic mice. DNA encoding a protein of interest, here the mouse MHC class II protein Eα, is purified and microinjected into the male pronuclei of fertilized eggs. The eggs are then implanted into pseudopregnant female mice. The resulting offspring are screened for the presence of the transgene in their cells, and positive mice are used as founders that transmit the transgene to their offspring, establishing a line of transgenic mice that carry one or more extra genes. The function of the Eα gene used here is tested by breeding the transgene into C57BL/6 mice that carry an inactivating mutation in their endogenous Eα gene.

mice are routinely prepared in F_2 embryos (that is, the embryo formed after the mating of two F_1 animals). The transgene must then be bred onto a well-characterized genetic background; this requires 10 generations of back-crossing with an inbred strain to assure that the integrated transgene is largely (>99%) free of heterogeneous genes from the founder mouse of the transgenic mouse line (Fig. A.44).

This technique allows one to study the impact of a newly discovered gene on development, to identify the regulatory regions of a gene required for its normal tissue-specific expression, to determine the effects of its over-expression or expression in inappropriate tissues, and to find out the impact of mutations on gene function. Transgenic mice have been particularly useful in studying the role of T-cell and B-cell receptors in lymphocyte development, as described in Chapter 7.

A-47 Gene knockout by targeted disruption.

In many cases, the functions of a particular gene can be fully understood only if a mutant animal that does not express the gene can be obtained. Whereas genes used to be discovered through identification of mutant phenotypes, it is now far more common to discover and isolate the normal gene and then determine its function by replacing it *in vivo* with a defective copy. This procedure is known as **gene knockout**, and it has been made possible by two fairly recent developments: a powerful strategy to select for targeted mutation by homologous recombination, and the development of continuously growing lines of **embryonic stem cells (ES cells)**. These are embryonic cells which, on implantation into a blastocyst, can give rise to all cell lineages in a chimeric mouse.

The technique of **gene targeting** takes advantage of the phenomenon known as **homologous recombination** (Fig. A.45). Cloned copies of the target gene are altered to make them nonfunctional and are then introduced into the ES cell where they recombine with the homologous gene in the cell's genome, replacing the normal gene with a nonfunctional copy. Homologous recombination is a rare event in mammalian cells, and thus a powerful selection strategy is required to detect those cells in which it has occurred. Most commonly, the introduced gene construct has its sequence disrupted by an inserted antibiotic-resistance gene such as that for neomycin resistance. If this construct undergoes homologous recombination with the endogenous copy of the gene, the endogenous gene is disrupted but the antibiotic-resistance gene remains functional, allowing cells that have incorporated the gene to be selected in culture for resistance to the neomycin-like drug G418. However, antibiotic resistance on its own shows only that the cells have taken up and integrated the neomycin-resistance gene. To be able to select for those cells in which homologous recombination has occurred, the ends of the construct usually carry the thymidine kinase gene from the herpes simplex virus

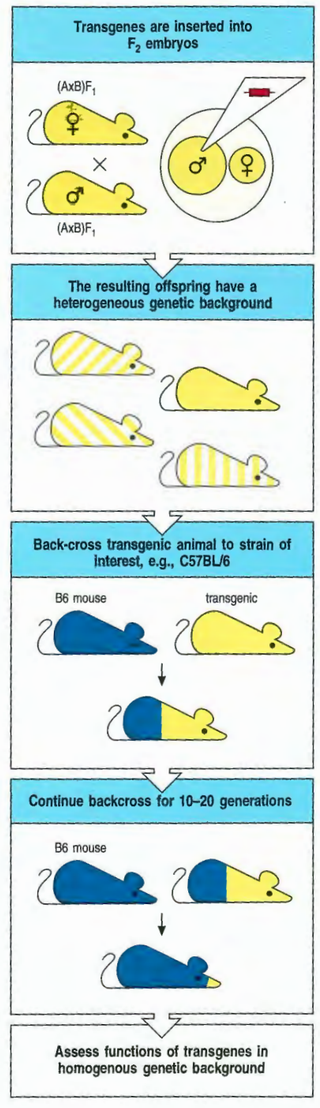


Fig. A.44 The breeding of transgenic co-isogeneic or congenic mouse strains. Transgenic mouse strains are routinely made in F2 mice. To produce mice on an inbred background, the transgene is introgressively back-crossed onto a standard strain, usually C57BL/6 (B6). The presence of the transgene is tracked by carrying out PCR on genomic DNA extracted from the tail of young mice. After 10 generations of back-crossing, mice are >99% genetically identical, so that any differences observed between the mice are likely to be due to the transgene itself. The same technique can be used to breed a gene knockout into a standard strain of mice, as most gene knockouts are made in the 129 strain of mice (see Fig. A.46). The mice are then intercrossed and homozygous knockout mice detected by an absence of an intact copy of the gene of interest (as determined by PCR).

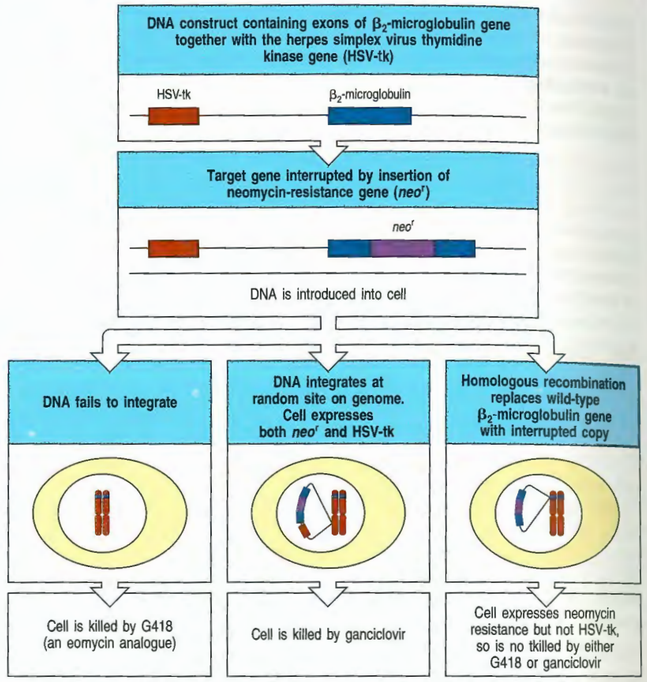


Fig. A.45 The deletion of specific genes can be accomplished by homologous recombination. When pieces of DNA are introduced into cells, they can integrate into cellular DNA in two different ways. If they randomly insert into sites of DNA breaks, the whole piece is usually integrated, often in several copies. However, extrachromosomal DNA can also undergo homologous recombination with the cellular copy of the gene, in which case only the central, homologous region is incorporated into cellular DNA. Inserting a selectable marker gene such as resistance to neomycin (neo^r) into the coding region of a gene does not prevent homologous recombination, and it achieves two goals. First, any cell that has integrated the injected DNA is protected from the neomycin-like antibiotic G418. Second, when the gene recombines with homologous cellular DNA, the neo^r gene disrupts the coding sequence of the modified cellular gene. Homologous recombinants can be discriminated from random insertions if the gene for herpes simplex virus thymidine kinase (HSV-tk) is placed at one or both ends of the DNA construct, which is often known as a 'targeting construct' because it targets the cellular gene. In random DNA integrations, HSV-tk is retained. HSV-tk renders the cell sensitive to the antiviral agent ganciclovir. However, as HSV-tk is not homologous to the target DNA, it is lost from homologous recombinants. Thus, cells that have undergone homologous recombination are uniquely both G418 and ganciclovir resistant, and survive in a mixture of the two antibiotics. The presence of the disrupted gene has to be confirmed by Southern blotting or by PCR using primers in the neor gene and in cellular DNA lying outside the region used in the targeting construct. By using two different resistance genes one can disrupt the two cellular copies of a gene, making a deletion mutant (not shown).

(HSV-tk). Cells that incorporate DNA randomly usually retain the entire DNA construct including HSV-tk, whereas homologous recombination between the construct and cellular DNA, the desired result, involves the exchange of homologous DNA sequences so that the nonhomologous HSV-tk genes at the ends of the construct are eliminated. Cells carrying HSV-tk are killed by the antiviral drug ganciclovir, and so cells with homologous recombinations have the unique feature of being resistant to both neomycin and ganciclovir, allowing them to be selected efficiently when these drugs are added to the cultures (see Fig. A.45).

This technique can be used to produce homozygous mutant cells in which the effects of knocking-out a specific gene can be analyzed. Diploid cells in which both copies of a gene have been mutated by homologous recombination can be selected after transfection with a mixture of constructs in which the gene to be targeted has been disrupted by one or other of two different antibiotic-resistance genes. Having obtained a mutant cell with a functional defect, the defect can be ascribed definitively to the mutated gene if the mutant phenotype can be reverted with a copy of the normal gene transfected into the mutant cell. Restoration of function means that the defect in the mutant gene has been complemented by the normal gene's function. This technique is very powerful as it allows the gene that is being transferred to be mutated in precise ways to determine which parts of the protein are required for function.

To knock out a gene *in vivo*, it is only necessary to disrupt one copy of the cellular gene in an ES cell. ES cells carrying the mutant gene are produced by

targeted mutation (see Fig. A.45), and injected into a blastocyst which is implanted into the uterus. The cells carrying the disrupted gene become incorporated into the developing embryo and contribute to all tissues of the resulting chimeric offspring, including those of the germline. The mutated gene can therefore be transmitted to some of the offspring of the original chimera, and further breeding of the mutant gene to homozygosity produces mice that completely lack the expression of that particular gene product (Fig. A.46). The effects of the absence of the gene's function can then be studied. In addition, the parts of the gene that are essential for its function can be identified by determining whether function can be restored by introducing different mutated copies of the gene back into the genome by transgenesis. The manipulation of the mouse genome by gene knockout and transgenesis is revolutionizing our understanding of the role of individual genes in lymphocyte development and function.

Because the most commonly used ES cells are derived from a poorly characterized strain of mice known as strain 129, the analysis of the function of a gene knockout often requires extensive back-crossing to another strain, just as in transgenic mice (see Fig. A.44). One can track the presence of the mutant copy of the gene by the presence of the *neo*^{r} gene. After sufficient back-crossing, the mice are intercrossed to produce mutants on a stable genetic background.

A problem with gene knockouts arises when the function of the gene is essential for the survival of the animal; in such cases the gene is termed a **recessive lethal gene** and homozygous animals cannot be produced. However, by making chimeras with mice that are deficient in B and T cells, it is possible to analyze the function of recessive lethal genes in lymphoid cells. To do this, ES cells with homozygous lethal loss-of-function mutations are injected into blastocysts of mice lacking the ability to rearrange their antigen-receptor genes because of a mutation in their recombinase-activating genes (*RAG* knockout mice). As these chimeric embryos develop, the *RAG*-deficient cells can compensate for any developmental failure resulting from the gene knockout in the ES cells in all except the lymphoid lineage. So long as the mutated ES cells can develop into hematopoietic progenitors in the bone marrow, the embryos will survive and all of the lymphocytes in the resulting chimeric mouse will be derived from the mutant ES cells (Fig. A.47).

A second powerful technique achieves tissue-specific or developmentally regulated gene deletion by employing the DNA sequences and enzymes used by bacteriophage P1 to excise itself from a host cell's genome. Integrated

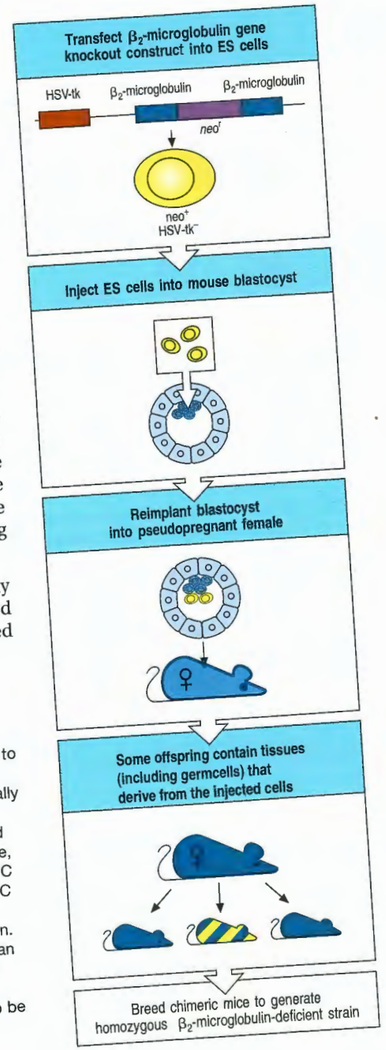


Fig. A.46 Gene knockout in embryonic stem cells enables mutant mice to be produced. Specific genes can be inactivated by homologous recombination in cultures of embryonic stem cells (ES cells). Homologous recombination is carried out as described in Fig. A.45. In this example, the gene for β_2-microglobulin in ES cells is disrupted by homologous recombination with a targeting construct. Only a single copy of the gene needs to be disrupted. ES cells in which homologous recombination has taken place are injected into mouse blastocysts. If the mutant ES cells give rise to germ cells in the resulting chimeric mice (striped in the figure), then the mutant gene can be transferred to their offspring. By breeding the mutant gene to homozygosity, a mutant phenotype is generated. These mutant mice are usually of the 129 strain as gene knockout is generally conducted in ES cells derived from the 129 strain of mice. In this case, the homozygous mutant mice lack MHC class I molecules on their cells, as MHC class I molecules have to pair with β_2-microglobulin for surface expression. The β_2-microglobulin-deficient mice can then be bred with mice transgenic for subtler mutants of the deleted gene, allowing the effect of such mutants to be tested *in vivo*.

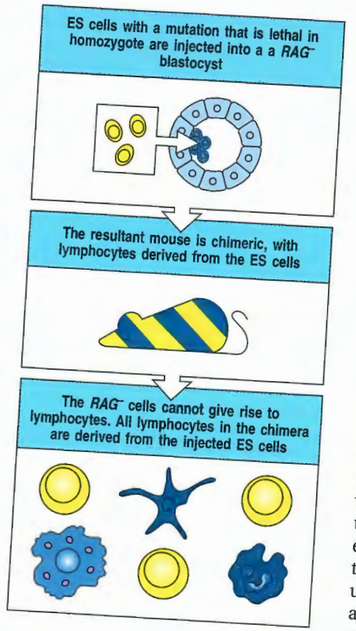
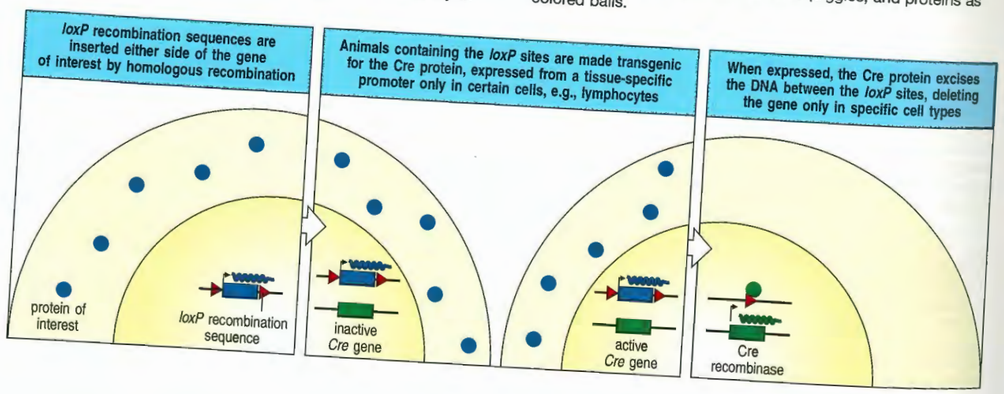


Fig. A.47 The role of recessive lethal genes in lymphocyte function can be studied using RAG-deficient chimeric mice. ES cells homozygous for the lethal mutation are injected into a RAG-deficient blastocyst (top panel). The RAG-deficient cells can give rise to all the tissues of a normal mouse except lymphocytes, and so can compensate for any deficiency in the developmental potential of the mutant ES cells (middle panel). If the mutant ES cells are capable of differentiating into hematopoietic stem cells, that is, if the gene function that has been deleted is not essential for this developmental pathway, then all the lymphocytes in the chimeric mouse will be derived from the ES cells (bottom panel), as RAG-deficient mice cannot make lymphocytes of their own.

bacteriophage P1 DNA is flanked by recombination signal sequences called *loxP* sites. A recombinase, Cre, recognizes these sites, cuts the DNA and joins the two ends, thus excising the intervening DNA in the form of a circle. This mechanism can be adapted to allow the deletion of specific genes in a transgenic animal only in certain tissues or at certain times in development. First, *loxP* sites flanking a gene, or perhaps just a single exon, are introduced by homologous recombination (Fig. A.48). Usually, the introduction of these sequences into flanking or intronic DNA does not disrupt the normal function of the gene. Mice containing such *loxP* mutant genes are then mated with mice made transgenic for the Cre recombinase, under the control of a tissue-specific or inducible promoter. When the Cre recombinase is active, either in the appropriate tissue or when induced, it excises the DNA between the inserted *loxP* sites, thus inactivating the gene or exon. Thus, for example, using a T-cell specific promoter to drive expression of the Cre recombinase, a gene can be deleted only in T cells, while remaining functional in all other cells of the animal. This is an extremely powerful genetic technique that while still in its infancy, was used to demonstrate the importance of B-cell receptors in B-cell survival. It is certain to yield exciting results in the future.

Fig. A.48 The P1 bacteriophage recombination system can be used to eliminate genes in particular cell lineages. The P1 bacteriophage protein Cre excises DNA that is bounded by recombination signal sequences called loxP sequences. These sequences can be introduced at either end of a gene by homologous recombination (left panel). Animals carrying genes flanked by loxP can also be made transgenic for the gene for the Cre protein, which is placed under the control of a tissue-specific promoter so that it is expressed only in certain cells or only at certain times during development (middle panel). In the cells in which the Cre protein is expressed, it recognizes the loxP sequences and excises the DNA lying between them (right panel). Thus, individual genes can be deleted only in certain cell types or only at certain times. In this way, genes that are essential for the normal development of a mouse can be analyzed for their function in the developed animal and/or in specific cell types. Genes are shown as boxes, RNA as squiggles, and proteins as colored balls.



APPENDICES II – V

Appendix II. CD antigens.

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD1a,b,c,d	Cortical thymocytes, Langerhans cells, dendritic cells, B cells (CD1c), intestinal epithelium, smooth muscle, blood vessels (CD1d)	43–49	MHC class I-like molecule, associated with β_2-microglobulin. Has specialized role in presentation of lipid antigens		Immunoglobulin
CD2	T cells, thymocytes, NK cells	45–58	Adhesion molecule, binding CD58 (LFA-3). Binds Lck intracellularly and activates T cells	T11, LFA-2	Immunoglobulin
CD3	Thymocytes, T cells	γ: 25–28 δ: 20 ε: 20	Associated with the T-cell antigen receptor (TCR). Required for cell-surface expression of and signal transduction by the TCR	T3	Immunoglobulin
CD4	Thymocyte subsets, T_H1 and T_H2 T cells (about two thirds of peripheral T cells), monocytes, macrophages	55	Co-receptor for MHC class II molecules. Binds Lck on cytoplasmic face of membrane. Receptor for HIV-1 and HIV-2 gp120	T4, L3T4	Immunoglobulin
CD5	Thymocytes, T cells, subset of B cells	67		T1, Ly1	Scavenger receptor
CD6	Thymocytes, T cells, B cells in chronic lymphatic leukemia	100–130	Binds CD166	T12	Scavenger receptor
CD7	Pluripotential hematopoietic cells, thymocytes, T cells	40	Unknown, cytoplasmic domain binds PI 3-kinase on cross-linking. Marker for T cell acute lymphatic leukemia and pluripotential stem cell leukemias		Immunoglobulin
CD8	Thymocytes subsets, cytotoxic T cells (about one third of peripheral T cells)	α: 32–34 β: 32–34	Co-receptor for MHC class I molecules. Binds Lck on cytoplasmic face of membrane	T8, Lyt2,3	Immunoglobulin
CD9	Pre-B cells, monocytes, eosinophils, basophils, platelets, activated T cells, brain and peripheral nerves, vascular smooth muscle	24	Mediates platelet aggregation and activation via FcγRIIa, may play a role in cell migration		Tetraspanning membrane protein, also called transmembrane 4 (TM4)
CD10	B- and T-cell precursors, bone marrow stromal cells	100	Zinc metalloproteinase, marker for pre-B acute lymphatic leukemia (ALL)	Neutral endopeptidase, common acute lymphocytic leukemia antigen (CALLA)	
CD11a	Lymphocytes, granulocytes, monocytes and macrophages	180	αL subunit of integrin LFA-1 (associated with CD18); binds to CD54 (ICAM-1), CD102 (ICAM-2), and CD50 (ICAM-3)	LFA-1	Integrin α

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD11b	Myeloid and NK cells	170	αM subunit of integrin CR3 (associated with CD18): binds CD54, complement component iC3b, and extracellular matrix proteins	Mac-1	Integrin α
CD11c	Myeloid cells	150	αX subunit of integrin CR4 (associated with CD18); binds fibrinogen	CR4, p150, 95	Integrin α
CD11d	Leukocytes	125	αD subunits of integrin; associated with CD18; binds to CD50		Integrin α
CDw12	Monocytes, granulocytes, platelets	90–120	Unknown		
CD13	Myelomonocytic cells	150–170	Zinc metalloproteinase	Aminopeptidase N	
CD14	Myelomonocytic cells	53–55	Receptor for complex of lipopoly-saccharide and lipopolysaccharide binding protein (LBP)		
CD15	Neutrophils, eosinophils, monocytes		Terminal trisaccharide expressed on glycolipids and many cell-surface glycoproteins	Lewis^x (Le^x)	
CD15s	Leukocytes, endothelium		Ligand for CD62E, P	Sialyl-Lewis^x (sLe^x)	poly-N-acetyl-lactosamine
CD15u			Sulphated CD15		Carbohydrate structures
CD16	Neutrophils, NK cells, macrophages	50–80	Component of low affinity Fc receptor, FcγRIII, mediates phagocytosis and antibody-dependent cell-mediated cytotoxicity	FcγRIII	Immunoglobulin
CDw17	Neutrophils, monocytes, platelets		Lactosyl ceramide, a cell-surface glycosphingolipid		
CD18	Leukocytes	95	Intergrin β2 subunit, associates with CD11a, b, c, and d		Integrin β
CD19	B cells	95	Forms complex with CD21 (CR2) and CD81 (TAPA-1); co-receptor for B cells—cytoplasmic domain binds cyto-plasmic tyrosine kinases and PI 3-kinase		Immunoglobulin
CD20	B cells	33–37	Oligomers of CD20 may form a Ca^{2+} channel; possible role in regulating B-cell activation		Contains 4 transmembrane segments
CD21	Mature B cells, follicular dendritic cells	145	Receptor for complement component C3d, Epstein–Barr virus. With CD19 and CD81, CD21 forms co-receptor for B cells	CR2	Complement control protein (CCP)
CD22	Mature B cells	α: 130 β: 140	Binds sialoconjugates	BL-CAM	Immunoglobulin
CD23	Mature B cells, activated macrophages, eosinophils, follicular dendritic cells, platelets	45	Low-affinity receptor for IgE, regulates IgE synthesis; ligand for CD19:CD21:CD81 co-receptor	FcεRII	C-type lectin
CD24	B cells, granulocytes	35–45	Unknown	Possible human homologue of mouse heat stable antigen (HSA)	
CD25	Activated T cells, B cells, and monocytes	55	IL-2 receptor α chain	Tac	CCP
CD26	Activated B and T cells, macrophages	110	Exopeptidase, cleaves N terminal X-Pro or X-Ala dipeptides from polypeptides	Dipeptidyl peptidase IV	Type II transmem-brane glycoprotein

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD27	Medullary thymocytes, T cells, NK cells, some B cells	55	Binds CD70; can function as a co-stimulator for T and B cells		TNF receptor
CD28	T-cell subsets, activated B cells	44	Activation of naive T cells, receptor for co-stimulatory signal (signal 2) binds CD80 (B7.1) and CD86 (B7.2)	Tp44	Immunoglobulin and CD86 (B7.2)
CD29	Leukocytes	130	Integrin β1 subunit, associates with CD49a in VLA-1 integrin		Integrin β
CD30	Activated T, B, and NK cells, monocytes	120	Binds CD30L (CD153); cross-linking CD30 enhances proliferation of B and T cells	Ki-1	TNF receptor
CD31	Monocytes, platelets, granulocytes, T-cell subsets, endothelial cells	130–140	Adhesion molecule, mediating both leukocyte–endothelial and endothelial–endothelial interactions	PECAM-1	Immunoglobulin
CD32	Monocytes, granulocytes, B cells, eosinophils	40	Low affinity Fc receptor for aggregated immunoglobulin:immune complexes	FcγRII	Immunoglobulin
CD33	Myeloid progenitor cells, monocytes	67	Binds sialoconjugates		Immunoglobulin
CD34	Hematopoietic precursors, capillary endothelium	105–120	Ligand for CD62L (L-selectin)		Mucin
CD35	Erythrocytes, B cells, monocytes, neutrophils, eosinophils, follicular dendritic cells	250	Complement receptor 1, binds C3b and C4b, mediates phagocytosis	CR1	CCP
CD36	Platelets, monocytes, endothelial cells	88	Platelet adhesion molecule; involved in recognition and phagocytosis of apoptosed cells	Platelet GPIV, GPIIIb	
CD37	Mature B cells, mature T cells, myeloid cells	40–52	Unknown, may be involved in signal transduction; forms complexes with CD53, CD81, CD82, and MHC class II		Transmembrane 4
CD38	Early B and T cells, activated T cells, germinal center B cells, plasma cells	45	NAD glycohydrolase, augments B cell proliferation	T10	
CD39	Activated B cells, activated NK cells, macrophages, dendritic cells	78	Unknown, may mediate adhesion of B cells		
CD40	B cells, macrophages, dendritic cells, basal epithelial cells	48	Binds CD154 (CD40L); receptor for co-stimulatory signal for B cells, promotes growth, differentiation, and isotype switching of B cells, and cytokine production by macrophages and dendritic cells		TNF receptor
CD41	Platelets, megakaryocytes	Dimer: GPIIba: 125 GPIIbb: 22	αIIb integrin, associates with CD61 to form GPIIb, binds fibrinogen, fibronectin, von Willebrand factor, and thrombospondin	GPIIb	Integrin α
CD42a,b,c,d	Platelets, megakaryocytes	a: 23 b: 135, 23 c: 22 d: 85	Binds von Willebrand factor, thrombin; essential for platelet adhesion at sites of injury	a: GPIX b: GPIbα c: GPIbβ d: GPV	Leucine-rich repeat
CD43	Leukocytes, except resting B cells	115–135 (neutrophils) 95–115 (T cells)	Has extended structure, approx. 45 nm long and may be anti-adhesive	Leukosialin, sialophorin	Mucin
CD44	Leukocytes, erythrocytes	80–95	Binds hyaluronic acid, mediates adhesion of leukocytes	Hermes antigen, Pgp-1	Link protein

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD45	All hematopoietic cells	180–240 (multiple isoforms)	Tyrosine phosphatase, augments signaling through antigen receptor of B and T cells, multiple isoforms result from alternative splicing (see below)	Leukocyte common antigen (LCA), T200, B220	Fibronectin type III
CD45RO	T-cell subsets, B-cell subsets, monocytes, macrophages	180	Isoform of CD45 containing none of the A, B, and C exons		Fibronectin type II
CD45RA	B cells, T-cell subsets (naive T cells), monocytes	205–220	Isoforms of CD45 containing the A exon		Fibronectin type II
CD45RB	T-cell subsets, B cells, monocytes, macrophages, granulocytes	190–220	Isoforms of CD45 containing the B exon	T200	Fibronectin type II
CD46	Hematopoietic and non-hematopoietic nucleated cells	56/66 (splice variants)	Membrane co-factor protein, binds to C3b and C4b to permit their degradation by Factor I	MCP	CCP
CD47	All cells	47–52	Adhesion molecule, thrombospondin receptor	IAP, MER6, OA3	Immunoglobulin superfamily
CD48	Leukocytes	40–47	Putative ligand for CD244	Blast-1	Immunoglobulin
CD49a	Activated T cells, monocytes, neuronal cells, smooth muscle	200	α1 integrin, associates with CD29, binds collagen, laminin-1	VLA-1	Integrin α
CD49b	B cells, monocytes, platelets, megakaryocytes, neuronal, epithelial and endothelial cells, osteoclasts	160	α2 integrin, associates with CD29, binds collagen, laminin	VLA-2, platelet GPIa	Integrin α
CD49c	B cells, many adherent cells	125, 30	α3 integrin, associates with CD29, binds laminin-5, fibronectin, collagen, entactin, invasin	VLA-3	Integrin α
CD49d	Broad distribution includes B cells, thymocytes, monocytes, granulocytes, dendritic cells	150	α4 integrin, associates with CD29, binds fibronectin, MAdCAM-1, VCAM-1	VLA-4	Integrin α
CD49e	Broad distribution includes memory T cells, monocytes, platelets	135, 25	α5 integrin, associates with CD29, binds fibronectin, invasin	VLA-5	Integrin α
CD49f	T lymphocytes, monocytes, platelets, megakaryocytes, trophoblasts	125, 25	α6 integrin, associates with CD29, binds laminin, invasin, merosine	VLA-6	Integrin α
CD50	Thymocytes, T cells, B cells, monocytes, granulocytes	130	Binds integrin CD11a/CD18	ICAM-3	Immunoglobulin
CD51	Platelets, megakaryocytes	125, 24	αV integrin, associates with CD61, binds vitronectin, von Willebrand factor, fibrinogen, and thrombospondin; may be receptor for apoptotic cells	Vitronectin receptor	Integrin α
CD52	Thymocytes, T cells, B cells (not plasma cells), monocytes, granulocytes, spermatozoa	25	Unknown, target for antibodies used therapeutically to deplete T cells from bone marrow	CAMPATH-1, HE5	
CD53	Leukocytes	35–42	Unknown	MRC OX44	Transmembrane 4
CD54	Hematopoietic and non-hematopoietic cells	75–115	Intercellular adhesion molecule (ICAM)-1 binds CD11a/CD18 integrin (LFA-1) and CD11b/CD18 integrin (Mac-1), receptor for rhinovirus	ICAM-1	Immunoglobulin
CD55	Hematopoietic and non-hematopoietic cells	60–70	Decay accelerating factor (DAF), binds C3b, disassembles C3/C5 convertase	DAF	CCP
CD56	NK cells	135–220	Isoform of neural cell-adhesion molecule (NCAM), adhesion molecule	NKH-1	Immunoglobulin
CD57	NK cells, subsets of T cells, B cells, and monocytes		Oligosaccharide, found on many cell-surface glycoproteins	HNK-1, Leu-7	

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD58	Hematopoietic and non-hematopoietic cells	55–70	Leukocyte function-associated antigen-3 (LFA-3), binds CD2, adhesion molecule	LFA-3	Immunoglobulin
CD59	Hematopoietic and non-hematopoietic cells	19	Binds complement components C8 and C9, blocks assembly of membrane-attack complex	Protectin, Mac inhibitor	Ly-6
CD60a			Disialyl ganglioside D3 (GD3)		Carbohydrate structures
CD60b			9-*O*-acetyl-GD3		Carbohydrate structures
CD60c			7-*O*-acetyl-GD3		Carbohydrate structures
CD61	Platelets, megakaryocytes, macrophages	110	Intergrin β3 subunit, associates with CD41 (GPIIb/IIIa) or CD51 (vitronectin receptor)		Integrin β
CD62E	Endothelium	140	Endothelium leukocyte adhesion molecule (ELAM), binds sialyl-Lewis^x, mediates rolling interaction of neutrophils on endothelium	ELAM-1, E-selectin	C-type lectin, EGF, and CCP
CD62L	B cells, T cells, monocytes, NK cells	150	Leukocyte adhesion molecule (LAM), binds CD34, GlyCAM, mediates rolling interactions with endothelium	LAM-1, L-selectin, LECAM-1	C-type lectin, EGF, and CCP
CD62P	Platelets, megakaryocytes, endothelium	140	Adhesion molecule, binds CD162 (PSGL-1), mediates interaction of platelets with endothelial cells, monocytes and rolling leukocytes on endothelium	P-selectin, PADGEM	C-type lectin, EGF, and CCP
CD63	Activated platelets, monocytes, macrophages	53	Unknown, is lysosomal membrane protein translocated to cell surface after activation	Platelet activation antigen	Transmembrane 4
CD64	Monocytes, macrophages	72	High-affinity receptor for IgG, binds IgG3>IgG1>IgG4>>>IgG2, mediates phagocytosis, antigen capture, ADCC	FcγRI	Immunoglobulin
CD65	Myeloid cells		Oligosaccharide component of a ceramide dodecasaccharide		
CD66a	Neutrophils	160–180	Unknown, member of carcino-embryonic antigen (CEA) family (see below)	Biliary glyco-protein-1 (BGP-1)	Immunoglobulin
CD66b	Granulocytes	95–100	Unknown, member of carcino-embryonic antigen (CEA) family	Previously CD67	Immunoglobulin
CD66c	Neutrophils, colon carcinoma	90	Unknown, member of carcino-embryonic antigen (CEA) family	Nonspecific cross-reacting antigen (NCA)	Immunoglobulin
CD66d	Neutrophils	30	Unknown, member of carcino-embryonic antigen (CEA) family		Immunoglobulin
CD66e	Adult colon epithelium, colon carcinoma	180–200	Unknown, member of carcino-embryonic antigen (CEA) family	Carcino-embryonic antigen (CEA)	Immunoglobulin
CD66f	Unknown		Unknown, member of carcino-embryonic antigen (CEA) family	Pregnancy specific glycoprotein	Immunoglobulin
CD68	Monocytes, macrophages, neutrophils, basophils, large lymphocytes	110	Unknown	Macrosialin	Mucin

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD69	Activated T and B cells, activated macrophages and NK cells	28, 32 homodimer	Unknown, early activation antigen	Activation inducer molecule (AIM)	C-type lectin
CD70	Activated T and B cells, and macrophages	75, 95, 170	Ligand for CD27, may function in co-stimulation of B and T cells	Ki-24	TNF
CD71	All proliferating cells, hence activated leukocytes	95 homodimer	Transferrin receptor	T9	
CD72	B cells (not plasma cells)	42 homodimer	Unknown	Lyb-2	C-type lectin
CD73	B-cell subsets, T-cell subsets	69	Ecto-5′-nucleotidase, dephosphorylates nucleotides to allow nucleoside uptake		
CD74	B cells, macrophages, monocytes, MHC class II positive cells	33, 35, 41, 43 (alternative initiation and splicing)	MHC class II-associated invariant chain	Ii, Iγ	
CD75	Mature B cells, T-cells subsets		Lactosamines, ligand for CD22, mediates B-cell–B-cell adhesion		
CD75s			α-2,6-sialylated lactosamines		Carbohydrate structures
CD77	Germinal center B cells		Neutral glycosphingolipid (Galα1→4Galβ1→4Galcβ1 →ceramide), binds Shiga toxin, cross-linking induces apoptosis	Globotriaocyl-ceramide (Gb3) Pk blood group	
CD79α,β	B cells	α: 40–45 β: 37	Components of B-cell antigen receptor analogous to CD3, required for cell-surface expression and signal transduction	Igα, Igβ	Immunoglobulin
CD80	B-cell subset	60	Co-stimulator, ligand for CD28 and CTLA-4	B7 (now B7.1), BB1	Immunoglobulin
CD81	Lymphocytes	26	Associates with CD19, CD21 to form B cell co-receptor	Target of anti-proliferative antibody (TAPA-1)	Transmembrane 4
CD82	Leukocytes	50–53	Unknown	R2	Transmembrane 4
CD83	Dendritic cells, B cells, Langerhans cells	43	Unknown	HB15	Immunoglobulin
CDw84	Monocytes, platelets, circulating B cells	73	Unknown	GR6	Immunoglobulin
CD85	Dendritic cells		ILT/LIR family	GR4	Immunoglobulin superfamily
CD86	Monocytes, activated B cells, dendritic cells	80	Ligand for CD28 and CTLA4	B7.2	Immunoglobulin
CD87	Granulocytes, monocytes, macrophages, T cells, NK cells, wide variety of nonhematopoietic cell types	35–59	Receptor for urokinase plasminogen activator	uPAR	Ly-6
CD88	Polymorphonuclear leukocytes, macrophages, mast cells	43	Receptor for complement component C5a	C5aR	G protein-coupled receptor
CD89	Monocytes, macrophages, granulocytes, neutrophils, B-cell subsets, T-cell subsets	50–70	IgA receptor	FcαR	Immunoglobulin
CD90	CD34+ prothymocytes (human), thymocytes, T cells (mouse)	18	Unknown	Thy-1	Immunoglobulin
CD91	Monocytes, many non-hematopoietic cells	515, 85	α2-macroglobulin receptor		EGF, LDL receptor

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD92	Neutrophils, monocytes, platelets, endothelium	70	Unknown	GR9	
CD93	Neutrophils, monocytes, endothelium	120	Unknown	GR11	
CD94	T-cell subsets, NK cells	43	Unknown	KP43	C-type lectin
CD95	Wide variety of cell lines, *in vivo* distribution uncertain	45	Binds TNF-like Fas ligand, induces apoptosis	Apo-1, Fas	TNF receptor
CD96	Activated T cells, NK cells	160	Unknown	T-cell activation increased late expression (TACTILE)	Immunoglobulin
CD97	Activated B and T cells, monocytes, granulocytes	75–85	Binds CD55	GR1	EGF, G protein-coupled receptor
CD98	T cells, B cells, natural killer cells, granulocytes, all human cell lines	80, 45 heterodimer	May be amino acid transporter	4F2, FRP-1	
CD99	Peripheral blood lymphocytes, thymocytes	32	Unknown	MIC2, E2	
CD100	Hematopoietic cells	150 homodimer	Unknown	GR3	Semaphorin
CD101	Monocytes, granulocytes, dendritic cells, activated T cells	120 homodimer	Unknown	BPC#4	Immunoglobulin
CD102	Resting lymphocytes, monocytes, vascular endothelium cells (strongest)	55–65	Binds CD11a/CD18 (LFA-1) but not CD11b/CD18 (Mac-1)	ICAM-2	Immunoglobulin
CD103	Intraepithelial lymphocytes, 2–6% peripheral blood lymphocytes	150, 25	αE integrin	HML-1, α6, αE integrin	Integrin α
CD104	CD4− CD8− thymocytes, neuronal, epithelial, and some endothelial cells, Schwann cells, trophoblasts	220	Integrin β4 associates with CD49f, binds laminins	β4 integrin	Integrin β
CD105	Endothelial cells, activated monocytes and macrophages, bone marrow cell subsets	90 homodimer	Binds TGF-β	Endoglin	
CD106	Endothelial cells	100–110	Adhesion molecule, ligand for VLA-4 ($\alpha_4\beta_1$ integrin)	VCAM-1	Immunoglobulin
CD107a	Activated platelets, activated T cells, activated neutrophils, activated endothelium	110	Unknown, is lysosomal membrane protein translocated to the cell surface after activation	Lysosomal associated membrane protein-1 (LAMP-1)	
CD107b	Activated platelets, activated T cells, activated neutrophils, activated endothelium	120	Unknown, is lysosomal membrane protein translocated to the cell surface after activation	LAMP-2	
CD108	Erythrocytes, circulating lymphocytes, lymphoblasts	80	Unknown	GR2, John Milton-Hagen blood group antigen	
CD109	Activated T cells, activated platelets, vascular endothelium	170	Unknown	Platelet activation factor, GR56	
CD110	Platelets		MPL, TPO R		
CD111	Myeloid cells		PPR1/Nectin1		
CD112	Myeloid cells		PRR2		
CD114	Granulocytes, monocytes	150	Granulocytes colony-stimulating factor (G-CSF) receptor		Immunoglobulin, fibronectin type III

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD115	Monocytes, macrophages	150	Macrophage colony-stimulating factor (M-CSF) receptor	M-CSFR, c-fms	Immunoglobulin, tyrosine kinase
CD116	Monocytes, neutrophils, eosinophils, endothelium	70–85	Granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor α chain	GM-CSFRα	Cytokine receptor, fibronectin type III
CD117	Hematopoietic progenitors	145	Stem-cell factor (SCF) receptor	c-Kit	Immunoglobulin, tyrosine kinase
CD118	Broad cellular expression		Interferon-α, β receptor	IFN-α, βR	
CD119	Macrophages, monocytes, B cells, endothelium	90–100	Interferon-γ receptor	IFN-γR	Fibronectin type III
CD120a	Hematopoietic and nonhematopoietic cells, highest on epithelial cells	50–60	TNF receptor, binds both TNF-α and TNF-β	TNFR-I	TNF receptor
CD120b	Hematopoietic and nonhematopoietic cells, highest on myeloid cells	75–85	TNF receptor, binds both TNF-α and TNF-β	TNFR-II	TNF receptor
CD121a	Thymocytes, T cells	80	Type I interleukin-1 receptor, binds IL-1α and IL-1β	IL-1R type I	Immunoglobulin
CDw121b	B cells, macrophages, monocytes	60–70	Type II interleukin-1 receptor, binds IL-1α and IL-1β	IL-1R type II	Immunoglobulin
CD122	NK cells, resting T-cell subsets, some B-cell lines	75	IL-2 receptor β chain	IL-2Rβ	Cytokine receptor, fibronectin type III
CD123	Bone marrow stem cells, granulocytes, monocytes, megakaryocytes	70	IL-3 receptor α chain	IL-3Rα	Cytokine receptor, fibronectin type III
CD124	Mature B and T cells, hematopoietic precursor cells	130–150	IL-4 receptor	IL-4R	Cytokine receptor, fibronectin type III
CD125	Eosinophils, basophils, activated B cells	55–60	IL-5 receptor	IL-5R	Cytokine receptor, fibronectin type III
CD126	Activated B cells and plasma cells (strong), most leukocytes (weak)	80	IL-6 receptor α subunit	IL-6Rα	Immunoglobulin, cytokine receptor, fibronectin type III
CD127	Bone marrow lymphoid precursors, pro-B cells, mature T cells, monocytes	68–79, possibly forms homodimers	IL-7 receptor	IL-7R	Fibronectin type III
CDw128	Neutrophils, basophils, T-cell subsets	58–67	IL-8 receptor	IL-8R	G protein-coupled receptor
CD129	Not yet assigned				
CD130	Most cell types, strong on activated B cells and plasma cells	130	Common subunit of IL-6, IL-11, oncostatin-M (OSM) and leukemia inhibitory factor (LIF) receptors	IL-6Rβ, IL-11Rβ, OSMRβ, LIFRβ, IFRβ	Immunoglobulin, cytokine receptor, fibronectin type III
CDw131	Myeloid progenitors, granulocytes	140	Common β subunit of IL-3, IL-5, and GM-CSF receptors	IL-3Rβ, IL-5Rβ, GM-CSFRβ	Cytokine receptor, fibronectin type III
CD132	B cells, T cells, NK cells, mast cells, neutrophils	64	IL-2 receptor γ chain, common subunit of IL-2, IL-4, IL-7, IL-9, and IL-15 receptors		Cytokine receptor
CD133	Stem/progenitor cells		AC133		
CD134	Activated T cells	50	May act as adhesion molecule costimulator	OX40	TNF receptor
CD135	Multipotential precursors, myelomonocytic and B-cell progenitors	130, 155	Growth factor receptor	FLK2, STK-1	Immunoglobulin, tyrosine kinase
CDw136	Monocytes, epithelial cells, central and peripheral nervous system	180	Chemotaxis, phagocytosis, cell growth, and differentiation	MSP-R, RON	Tyrosine kinase

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CDw137	T and B lymphocytes, monocytes, some epithelial cells		Co-stimulator of T-cell proliferation	ILA (induced by lymphocyte activation), 4-1BB	TNF receptor
CD138	B cells		Heparan sulphate proteoglycan binds collagen type I	Syndecan-1	
CD139	B cells	209, 228	Unknown		
CD140a,b	Stromal cells, some endothelial cells	a: 180 b: 180	Platelet derived growth factor (PDGF) receptor α and β chains		
CD141	Vascular endothelial cells	105	Anticoagulant, binds thrombin, the complex then activates protein C	Thrombomodulin fetomodulin	C-type lectin, EGF
CD142	Epidermal keratinocytes, various epithelial cells, astrocytes, Schwann cells. Absent from cells in direct contact with plasma unless induced by inflammatory mediators	45–47	Major initiating factor of clotting. Binds Factor VIIa; this complex activates Factors VII, IX, and X	Tissue factor, thromboplastin	Fibronectin type III
CD143	Endothelial cells, except large blood vessels and kidney, epithelial cells of brush borders of kidney and small intestine, neuronal cells, activated macrophages and some T cells. Soluble form in plasma	170–180	Zn^{2+} metallopeptidase dipeptidyl peptidase, cleaves angiotensin I and bradykinin from precursor forms	Angiotensin converting enzyme (ACE)	
CD144	Endothelial cells	130	Organizes adherens junction in endothelial cells	Cadherin-5, VE-cadherin	Cadherin
CD145	Endothelial cells, some stromal cells	25, 90, 110	Unknown		
CD146	Endothelium	130	Potential adhesion molecule, localized at cell–cell junctions	MCAM, MUC18, S-ENDO	Immunoglobulin
CD147	Leukocytes, red blood cells, platelets, endothelial cells	55–65	Potential adhesion molecule	M6, neurothelin, EMMPRIN, basigin, OX-47	Immunoglobulin
CD148	Granulocytes, monocytes, dendritic cells, T cells, fibroblasts, nerve cells	240–260	Contact inhibition of cell growth	HPTPη	Fibronectin type III, protein tyrosine phosphatase
CD150	Thymocytes, activated lymphocytes	75–95	Unknown	SLAM	Immunoglobulin
CD151	Platelets, megakaryocytes, epithelial cells, endothelial cells	32	Associates with β1 integrins	PETA-3, SFA-1	Transmembrane 4
CD152	Activated T cells	33	Receptor for B7.1 (CD80), B7.2 (CD86); negative regulator of T-cell activation	CTLA-4	Immunoglobulin
CD153	Activated T cells, activated macrophages, neutrophils, B cells	38–40	Ligand for CD30, may co-stimulate T cells	CD30L	TNF
CD154	Activated CD4 T cells	30 trimer	Ligand for CD40, inducer of B cell proliferation and activation	CD40L, TRAP, T-BAM, gp39	TNF receptor
CD155	Monocytes, macrophages, thymocytes, CNS neurons	80–90	Normal function unknown; receptor for poliovirus	Poliovirus receptor	Immunoglobulin
CD156a	Neutrophils, monocytes	69	Unknown, may be involved in integrin leukocyte extravasation	MS2, ADAM 8 (A disintegrin and metallo-protease)	
CD156b			TACE/ADAM17. Adhesion structures		
CD157	Granulocytes, monocytes, bone marrow stromal cells, vascular endothelial cells, follicular dendritic cells	42–45 (50 on monocytes)	ADP-ribosyl cyclase, cyclic ADP-ribose hydrolase	BST-1	
CD158	NK cells		KIR family		

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD158a	NK-cell subsets	50 or 58	Inhibits NK cell cytotoxicity on binding MHC class I molecules	p50.1, p58.1	Immunoglobulin
CD158b	NK-cell subsets	50 or 58	Inhibits NK cell cytotoxicity on binding HLA-Cw3 and related alleles	p50.2, p58.2	Immunoglobulin
CD159a	NK cells		Binds CD94 to form NK receptor; inhibits NK cell cytotoxicity on binding MHC class I molecules	NKG2A	
CD160	T cells			BY55	
CD161	NK cells, T cells	44	Regulates NK cytotoxicity	NKRP1	C-type lectin
CD162	Neutrophils, lymphocytes, monocytes	120 homodimer	Ligand for CD62P	PSGL-1	Mucin
CD162R	NK cells			PEN5	
CD163	Monocytes, macrophages	130	Unknown	M130	
CD164	Epithelial cells, monocytes, bone marrow stromal cells	80	Unknown	MUC-24 (multi-glycosylated protein 24)	Mucin
CD165	Thymocytes, thymic epithelial cells, CNS neurons, pancreatic islets, Bowman's capsule	37	Adhesion between thymocytes and thymic epithelium	Gp37, AD2	
CD166	Activated T cells, thymic epithelium, fibroblasts, neurons	100–105	Ligand for CD6, involved integrin neurite extension	ALCAM, BEN, DM-GRASP, SC-1	Immunoglobulin
CD167a	Normal and transformed epithelial cells	63, 64 dimer	Binds collagen	DDR1, trkE, cak, eddr1	Receptor tyrosine kinase, discoidin-related
CD168	Breast cancer cells	Five isoforms: 58, 60, 64, 70, 84	Adhesion molecule. Receptor for hyaluronic acid-mediated motility—mediated cell migration	RHAMM	
CD169	Subsets of macrophages	185	Adhesion molecule. Binds sialylated carbohydrates. May mediate macrophage binding to granulocytes and lymphocytes	Sialoadhesin	Immunoglobulin superfamily, sialoadhesin family
CD170	Neutrophils	67 homodimer	Adhesion molecule. Sialic acid-binding Ig-like lectin (Siglec). Cytoplasmic tail contains ITIM motifs	Siglec-5, OBBP2, CD33L2	Immunoglobulin superfamily, sialoadhesin family
CD171	Neurons, Schwann cells, lymphoid and myelomonocytic cells, B cells, CD4 T cells (not CD8 T cells)	200–220, exact MW varies with cell type	Adhesion molecule, binds CD9, CD24, CD56, also homophilic binding	L1, NCAM-L1	Immunoglobulin superfamily
CD172a		115–120	Adhesion molecule; the transmembrane protein is a substrate of activated receptor tyrosine kinases (RTKs) and binds to SH2 domains	SIRP, SHPS1, MYD-1, SIRP-α-1, protein tyrosine phosphatase, non-receptor type substrate 1 (PTPNS1)	Immunoglobulin superfamily
CD173	All cells		Blood group H type 2. Carbohydrate moiety		
CD174	All cells		Lewis y Blood group. Carbohydrate moiety		
CD175	All cells		Tn Blood group. Carbohydrate moiety		
CD175s	All cells		Sialyl-Tn Blood group. Carbohydrate moiety		
CD176	All cells		TF Blood group. Carbohydrate moiety		

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD177	Myeloid cells	56–64	NB1 is a GPI-linked neutrophil-specific antigen, found on only a subpopulation of neutrophils present in NB1-positive adults (97% of healthy donors) NB1 is first expressed at the myelocyte stage of myeloid differentiation	NB1	
CD178	Activated T cells	38–42	Fas ligand; binds to Fas to induce apoptosis	FasL	TNF superfamily
CD179a	Early B cells	16–18	Immunoglobulin iota chain associates non-covalently with CD179b to form a surrogate light chain which is a component of the pre-B-cell receptor that plays a critical role in early B-cell differentiation	VpreB, IGVPB, IGι	Immunoglobulin superfamily
CD179b	B cells	22	Immunoglobulin λ-like polypeptide 1 associates noncovalently with CD179a to form a surrogate light chain that is selectively expressed at the early stages of B-cell development. Mutations in the CD179b gene have been shown to result in impairment of B-cell development and agammaglobulinemia in humans	IGLL1, λ5 (IGL5), IGVPB, 14.	Immunoglobulin-superfamily
CD180	B cells	95–105	Type 1 membrane protein consisting of extracellular leucine-rich repeats (LRR). Is associated with a molecule called MD-1 and forms the cell-surface receptor complex, RP105/MD-1, which by working in concert with TLR4, controls B-cell recognition and signaling of lipopolysaccharide (LPS)	LY64, RP105	Toll-like receptors (TLR)
CD183	Particularly on malignant B cells from chronic lymphoproliferative disorders	46–52	CXC chemokine receptor involved in chemotaxis of malignant B lymphocytes. Binds INP10 and MIG[3]	CXCR3, G protein-coupled receptor 9 (GPR 9)	Chemokine receptors, G protein-coupled receptor superfamily
CD184	Preferentially expressed on the more immature CD34+ haematopoietic stem cells	46–52	Binding to SDF-1 (LESTR/fusin); acts as a cofactor for fusion and entry of T-cell line; trophic strains of HIV-1	CXCR4, NPY3R, LESTR, fusin, HM89	Chemokine receptors, G protein-coupled receptor superfamily
CD195	Promyelocytic cells	40	Receptor for a CC type chemokine. Binds to MIP-1α, MIP-1β and RANTES. May play a role in the control of granulocytic lineage proliferation or differentiation. Acts as co-receptor with CD4 for primary macrophage-tropic isolates of HIV-1	CMKBR5, CCR5, CKR-5, CC-CKR-5, CKR5	Chemokine receptors, G protein-coupled receptor superfamily
CDw197	Activated B and T lymphocytes, strongly upregulated in B cells infected with EBV and T cells infected with HHV6 or 7	46–52	Receptor for the MIP-3β chemokine; probable mediator of EBV effects on B lymphocytes or of normal lymphocyte functions	CCR7. EBI1 (Epstein–Barr virus induced gene 1), CMKBR7, BLR2	Chemokine receptors, G protein-coupled receptor superfamily
CD200	Normal brain and B-cell lines	41 (rat thymocytes) 47 (rat brain)	Antigen identified by MoAb MRC OX-2. Nonlineage molecules. Function unknown	MOX-2, MOX-1	Immunoglobulin superfamily
CD201	Endothelial cells	49	Endothelial cell-surface receptor (EPCR) that is capable of high-affinity binding of protein C and activated protein C. It is downregulated by exposure of endothelium to tumor necrosis factor	EPCR	CD1major histocompatibility complex family
CD202b	Endothelial cells	140	Receptor tyrosine kinase, binds angiopoietin-1; important in angiogenesis, particularly for vascular network formation in endothelial cells. Defects in TEK are associated with inherited venous malformations; the TEK signaling pathway appears to be critical for endothelial cell–smooth muscle cell communication in venous morphogenesis	VMCM, TEK (tyrosine kinase, endothelial), TIE2 (tyrosine kinase with Ig and EGF homology domains), VMCM1	Immunoglobulin superfamily, tyrosine kinase

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD203c	Myeloid cells (uterus, basophils, and mast cells)	101	Belongs to a series of ectoenzymes that are involved in hydrolysis of extracellular nucleotides. They catalyze the cleavage of phosphodiester and phosphosulfate bonds of a variety of molecules, including deoxynucleotides, NAD, and nucleotide sugars	NPP3, B10, PDNP3, PD-Iβ, gp130RB13-6	Type II transmembrane proteins, Ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) family
CD204	Myeloid cells	220	Mediate the binding, internalization, and processing of a wide range of negatively charged macromolecules. Implicated in the pathologic deposition of cholesterol in arterial walls during atherogenesis	Macrophage scavenger R (MSR1)	Scavenger receptor family, collagen-like
CD205	Dendritic cells	205	Lymphocyte antigen 75; putative antigen-uptake receptor on dendritic cells	LY75, DEC-205, GP200-MR6	Type I transmembrane protein
CD206	Macrophages, endothelial cells	175–190	Type I membrane glycoprotein; only known example of a C-type lectin that contains multiple C-type CRDs (carbohydrate-recognition domains); it binds high-mannose structures on the surface of potentially pathogenic viruses, bacteria, and fungi	Macrophage mannose receptor (MMR), MRC1	C-type lectin superfamily
CD207	Langerhans cells	40	Type II transmembrane protein; Langerhans cell specific C-type lectin; potent inducer of membrane superimposition and zippering leading to BG (Birbeck granules) formation	Langerin	C-type lectin superfamily
CD208	Interdigitating dendritic cells in lymphoid organs	70–90	Homologous to CD68, DC-LAMP is a lysosomal protein involved in remodeling of specialized antigen-processing compartments and in MHC class II-restricted antigen presentation. Upregulated in mature DCs induced by CD40L, TNF-α and LPS.	D lysosome-associated membrane protein, DC-LAMP	Major histocompatibility complex family
CD209	Dendritic cells	44	C-type lectin; binds ICAM3 and HIV-1 envelope glycoprotein gp120 enables T-cell receptor engagement by stabilization of the DC/T-cell contact zone, promotes efficient infection in *trans* cells that express CD4 and chemokine receptors; type II transmembrane protein	DC-SIGN (dendritic cell-specific ICAM3-grabbing non-integrin)	C-type lectin superfamily
CDw210	B cells, T helper cells, and cells of the monocyte/macrophage lineage	90–110	Interleukin 10 receptor α and β	IL-10Rα, IL-10RA, HIL-10R, IL-10Rβ, IL-10RB, CRF2-4, CRFB4	Class II cytokine receptor family
CD212	Activated CD4, CD8, and NK cells	130	IL-12 receptor β chain; a type I transmembrane protein involved in IL-12 signal transduction.	IL-12R IL-12RB	Haemopoietin cytokine receptor superfamily
CD213a1	B cells, monocytes, fibroblasts, endothelial cells	60–70	Receptor which binds Il-13 with a low affinity; together with IL-4Rα can form a functional receptor for IL-13, also serves as an alternate accessory protein to the common cytokine receptor gamma chain for IL-4 signaling	IL-13Rα 1, NR4, IL-13Ra	Haemopoietic cytokine receptor superfamily
CD213a2	B cells, monocytes, fibroblasts, endothelial cells		IL-13 receptor which binds as a monomer with high affinity to interleukin-13 (IL-13), but not to IL-4; human cells expressing IL-13RA2 show specific IL-13 binding with high affinity	IL-13Rα 2, IL-13BP	Haemopoietic cytokine receptor superfamily
CDw217	Activated memory T cells	120	Interleukin 17 receptor homodimer	IL-17R, CTLA-8	Chemokine/ cytokine receptors

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD220	Nonlineage molecules	α: 130 β: 95	Insulin receptor; integral transmembrane glycoprotein comprised of two α and two β subunits; this receptor binds insulin and has a tyrosine-protein kinase activity—autophosphorylation activates the kinase activity	Insulin receptor	Insulin receptor family of tyrosine-protein kinases, EGFR family
CD221	Nonlineage molecules	α: 135 β: 90	Insulin-like growth factor I receptor binds insulin-like growth factor with a high affinity. It has tyrosine kinase activity and plays a critical role in transformation events. Cleavage of the precursor generates α and β subunits.	IGF1R, JTK13	Insulin receptor family of tyrosine-protein kinases, EGFR family
CD222	Nonlineage molecules	250	Ubiquitously expressed multifunctional type I transmembrane protein. Its main functions include internalization of IGF-II, internalization or sorting of lysosomal enzymes and other M6P-containing proteins	IGF2R, CIMPR, CI-MPR, IGF2R, M6P-R (Mannose-6-phosphate receptor)	Mammalian lectins
CD223	Activated T and NK cells	70	Involved in lymphocyte activation; binds to HLA class-II antigens; role in down-regulating antigen specific response; close relationship of LAG3 to CD4	Lymphocyte-activation gene 3 LAG-3	Immunoglobulin superfamily
CD224	Nonlineage molecules	62 (unprocessed precursor)	Predominantly a membrane-bound enzyme; plays a key role in the γ-glutamyl cycle, a pathway for the synthesis and degradation of glutathione. This enzyme consists of two polypeptide chains, which are synthesized in precursor form from a single polypeptide	γ-glutamyl transferase, GGT1, D22S672 D22S732	γ-glutamyl transferase protein family
CD225	Leukocytes and endothelial cells	16–17	Interferon-induced transmembrane protein 1 is implicated in the control of cell growth. It is a component of a multimeric complex involved in the transduction of antiproliferative and homotypic adhesion signals	Leu 13, IFITM1, IFI17	IFN-induced transmembrane proteins
CD226	NK cells, platelets, monocytes, and a subset of T cells	65	Adhesion glycoprotein; mediates cellular adhesion to other cells bearing an unidentified ligand and cross-linking CD226 with antibodies causes cellular activation	DNAM-1 (PTA1), DNAX, TLiSA1	Immunoglobulin superfamily
CD227	Human epithelial tumors, such as breast cancer	122 (non-glycosylated)	Epithelial mucin containing a variable number of repeats with a length of twenty amino acids, resulting in many different alleles. Direct or indirect interaction with actin cytoskeleton.	PUM (peanut-reactive urinary mucin), MUC.1, mucin 1	Mucin
CD228	Predominantly in human melanomas	97	Tumor-associated antigen (melanoma) identified by monoclonal antibodies 133.2 and 96.5,involved in cellular iron uptake.	Melanotransferrin, P97	Transferrin superfamily
CD229	Lymphocytes	90–120	May participate in adhesion reactions between T lymphocytes and accessory cells by homophilic interaction	Ly9	Immunoglobulin superfamily (CD2 subfamily)
CD230	Expressed both in normal and infected cells	27–30	The function of PRP is not known. It is encoded in the host genome found in high quantity in the brain of humans and animals infected with neurodegenerative diseases known as transmissible spongiform encephalopathies or prion diseases (Creutzfeld–Jakob disease, Gerstmann–Strausler–Scheinker syndrome, fatal familial insomnia)	CJD, PRIP, prion protein (p27-30)	Prion family

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD231	T-cell acute lymphoblastic leukemia, neuroblastoma cells and normal brain neuron	150	The function of CD231 is currently unknown. It is cell-surface glycoprotein which is a specific marker for T-cell acute lymphoblastic leukemia. Also found on neuroblastomas	TALLA-1, TM4SF2, A15, MXS1, CCG-B7	Transmembrane 4 superfamily (TM4SF also known as tetraspanins)
CD232	Nonlineage molecules	200	Receptor for an immunologically active semaphorin (virus-encoded semaphorin protein receptor)	VESPR, PLXN, PLXN-C1	Plexin family
CD233	Erythroid cells	93	Band 3 is the major integral glycoprotein of the erythrocyte membrane. It has two functional domains. Its integral domain mediates a 1:1 exchange of inorganic anions across the membrane, whereas its cytoplasmic domain provides binding sites for cytoskeletal proteins, glycolytic enzymes, and hemoglobin. Multifunctional transport protein	SLC4A1, Diego blood group, D1, AE1, EPB3	Anion exchanger family
CD234	Erythroid cells and nonerythroid cells	35	Fy-glycoprotein; Duffy blood group antigen; nonspecific receptor for many chemokines such as IL-8, GRO, RANTES, MCP-1 and TARC. It is also the receptor for the human malaria parasites *Plasmodium vivax* and *Plasmodium knowlesi* plays a role in inflammation and in malaria infection	GPD, CCBP1, DARC (duffy antigen/receptor for chemokines)	Family 1 of G protein-coupled receptors, chemokine receptors superfamily
CD235a	Erythroid cells	31	Major carbohydrate rich sialoglycoprotein of human erythrocyte membrane which bears the antigenic determinants for the MN and Ss blood groups. The N-terminal glycosylated segment, which lies outside the erythrocyte membrane, has MN blood group receptors and also binds influenza virus	Glycophorin A, GPA, MNS	Glycophorin A family
CD235b	Erythroid cells	GYPD is smaller than GYPC (24 kD vs 32 kD)	This protein is a minor sialoglycoprotein in human erythrocyte membranes. Along with GYPA, GYPB is responsible for the MNS blood group system. The Ss blood group antigens are located on glycophorin B	Glycophorin B, MNS, GPB	Glycophorin A family
CD236	Erythroid cells	24	Glycophorin C (GPC) and glycophorin D (GPD) are closely related sialoglycoproteins in the human red blood cell (RBC) membrane. GPD is a ubiquitous shortened isoform of GPC, produced by alternative splicing of the same gene. The Webb and Duch antigens, also known as glycophorin D, result from single point mutations of the glycophorin C gene	Glycophorin D, GPD, GYPD	Type III membrane proteins
CD236R	Erythroid cells	32	Glycophorin C (GPC) is associated with the Gerbich (Ge) blood group deficiency. It is a minor red cell-membrane component, representing about 4% of the membrane sialoglycoproteins, but shows very little homology with the major red cell-membrane glycophorins A and B. It plays an important role in regulating the mechanical stability of red cells and is a putative receptor for the merozoites of *Plasmodium falciparum*	Glycophorin C, GYPC, GPC	Type III membrane proteins
CD238	Erythroid cells	93	KELL blood group antigen; homology to a family of zinc metalloglycoproteins with neutral endopeptidase activity, type II transmembrane glycoprotein	KELL	Belongs to peptidase family m13 (zinc metalloprotease); also known as the neprilysin subfamily

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD239	Erythroid cells	78	A type I membrane protein.The human F8/G253 antigen, B-CAM, is a cell surface glycoprotein that is expressed with restricted distribution pattern in normal fetal and adult tissues, and is upregulated following malignant transformation in some cell types. Its overall structure is similar to that of the human tumor marker MUC 18 and the chicken neural adhesion molecule SC1	B-CAM (B-cell adhesion molecule), LU, Lutheran blood group	Immunoglobulin superfamily
CD240CE	Erythroid cells	45.5	Rhesus blood group, CcEe antigens. May be part of an oligomeric complex which is likely to have a transport or channel function in the erythrocyte membrane. It is highly hydrophobic and deeply buried within the phospholipid bilayer	RHCE, RH30A, RHPI, Rh4	Rh family
CD240D	Erythroid cells	45.5 (product—30)	Rhesus blood group, D antigen. May be part of an oligomeric complex which is likely to have a transport or channel function in the erythrocyte membrane. Absent in the Caucasian RHD-negative phenotype	RhD, Rh4, RhPI, RhII, Rh30D	Rh family
CD241	Erythroid cells	50	Rhesus blood group-associated glycoprotein RH50, component of the RH antigen multisubunit complex; required for transport and assembly of the Rh membrane complex to the red blood cell surface. Highly homologous to RH, 30kD components. Defects in RhAg are a cause of a form of chronic hemolytic anemia associated with stomatocytosis, and spherocytosis, reduced osmotic fragility, and increased cation permeability	RhAg, RH50A	Rh family
CD242	Erythroid cells	42	Intercellular adhesion molecule 4, Landsteiner-Wiener blood group. LW molecules may contribute to the vaso-occlusive events associated with episodes of acute pain in sickle cell disease	ICAM-4, LW	Immunoglobulin superfamily, intercellular adhesion molecules (ICAMs)
CD243	Stem/progenitor cells	170	Multidrug resistance protein 1 (P-glycoprotein). P-gp has been shown to utilise ATP to pump hydrophobic drugs out of cells, thus increasing their intracellular concentration and hence their toxicity. The MDR 1 gene is amplified in multidrug resistant cell lines	MDR-1, p-170	ABC superfamily of ATP-binding transport proteins
CD244	NK cells	66	2B4 is a cell-surface glycoprotein related to CD2 and implicated in the regulation of natural killer and T-lymphocyte function. It appears that the primary function of 2B4 is to modulate other receptor–ligand interactions to enhance leukocyte activation	2B4, NK cell activation inducing ligand (NAIL)	Immunoglobulin superfamily
CD245	T cells	220–240	Cyclin E/Cdk2 interacting protein p220. NPAT is involved in a key S phase event and links cyclical cyclin E/Cdk2 kinase activity to replication-dependent histone gene transcription. NPAT gene may be essential for cell maintenance and may be a member of the housekeeping genes	NPAT	

CD antigen	Cellular expression	Molecular weight (kDa)	Functions	Other names	Family relationships
CD246	Expressed in the small intestine, testis, and brain but not in normal lymphoid cells	177 kDa; after glycosylation, produces a 200 kDa mature glycoprotein	Anaplastic (CD30+ large cell) lymphoma kinase; plays an important role in brain development, involved in anaplastic nodal non Hodgkin lymphoma or Hodgkin's disease with translocation t(2;5)(p23;q35) or inv2(23;q35). Oncogenesis via the kinase function is activated by oligomerization of NPM1-ALK mediated by the NPM1 part	ALK	Insulin receptor family of tyrosine-protein kinases
CD247	T cells, NK cells	16	T-cell receptor ζ; has a probable role in assembly and expression of the TCR complex as well as signal transduction upon antigen triggering. TCRζ together with TCRα:β and γ:δ heterodimers and CD3-γ, -δ, and -ε, forms the TCR-CD3 complex. The ζ chain plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways. Low expression of the antigen results in impaired immune response	ζ chain, CD3Z	Immunoglobulin superfamily

Compiled by Laura Herbert, Royal Free Hospital, London. Data based on CD designations made at the 7th Workshop on Human Leucocyte Differentiation Antigens, provided by Protein Reviews on the Web (www.ncbi.nlm.nih.gov/prow/).