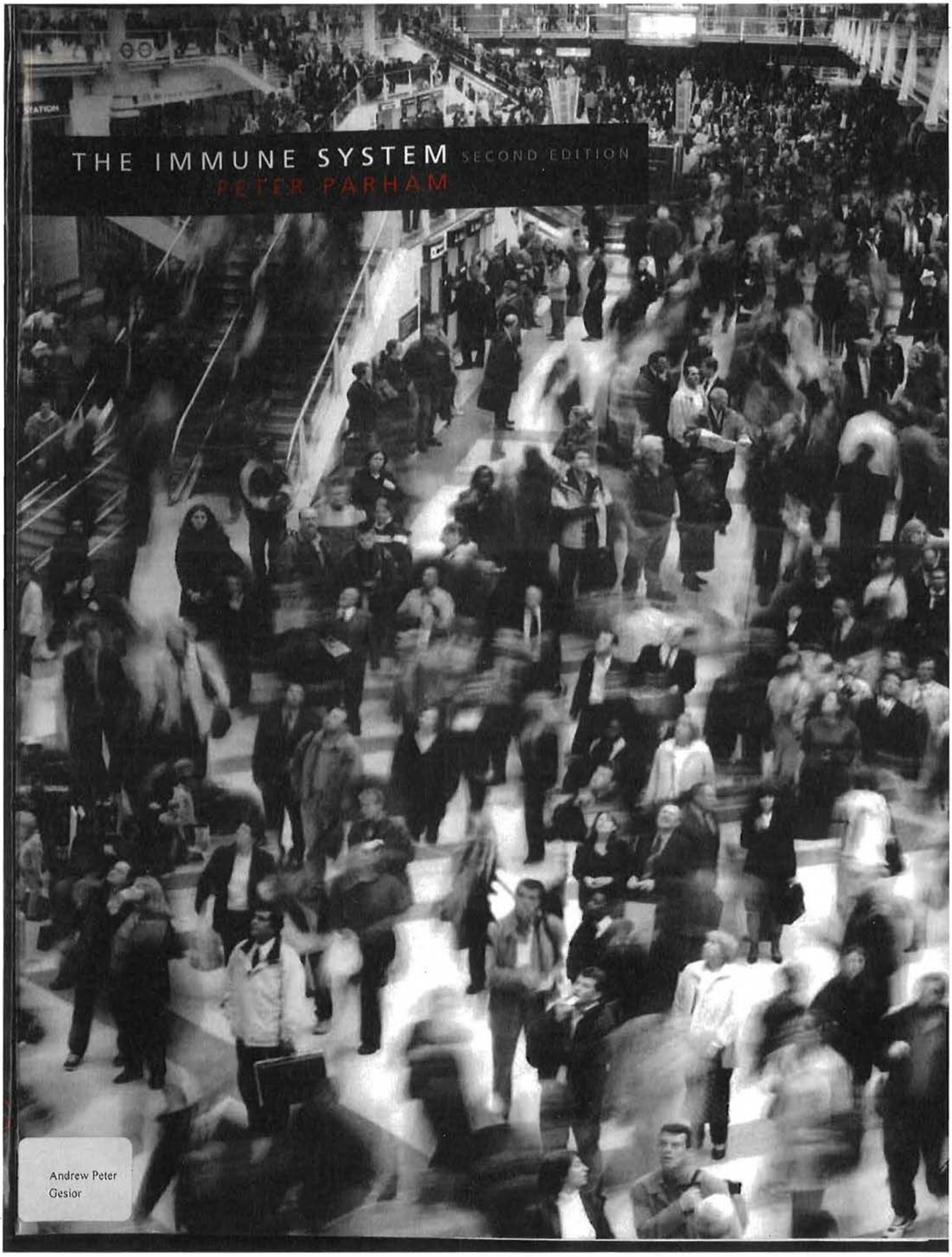


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THE IMMUNE SYSTEM SECOND EDITION

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Preface

In the twentieth century immunology was often taught as a form of epic saga, a progression of elegant experiments, breakthrough discoveries, and intrepid investigators. The merit of the approach was its simplicity, as in tracing history using the dates of monarchs, ministers, conflicts, and conquests. It also added spice and personality in an era when knowledge was hard won, incomplete and only tenuously related to the human condition. That situation has clearly changed, the direct consequence of technical innovations implemented during the latter part of the twentieth century. Today new information literally pours from the research pipeline, onto the printed page and into the World Wide Web. Elegant experiments, breakthrough discoveries, and intrepid investigators are now in such abundance that they in themselves can no longer provide a simplifying principle for explaining immunology. However, as a result of all this industry immunologists now have a more coherent and intellectually more satisfying picture of the immune system and how it works, not always successfully, to protect the body from infection.

This book is aimed at students who are coming to immunology for the first time. Throughout the book the emphasis is upon the human immune system and how its successes, failures, and compromises affect the lives of each and every one of us. The first eight chapters of the book describe the cells and molecules of the immune system and how they work together in providing defenses against invading microorganisms. This part of the book is built around the biology of the B and T lymphocytes, the cells that adapt to infection and provide long-lasting protective immunity, but also emphasizes the importance of other cells—macrophages, dendritic cells, granulocytes, mast cells, and natural killer cells—that provide both the front-line defenses of innate immunity and the inflammation necessary to stimulate B and T cells. The first chapter introduces the different types of cells that make up the immune system and the functions they perform. Antigen recognition, repertoire development, and effector functions of B and T cells are dealt with in turn in the next six chapters. Completing this part of the book is a chapter describing how innate and adaptive immunity work together to battle common types of infection.

The next three chapters focus upon diseases that arise from inadequacies of the human immune system. The first describes situations where infections fail to be controlled, often because the microorganism actively evades, exploits, or subverts the immune response. The following chapter examines conditions in which the immune system overreacts to innocuous substances in the environment and causes chronic inflammatory diseases such as allergies and asthma. The third of these chapters examines the autoimmune diseases, such as Graves' disease, insulin-dependent diabetes, and rheumatoid arthritis, in which the immune system attacks healthy cells and causes tissue damage and loss of function. The last chapter of the book illustrates how the immune system can be manipulated to improve human health, by consideration of the well-established practices of vaccination and transplantation as well as the emerging field of cancer immunology.

In this second edition of *The Immune System* the changes made to the text and figures of the first edition had two aims. First were changes aimed at improving the clarity and coherence of the material presented in the first edition and stem in large part from the suggestions and criticisms of instructors

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using the book. An almost universal request was for questions that would help students to learn and revise the material. Such questions are now included at the end of each chapter with a full set of answers included at the back of the book. In making revisions one guiding principle was to replace discussion of animal models with examples drawn from human immunology and clinical practice; a second was to include more of the genetics of human immunity. On a more specific issue the text pertaining to the complement system in chapters 7 and 8 has been rewritten and many figures have been redrawn using a modified iconography. The second category of revision embraces advances in knowledge and understanding of the immune system. Amongst the many new sections are ones describing pathogen receptors of innate immune cells, the role of dendritic cells in triggering adaptive immunity, mechanisms that contribute to immunological self-tolerances, and autoimmunity including regulatory T cells and rational approaches to cancer vaccination. As well as adding such new material, the revision has involved the elimination of text or figures that were judged unnecessary or digressive.

The end-of-chapter questions new to this edition, as well as the answers to those questions, were composed by Sheryl L. Fuller-Espie of Cabrini College, Radnor, Pennsylvania. I am grateful for Sherry's contribution to the book. She wrote the first draft of questions, made significant collaborations during the revision process, and was meticulous in editing the final drafts.

I thank and acknowledge the authors of *Immunobiology* and of *Case Studies in Immunology* for giving me license with the text and figures of their books. I am most grateful to the many instructors and their students whose encouragement and criticism prompted this revision of *The Immune System.* In particular, Frances Brodsky made innumerable and ineffable contributions at each stage of the revision. Eleanor Lawrence not only edited all aspects of the manuscript and figures but contributed much more. Her unwavering sympathy for the prospective reader ensured that much jargon, complexity and other nonsense never made it to the printed page. Nigel Orme created many new illustrations for this edition, as well as the image for the front cover photographed by Richard Denyer. Emma Hunt was creative with the layout and assiduous in proofing and production. The orchestration of all these efforts has been most ably and good humoredly done by Mike Morales. I am indebted to Denise Schanck for her consistent support and encouragement of this project. Finally, I would like to thank my sister Joyce and her husband Rowland for providing a welcoming place to stay in London when I have been working there on the book.

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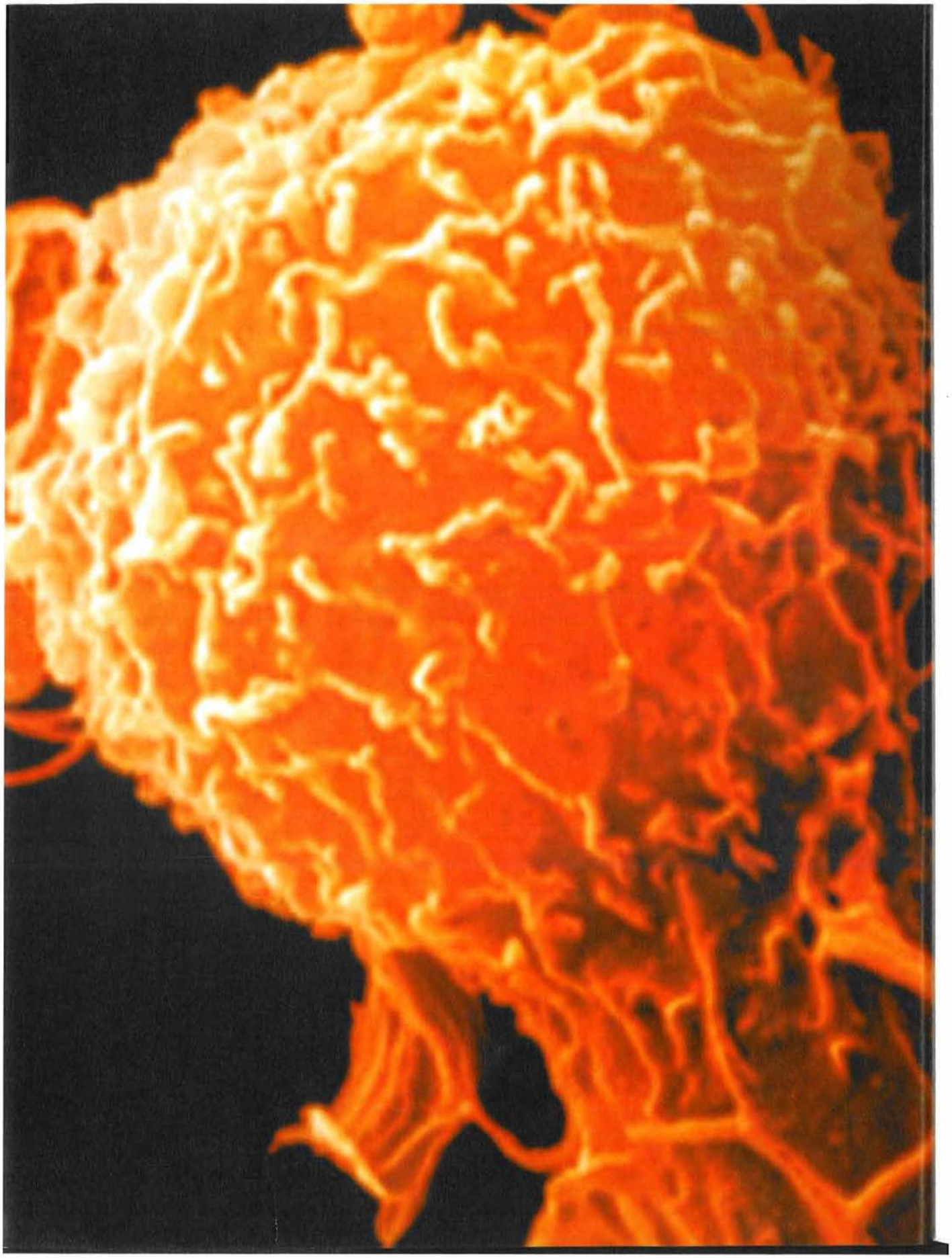
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Chapter 1

Elements of the Immune System and their Roles in Defense



Immunology is the study of the physiological mechanisms that humans and other animals use to defend their bodies from invasion by other organisms. The origins of the subject lie in the practice of medicine and in historical observations that people who survived the ravages of epidemic disease were untouched when faced with that same disease again—they had become **immune** to infection. Infectious diseases are caused by microorganisms, which have the advantage of reproducing and evolving much more rapidly than do their human hosts. During the course of an infection, the microorganism can pit enormous populations of its species against an individual *Homo sapiens*. In response, the human body invests heavily in cells dedicated to defense, which collectively form the **immune system**.

The immune system is crucial to human survival. In the absence of a working immune system, even minor infections can take hold and prove fatal. Without intensive treatment, children born without a functional immune system die in early childhood from the effects of common infections. However, in spite of their immune systems, all humans suffer from infectious diseases, especially when young. This is because the immune system takes time to build up a strong response to an invading microorganism, time during which the invader can multiply and cause disease. To provide **protective immunity** for the future, the immune system must first do battle with the microorganism. This places people at highest risk during their first infection with a microorganism and, in the absence of modern medicine, leads to substantial child mortality, as witnessed in the developing world. When entire populations face a completely new infection, the outcome can be catastrophic, as experienced by indigenous Americans who were decimated by European diseases to which they were suddenly exposed after 1492. Today, infection with human immunodeficiency virus (HIV) and the acquired immune deficiency syndrome (AIDS) it causes are having a similarly tragic impact on the populations of several African countries.

In medicine the greatest triumph of immunology has been **vaccination**, or **immunization**, a procedure whereby severe disease is prevented by prior exposure to the infectious agent in a form that cannot cause disease. Vaccination provides the opportunity for the immune system to gain the experience needed to make a protective response with little risk to health or life. Vaccination was first used against smallpox, a viral scourge that once ravaged populations and disfigured the survivors. In Asia, small amounts of smallpox virus had been used to induce protective immunity for hundreds of years before 1721, when Lady Mary Wortley Montagu introduced the method into

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western Europe. Subsequently, in 1796, Edward Jenner, a doctor in rural England, showed how inoculation with cowpox virus offered protection against the related smallpox virus with less risk than the earlier methods. Jenner called his procedure vaccination, after *vaccinia*, the name given to the mild disease produced by cowpox, and he is generally credited with its invention. Since his time, vaccination dramatically reduced the incidence of smallpox worldwide, with the last cases being seen by physicians in the 1970s (Figure 1.1).

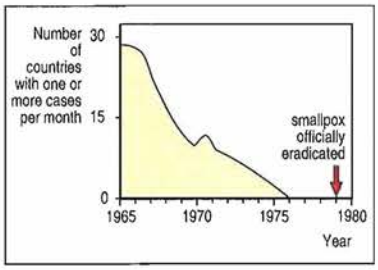


Figure 1.1 The eradication of smallpox by vaccination. Smallpox vaccination was started in 1796. In 1979, after 3 years in which no case of smallpox was recorded, the World Health Organization announced that the virus had been eradicated. Since then the proportion of the human population that has been vaccinated against smallpox, or has acquired immunity from an infection, has steadily decreased. The result is that the human population has become increasingly vulnerable should the virus emerge again, either naturally or as a deliberate act of human malevolence.

Effective vaccines have been made from only a fraction of the agents that cause disease and some are of limited availability because of their cost. Most of the widely used vaccines were first developed many years ago by processes of trial and error, before very much was known about the workings of the immune system. That approach is no longer so successful for making new vaccines, perhaps because all the easily won vaccines have been made. But deeper understanding of the mechanisms of immunity is spawning new ideas for vaccines against infectious diseases and even against other types of disease such as cancer. Much is now known about the molecular and cellular components of the immune system and what they can do in the laboratory. Current research aims at understanding their contributions to fighting infections in the world at large. The new knowledge is also being used to find better ways of manipulating the immune system to prevent the unwanted immune responses that cause allergies, autoimmune diseases, and rejection of organ transplants.

In the first part of this chapter we consider the microorganisms that infect human beings and the defenses they must overcome to start an infection. These include physical barriers, chemical barriers, and the fixed defenses of innate immunity, which are ready and waiting to halt infections before they can barely start. The individual cells and tissues of the immune system will also be described, and how they integrate their functions with the rest of the human body. The second part of the chapter focuses on the more flexible and forceful defenses of adaptive immunity. These mechanisms are only brought into play if and when an infection is established. The adaptive response is always targeted to the specific problem at hand and is made and refined during the course of the infection. When successful, it clears the infection and provides long-lasting immunity that prevents its recurrence.

Defenses facing invading pathogens

The purpose of the vertebrate immune system is to recognize invading foreign organisms, to prevent their spread, and ultimately to clear them from the body. It consists of billions of cells of various types, which interact with the infectious agent and with each other to fight the infection. All cells of the immune system originate in the **bone marrow**, but at some time in their life they leave that site to circulate in the blood, to enter other tissues, and to form part of specialized **lymphoid** tissues. This part of the chapter introduces the cells and tissues of the immune system and some of the ways in which they carry out their functions. But first we shall consider the organisms and infections that the immune system evolved to protect us against.

1-1 Pathogens are infectious organisms that cause disease

Numerous species of microorganism colonize the human body in large numbers and rarely produce symptoms of disease, for example the benign strains of the bacterium *Escherichia coli* that normally inhabit the gastrointestinal tract. However, for some microorganisms, such as the influenza virus or the

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The immune system protects against four classes of pathogen		
Type of pathogen	**Examples**	**Diseases**
Bacteria	*Salmonella enteritidis* *Mycobacterium tuberculosis*	Food poisoning Tuberculosis
Viruses	Variola Influenza HIV	Smallpox Flu AIDS
Fungi	*Epidermophyton floccosum* *Candida albicans*	Ringworm Thrush, systemic candidiasis
Parasites protozoa worms	*Trypanosoma brucei* *Leishmania donovani* *Plasmodium falciparum* *Ascaris lumbricoides* *Schistosoma mansoni*	Sleeping sickness Leishmaniasis Malaria Ascariasis Schistosomiasis

Figure 1.2 The four kinds of pathogen that cause human disease. Examples of the types of pathogen are listed, along with the diseases they cause.

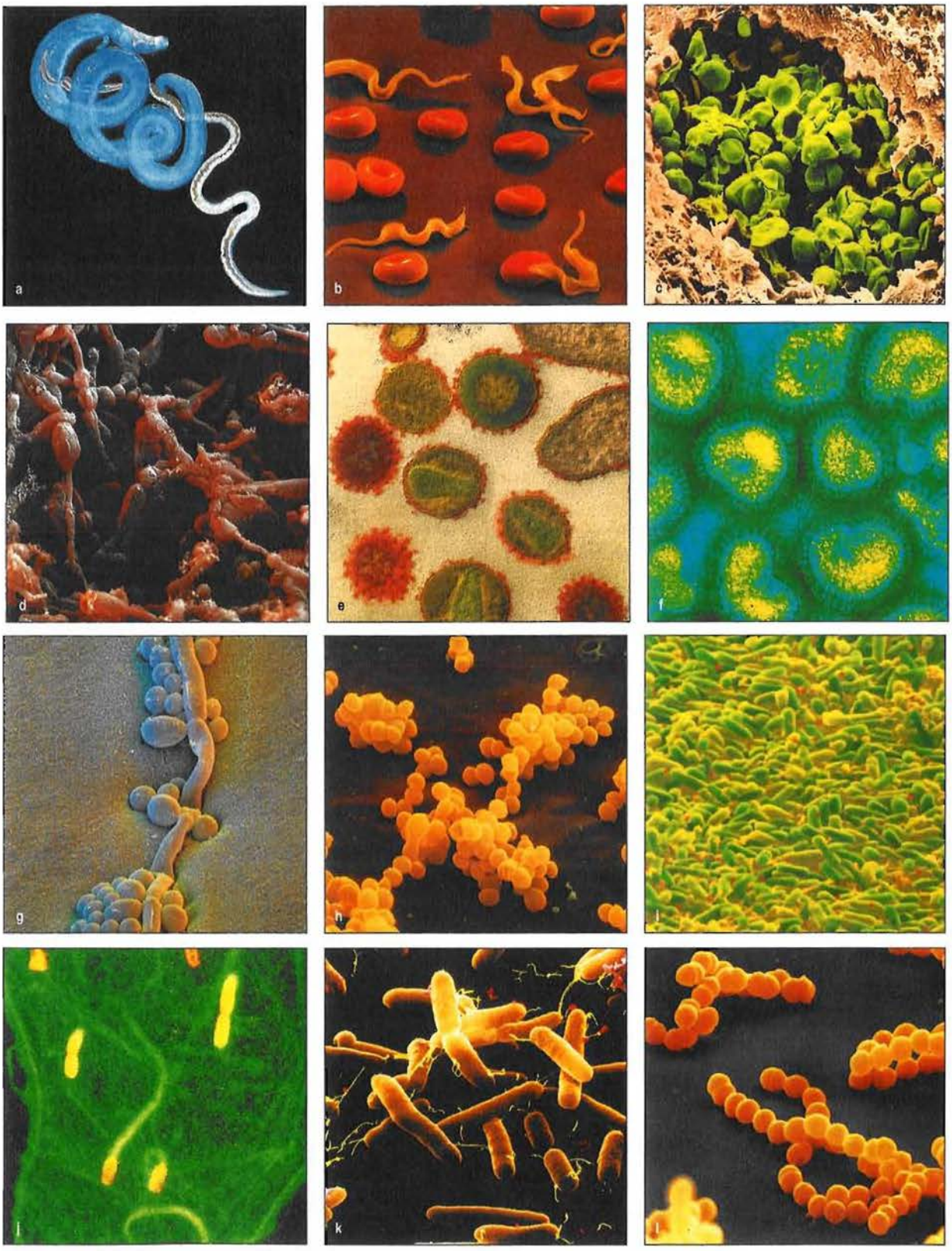
typhoid bacillus, infection habitually causes a disease. Any organism with the potential to cause disease is known as a **pathogen**. This definition includes not only microorganisms that generally cause disease if they enter the body, but also ones that can colonize the human body to no ill effect for much of the time, but cause illness if the body's defenses are weakened or if the microbe gets into the 'wrong' place. The latter kinds of pathogen are known as **opportunistic pathogens**.

Pathogens can be divided into four kinds: **bacteria**, **viruses**, and **fungi**, which are each a group of related microorganisms, and internal **parasites**, a less precise term used to embrace a heterogeneous collection of unicellular protozoa and multicellular invertebrates, mainly worms (Figure 1.2). In this book we consider the functions of the human immune system principally in the context of controlling infections. For some pathogens this necessitates their complete elimination, but for others it is sufficient to limit the size and location of the pathogen population within the human host. Figure 1.3 gives examples of pathogens from the four classes.

Over evolutionary time, the relationship between a pathogen and its human hosts can change, affecting the severity of the disease produced. Most pathogenic organisms have evolved special adaptations that enable them to invade their hosts, replicate in them, and be transmitted. However, the rapid death of its host is rarely in a microbe's interest, because this destroys both its home and its source of food. Consequently, those organisms with the potential to cause severe and rapidly fatal disease often tend to evolve towards an accommodation with their hosts. In complementary fashion, host populations have evolved a degree of in-built genetic resistance to common disease-causing organisms, as well as acquiring lifetime immunity to endemic diseases as a result of infection in childhood. Because of the interplay between host and pathogen, the nature and severity of infectious diseases in the human population are always changing.

Influenza is a good example of a common viral disease that, although severe in its symptoms, is usually overcome successfully by the immune system. The fever, aches, and lassitude that accompany infection can be overwhelming, and it is difficult to imagine overcoming foes or predators at the peak of a bout of influenza. However, despite the severity of the symptoms, most strains of influenza pose no great danger to healthy people in populations where

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Figure 1.3 The diversity of human pathogens. Panel a: light micrograph of *Schistosoma mansoni*, the helminth worm that causes schistosomiasis. The adult intestinal blood fluke form is shown: the male is thick and bluish, the female white and thread-like. Magnification × 5. Panel b: false-color scanning electron micrograph of red blood cells and *Trypanosoma brucei*, a protozoan of the type that causes African sleeping sickness. Magnification × 1750. Panel c: false-color scanning electron micrograph of *Pneumocystis carinii*, the fungus that causes opportunistic infections in patients whose immune system is suppressed by disease or drugs. The view is of a lung alveolus from a monkey who is immunosuppressed because of infection with a simian virus that causes an acquired immune deficiency syndrome (AIDS). The fungal cells have been colored green. Magnification × 720. Panel d: scanning electron micrograph of *Epidermophyton floccosum*, the dermatophyte fungus that causes ringworm. Pear-shaped spore-producing structures (macronidia) are seen connected by filaments (hyphae). Magnification × 500. Panel e: false-color transmission electron micrograph of human immunodeficiency virus (HIV), the cause of AIDS. Magnification × 80,000. Panel f: false-color transmission electron micrograph of influenza virus, an orthomyxovirus that causes influenza. Magnification × 40,000. Panel g: false-color scanning electron micrograph of the fungus *Candida albicans*, a normal inhabitant of the human body that occasionally causes thrush and more severe systemic infections. Pseudohyphae are visible in a row. At the junctions of the hyphae, rounded yeast-like cells (blastospores) are budded off into colonies. Magnification × 1400. Panel h: false-color scanning electron micrograph of *Staphylococcus aureus*, a Gram-positive bacterium that colonizes human skin and is the common cause of pimples and boils. Some strains, however, cause food poisoning. The small spherical cells (cocci) typically form grape-like clusters. Magnification × 5000. Panel i: false-color scanning electron micrograph of *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis. Magnification × 15,000. Panel j: false-color transmission electron micrograph of a human cell (colored green) and bacteria of the species *Listeria monocytogenes*, a Gram-positive coccobacillus that can contaminate processed food, causing disease (listeriosis) in immunocompromised individuals and pregnant women. Magnification × 1250. Panel k: false-color scanning electron micrograph of *Salmonella enteritidis*, a Gram-negative, rod-shaped bacterium that is a common cause of food poisoning. The hair-like flagella enable the bacteria to move. Magnification × 6500. Panel l: false-color scanning electron micrograph of *Streptococcus pyogenes*, a Gram-positive bacterium that is the principal cause of tonsilitis and scarlet fever, and can also cause ear infections. It has rounded or spherical cells that sometimes form chains as seen here. Magnification × 6500.

influenza is endemic. Warm, well-nourished, and otherwise healthy people usually recover in a couple of weeks and take it for granted that their immune system will accomplish this task. Pathogens new to the human population, in contrast, often cause high mortality in those infected—between 60% and 75% in the case of the Ebola virus.

1-2 The skin and mucosal surfaces form physical barriers against infection

The skin is the human body's first defense against infection. It forms a tough impenetrable barrier of epithelium protected by layers of keratinized cells. This barrier can be breached by physical damage, such as wounds, burns, or surgical procedures, which exposes soft tissues and renders them vulnerable to infection. Until the adoption of antiseptic procedures in the nineteenth century, surgery was a very risky business, principally because of the life-threatening infections that the procedures introduced. For the same reason,

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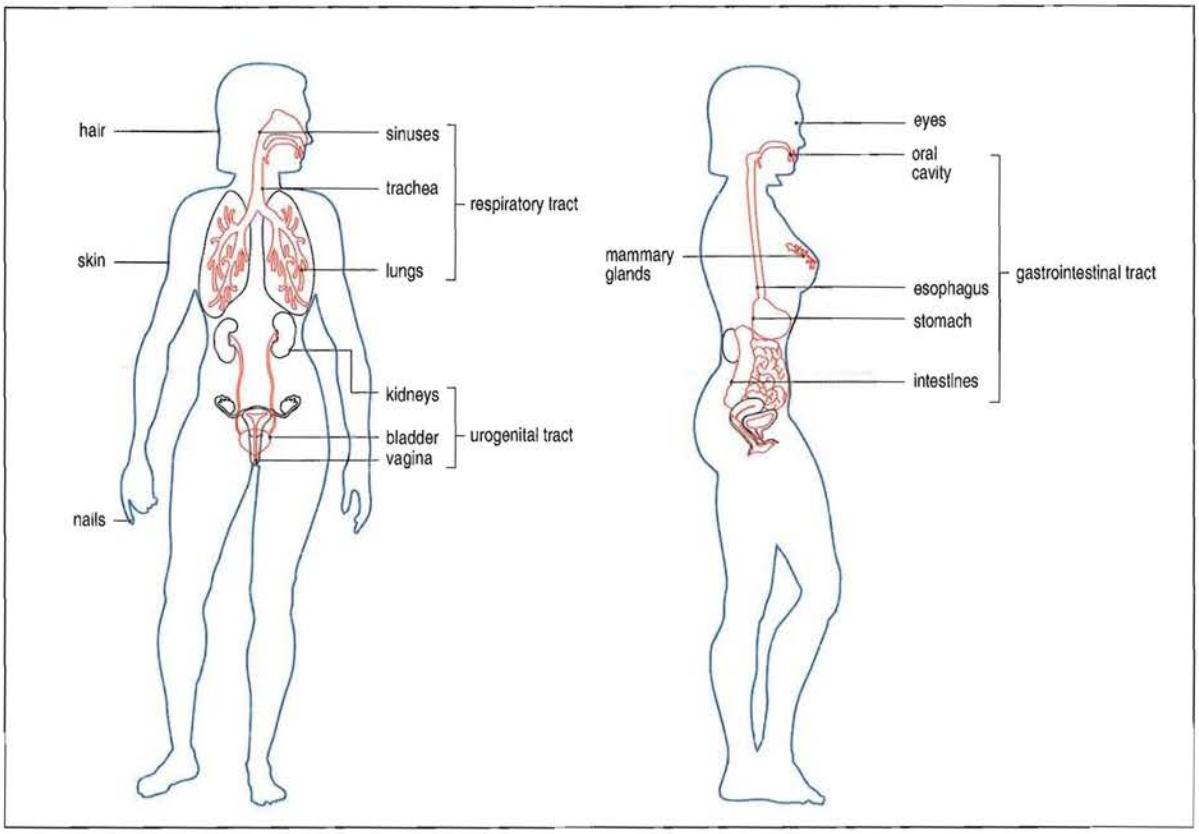


Figure 1.4 The physical barriers that separate the body from its external environment. In these images of a woman, the strong barriers to infection provided by the skin, hair, and nails are colored blue and the more vulnerable mucosal membranes are colored red.

far more soldiers have died from infection acquired on the battlefield than from the direct effects of enemy action. Ironically, the need to conduct increasingly sophisticated and wide-ranging warfare has been the major force driving improvements in surgery and medicine. As an example from immunology, the burns suffered by fighter pilots during World War II stimulated studies on skin transplantation that led directly to the understanding of the cellular basis of the immune response.

Continuous with the skin are the epithelia lining the respiratory, gastrointestinal, and urogenital tracts (Figure 1.4). On these internal surfaces, the impermeable skin gives way to tissues that are specialized for communication with their environment and are more vulnerable to microbial invasion. Such surfaces are known as **mucosal surfaces** or **mucosae** as they are continually bathed in the **mucus** that they secrete. This thick fluid layer contains glycoproteins, proteoglycans, and enzymes that protect the epithelial cells from damage and help to limit infection. The enzyme lysozyme in tears and saliva is one of a number of antibacterial substances in secretions from mucosal surfaces. In the respiratory tract, mucus is continuously removed through the action of epithelial cells bearing beating cilia and is replenished by mucus-secreting goblet cells. The respiratory mucosa is thus continually cleansed of unwanted material, including infectious microorganisms that have been breathed in. Microorganisms are also deterred by the acidic environments of the stomach, the vagina, and the skin. With such defenses, skin and mucosa provide a well-maintained physical and chemical barrier that prevents most pathogens from gaining access to the cells and tissues of the body. When that barrier is breached and pathogens gain entry to the body's soft tissues, the fixed defenses of the innate immune system are brought into play.

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1-3 The innate immune response causes inflammation at sites of infection

Cuts, abrasions, bites, and wounds provide routes for pathogens to get through the skin. Touching, rubbing, picking, and poking of the eyes, nose, and mouth help pathogens to breach mucosal surfaces, as does breathing polluted air, eating contaminated food, and being around infected people. With very few exceptions, infections remain highly localized and are extinguished within a few days without illness or incapacitation. Such infections are controlled and terminated by immune mechanisms that are ready to react quickly to infection. This response consists of two parts (Figure 1.5). First is recognition that a pathogen is present. This involves soluble proteins and cell-surface receptors that bind either to the pathogen and its products or to human cells and serum proteins that become altered in the presence of the pathogen. Once the pathogen has been recognized, the second part of the response involves the recruitment of destructive **effector mechanisms** that kill and eliminate it. The effector mechanisms are provided by **effector cells** that engulf bacteria, kill virus-infected cells, or attack protozoan parasites, and a battery of serum proteins called **complement** that help the effector cells by marking pathogens with molecular flags, but also attack pathogens in their own right. Collectively, these mechanisms are called **innate immunity** or the **innate immune response**. The word 'innate' refers to the fact that all these mechanisms are determined entirely by the genes a person inherits from their parents. Many families of receptor proteins contribute to the recognition of pathogens in the innate immune response. They are of several different structural types and bind to chemically diverse ligands: peptides, proteins, glycoproteins, proteoglycans, peptidoglycan, carbohydrates, glycolipids, phospholipids and nucleic acids.

An infection that would typically be cleared by innate immunity is of the sort experienced by skateboarding adolescents when they tumble onto a San Francisco sidewalk. On returning home the graze is washed, which removes most of the dirt and the associated pathogens of human, soil, pigeon, dog, cat, raccoon, skunk, and possum origin. Of the bacteria that remain, some begin to divide and set up an infection. Cells and proteins in the damaged tissue sense the presence of the bacteria and the cells send out soluble proteins called **cytokines** that interact with other cells to trigger the innate immune response. The overall effect of the innate immune response is to induce a state of **inflammation** in the infected tissue. Inflammation is an ancient concept in medicine that has traditionally been defined by the Latin words *calor*, *dolor*, *rubor*, and *tumor*, for heat, pain, redness, and swelling, respectively. These symptoms, which are part of everyday human experience, are not due to the infection itself but to the immune system's response to it.

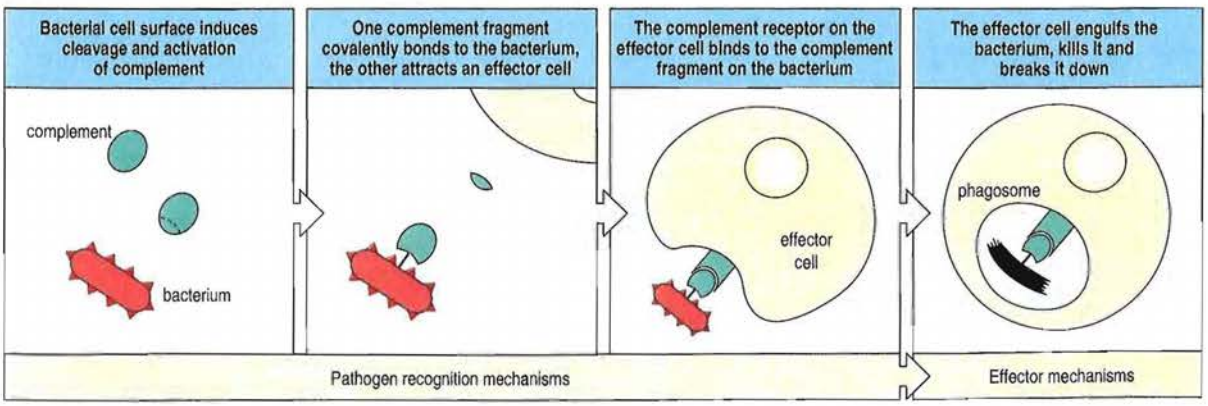


Figure 1.5 Immune defense involves recognition of pathogens followed by their destruction. Almost all components of the immune system contribute to mechanisms for either recognizing pathogens or destroying pathogens, or to mechanisms for communicating between these two activities. This is illustrated here by the most basic process of innate immunity, around which the entire immune system is built. Serum proteins of the complement system (turquoise) are activated in the presence of a pathogen (red) to form a covalent bond between a fragment of complement protein and the pathogen. The attached piece of complement marks the pathogen as dangerous. The soluble complement fragment summons a phagocytic white blood cell to the site of complement activation. The effector cell has a surface receptor that binds to the complement fragment attached to the pathogen. The receptor and its bound ligand are taken up into the cell by endocytosis, which delivers the pathogen to an intracellular vesicle called a phagosome, where it is destroyed.

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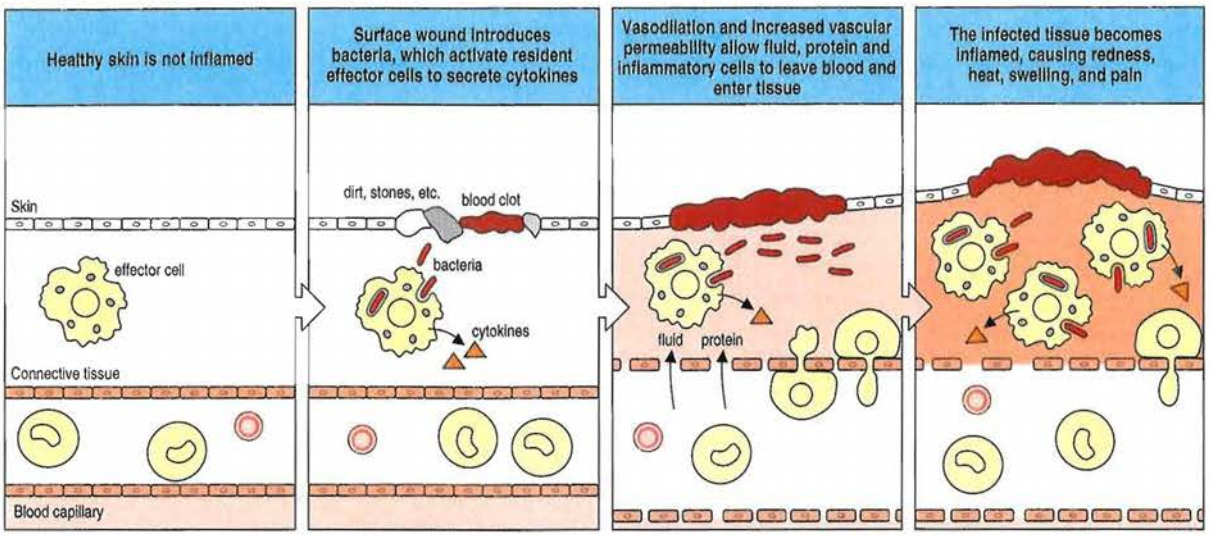


Figure 1.6 Innate immune mechanisms establish a state of inflammation at sites of infection. Illustrated here are the events following an abrasion of the skin that leads to bacteria invading the underlying connective tissue and stimulating the innate immune response.

Cytokines induce the local dilation of blood capillaries, which by increasing the blood flow causes the skin to warm and redden. Vascular dilation (vasodilation) introduces gaps between the endothelial cells, increasing the leak of blood plasma into the connective tissue. Such expansion of the local fluid volume causes edema or swelling, putting pressure on nerve endings and causing pain. Cytokines also change the adhesive properties of the vascular endothelium, inviting white blood cells to attach to it and move from the blood to the inflamed tissue (Figure 1.6). White blood cells that are usually present in inflamed tissues and release substances that contribute to the inflammation are called **inflammatory cells**. Infiltration of cells into the inflamed tissue increases the swelling, and some of the molecules they release contribute to the pain. The benefit of the discomfort and disfigurement is that inflammation enables cells and molecules of the immune system to be brought rapidly and in large numbers into the infected tissue.

1-4 The adaptive immune response adds to an ongoing innate immune response

Human beings are exposed to pathogens on a daily basis. The intensity of exposure and the diversity of the pathogens encountered increases with crowded city living and the daily input of people and pathogens from international airports. Despite this exposure, innate immunity keeps most people healthy for most of the time. Nevertheless, some infections outrun the innate immune response, an event more likely in people who are malnourished, poorly housed, deprived of sleep, or stressed in other ways. When this occurs, the innate immune response works to slow the spread of infection while it calls upon white blood cells called **lymphocytes** that increase the power and focus of the immune response. This contribution to defense is called the **adaptive immune response** because it is organized around an ongoing infection and adapts to the nuances of the infecting pathogen. Consequently, **adaptive immunity** provides a highly specialized defense against one pathogen that is of little effect against infection by a different pathogen.

The effector mechanisms used in the adaptive immune response are similar to those used in the innate immune response; the important difference is in the cell-surface receptors used by lymphocytes to recognize pathogens (Figure 1.7). In contrast to the receptor molecules of innate immunity, the receptor molecules of adaptive immunity are all of the same basic type. They are

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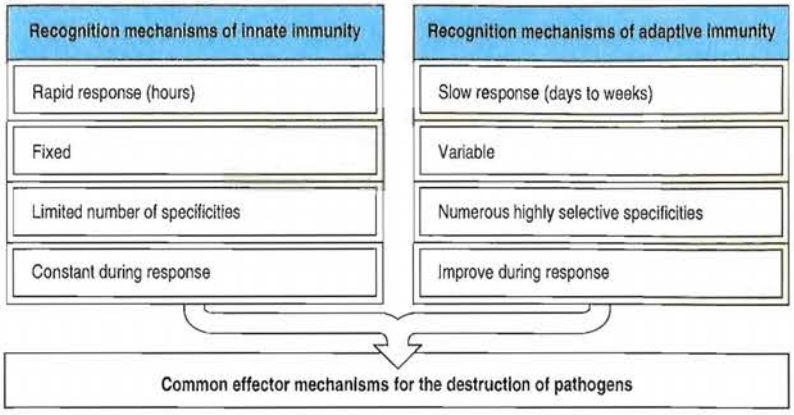


Figure 1.7 The principal characteristics of innate and adaptive immunity.

not encoded by conventional genes but by genes that are cut, spliced, and modified to produce billions of variants of the basic type, each of which is borne by a different subpopulation of cells. During infection only those lymphocytes bearing receptors that recognize the pathogen are selected to participate in the adaptive response, and their numbers are expanded and matured to produce large numbers of effector cells (Figure 1.8). As this process takes time, the benefit of the adaptive immune response only begins to be felt about a week after the infection began.

The value of the adaptive immune response is well illustrated in influenza, which is caused by infection of the epithelial cells in the lower respiratory tract with influenza virus. The debilitating symptoms start 3 or 4 days after the start of infection, when the virus has begun to outrun the innate immune response. The disease persists for 5–7 days while the adaptive immune response is being organized and put to work. As the adaptive immune response gains the upper hand, fever subsides and a gradual convalescence begins in the second week after infection.

The lymphocyte populations that expand during an adaptive immune response persist in the body and provide long-term **immunological memory** of the pathogen. These memory cells allow subsequent encounters with the same pathogen to elicit a stronger and faster adaptive immune response, which terminates infection with minimal illness. The adaptive immunity provided by immunological memory is also called **acquired immunity** or **protective immunity**. For some pathogens like measles virus, one full-blown infection can provide immunity for decades, whereas for influenza the effect seems more short-lived. This is not because the immunological memory is faulty but because the influenza virus changes on a yearly basis to escape the immunity acquired by its human hosts. The first time that an adaptive

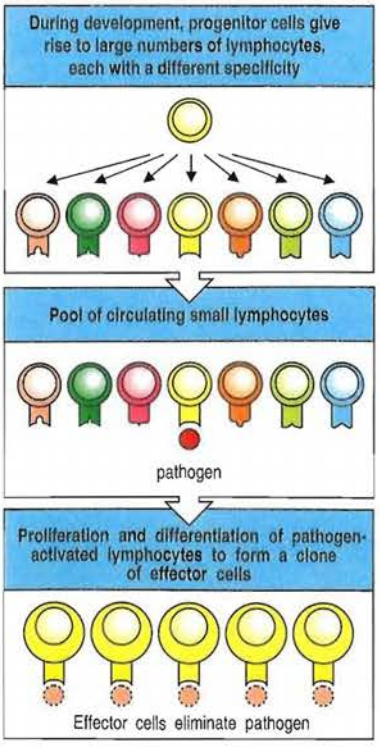
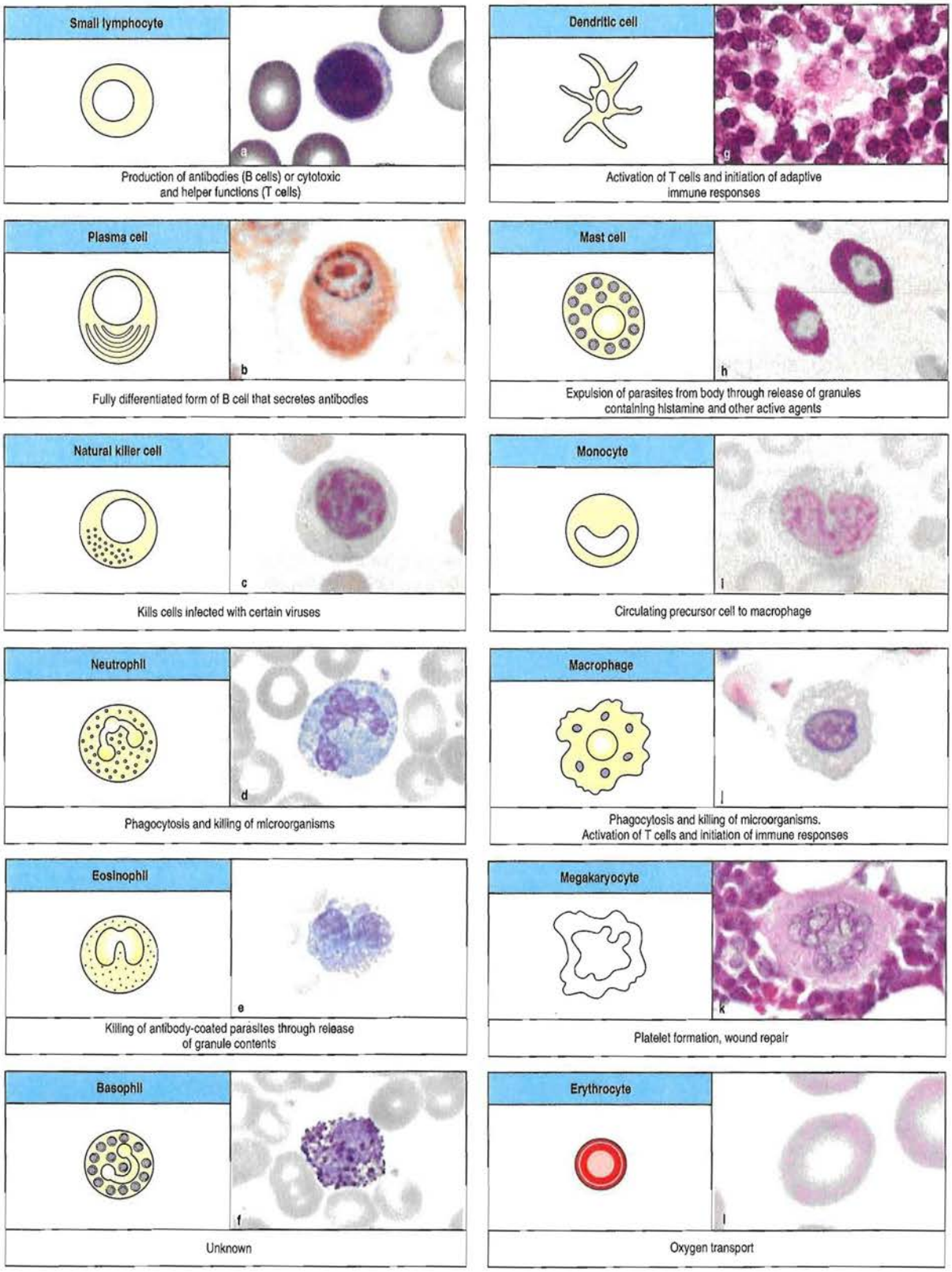


Figure 1.8 Selection of lymphocytes by a pathogen. During development, each lymphocyte is programmed to make a single species of cell-surface antigen receptor. The population of circulating lymphocytes embraces many millions of receptor species, which enables all possible pathogens to be recognized. In the panels, lymphocytes with different receptors are represented by the different colors. On infection by a particular pathogen, the small subsets of lymphocytes having receptors that bind to the pathogen or its components are stimulated to divide and differentiate, thereby producing expanded populations of effector cells from each antigen-binding lymphocyte. In the second panel the lymphocyte subpopulation selected by the pathogen is represented by that colored yellow. After activation and cell division, it gives rise to the effector lymphocytes shown in the third panel.

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Figure 1.9 Types of hematopoietic cell. The different types of hematopoietic cell are depicted in schematic diagrams, which indicate their characteristic morphological features, and in accompanying light micrographs. Their main functions are indicated. We shall use these schematic representations for these cells throughout the book. Red blood cells (erythrocytes) are seen in most of the pictures and are shown alone in panel l. They are smaller than the white blood cells and have no nucleus. Megakaryocytes (k) reside in bone marrow and release tiny non-nucleated fragments of cytoplasm, which circulate in the blood and are known as platelets. Photographs courtesy of N. Rooney (a, d, e, f, g, j), D. Friend (b, c, h, i), and L. Slomianka (k, l).

immune response is made to a given pathogen it is called the **primary response**. The second and subsequent times that an adaptive immune response is made, and when immunological memory applies, it is called a **secondary response**. The purpose of vaccination is to induce immunological memory to a pathogen so that subsequent infection with the pathogen elicits a strong fast-acting adaptive response. As all adaptive immune responses are contingent upon an innate immune response, vaccines must also induce both innate and adaptive immune responses.

1-5 Immune system cells with different functions all derive from hematopoietic stem cells

The cells of the immune system are principally the white blood cells or **leukocytes**, and the tissue cells related to them. These cells derive from a common progenitor called the **pluripotent hematopoietic stem cell**, which also gives rise to **erythrocytes**, the red blood cells, and to the cells called **megakaryocytes**. All these cell types are collectively called **hematopoietic cells** (Figure 1.9). The developmental process by which hematopoietic stem cells give rise to this variety of hematopoietic cells is called **hematopoiesis**. The anatomical site for hematopoiesis changes with age (Figure 1.10). In the early embryo, blood cells are first produced in the yolk sac and later in the fetal liver. From the third to the seventh month of fetal life the spleen is the major site of hematopoiesis. As the bones develop during the fourth and fifth months of fetal growth, hematopoiesis begins to shift to the bone marrow and by birth this is where practically all hematopoeisis takes place. In adults hematopoiesis occurs mainly in the bone marrow of the skull, ribs, sternum, vertebral column, pelvis, and femurs. Because blood cells are both vital and short-lived, hematopoiesis is active throughout life.

Hematopoietic stem cells can divide to give further hematopoietic stem cells, a process called **self renewal**; they can also become more mature stem cells that commit to one of three cell lineages: the erythroid, myeloid, and lymphoid lineages (Figure 1.11). The erythroid progenitor gives rise to the erythroid lineage of blood cells—the oxygen-carrying red blood cells (erythrocytes) and the platelet-producing megakaryocytes. Platelets are small non-nucleated cells of plate-like shape that maintain the integrity of blood vessels. A principal function of platelets is to initiate and participate in the clotting reactions that block up badly damaged blood vessels to prevent blood loss. Megakaryocytes are giant cells that arise from the fusion of multiple precursor cells and have nuclei containing multiple sets of chromosomes (megakaryocyte means cell with giant nucleus). Megakaryocytes are permanent residents of the bone marrow.

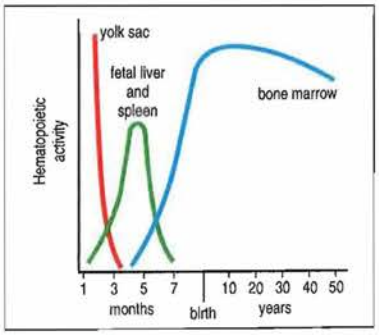


Figure 1.10 The site of hematopoiesis in humans changes during development.

The **myeloid progenitor** gives rise to the **myeloid lineage** of cells. One group of myeloid cells are called **granulocytes** because of their prominent cytoplasmic granules, which contain reactive substances that kill microorganisms and enhance inflammation. Because granulocytes have irregularly shaped nuclei

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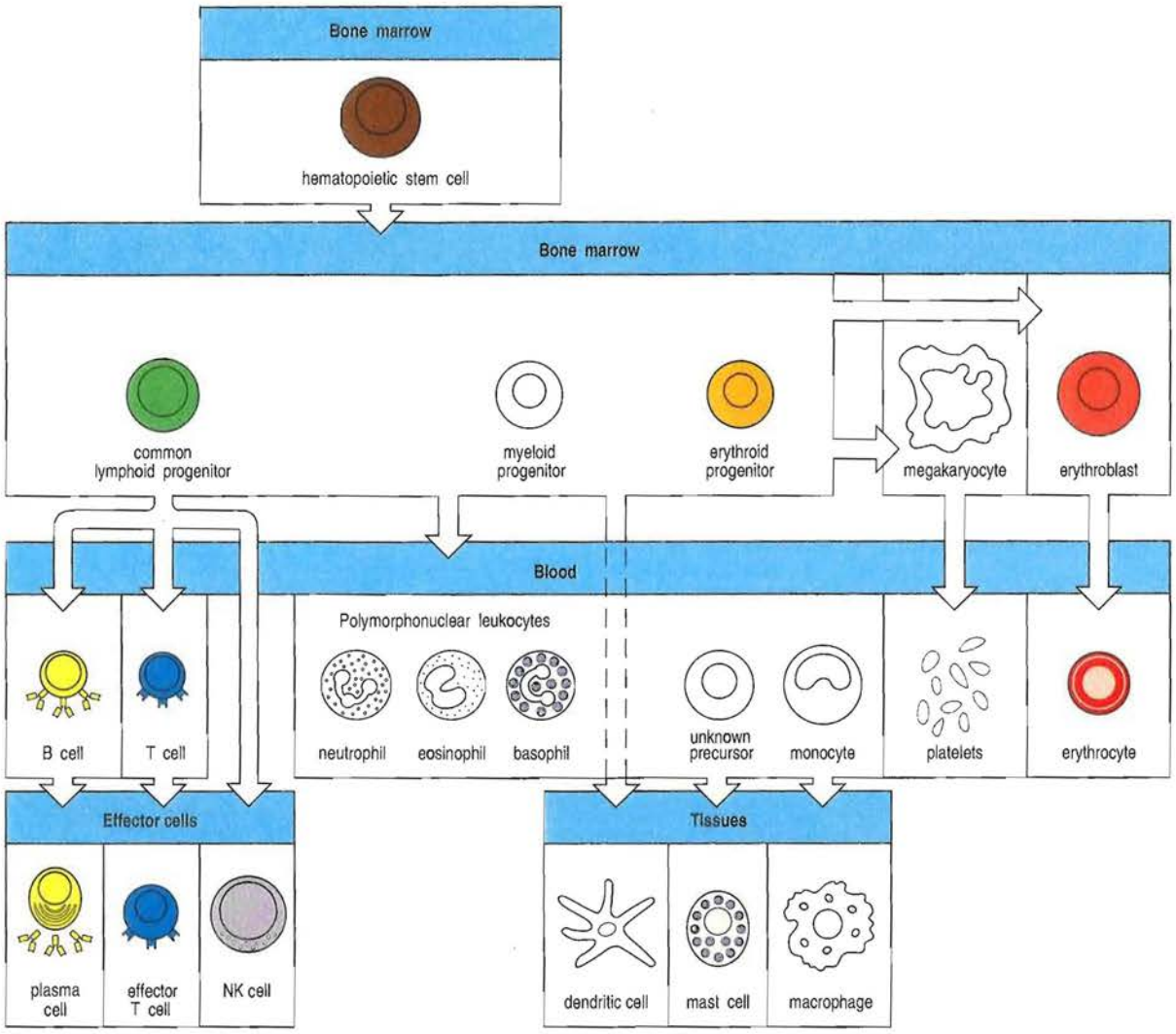


Figure 1.11 Blood cells and certain tissue cells derive from a common hematopoietic stem cell. The pluripotent stem cell (brown) divides and differentiates into more specialized progenitor cells that give rise to the lymphoid lineage, the myeloid lineage, and the erythroid lineage. The common lymphoid progenitor divides and differentiates to give B cells (yellow), T cells (blue), and NK cells (purple). On activation by infection, B cells divide and differentiate into plasma cells, whereas T cells differentiate into various types of activated effector T cell. The myeloid progenitor cell divides and differentiates to produce at least six cell types. These are: the three types of granulocyte—the neutrophil, the eosinophil, and the basophil; the mast cell, which takes up residence in connective and mucosal tissues; the circulating monocyte, which gives rise to the macrophages resident in tissues; and the dendritic cell. The word myeloid means 'of the bone marrow.' The myeloid lineage of cells was named for the neutrophil, which is the most abundant cell in the bone marrow. The erythroid progenitor gives rise to erythrocytes and megakaryocytes.

with two to five lobes, they are also called **polymorphonuclear leukocytes**. Most abundant of the granulocytes, and of all white blood cells, is the neutrophil (Figure 1.12) which is specialized in the capture, engulfment and killing of microorganisms. Cells with this function are called **phagocytes**, of which neutrophils are the most numerous and lethal. **Neutrophils** are effector cells of innate immunity that are rapidly mobilized to enter sites of infection and can work in the anaerobic conditions that often prevail in damaged tissue. Neutrophils are short-lived and die at the site of infection, forming **pus** (Figure 1.13). The second most abundant granulocyte is the **eosinophil**, which defends against helminth worms and other intestinal parasites. The

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least abundant granulocyte, the **basophil**, is so rare that little is known of its contribution to the immune response. The names of the granulocytes refer to the staining of their cytoplasmic granules with the commonly used histological stains: the eosinophil's granules contain basic substances that bind the acidic stain eosin, the basophil's granules contain acidic substances that bind basic stains such as hematoxylin, and the contents of the neutrophil's granules bind to neither acidic nor basic stains.

The second group of myeloid cells consists of monocytes, macrophages, and dendritic cells. **Monocytes** are leukocytes that circulate in the blood. They are distinguished from the granulocytes by being bigger, by having a distinctive indented nucleus, and by all looking the same: hence the name monocyte. Monocytes are mobile progenitors of the sedentary tissue macrophage; they travel in the blood to tissues, where they mature to **macrophages** and take up residence. The name macrophage means large phagocyte, and like the neutrophil, which was once called the microphage, the macrophage is well equipped for phagocytosis. Tissue macrophages are large irregularly shaped cells characterized by an extensive cytoplasm with numerous vacuoles, often containing engulfed materials (Figure 1.14). They are the general scavenger cells of the body, phagocytosing and disposing of dead cells and cell debris as well as invading microorganisms.

If neutrophils are the short-lived infantry of innate immunity, then macrophages are the long-lived commanders who provide intelligence to other cells and orchestrate the local response to infection. Macrophages present in the infected tissues are generally the first phagocytic cell to sense an invading microorganism. As part of their response to the pathogen, macrophages secrete the cytokines that recruit neutrophils and other leukocytes into the infected area.

Myeloid **dendritic cells** are resident in the body's tissues and have a distinctive star-shaped morphology. Although they have many properties in common with macrophages, their unique function is to act as cellular messengers that are sent to call up an adaptive immune response when it is needed. At such times, dendritic cells present in the infected tissue will leave the tissue with a cargo of degraded and intact pathogens and take it to one of several lymphoid organs that specialize in making adaptive immune responses.

The last type of myeloid cell is the mast cell, which is resident in all connective tissues. Although having granules like those of the basophil, the mast cell

Cell type	Proportion of leukocytes (%)
Neutrophil	40–75
Eosinophil	1–6
Basophil	<1
Monocyte	2–10
Lymphocyte	20–50

Figure 1.12 The abundance of leukocytes in human peripheral blood. The relative proportion of each cell type in the leukocyte population of peripheral blood taken from a vein from healthy donors is given.

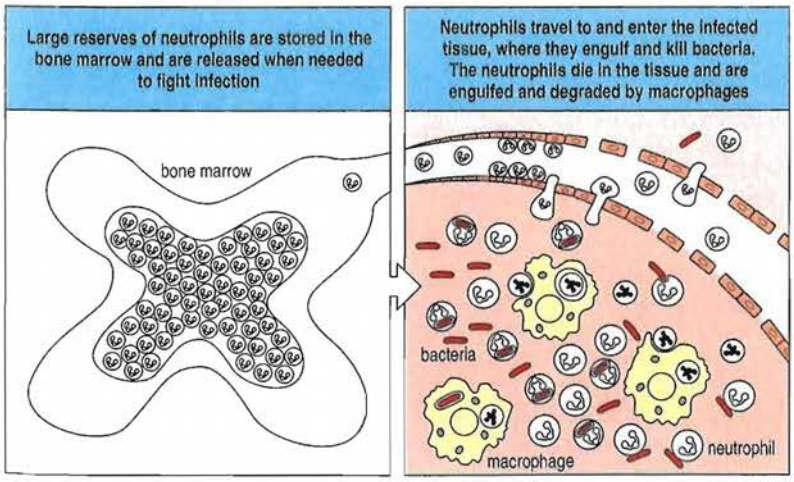


Figure 1.13 Neutrophils are stored in the bone marrow and move in large numbers to sites of infection, where they act and then die. After one round of ingestion and killing of bacteria, a neutrophil dies. The dead neutrophils are eventually mopped up by long-lived tissue macrophages, which break them down. The creamy material known as pus is composed of dead neutrophils.

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is not derived from the basophil and the nature of its blood-borne progenitor is not yet known. The activation and degranulation of mast cells at sites of infection make a major contribution to inflammation.

The **lymphoid progenitor** gives rise to the **lymphoid lineage** of white blood cells. Two populations of blood lymphocytes are distinguished morphologically: large lymphocytes with a granular cytoplasm and small lymphocytes with almost no cytoplasm. The **large granular lymphocytes** are effector cells of innate immunity called **natural killer cells** or **NK cells**. NK cells are important in the defense against viral infections. They enter infected tissues, where they prevent the spread of infection by killing virus-infected cells and secreting cytokines that impede viral replication in infected cells. The **small lymphocytes** are the cells responsible for the adaptive immune response. They are small because they circulate in a quiescent and immature form that is functionally inactive. Recognition of a pathogen by small lymphocytes drives a process of lymphocyte selection, growth and differentiation that after 1–2 weeks produces a powerful response tailored to the invading organism.

The small lymphocytes, although morphologically indistinguishable from each other, comprise several sublineages that are distinguished by their cell-surface receptors and the functions that they are programmed to perform. The most important difference is between **B lymphocytes** or **B cells** and **T lymphocytes** or **T cells**. For B cells the cell-surface receptors for pathogens are **immunoglobulins**, whereas those of T cells are known as **T-cell receptors**. Immunoglobulins and T-cell receptors are structurally similar molecules that are the products of genes that are cut, spliced, and modified during lympho-

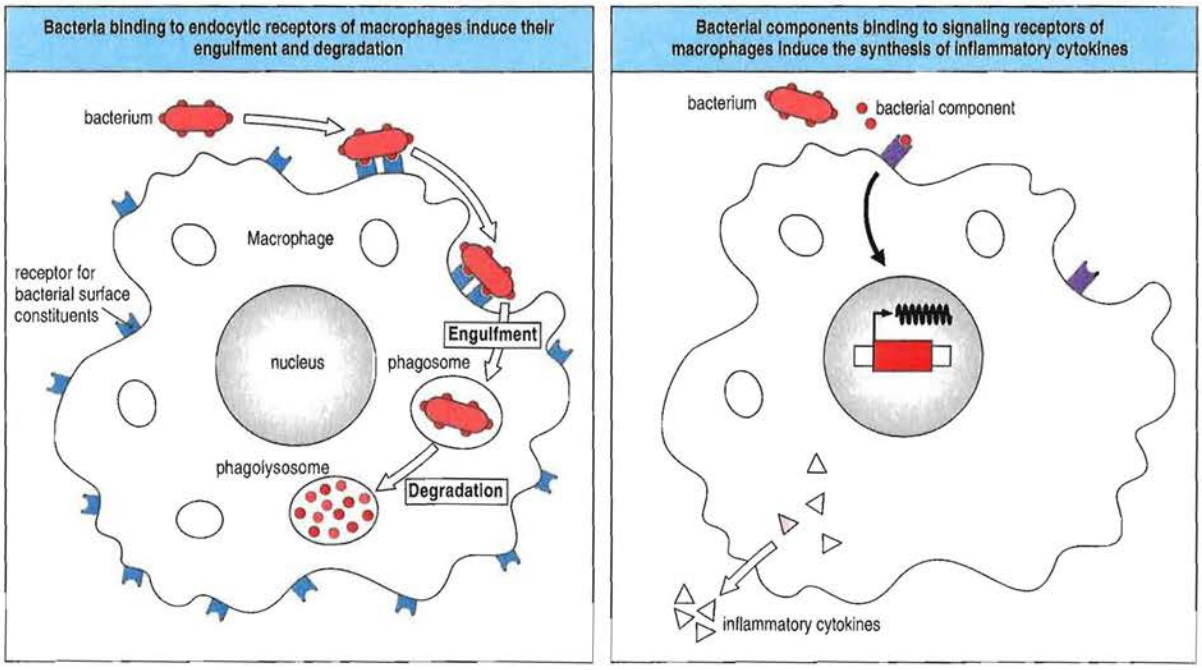


Figure 1.14 Macrophages respond to pathogens by using different receptors to stimulate phagocytosis and cytokine secretion. The left panel shows receptor-mediated phagocytosis of bacteria by a macrophage. The bacterium (red) binds to cell-surface receptors of the macrophage (blue), inducing engulfment of the bacterium into an endocytic vesicle called a phagosome within the cytoplasm of the macrophage. Fusion of the phagosome with lysosomes forms an acidic vesicle called a phagolysosome, which contains toxic small molecules and hydrolytic enzymes that kill and degrade the bacterium. The right panel shows how a bacterial component binding to a cell-surface receptor sends a signal to the macrophage's nucleus that initiates the transcription of genes for inflammatory cytokines. The cytokines are synthesized and secreted into the extracellular fluid.

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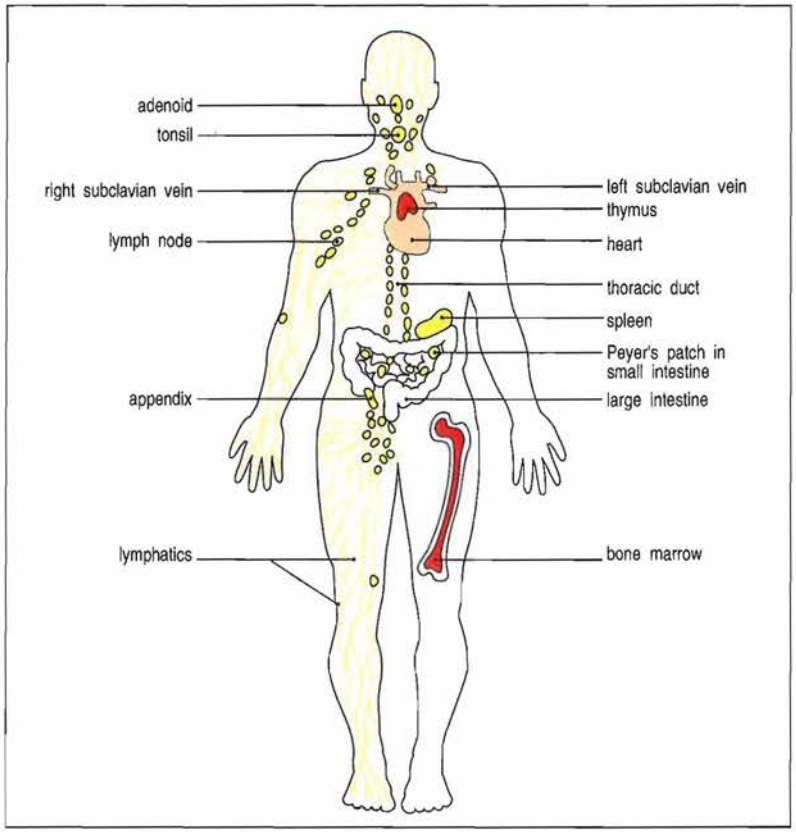


Figure 1.15 The sites of the principal lymphoid tissues within the human body. Lymphocytes arise from stem cells in the bone marrow. B cells complete their maturation in the bone marrow, whereas T cells leave at an immature stage and complete their development in the thymus. The bone marrow and the thymus are the primary lymphoid tissues and are shown in red. The secondary lymphoid tissues and the lymphatics are shown in yellow. Plasma that has leaked from the blood is collected by the lymphatics and returned to the blood supply via the thoracic duct, which empties into the left subclavian vein.

cyte development. As a consequence of these processes each B cell expresses a single type of immunoglobulin and each T cell expresses a single T-cell receptor. Within the small lymphocyte population billions of different immunoglobulins and T-cell receptors are represented.

1-6 Most lymphocytes are present in specialized lymphoid tissues

As well as circulating in the blood, lymphocytes congregate in specialized tissues known as **lymphoid tissues** or **lymphoid organs**. The major lymphoid tissues are bone marrow, thymus, spleen, adenoids, tonsils, appendix, lymph nodes, and Peyer's patches. Lymphoid tissues are also found lining the mucosal surfaces of the respiratory, gastrointestinal, and urogenital tracts. The lymphoid tissues are functionally divided into two types. **Primary** or **central lymphoid tissues** are where lymphocytes develop and mature to the stage at which they are able to respond to a pathogen. The bone marrow and the **thymus** are the primary lymphoid tissues. All other lymphoid tissues are known as **secondary** or **peripheral** lymphoid tissues; they are where mature lymphocytes become stimulated to respond to invading pathogens (Figure 1.15).

Lymph nodes lie at the junctions of an anastomosing network of **lymphatic vessels** or **lymphatics**, which originate in the connective tissues and collect the plasma that continually leaks out of blood vessels and forms the extracellular fluid. The lymphatics eventually return this fluid to the blood, chiefly via the thoracic duct, which empties into the left subclavian vein in the neck. Mature lymphocytes leave the primary lymphoid tissues and enter the bloodstream, whereupon they travel between the blood, the secondary lymphoid tissues, and the lymphatics, eventually being returned to the blood. The mix-

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ture of fluid and cells that flows through the lymphatics is called **lymph**. These movements are called **recirculation** of lymphocytes. An exception to this pattern is the spleen, which has no connections to the lymphatic system. Lymphocytes both enter and leave the spleen in the blood. At any one time only a very small fraction of lymphocytes are in the blood and lymph; the majority are in lymphoid organs and tissues.

Both B and T lymphocytes originate from lymphoid precursors in the bone marrow. But whereas B cells complete their maturation in the bone marrow before entering the circulation, T cells leave the bone marrow at an immature stage and migrate in the blood to the thymus, where they complete their maturation. Lymphocyte maturation is a highly selective process in which the vast majority of immature lymphocytes are destroyed because they fail to develop immunoglobulins or T-cell receptors that are useful to the immune system. The small fraction of immature B and T cells that successfully complete development depart from the primary lymphoid organs and enter the circulation.

1-7 Lymphocytes are activated in the secondary lymphoid tissues

Secondary lymphoid tissues provide meeting places where lymphocytes circulating in the blood encounter pathogens and their products brought from a site of infection. Precisely how this works depends on where an infection occurs. Frequent sites of infection are the connective tissues, which pathogens penetrate as a result of skin wounds. From such sites pathogens are carried by the lymphatics to the nearest **lymph node** (Figure 1.16). The

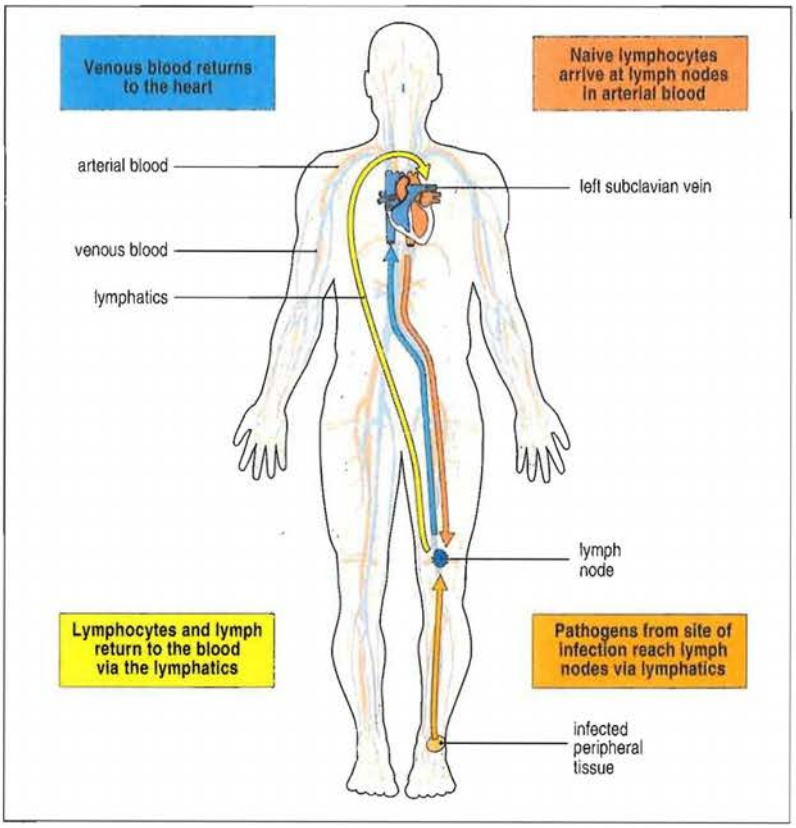


Figure 1.16 Circulating lymphocytes meet lymph-borne pathogens in draining lymph nodes. Lymphocytes leave the blood and enter lymph nodes, where they can be activated by pathogens draining in the afferent lymph from a site of infection. The circulation pertaining to a site of infection in the left foot is shown. When activated by pathogens, lymphocytes stay in the node to divide and differentiate into effector cells. If lymphocytes are not activated, they leave the node in the efferent lymph and are carried by the lymphatics to the thoracic duct (see Figure 1.15), which empties into the blood at the left subclavian vein. Lymphocytes recirculate all the time, irrespective of infection. Every minute 5×10^6 lymphocytes leave the blood and enter secondary lymphoid tissues.

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lymph node receiving the fluid collected at an infected site is called the **draining lymph node**. Also traveling along this route are dendritic cells that have been activated by the infection and are carrying pathogens and their components.

Unlike the blood, the lymph is not driven by a dedicated pump and its flow is comparatively sluggish. One-way valves within lymphatic vessels and the lymph nodes placed at their junctions ensure that net movement of the lymph is always in a direction away from the peripheral tissues and towards the ducts in the upper body where the lymph empties into the blood. The flow of lymph is driven by the continual movements of one part of the body with respect to another. In the absence of such movement, as when a patient is confined to bed for a long time, lymph flow slows and fluid accumulates in tissues, causing the swelling known as **edema**.

Pathogens and dendritic cells arrive at a lymph node in **afferent lymphatic vessels**, several of which unite at the node to leave it as a single **efferent lymphatic vessel**. The anatomy of a lymph node is shown in Figure 1.17. As the lymph passes through the node, pathogens and other extraneous materials are filtered out by macrophages. This process prevents infectious organisms from reaching the blood and provides a depot of pathogen within the lymph node that can be used to activate lymphocytes. More important for this function are the dendritic cells that take up residence in parts of the lymph node

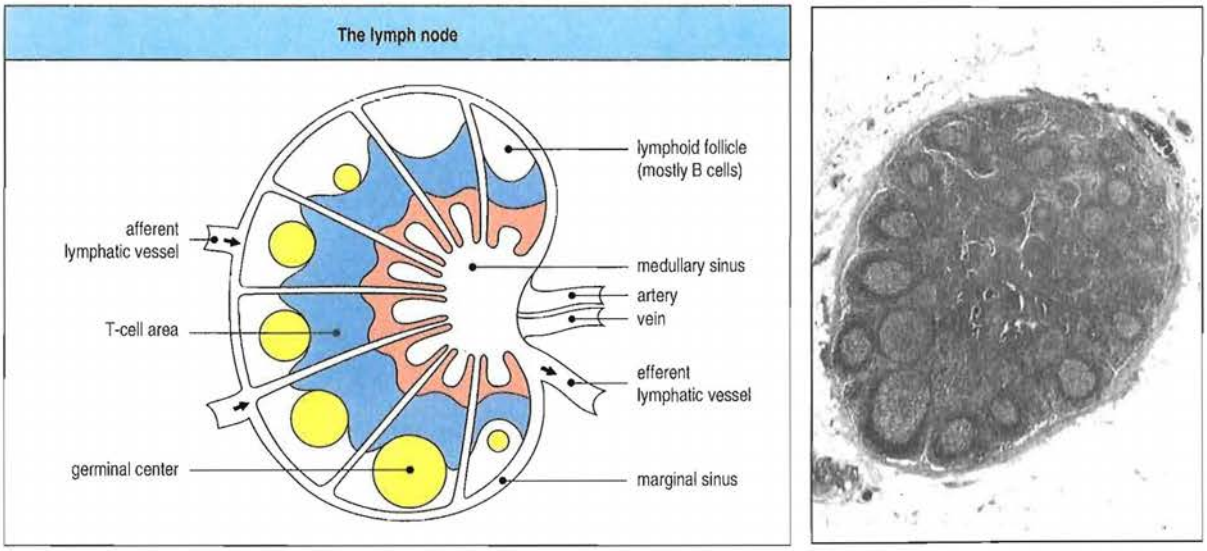


Figure 1.17 Architecture of the lymph node, the site where blood-borne lymphocytes respond to lymph-borne pathogens. Human lymph nodes are small kidney-shaped organs weighing 1 gram or less that form junctions where a number of afferent lymph vessels bringing lymph from the tissues unite to form a single, larger efferent lymph vessel. During infection, pathogens and dendritic cells carrying pathogens arrive in draining afferent lymph. The lymph node is packed with lymphocytes, macrophages and other cells of the immune system through which the lymph percolates. Dendritic cells settle in the lymph node; pathogens are picked up by the resident macrophages and then degraded. Both dendritic cells and macrophages are important stimulators of lymphocytes. Lymphocytes arrive at lymph nodes in the arterial blood. They enter the node by passing between the endothelial cells that line the fine capillaries within the lymph node (not shown). The population of lymphocytes within a node is in a continual state of flux, with new lymphocytes entering from the blood while others leave in the efferent lymph. Within the lymph node there are anatomically discrete areas where B or T cells tend to congregate. A lymph node draining a site of infection increases in size owing to the proliferation of activated lymphocytes, a phenomenon sometimes referred to as 'swollen glands.' The expansion of lymphocyte populations takes place in spherical lymphoid follicles present in the lymph node cortex. As lymphocyte division and differentiation proceed, the follicle morphology changes and it is then called a germinal center. The photograph shows a section through a lymph node in which there are prominent germinal centers. Photograph (× 7) courtesy of N. Rooney.

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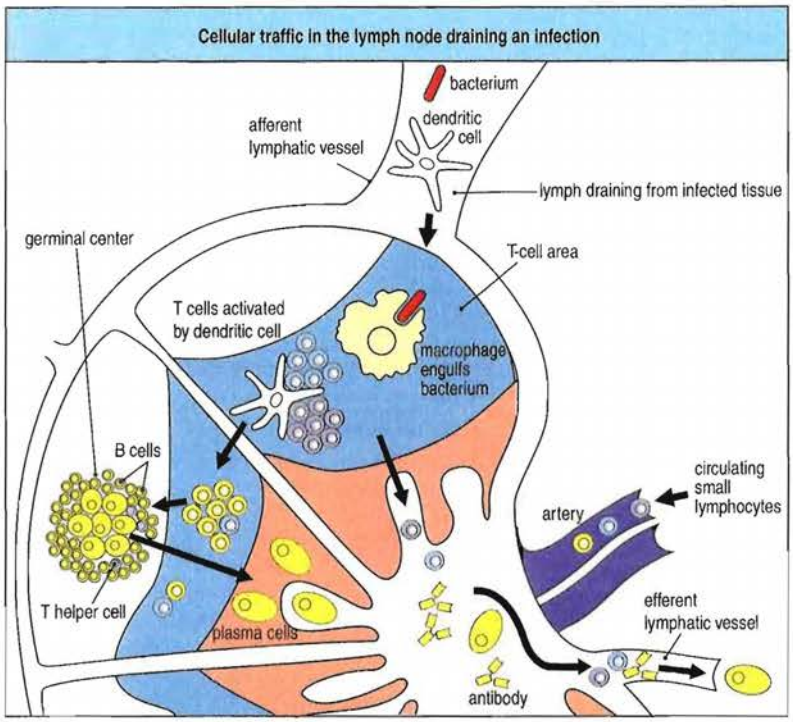


Figure 1.18 Cellular traffic in the lymph node draining an infection. Pathogens and dendritic cells carrying pathogens and their antigens arrive in the afferent lymph draining the site of infection. Free pathogens and debris are removed by macrophages; dendritic cells move to the T-cell areas, where they meet small lymphocytes that have entered the node from the blood. The dendritic cells stimulate the division and differentiation of pathogen-specific small lymphocytes into effector lymphocytes. Some helper T cells (blue) and cytotoxic T cells (purple) leave in the efferent lymph and travel to the infected tissue via the lymph and blood. Other helper T cells (blue) remain in the lymph node and stimulate the division and differentiation of pathogen-specific B cells (yellow) into plasma cells. Plasma cells move to the medulla of the lymph node, where they secrete pathogen-specific antibody, which is taken to the site of infection by the efferent lymph and blood. Some plasma cells leave the lymph node and travel via the efferent lymph and the blood to the bone marrow, where they continue to secrete antibody.

where the lymphocytes are predominantly T cells. These parts of the lymph node are called the **T-cell areas**.

Small lymphocytes recirculate through the body in both the blood and the lymph. When they reach the blood capillaries running through a lymph node, lymphocytes can leave the blood and enter the node. Within the node T cells move into the T-cell areas, where they form transient interactions with dendritic cells (Figure 1.18). If the receptors on the T-cell surface bind to pathogen components displayed by a dendritic cell, then the T cell is signaled to divide and differentiate into functional **effector cells**. Some T cells differentiate into **helper T cells** that stay in the lymph node and provide soluble proteins and intercellular contacts that drive the differentiation of B cells possessing immunoglobulin receptors that bind the pathogen. Effector B cells, called **plasma cells**, either stay in the lymph node or migrate to the bone marrow via lymph and blood. Plasma cells make and secrete large amounts of **antibody**, a soluble form of their cell-surface immunoglobulin. A second kind of T cell differentiates into helper T cells that leave the lymph node and travel via the efferent lymph and the blood to the infected tissue. These helper T cells interact with macrophages and secrete soluble cytokines to amplify the inflammation. A third type of effector T cell is called the **cytotoxic T cell** because these cells kill cells infected with viruses or other intracellular pathogens. Only a very small minority of the circulating small lymphocytes will be activated by any given pathogen; the rest will pass through the lymph node and leave in the efferent lymph to continue recirculation.

Pathogens can enter the blood directly, as occurs when blood-feeding insects transmit disease or when lymph nodes have failed to remove microorganisms from the lymph returned to the blood. The **spleen** is the lymphoid organ that serves as a filter for the blood. One purpose of the filtration is to remove damaged or old red cells; the second function of the spleen is that of a secondary lymphoid tissue—infectious agents are removed and used to activate lym-

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phocytes. In this latter role, the spleen functions in a similar fashion to the lymph node, the only difference being that both pathogens and lymphocytes enter and leave the spleen in the blood (Figure 1.19).

The parts of the body that harbor the largest and most diverse populations of microorganisms are the respiratory and gastrointestinal tracts. The most heavily infested site is the oral cavity. The extensive mucosal surfaces of these tissues make them particularly vulnerable to infection and they are therefore heavily invested with secondary lymphoid tissue. The **gut-associated lymphoid tissues** (**GALT**) include the **tonsils**, **adenoids**, **appendix**, and the **Peyer's patches** which line the small intestine (see Figure 1.15). Similar but less organized aggregates of secondary lymphoid tissue line the respiratory epithelium, where they are called **bronchial-associated lymphoid tissue** (**BALT**), and other mucosal surfaces, including the gastrointestinal tract. The more diffuse mucosal lymphoid tissues are known generally as **mucosa-associated lymphoid tissue** (**MALT**).

Although different from the lymph nodes or spleen in outward appearance, the mucosal lymphoid tissues are similar to them in their microanatomy (Figure 1.20) and in their function of trapping pathogens to activate lymphocytes. The differences are chiefly in the route of pathogen entry and the migration patterns of their lymphocytes. Pathogens arrive at mucosa-associated lymphoid tissues by direct delivery across the mucosa, mediated by specialized cells of the mucosal epithelium called **M cells**. Lymphocytes first enter mucosal lymphoid tissue from the blood and, if not activated, leave via lymphatics that connect the mucosal tissues to draining lymph nodes. Lymphocytes activated in mucosal tissues tend to stay within the mucosal system, moving out from the lymphoid tissue into the lamina propria and the mucosal epithelium, where they perform their effector actions.

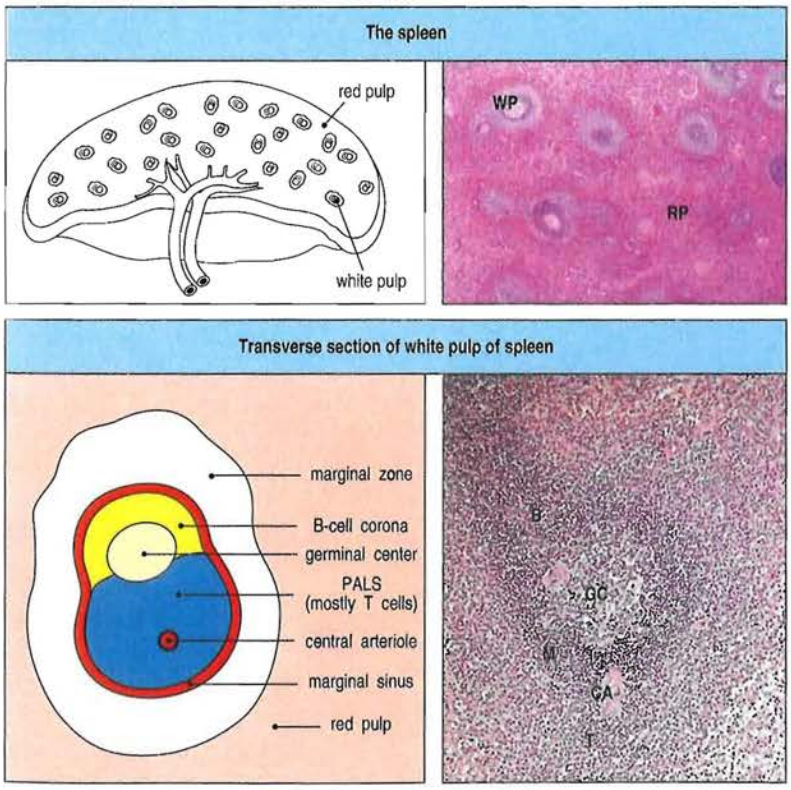


Figure 1.19 The spleen has aggregations of lymphocytes similar to those in lymph nodes. The human spleen is a large lymphoid organ in the upper left part of the abdomen, weighing about 150 grams. The upper diagram depicts a section of spleen in which nodules of white pulp are scattered within the more extensive red pulp. The red pulp (RP) is where old or damaged red cells are removed from the circulation; the white pulp (WP) is secondary lymphoid tissue, in which lymphocyte responses to blood-borne pathogens are made. The bottom diagram shows a nodule of white pulp in transverse section. It consists of a sheath of lymphocytes surrounding a central arteriole (CA). The sheath is called the periarteriolar lymphoid sheath (PALS). The lymphocytes closest to the arteriole are mostly T cells (T); B cells are placed more peripherally, forming a B-cell corona (B). Lymphoid follicles and germinal centers (GC) lie between the B- and T-cell zones. The marginal zone (M) contains differentiating B cells. Photographs courtesy of H.G. Burkitt and B. Young (top) and N. Rooney (bottom).

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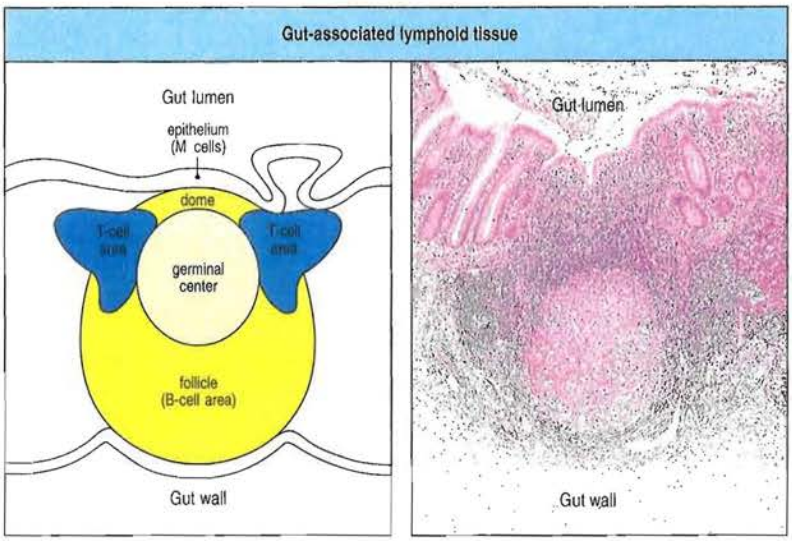


Figure 1.20 A typical region of gut-associated lymphoid tissue. A schematic diagram (left panel) and a light micrograph (right panel) of a typical region of gut-associated lymphoid tissue. M cells of the gut epithelial wall deliver pathogens from the luminal side of the gut mucosa to the lymphoid tissue within the gut wall. These areas are organized similarly to the lymph node and the white pulp of the spleen, with distinctive B- and T-cell zones, lymphoid follicles, and germinal centers. Photograph courtesy of N. Rooney.

Summary

To restrict the nature, size, and location of microbial infection, animals have evolved a variety of defense mechanisms. The skin and contiguous mucosal surfaces provide physical and chemical barriers that confine microorganisms to the external surfaces of the body. In addition, cells and molecules of the innate and adaptive immune systems identify and eliminate microorganisms that have penetrated the physical and chemical barriers and gained entry to the soft tissues. The cells that mediate both innate and adaptive immune responses are principally the various types of leukocyte and allied tissue cells, which derive from the bone marrow. In responding to infection, the immune system uses innate mechanisms that are fast but limited, and adaptive mechanisms that are slow to start but eventually become both powerful and quick to recall. The cells and molecules of innate immunity identify common classes of pathogen and destroy them. Four key elements of innate immunity are: molecules that noncovalently bind to surface macromolecules of pathogens; molecules that covalently bond to pathogen surfaces, forming ligands for phagocyte receptors; phagocytic cells that engulf and kill pathogens; and cytotoxic cells that kill virus-infected cells. Vertebrates have evolved the additional defenses of adaptive immune responses, which involve the T and B lymphocytes. Adaptive immune responses are initiated in lymphoid tissues such as lymph nodes and spleen, in which pathogen-specific lymphocytes encounter and are activated by pathogen antigens.

Principles of adaptive immunity

In the innate immune response the diversity of pathogens that a person might encounter in a lifetime is faced by a repertoire of receptors that are always available. Some of these recognize structures shared by many different pathogens, and others recognize alterations to human cells that are commonly induced by the presence of pathogens. The adaptive immune response uses a quite different strategy in which receptors that uniquely define an infecting pathogen are first selected then mass produced on demand. Millions of different immunoglobulins and T-cell receptors are made by the B and T lymphocytes of the human immune system, and each recognizes a different molecular structure. On infection with a pathogen, only those B and T

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cells making recognition molecules that can bind to constituents of that pathogen are stimulated to divide, proliferate, and differentiate into effector lymphocytes.

1-8 Immunoglobulins and T-cell receptors are the highly variable recognition molecules of adaptive immunity

The molecules and mechanisms of adaptive immunity are restricted to vertebrate animals, suggesting that their evolution became feasible or advantageous only with the degree of cellular and anatomical complexity attained by the vertebrates. Immunoglobulins are expressed on the surface of B cells, where they can bind pathogens. Effector B cells, called plasma cells, secrete soluble forms of these immunoglobulins, which are known as antibodies. In contrast, T-cell receptors are only ever expressed as cell-surface recognition molecules, never as soluble proteins.

Any molecule, macromolecule, virus particle or cell that contains a structure recognized and bound by an immunoglobulin or T-cell receptor is known as its corresponding **antigen**. Surface immunoglobulins and T-cell receptors are thus also referred to as the **antigen receptors** of lymphocytes. The particular part of the antigen bound by the immunoglobulin or T-cell receptor is known as the **antigenic determinant** or **epitope**. Immunoglobulins can bind to a vast variety of different chemical structures, whereas T-cell receptors recognize a more limited range of epitopes. Immunoglobulins and T-cell receptors are said to be **specific**, or to have **specificity**, for the antigens they bind.

Immunoglobulins and T-cell receptors are structurally related molecules whose diversity of antigen-recognition sites is generated by similar genetic mechanisms. Immunoglobulins are formed from two different polypeptides called the heavy and light chains; each Y-shaped immunoglobulin molecule consists of two identical heavy chains and two identical light chains. Both types of polypeptide have an amino-terminal **variable region** that differs in amino-acid sequence from one immunoglobulin to the next, and a **constant region** that is identical in amino-acid sequence from one immunoglobulin to another (Figure 1.21). The variable regions contain the sites that bind antigens. Surface immunoglobulin is anchored in the membrane by transmembrane regions at the carboxyl ends of the heavy chains. Antibodies are a

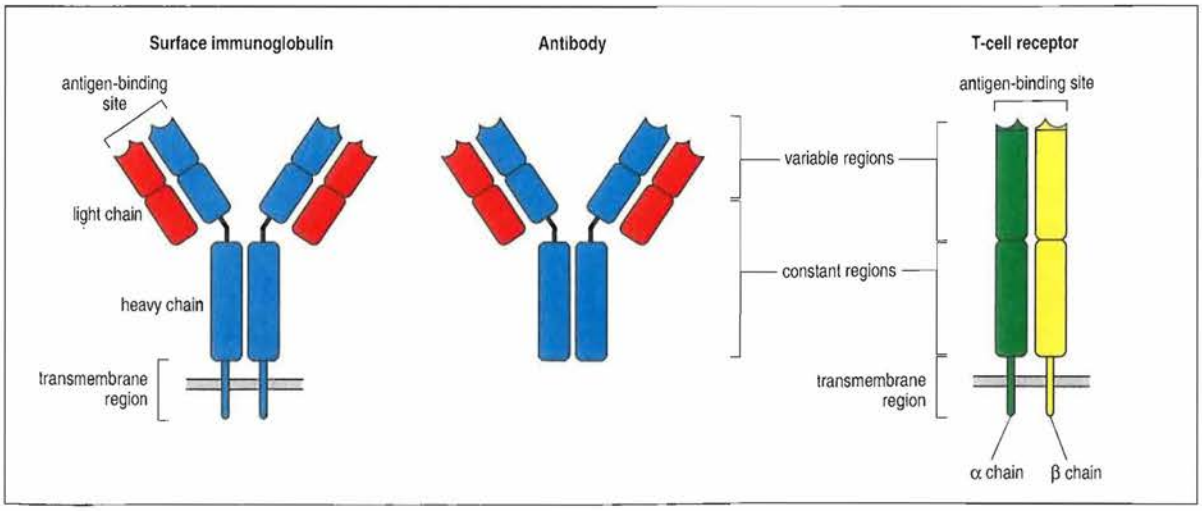


Figure 1.21 Comparison of the basic structures of surface immunoglobulin, antibody, and the T-cell receptor. The heavy chains of surface immunoglobulin and antibody are shown in blue, the light chains in red.

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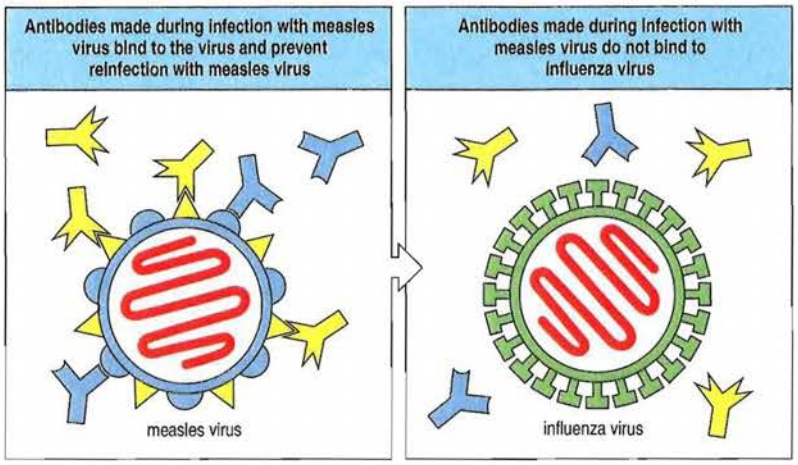


Figure 1.22 The antibodies made against a pathogen are highly specific for that pathogen. After having recovered from infection with measles virus a person's body fluids contain many different antibodies that can bind to the measles virus and prevent reinfection. None of these antibodies binds to an unrelated virus such as influenza.

secreted form of immunoglobulin that lack these transmembrane regions but are otherwise identical to surface immunoglobulins (see Figure 1.21).

A typical T-cell receptor consists of an α chain and a β chain, both anchored in the T-cell membrane. Like the light chains and heavy chains of immunoglobulins, the α and β chains of T-cell receptors each consist of a variable region and a constant region, with the variable regions forming an antigen-binding site (see Figure 1.21).

The differences in the amino-acid sequences of the variable regions of immunoglobulins and T-cell receptors create a vast variety of binding sites that are specific for different antigens, and thus for different pathogens. This is one of the hallmarks of an adaptive immune response. Antibodies secreted in response to a measles infection bind to measles virus but not to influenza virus (Figure 1.22); conversely, antibodies specific for influenza virus do not bind to measles virus.

The constant regions of antibodies contain binding sites for cell-surface receptors on phagocytes and inflammatory cells and also for complement proteins. The secreted antibody thus acts as a molecular adaptor or bridge. It binds to pathogens with one type of site and by another site to effector cells and molecules that destroy the pathogen. For immunoglobulins, but not T-cell receptors, there are several different types of constant region, known as **isotypes**, which confer different effector functions on the secreted antibody and can also target them to different anatomical sites. Every person can make antibodies of all isotypes.

1-9 The diversity of immunoglobulins and T-cell receptors is generated by gene rearrangement

The extensive diversity of variable regions in the antigen receptors of B and T cells is produced by a genetic mechanism unique to the immunoglobulin and T-cell receptor genes. In the genome, the variable regions of immunoglobulin and T-cell receptor chains are encoded in a series of separate gene segments. For a functional gene to be made, the appropriate gene segments need to be brought together by a physical rearrangement of the DNA (Figure 1.23). The immunoglobulin and T-cell receptor genes are quite unlike other human genes in this respect. Rearrangements at the heavy- and light-chain gene loci occur only in B cells, whereas rearrangements at the α- and β-chain gene loci

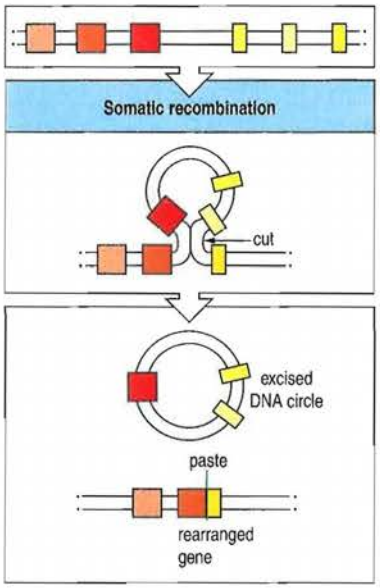


Figure 1.23 Gene rearrangement of the type occurring in immunoglobulin and T-cell receptor genes. In the unrearranged DNA there are three alternative 'red' segments and three alternative 'yellow' segments. A functional gene consists of one red segment joined to one yellow segment. This rearrangement is achieved by a process of 'cut and paste' in which the intervening DNA is removed as a circle. Different combinations of red and yellow segments can be brought together. In the example shown, the second red segment is brought together with the third yellow segment (counting from left to right), but other combinations of a red and a yellow segment would have been equally possible.

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occur only in T cells. Before any rearrangement, the immunoglobulin and T-cell receptor genes are said to be in the **germline configuration** because that is how they are present in germ cells: eggs and sperm. The process of gene rearrangement in B and T cells is called **somatic recombination** because it recombines gene segments and occurs in the soma—those cells of the body that are not germ cells.

Many alternative forms of each gene segment are present within the unrearranged DNA and they can be rearranged in numerous different combinations. This is the basis for the diversity of the variable regions. Further diversity is introduced as a result of imprecision in the enzymatic machinery used to cut and splice the DNA during gene rearrangement. Its effect is to introduce additional nucleotides into the joints between gene segments. These processes create sequence diversity in the individual chains of the immunoglobulin and T-cell receptor polypeptides. Additional diversity in the antigen-binding sites arises from the association of different heavy and light chains in immunoglobulins and of different α and β chains in T-cell receptors.

So far we have considered the mechanisms that produce diversity in lymphocyte receptors during their development in the primary lymphoid tissues. In B cells, but not in T cells, an additional mechanism for diversification is brought into play after activation by pathogens. Activated dividing B cells in secondary lymphoid tissues initiate a process of **somatic hypermutation**, which introduces nucleotide substitutions into the immunoglobulin heavy- and light-chain genes. Some of the variant immunoglobulins produced by somatic hypermutation bind the pathogen more tightly and the cells producing them are preferentially chosen to become antibody-secreting plasma cells.

1-10 B cells recognize intact pathogens, whereas T cells recognize pathogen-derived peptides bound to proteins of the major histocompatibility complex

Antibodies made by B cells are the soluble pathogen-binding molecules of adaptive immunity. By circulating in the body fluids, antibodies can bind to bacterial cells and intact viral particles in extracellular spaces, targeting them for phagocytosis. To fulfill this function, the binding sites of antibodies interact with intact components of the pathogen surface such as glycoproteins and proteoglycans. The epitopes bound by antibodies most commonly include carbohydrate groups, or clusters of amino acids on the protein surface, or combinations of the two.

Whereas antibodies bind directly to the native structures of biological macromolecules, T-cell receptors can bind only short peptides that have been assembled into a complex with a membrane glycoprotein called a **major histocompatibility complex (MHC) molecule** (Figure 1.24). T-cell antigens are therefore peptides. They are produced within human cells by the breakdown of pathogens or their protein products, a process called **antigen processing**.

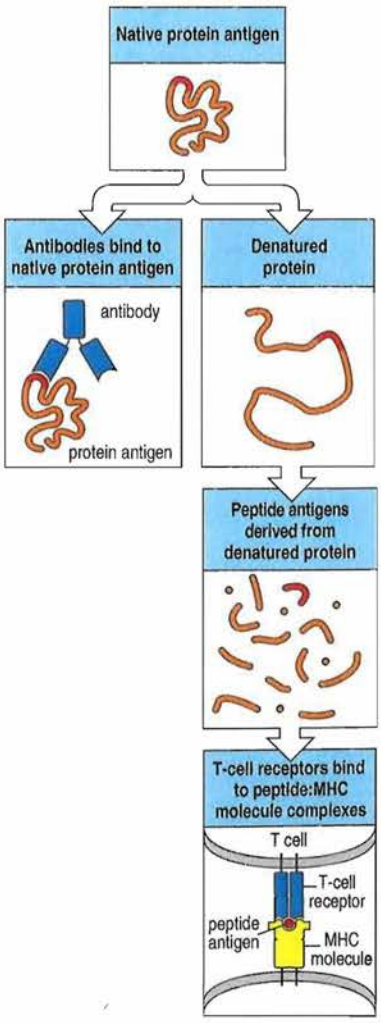


Figure 1.24 B cells recognize native proteins, whereas T cells recognize degraded proteins bound by major histocompatibility complex (MHC) molecules. The surface immunoglobulins of B cells and secreted antibodies bind to the native protein (left panel). To be recognized by a T-cell receptor, the protein must first be processed into peptide antigens by denaturation and proteolytic degradation (two center panels). Second, the peptide antigens must be bound to MHC molecules. Only then can the antigen be presented to the T cell (right panel).

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Assembly of the peptide:MHC molecule complex takes place in the cell where the antigen was processed and, once formed, the complex is transported to the cell surface. Here it is accessible to T-cell receptors on the surface of T lymphocytes. MHC molecules are therefore said to **present** antigens to T cells, and cells carrying antigen:MHC complexes are known as **antigen-presenting cells**.

There are two classes of MHC molecule: class I and class II (Figure 1.25). **MHC class I molecules** present peptide antigens derived from pathogens that replicate intracellularly, such as viruses and some bacteria, and whose proteins are present in the cytosol of the infected cell (Figure 1.26). MHC class I molecules present peptides to cytotoxic T cells that function by killing infected cells and are distinguished by the presence of the CD8 glycoprotein on their surfaces. As all nucleated cells can be infected by viruses, MHC class I molecules are present on almost all cell types.

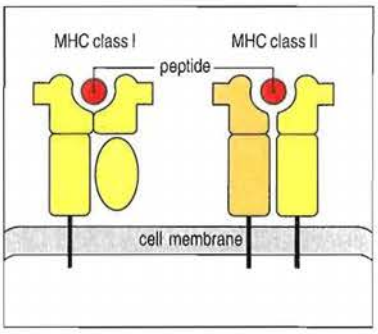


Figure 1.25 There are two types of MHC molecule, MHC class I and MHC class II.

MHC class II molecules present peptides obtained from pathogens and their products that are present in the extracellular milieu and have been taken up into the endocytic vesicles of phagocytic cells (Figure 1.27). The MHC class II molecules of dendritic cells present peptides to helper T cells, which then go on to activate B cells or macrophages. Helper T cells are distinguished by the presence of the CD4 glycoprotein on their surfaces. Because helper T cells interact only with immune system cells that are specialized in the uptake and processing of pathogens, MHC class II molecules are present on only a few cell types. These **professional antigen-presenting cells** are dendritic cells, macrophages, and B cells. The CD4 T cells that interact with macrophages or B cells are distinguished by the cytokines they secrete and are called T_H1 and T_H2 (where the 'H' stands for helper) cells respectively.

In human populations there are many different genetic variants of MHC molecules; this **polymorphism** is the chief cause of rejection of tissue transplants. When donors and recipients are of different MHC types, the immune system of the recipient makes a vigorous immune response against the donor's MHC molecules, which it perceives as 'foreign.' It was this phenomenon that led to the discovery of the MHC and its original naming as a complex of genes that governed the compatibility of tissues (Greek *histo*) on transplantation.

1-11 Clonal selection of B and T lymphocytes is the guiding principle of the adaptive immune response

A direct consequence of the mechanisms of immunoglobulin and T-cell receptor gene rearrangement is that each lymphocyte expresses immunoglobulin or T-cell receptors of a single specificity. As a population, however, the lymphocytes of the human immune system make millions of different immunoglobulins and T-cell receptors. This hierarchy, whereby individual lymphocytes make a single type of recognition molecule and diversity is

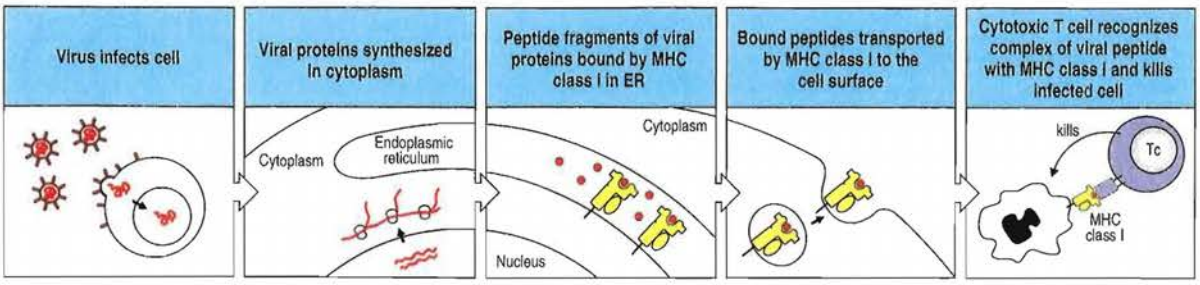


Figure 1.26 The MHC class I pathway presents antigens derived from intracellular infections to cytotoxic T cells. In virus-infected cells, new viral proteins are made on cellular ribosomes in the cytoplasm. Some of these proteins are degraded in the cytoplasm and the resultant peptides are transported into the endoplasmic reticulum (ER). MHC class I molecules bind peptides in the ER and then transport them to the infected cell's surface, where they can be recognized by a virus-specific cytotoxic T cell. The cytotoxic T cell kills the virus-infected cell.

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expressed at the level of lymphocyte populations, enables the lymphocyte response to be tailored toward particular pathogens. On infection, only the very small proportion of lymphocytes that have receptors that recognize the particular pathogen will be activated to divide and differentiate into effector cells. Consequently, each lymphocyte stimulated by the pathogen gives rise to a clonal population of cells all expressing the identical immunoglobulin or T-cell receptor. The process whereby pathogens select particular clones of lymphocyte for expansion is called **clonal selection** (see Figure 1.8). The use of a minute fraction of the total lymphocyte repertoire to respond to each pathogen ensures that the adaptive response is highly specific for the infection at hand.

Clonal selection is also an important influence on the development of B and T lymphocytes. During the maturation of B cells in the bone marrow, clonal selection is used to prevent the emergence of cells and antibodies that could attack the cells and tissues of the body. Clones of B cells with immunoglobulin receptors that bind strongly to the constituents of bone marrow tissue are signaled to die by programmed cell death, or **apoptosis**. During T-cell development in the thymus, clonal selection is more elaborate. First, there is positive selection, which saves those T cells that have receptors that interact well with a person's own MHC class I and class II variants (called self MHC) and discards all other T cells. Second, there is negative selection, which induces apoptosis in T cells that have receptors that bind too well to self MHC and

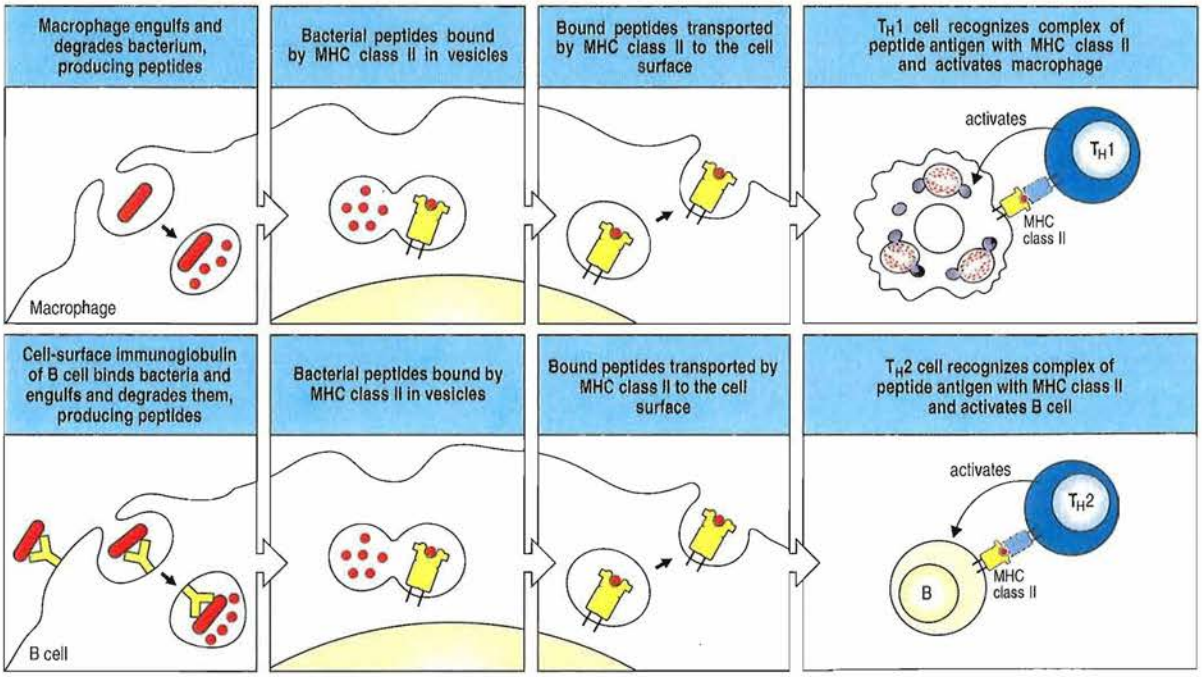


Figure 1.27 The MHC class II pathway presents antigens derived from extracellular infections to T_H1 cells. The upper panels show a macrophage phagocytosing extracellular bacteria and degrading their proteins in endocytic vesicles. MHC class II molecules bind peptides in endocytic vesicles and transport the peptides to the cell surface, where they are recognized by a T_H1 cell. Through cell contact and secretion of cytokines the T_H1 cell activates the macrophage. The lower panels show a B cell binding an extracellular bacterium with its cell-surface immunoglobulin. The bacterium bound to the immunoglobulin is endocytosed and its proteins are degraded into peptides. Some of these are bound by MHC class II molecules and transported to the cell surface, where they are recognized by a T_H2 cell. Through cell contact and secretion of cytokines the T_H2 cell activates the B cell. This will divide and differentiate into plasma cells secreting bacterium-specific antibody (not shown).

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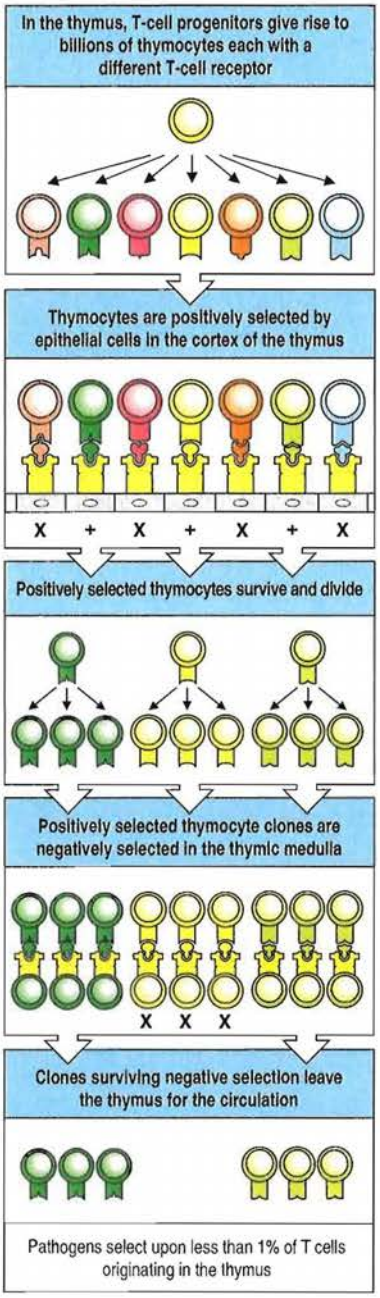


Figure 1.28 Stringent processes of positive and negative selection in the thymus determine the small fraction of T cells that mature and enter the peripheral circulation. Different clones of T cells are represented by different colors. Positive and negative selection are mediated by complexes of peptide with MHC class I and MHC class II molecules (the two classes of MHC molecule are not distinguished in this figure for simplicity). The epithelial cells in the cortex of the thymus that are involved in positive selection are not of hematopoietic origin; the cells of the thymic medulla that are involved in negative selection are of hematopoietic origin and include macrophages and thymocytes.

might thus attack normal healthy tissues. These selections are so stringent that fewer than 1% of thymocytes mature to become circulating T cells (Figure 1.28). The negative selection imposed on potentially self-reactive lymphocytes helps to render the circulating pool of mature B and T cells nonresponsive to, or **tolerant** of, the normal components of the body. The lymphocyte repertoire is thus said to be **self-tolerant**.

In the immune system, apoptosis is a common feature of pathways of cell development, activation and effector function. Individual cells are induced to commit a tidy form of cell suicide, where there is no generalized and messy tissue damage of the sort that cell death by necrosis produces. Membrane changes in cells undergoing apoptosis lead to their rapid phagocytosis by macrophages.

1-12 Extracellular pathogens and their toxins are eliminated by antibodies

Immunoglobulins are divided into five **classes** or isotypes, which differ in their heavy-chain constant regions and have specialized effector functions when secreted as antibodies. The isotypes are called **IgA**, **IgD**, **IgE**, **IgG**, and **IgM**, where Ig stands for immunoglobulin. Cell-surface IgM and IgD are the antigen receptors on circulating B cells that have yet to encounter antigen. IgM is always the first antibody to be secreted in the immune response (IgD antibody is also made but in negligible amounts). As the immune response matures, antibodies of other isotypes emerge. Immunity due to antibodies and their actions is often known as **humoral immunity**. This is because antibodies were first discovered circulating in body fluids (*humors*) such as blood and lymph.

IgM, IgA, and IgG are the main antibodies present in blood, lymph, and the fluid in connective tissues. Antibodies secreted into the blood from lymph nodes, spleen, and bone marrow circulate in the bloodstream. At sites of infection and inflammation, blood vessels dilate and become permeable, increasing the flow of fluid into the infected tissue and with it the supply of antibodies for binding to extracellular pathogens (bacteria and virus particles) and the protein toxins that many bacterial pathogens secrete. IgA is also made in the lymphoid tissues underlying mucosa and then selectively transported across the mucosal epithelium to bind extracellular pathogens and their toxins on the mucosal surfaces.

One way in which antibodies reduce infection is by binding tightly to a site on a pathogen so as to inhibit pathogen growth, replication, or interaction with human cells. This mechanism is called **neutralization**. For example, certain antibodies when bound to influenza virions prevent the virus from infecting human cells. Similarly, the lethal actions of bacterial toxins can be prevented by a bound antibody (Figure 1.29).

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The most important function of IgG antibodies is to facilitate the engulfment and destruction of extracellular microorganisms and toxins by phagocytes. Neutrophils and macrophages have cell-surface receptors that bind to the constant regions of the IgG heavy chains. A bacterium coated with IgG is more efficiently phagocytosed than an uncoated bacterium, a phenomenon called **opsonization** (see Figure 1.29). Opsonization can also be achieved by a coating of complement. There are no receptors on phagocytes for the constant region of IgM antibodies, but IgM bound to a pathogen's surface typically activates the complement system and the pathogen becomes coated with complement. This facilitates its uptake and phagocytosis by macrophages via another set of receptors that bind complement. A combination of IgG antibody and complement produces the greatest stimulation of phagocytosis because macrophage receptors for IgG constant regions and complement both participate in the process (see Figure 1.29).

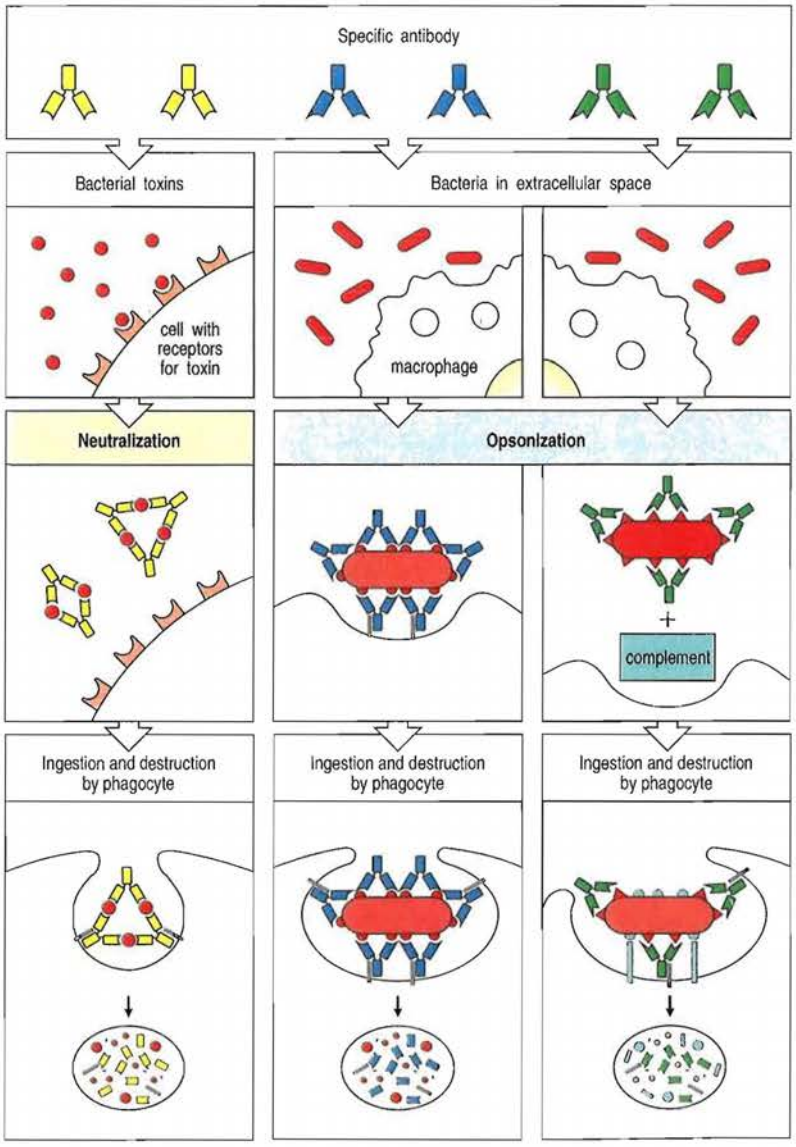


Figure 1.29 Mechanisms by which antibodies combat infection. Left panels: antibodies bind to a bacterial toxin and neutralize its toxic activity by preventing the toxin from interacting with its receptor on human cells. The complex of toxin and antibodies binds to macrophage receptors through the antibody's constant region. Finally the macrophage ingests and degrades the complex. Center panels: the opsonization of a bacterium by coating with antibody. When the bacterium is coated with IgG molecules, their constant regions point outward and can bind to the receptors on a macrophage, which then ingests and degrades the bacterium. Right panels: opsonization of a bacterium by a combination of antibody and complement. The bacterium is first coated with IgG molecules, which activate complement cleavage. Fragments of complement on the bacterial surface provide ligands for the complement receptor of macrophages. The combined interaction of macrophage receptors for complement and for the constant region of IgG makes for efficient phagocytosis.

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The constant regions of IgE antibodies bind tightly to receptors on the surface of mast cells; most of the IgE antibody in the body is in this form, rather than circulating freely in blood or lymph like IgM and IgG. In response to worms and other parasitic infestations, the IgE bound to mast cells triggers strong inflammatory reactions that are thought to help expel or destroy the parasites. However, in developed countries, where parasitic infections are not a major health problem, IgE is most often encountered as the antibody involved in unwanted allergic reactions to innocuous substances.

1-13 Adaptive immune responses generally give rise to long-lived immunological memory and protective immunity

Clonal selection by pathogens is the guiding principle of adaptive immunity and explains the features of immunity that perplexed physicians in the past. The severity of a first encounter with an infectious disease arises because the **primary immune response** is developed from very few lymphocytes; the time taken to expand their number provides an opportunity for the pathogen to establish an infection to the point of causing disease. The clones of lymphocytes produced in a primary response include long-lived **memory cells**, which can respond more quickly and forcefully to subsequent encounters with the same pathogen.

The potency of such **secondary immune responses** can be sufficient to repel the pathogen before there is any detectable symptom of disease. The individual therefore appears immune to that disease. The striking differences between a primary and secondary response are illustrated in Figure 1.30. The immunity due to a secondary immune response is absolutely specific for the pathogen that provoked the primary response. It is the difference between the primary and the secondary response that has made vaccination so successful in the prevention of disease. Figure 1.31 shows how the introduction and availability of vaccines against diphtheria, polio, and measles has dramatically reduced the incidence of these diseases. In the case of measles there is also a corresponding reduction of subacute sclerosing panencephalitis, an infrequent but fatal spasticity caused by persisting measles virus that becomes manifest 7–10 years after measles infection and usually affects children who were infected as infants. When vaccination programs are successful

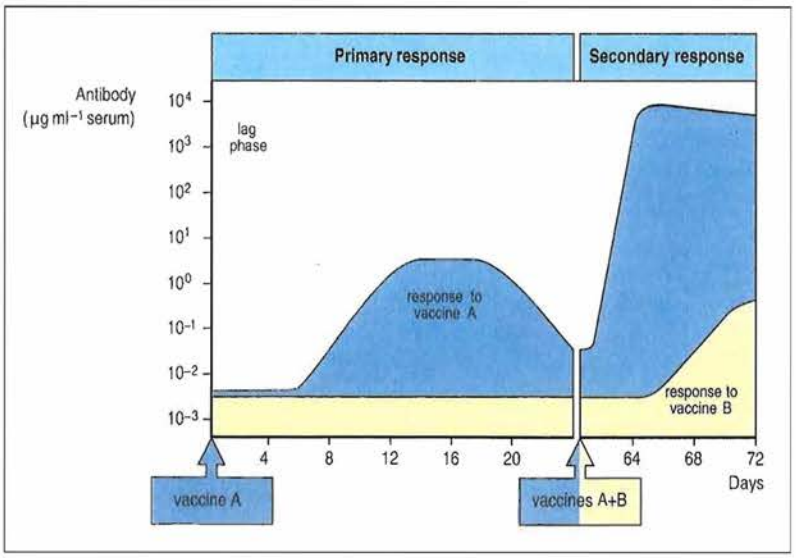


Figure 1.30 Comparison of a primary and secondary immune response. This diagram shows how the immune response develops during an experimental immunization of a laboratory animal. The response is measured in terms of the amount of pathogen-specific antibody present in the animal's blood serum, shown on the vertical axis, with time being shown on the horizontal axis. On the first day the animal is immunized with a vaccine against pathogen A. The levels of antibodies against pathogen A are shown in blue. The primary response reaches its maximum level 2 weeks after immunization. After the primary response has subsided, a second immunization with vaccine A on day 60 produces an immediate secondary response, which in 5 days is orders of magnitude greater than the primary response. In contrast, a vaccine against pathogen B, which was also given on day 60, produces a typical primary response to pathogen B as shown in yellow, demonstrating the specificity of the secondary response to vaccine A.

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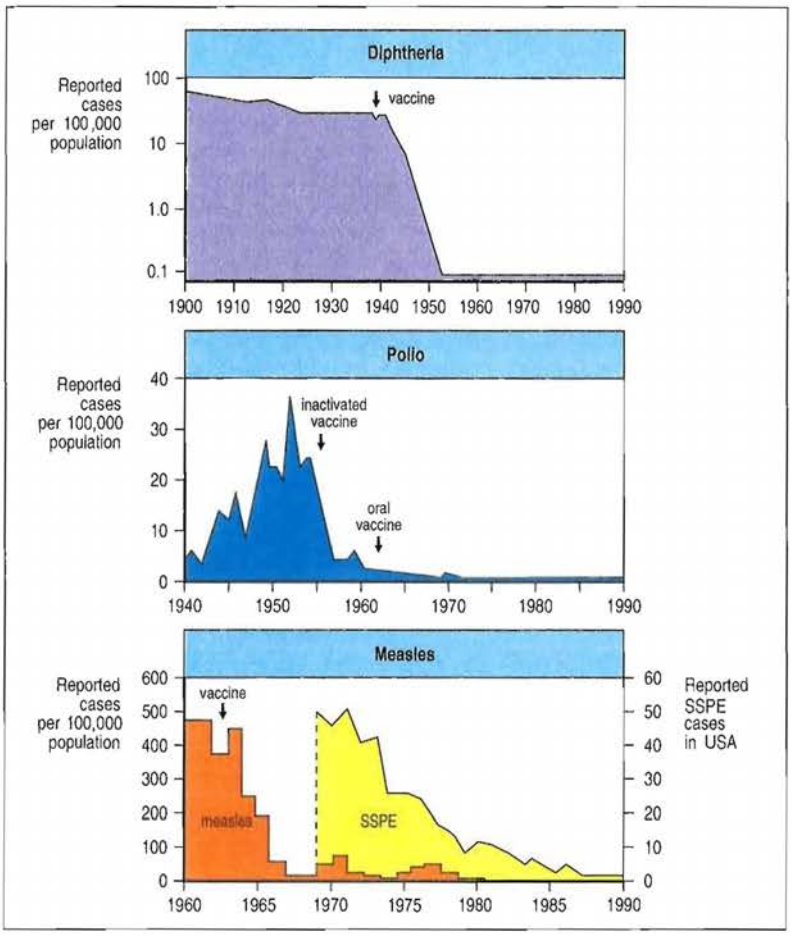


Figure 1.31 Successful vaccination campaigns. Diphtheria, poliomyelitis and measles have been virtually eliminated from the USA, as shown by these three graphs. The arrows indicate when the vaccination campaigns began. Subacute sclerosing panencephalitis (SSPE) is a brain disease that is a late consequence of measles infection for a minority of patients. Reduction of measles was paralleled by a reduction in SSPE 15 years later. Because these diseases have not been eradicated worldwide and the volume of international travel is so high, immunization must be maintained in much of the population to prevent disease recurrence.

to the point where the disease is unfamiliar to physicians and public alike, then concerns can arise with real or perceived side effects of a vaccine that affect a very small minority of those vaccinated. Such concerns lead to fewer children being vaccinated and to increasing occurrence of the disease.

1-14 The immune system can be compromised by inherited immunodeficiencies or by the actions of certain pathogens

When components of the immune system are either missing or do not work properly, this generally leads to increased susceptibility to microbial infection. One cause of defective immune responses is inherited mutations in genes encoding proteins that contribute to immunity. Most people who carry a mutant gene are healthy because their other, normal, copy of the gene provides sufficient functional protein; the small and unfortunate minority who carry two mutant copies of the gene lack the function encoded by that gene. Such deficiencies lead to varying degrees of failure of the immune system and a wide range of **immunodeficiency diseases**. In some of the immunodeficiency diseases, only one aspect of the immune response is affected, leading to susceptibility to particular kinds of infection; in others, adaptive immunity is completely absent, leading to a devastating vulnerability to all infections. These latter gene defects are rare, showing how vital is the protection normally afforded by the immune system. The discovery and study of immunodeficiency diseases has largely been the work of pediatricians, because such

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conditions usually show up early in life. Before the advent of antibiotics and, more recently, the possibility of bone marrow transplants and other replacement therapies, immunodeficiencies would usually have caused death in infancy.

Immunodeficiency states are caused not only by nonfunctional genes but also by pathogens that subvert the human immune system. An extreme example of an immunodeficiency due to disease is the **acquired immune deficiency syndrome** (**AIDS**), which is caused by infection with the **human immunodeficiency virus** (**HIV**). Although this disease has only been recognized by clinicians in the past 25 years, it is now at epidemic proportions, with more than 40 million people infected worldwide. HIV infects the CD4 T lymphocyte, a cell type essential for adaptive immunity. During the course of an extended infection, which can last for up to 20 years, the population of CD4 T cells gradually diminishes, eventually leading to collapse of the immune system. Patients with AIDS become increasingly susceptible to a range of infectious microorganisms, many of which rarely trouble uninfected people. Death usually results from the effects of one of these opportunistic infections rather than from the direct effects of HIV infection.

1-15 Unwanted effects of adaptive immunity cause allergy, autoimmune disease, and rejection of transplanted tissues

The sensitivity and force of the adaptive immune response, which are helpful traits in battling infection, can under some circumstances become misdirected toward innocuous materials or normal components of the human body. When this happens, various kinds of chronic and noninfectious disease emerge.

A state of **allergy** or **hypersensitivity** arises when antibodies of the IgE isotype are made against innocuous substances in the environment, for example foods, grass pollens, house dust, or dander from pets. Once such antibodies are made they circulate and become bound by their constant regions to mast cells throughout the body's tissues. On repeat exposure to the inducing antigen, called the **allergen**, it is bound by the IgE on mast cells. The combination of IgE and allergen on mast-cell surfaces triggers a response that produces symptoms (Figure 1.32) that sometimes lead to violent and life-threatening reactions such as those of asthma and systemic anaphylaxis. The damage and incapacitation caused by allergic reactions is greatly out of proportion to the threat posed by the allergen. Not all hypersensitivity diseases are caused by IgE; some are mediated by IgG, CD4 T_H1 cells or CD8 cytotoxic T cells.

Another class of noninfectious immunological disease is caused by chronic immune responses that gradually erode a target tissue. These conditions are called **autoimmune diseases** and result from **autoimmune responses** directed toward normal components of healthy human cells. In insulin-dependent diabetes, for example, the insulin-secreting β cells of the islets of Langerhans in the pancreas are the target of an autoimmune response. Because the human pancreas can produce insulin well in excess of what is normally needed, symptoms of disease do not show themselves until long after the start of the destructive response. Pancreatic β-cell destruction is believed to be due to B and T cells that respond to proteins made only in β cells. Other autoimmune diseases are rheumatoid arthritis, multiple sclerosis, myasthenia gravis, celiac disease, and Graves' disease. Autoimmune responses often seem to be by-products of a protective immune response made against an acute microbial infection. Once activated by pathogen-derived antigens, certain B or T cells cross-react with self components to which they were previously tolerant (Figure 1.33).

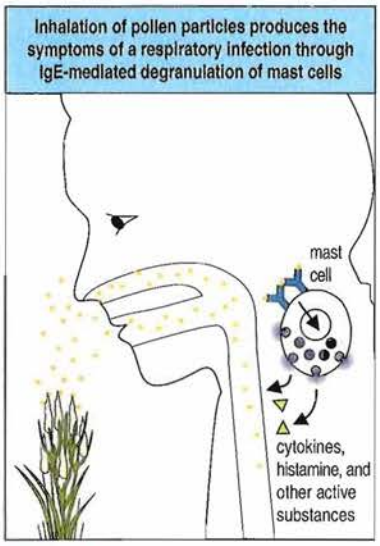


Figure 1.32 Many allergies are caused when antibodies of the IgE isotype are made against innocuous substances. The example shown here is of a pollen allergy, such as that to ragweed. For genetic and environmental reasons some members of the population make an antibody response to pollen that is of the IgE isotype. Once made, this antibody becomes bound to tissue mast cells, where it acts as a receptor. On subsequent exposure to pollen it binds to and cross-links the IgE, which is the signal for the mast cell to degranulate. The mast cell granules contain cytokines and other substances that induce inflammation, sneezing, and other symptoms that are usually associated with infectious diseases.

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Over the past century, hygiene, vaccination, and antimicrobial drugs have all served to reduce mortality from infectious disease in the developed countries, particularly for children. In these populations the treatment of chronic and malignant diseases has become of growing importance in medicine. For an increasing number of these diseases, the replacement of tissues or organs by **transplantation** is now being used to restore health and prolong life. A major factor limiting the benefits of transplantation are the tissue incompatibilities caused by the extensive polymorphism of MHC class I and class II genes in the human population. MHC-compatible donors can be found within families, but only for some of the patients needing a transplant. For patients seeking a transplant from an unrelated donor, the chances of finding one who is MHC identical are low. In practice, therefore, the majority of clinical transplants are performed across MHC differences that have the potential to stimulate strong immune responses. Currently, the only means of preventing transplant rejection is the prolonged use of drugs that suppress adaptive immunity. Although facilitating graft acceptance, such drugs have toxic effects and also render the transplant patient more vulnerable to infection and cancer.

Many of the unwanted actions of adaptive immunity in hypersensitivity diseases, autoimmune diseases, and in transplant recipients are due to chronic states of inflammation that facilitate a persistent response by inflammatory cells of adaptive immunity: CD4 T_H1 cells, CD8 cytotoxic T cells and mast cells charged with IgE (Figure 1.34). The overall incidence of hypersensitivity and autoimmune diseases is increasing in the richer countries, a trend that the **hygiene hypothesis** attributes to the widespread practice of hygiene, vaccination, and antibiotic therapy. It is argued that in growing up in this environment children's immune systems become less able to deal with proper infections and more likely to charge after imaginary foes in ways that, at best, are not helpful and, at worst, are literally self-defeating.

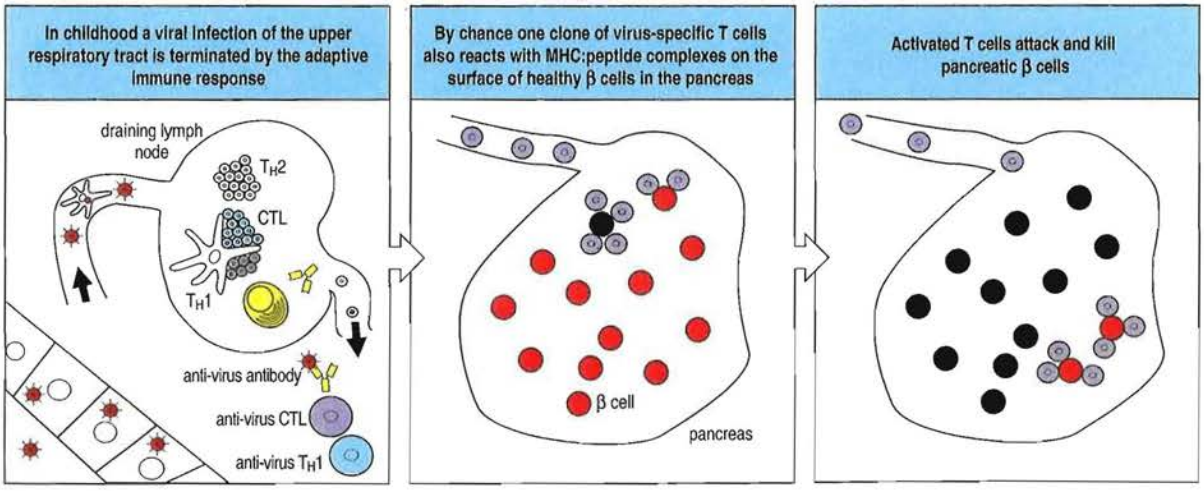


Figure 1.33 The adaptive immune response to a pathogen can sometimes trigger autoimmune reactions that eventually cause disease. Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease in which the insulin-producing β cells of the pancreas are slowly destroyed. Because the healthy pancreas can make much more insulin than is normally required, symptoms need not occur until many years after the start of the autoimmunity. A history of viral infection, for example with the Coxsackie viruses, has been correlated with IDDM and the autoimmunity may be a secondary consequence of the adaptive immune response to the virus. This could happen if a clone of T cells activated by the virus has, by chance, a T-cell receptor that reacts with an MHC:peptide complex present on the surface of pancreatic β cells. This MHC:peptide complex will then continually re-stimulate the T cells, which in turn persistently attack the β cells.

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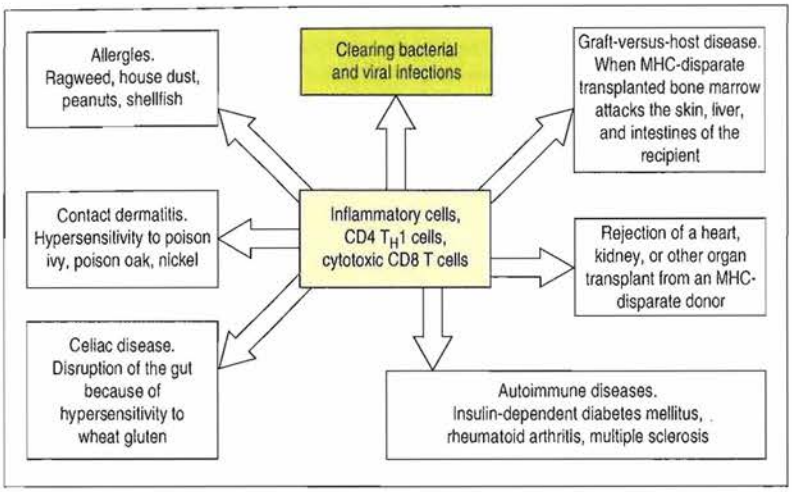


Figure 1.34 Inflammatory adaptive immune responses can be both beneficial and harmful. People in rich industrialized countries are experiencing an increase in inflammatory diseases caused by hypersensitivity to innocuous environmental antigens or by autoimmune destruction of healthy tissues of the body. Another situation in which inflammatory responses are unwanted is in transplantation of tissues and organs, which is increasingly used as therapy for inherited immunodeficiencies, malignant disease, and organ failure in the industrialized countries. In transplantation, the major complications are of inflammatory immune responses that either reject the transplant or attack the recipient's tissue to cause severe graft-versus-host disease. Many of these unwanted immune reactions are caused by inflammatory cells of adaptive immunity: T_H1 CD4 T cells, cytotoxic CD8 T cells, or mast cells charged with IgE.

Summary

The mechanisms of adaptive immunity are ones that improve pathogen recognition rather than pathogen destruction. Adaptive immune responses are due to the lymphocytes of the immune system, which collectively have the ability to recognize a vast array of antigens. Somatic gene rearrangement and somatic mutation in the genes for antigen receptors provide the lymphocyte population with a set of highly diverse antigen receptors—immunoglobulins on B lymphocytes, and T-cell receptors on T lymphocytes. An individual lymphocyte expresses receptors of a single and unique antigen-binding specificity. A pathogen therefore stimulates only the small subset of lymphocytes that express receptors for the pathogen antigens, focusing the adaptive immune response on that pathogen.

One major difference between B and T cells is the type of antigens they recognize. Whereas the immunoglobulin receptors of B cells bind whole molecules and intact pathogens, T-cell receptors recognize only short peptide antigens that are bound to major histocompatibility complex molecules on cell surfaces. A second major difference is that all B cells have the same general function of making immunoglobulins, whereas T cells can have distinctive functions. CD8 cytotoxic T cells kill cells infected with viruses by recognizing viral peptide antigens presented by MHC class I molecules; CD4 T cells help macrophages and B cells become activated by recognizing peptides presented by MHC class II molecules. The CD4 T cells are further subdivided into T_H1 cells, which help macrophages, and T_H2 cells, which help B cells. Before they can perform these functions all T cells must themselves be activated within a secondary lymphoid tissue through recognition of their specific antigen on a professional antigen-presenting cell.

B cells differentiate into plasma cells that make antibody, a secreted form of the cell-surface immunoglobulin. The production of antibodies by a B cell usually requires help from a CD4 T_H2 cell that responds to the same antigen and activates the B cell. Antibodies bind to extracellular pathogens and their toxins and deliver them to phagocytes and other effector cells for destruction. Immunity due to antibodies and their actions is known as humoral immunity. Whereas CD4 T_H2 cells work within secondary lymphoid tissues, CD8 cytotoxic T cells and CD4 T_H1 cells must travel to the infected site to perform their functions. Immunity that is due principally to CD8 cytotoxic T cells and/or CD4 T_H1 cells is known as cell-mediated immunity.

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When successful, an adaptive immune response terminates infection and provides long-lasting protective immunity against the pathogen that provoked the response. Failures to develop a successful response can arise from inherited deficiencies in the immune system or from the pathogen's ability to escape, avoid, or subvert the immune response. Such failures can lead to debilitating chronic infections or death. Another category of failure of adaptive immunity is the chronic disease caused by a misdirected response. Allergy is the consequence of a strong response to a harmless substance, whereas autoimmune disease is caused when the destructive potential of the immune system is directed at one or more of the body's own tissues. A further medical context for unwanted adaptive immunity is transplantation. Here the differences in MHC type between donor and recipient trigger an immune response that if left unchecked will reject the transplanted tissue.

Summary to Chapter 1

Throughout their evolutionary history, multicellular animals have been infected by microorganisms. In response, the animals evolved a series of defenses, which humans use today. Barriers confine microorganisms to the outer surfaces of the body, and when pathogens manage to breach the barriers they are sought out and destroyed by the immune system. In responding to microbial attack, the immune system starts with innate immunity, whose mechanisms are fast, fixed in their mode of action, and effective in stopping most infections at an early stage. The mechanisms of adaptive immunity are slow to start, but they eventually become powerful enough to terminate almost all of the infections that eluded innate immunity. The cells and molecules of innate immunity have counterparts in vertebrates and invertebrates. They identify common classes of pathogen and destroy them by using mechanisms that have stood the test of evolutionary time and are continuously useful. Mechanisms of adaptive immunity are found only in vertebrates and are distinguished by elaborate and highly specific ways of recognizing pathogens. They build on the mechanisms of innate immunity to provide a powerful response that is tailored to the pathogen at hand and can be rapidly reactivated on future challenge with that same pathogen, providing lifelong immunity to many common diseases. Adaptive immunity is an evolving process within a person's lifetime, in which each infection changes the makeup of that individual's lymphocyte population. These changes are neither inherited nor passed on but, during the course of a lifetime, they determine a person's fitness and their susceptibility to disease. The strategy of vaccination aims at circumventing the risk of a first infection, and in the twentieth century successful campaigns of vaccination were waged against several diseases that were once both familiar and feared. Through the use of vaccination and antimicrobial drugs, as well as better sanitation and nutrition, infectious disease has become a less common cause of death in many countries. In such situations, diseases due to unwanted activities of the immune system take on more importance. These include chronic conditions such as allergy and autoimmunity as well as the rejection of transplanted organs and tissues.

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Questions

Question 1–1

A. Identify the four classes of pathogen that provoke immune responses in our bodies.

B. Name two pathogens for which vaccines have been developed and discuss why vaccines provide protective immunity. (Refer to Figures 1.2, 1.3, and 1.31.)

Question 1–2

Discuss the properties of skin and mucosal surfaces that make them such important components of the body's first line of defense against invading pathogens. (Refer to Figure 1.4.)

Question 1–3

Phagocytosis of microbes or microbial constituents by macrophages is followed by macrophage activation and secretion of cytokines and other bioactive molecules. What are the main effects of these molecules? (Refer to Figure 1.6.)

Question 1–4

Define the Latin words *calor*, *dolor*, *rubor*, and *tumor* in the context of inflammation. (Refer to Figure 1.6.)

Question 1–5

What are the main differences between innate immunity and adaptive immunity? (Refer to Figure 1.7.)

Question 1–6

A. Identify the two major progenitor subsets of leukocytes.

B. Where do they originate in adults?

C. Name the white blood cells that differentiate from these two progenitor lineages. (Refer to Figures 1.9–1.11 and 1.15.)

Question 1–7

A. Name the primary lymphoid tissues in mammals and the main types of secondary lymphoid tissue.

B. What is the difference in function between primary and secondary lymphoid tissues and what are the principal events that take place in each?

C. In what way is the spleen different from the other secondary lymphoid tissues? (Refer to Figures 1.15 and 1.17–1.20.)

Question 1–8

A. What molecules do (i) B cells and (ii) T cells use to recognize foreign antigens?

B. Draw simple schematics of these molecules and contrast their different antigen-recognition properties.

C. Explain how antigen is prepared for T-cell recognition. (Refer to Figures 1.21, 1.24, 1.25.)

Question 1–9

Describe three distinct mechanisms by which antibodies eradicate infection. (Refer to Figure 1.29.)

Question 1–10

A. What is meant by the term immunodeficiency disease?

B. Why is an individual's ability to combat infection compromised if they have an immunodeficiency disease?

C. What causes an immunodeficiency disease?

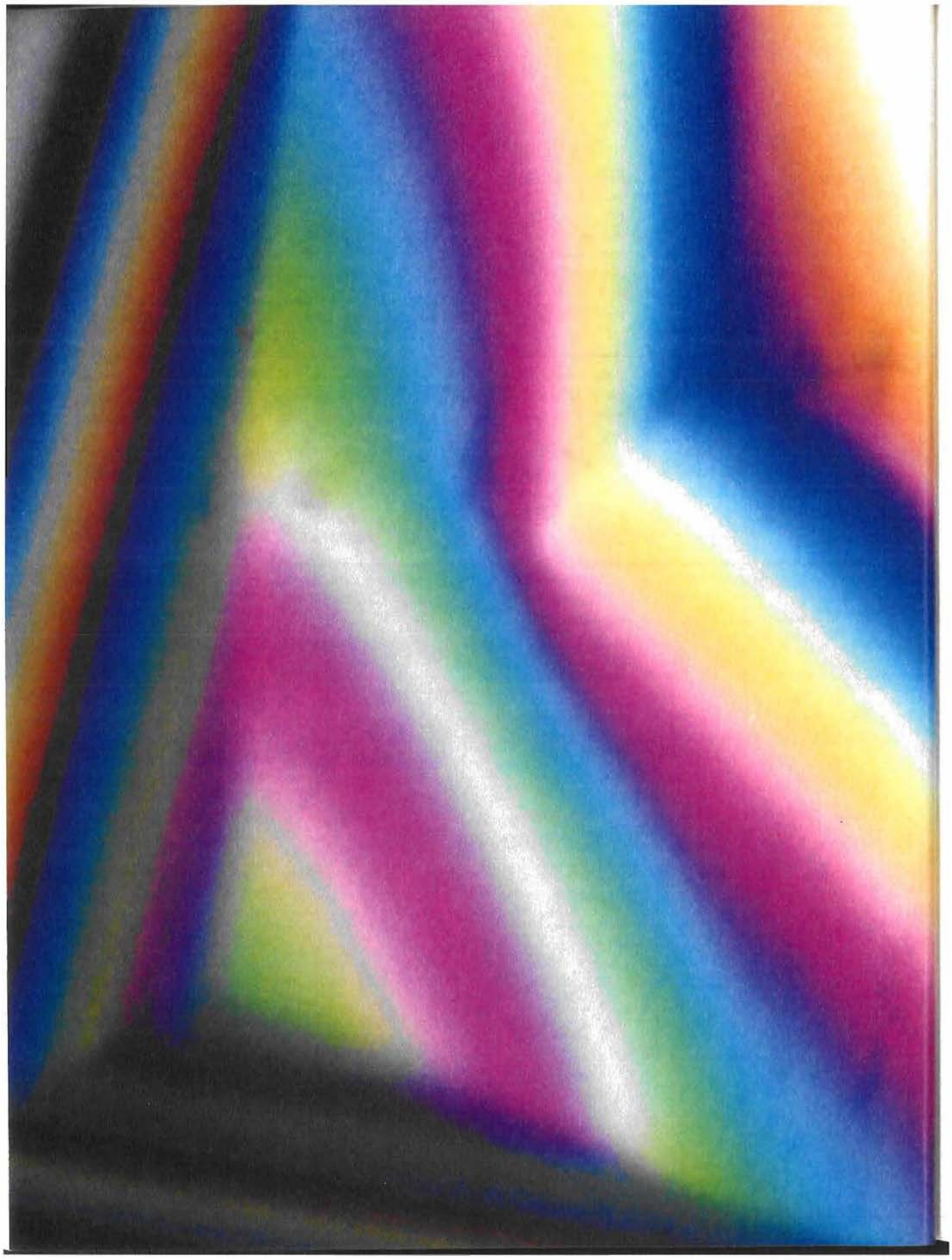
Question 1–11

Describe three types of unwanted and potentially harmful immune responses. (Refer to Figures 1.32, 1.33, and 1.34.)

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Chapter 2

Antibody Structure and the Generation of B-Cell Diversity



Antibodies are variable proteins produced by the B lymphocytes of the immune system in response to infection. They circulate as a major component of the plasma in blood and lymph. Their function is to bind to pathogenic microorganisms and their toxins when these are encountered in the extracellular spaces of the body. The molecules to which antibodies bind are, by definition, called **antigens**. All types of biological macromolecule serve as antigens, but proteins and carbohydrates are the most common. Binding of antibody to a pathogen can disable the pathogen and also renders it susceptible to destruction by other components of the immune system. Most vaccines provide their protection through stimulating the production of antibodies.

Individual antibodies are **specific**; each antibody can bind to only one or a very small number of different antigens. For this reason, immunity to the measles virus, for example, provides no protection against the influenza virus, nor does immunity to influenza protect against measles. The immune system of an individual has the potential to make antibodies against a vast number of different substances, paralleling the many different foreign antigens that a person is likely to be exposed to during their lifetime. Collectively, therefore, antibodies are diverse in their antigen-binding specificities; the total number of different specific antibodies that can be made by an individual is known as the **antibody repertoire** and it might be as high as 10^{16}.

Antibodies are the secreted form of proteins known more generally as **immunoglobulins** (**Ig**). Before it has encountered antigen, a mature B cell expresses immunoglobulin in a membrane-bound form that serves as the B cell's receptor for antigen. The development of B cells involves stages in which different components of the B-cell receptor are assembled. In this process each B cell becomes committed to expressing just one form of the B-cell receptor, but this form varies from one cell to the next. When antigen binds to this receptor, the B cell is stimulated to proliferate and to differentiate into **plasma cells**, which then secrete antibodies of the same specificity as that of the membrane-bound immunoglobulin (Figure 2.1). Antibody production is the single effector function of the B lymphocytes of the immune system.

The first part of this chapter describes the general structure of immunoglobulins, and the second part considers how immunoglobulin diversity is generated in developing B cells. After a mature B cell has encountered its specific antigen, changes are made in the specificity and effector functions of the antibodies that it produces; these are discussed in the final part of the chapter.

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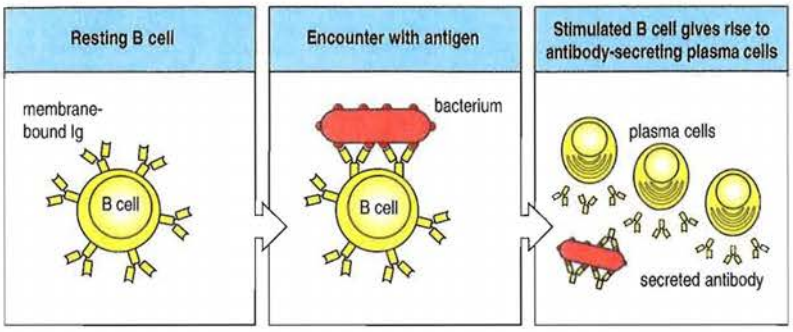


Figure 2.1 Plasma cells secrete antibody of the same antigen specificity as the membrane-bound immunoglobulin expressed by their B-cell precursor. A mature B cell expresses membrane-bound immunoglobulin (Ig) of a single antigen specificity. When a foreign antigen first binds to this immunoglobulin, the B cell is stimulated to proliferate. Its progeny differentiate into plasma cells that secrete antibody of the same specificity as the membrane-bound immunoglobulin.

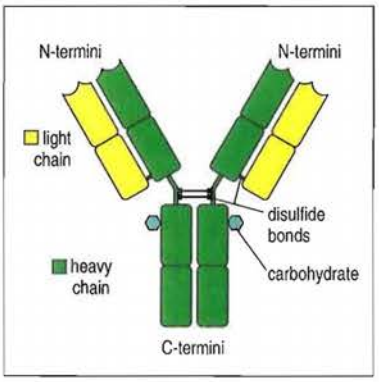
The structural basis of antibody diversity

The function of an antibody molecule in host defense is to recognize and bind its corresponding antigen, and to target the bound antigen to other components of the immune system. These then destroy the antigen or clear it from the body. Antigen binding and interaction with other immune system cells and molecules are carried out by different parts of the antibody molecule. One part is highly variable in that its amino-acid sequence differs considerably from antibody to antibody. This variable part contains the site of antigen binding and confers specificity to the antibody. The rest of the molecule is far less variable in its amino-acid sequence. This constant part of the antibody interacts with other immune system components.

Immunoglobulins are split into five classes—IgA, IgD, IgE, IgG, and IgM. They are distinguished on the basis of structural differences in the constant part of the molecule and have different effector functions. We shall first consider the general structure of immunoglobulins and then look more closely at the structural features that confer antigen specificity. IgG, the most abundant antibody in blood and lymph, will be used to illustrate the common structural features of immunoglobulins.

2-1 Antibodies are composed of polypeptides with variable and constant regions

Antibodies are glycoproteins that are built from a basic unit of four polypeptide chains. This unit consists of two identical **heavy chains** (**H chains**) and two identical, smaller, **light chains** (**L chains**), which are assembled into a structure that looks like the letter Y (Figure 2.2, top panel). An IgG molecule has a molecular weight of about 150 kDa, to which each heavy chain contributes approximately 50 kDa and each light chain approximately 25 kDa. Each arm of the Y is made up of a complete light chain paired with the amino-terminal (N-terminal) part of a heavy chain, covalently linked by a disulfide bond. The stem of the Y consists of the paired carboxy-terminal (C-terminal)



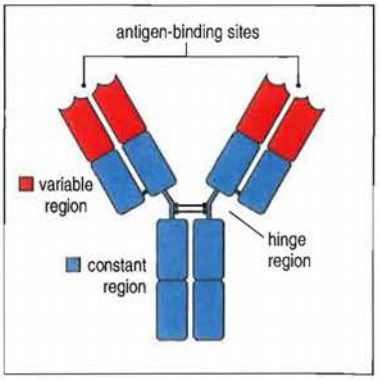


Figure 2.2 The immunoglobulin G (IgG) molecule. As shown in the top panel, each IgG molecule is made up of two identical heavy chains (green) and two identical light chains (yellow). Carbohydrate (turquoise) is attached to the heavy chains. The lower panel shows the location of the variable (V) and constant (C) regions in the IgG molecule. The amino-terminal regions (red) of the heavy and light chains are variable in sequence from one IgG molecule to another; the remaining regions are constant in sequence (blue). The carbohydrate is omitted from this panel and from most subsequent figures for simplicity. In IgG a flexible hinge region is located between the two arms and the stem of the Y.

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portions of the two heavy chains. The two heavy chains are also linked to each other by disulfide bonds.

The polypeptide chains of different antibodies vary greatly in amino-acid sequence, and the sequence differences are concentrated in the amino-terminal region of each type of chain; this is known as the **variable region** or **V region**. This variability is the reason for the great diversity of antigen-binding specificities among antibodies because the paired V regions of a heavy and a light chain form the **antigen-binding site** (Figure 2.2, bottom panel). Every Y-shaped antibody molecule, therefore, has two identical antigen-binding sites, one at the end of each arm. The remaining parts of the light chain and the heavy chain have much more limited variation in amino-acid sequence between different antibodies and are, therefore, known as the **constant regions** or **C regions**.

In IgG, a relatively unstructured portion in the middle of the heavy chain forms a flexible hinge region at which the molecule can be cleaved to produce defined antibody fragments. These fragments were instrumental in providing information about antibody structure and function because they can be used for separate analysis of the antigen-binding and effector functions of the molecule. Digestion with the plant protease papain produces three fragments, corresponding to the two arms and the stem (Figure 2.3). The fragments corresponding to the arms are called **Fab** (**F**ragment **a**ntigen **b**inding) because they bind antigen. The fragment corresponding to the stem is called **Fc** (**F**ragment **c**rystallizable) because it was seen to crystallize in the first experiments

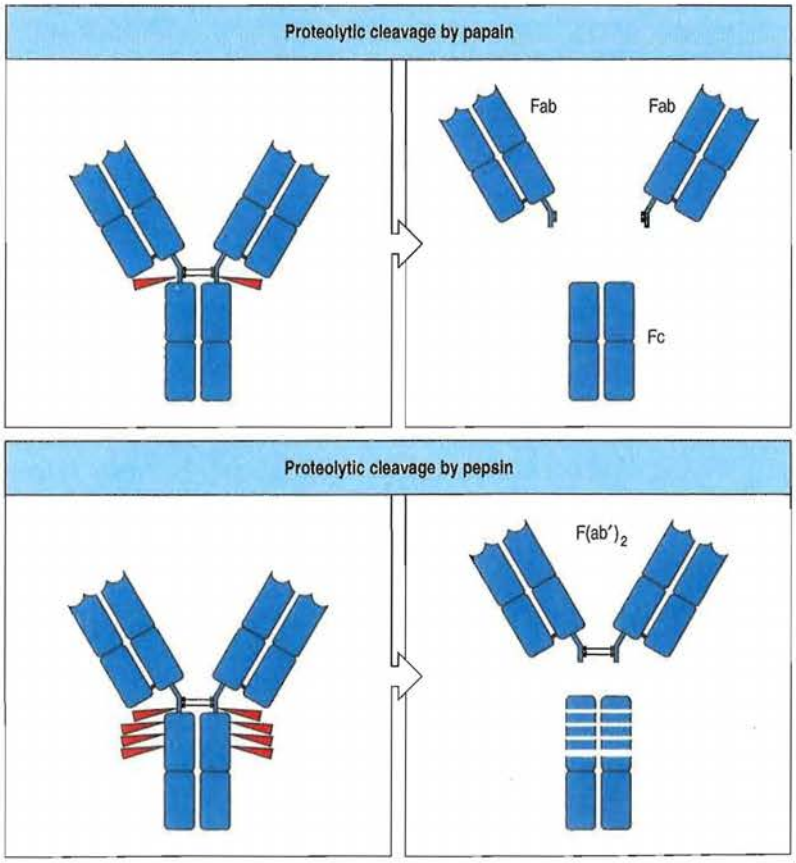


Figure 2.3 The Y-shaped immunoglobulin molecule can be dissected by using proteases. The protease papain cleaves the IgG molecule into three pieces: two Fab fragments and one Fc fragment (upper panels). The protease pepsin cleaves IgG to yield one $F(ab')_2$ fragment. The Fc fragment is not stable to pepsin degradation and is broken into a number of smaller pieces (lower panels). Red arrowheads denote the sites of protease attack.

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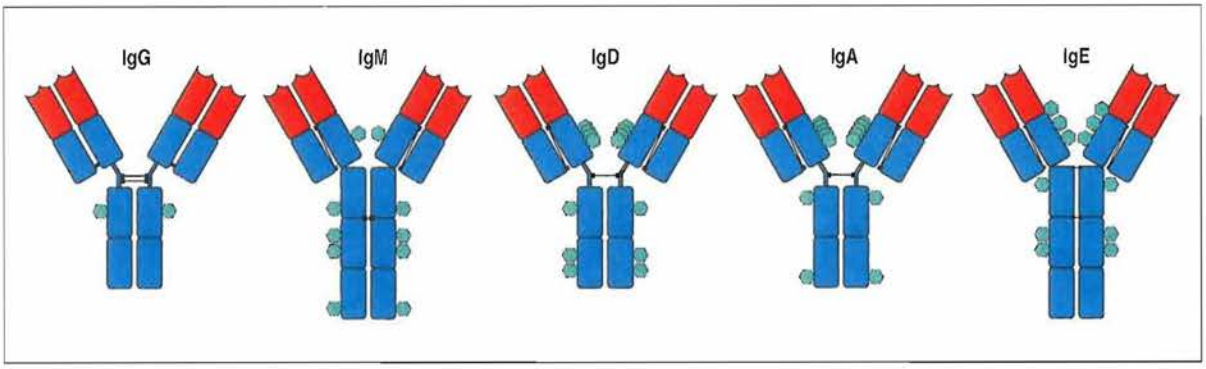


Figure 2.4 The structural organization of the human immunoglobulin isotypes. In particular, note the differences in length of the heavy-chain C regions, the locations of the disulfide bonds linking the chains, and the hinge region present in IgG, IgA, and IgD but not in IgM and IgE. The isotypes also differ in the distribution of *N*-linked carbohydrate groups, as shown in turquoise. All these immunoglobulins occur as monomers in their membrane-bound form. In their soluble, secreted form IgD, IgE and IgG are always monomers, IgA forms monomers and dimers and IgM forms only pentamers.

of this sort. The stem of a complete antibody molecule is, thus, often known as the **Fc region** or **Fc piece**, and the arms as Fab. Digestion of IgG with the gut protease pepsin produces a different fragment, **F(ab′)$_2$**, in which the two arms remain linked by disulfide bonds between the heavy chains.

Differences in the heavy-chain C regions define five main **isotypes** or **classes** of immunoglobulin, which have different functions in the immune response. They are **immunoglobulin A (IgA)**, **immunoglobulin D (IgD)**, **immunoglobulin E (IgE)**, **immunoglobulin G (IgG)**, and **immunoglobulin M (IgM)** (Figure 2.4). Their heavy chains are denoted by the corresponding lower-case Greek letter (α, δ, ε, γ, and μ, respectively).

The light chain has only two isotypes or classes, which are termed **kappa (κ)** and **lambda (λ)**. No functional difference has been found between antibodies carrying κ light chains and those carrying λ light chains; light chains of both isotypes are found associated with all the heavy-chain isotypes. Each antibody, however, contains either κ or λ light chains, not both. The relative abundances of κ and λ light chains vary with the species of animal. In humans, two-thirds of the antibody molecules contain κ chains and one-third have λ chains.

2-2 Immunoglobulin chains are folded into compact and stable protein domains

Antibodies function in extracellular environments in the presence of infection, where they can encounter variations in pH, salt concentration, proteolytic enzymes, and other potentially destabilizing factors. Their structure, however, helps them to withstand such harsh conditions. Heavy and light chains each consist of a series of similar sequence motifs; a single motif is about 100–110 amino acids long and folds up into a compact and exceptionally stable protein domain called an **immunoglobulin domain**. Each immunoglobulin chain is composed of a linear series of these domains.

The V region at the amino-terminal end of each heavy or light chain is composed of a single **variable domain (V domain)**: V_H in the heavy chain and V_L in the light chain. A V_H and a V_L domain together form an antigen-binding site. The other domains have little or no sequence diversity within a particular isotype and are termed the **constant domains (C domains)**, which make up the C regions. The constant region of a light chain is composed of a single C_L domain, whereas the constant region of a heavy chain is composed of three or four C domains, depending on the isotype. The γ heavy chains of IgG have three domains—C_H1, C_H2, and C_H3. Some other isotypes have four C domains (see Figure 2.4). In the complete IgG molecule the pairing of the four polypeptide chains produces three globular regions, corresponding to the

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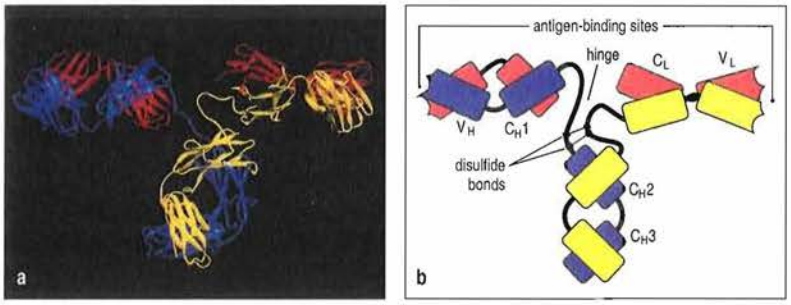


Figure 2.5 The heavy and light chains of an immunoglobulin molecule are made from a series of similar protein domains. Panel a shows a ribbon diagram tracing the course of the polypeptide backbones of chains in an IgG molecule. The heavy chains are shown in yellow and purple, the light chains in red. The molecule is composed of three similarly sized globular portions, which correspond to the Fc stem and the two Fab arms. Each of these globular portions is made up of four immunoglobulin domains. The schematic diagram in panel b shows the domain structure of an IgG molecule. The light chain (red) has one variable domain (V_L) and one constant domain (C_L). The γ heavy chain (one yellow and one purple) has a variable domain (V_H) and three constant domains (C_H1, C_H2, and C_H3). Panel a courtesy of L. Harris.

two Fab arms and the Fc stem, respectively, each of which is composed of four immunoglobulin domains (Figure 2.5).

The structure of a single immunoglobulin domain can be compared to a bulging sandwich in which two β sheets (the slices of bread) are held together by strong hydrophobic interactions between their constituent amino acid side chains (the filling). The structure is stabilized by a disulfide bond between the two β sheets. The adjacent strands within the β sheets are connected by loops of the polypeptide chain. This arrangement of β sheets provides the stable structural framework of all immunoglobulin domains, whereas the amino-acid sequence of the loops can be varied to confer different binding properties on the domain. The structural similarities and differences between the C and V domains are illustrated for the light chain in Figure 2.6.

Although immunoglobulin domains were first discovered in antibodies, very similar domains, known generally as **immunoglobulin-like domains**, have subsequently been found in many other proteins, and are particularly common in cell-surface and secreted proteins of the immune system. Such proteins collectively form the **immunoglobulin superfamily**.

2-3 An antigen-binding site is formed from the hypervariable regions of a heavy-chain and a light-chain V domain

A comparison of the V domains of heavy and light chains from different antibody molecules shows that the differences in amino-acid sequence are concentrated within particular regions called **hypervariable regions** (**HV**), which

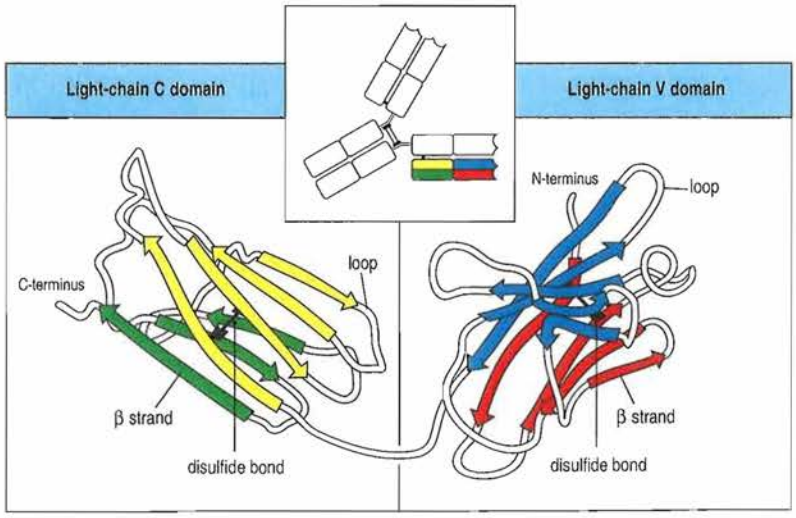


Figure 2.6 The three-dimensional structure of immunoglobulin C and V domains. An individual light chain is folded into a single C domain (left panel) and a single V domain (right panel). The inset shows the location of this light chain in an IgG molecule. The folding of the polypeptide chain backbone is depicted as ribbon diagrams in which the thick arrows correspond to the parts of the chain that form the β strands of the β sheet. The arrows point from the amino terminus of the chain to the carboxy terminus. Adjacent strands in each β sheet run in opposite directions (antiparallel), as shown by the arrows, and are connected by loops. In a C domain there are four β strands in the upper β sheet (yellow) and three in the lower sheet (green). In a V domain there are five strands in the upper sheet (blue) and four in the lower (red).

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are flanked by much less variable **framework regions** (Figure 2.7, top panel). Three hypervariable regions are found in each V domain. When mapped onto the folded structure of a V domain, the hypervariable regions locate to three of the loops that are exposed at the end of the domain farthest from the constant region. The framework regions correspond to the β strands and the remaining loops. This relationship is illustrated for the light-chain V domain in Figure 2.7 (center panel). Thus, the structure of the immunoglobulin domain provides the capacity for localized variability within a structurally constant framework.

The pairing of a heavy and a light chain in an antibody molecule brings together the hypervariable loops from each V domain to create a composite hypervariable surface, which forms the antigen-binding site at the tip of each Fab arm (see Figure 2.7, bottom panel). The differences in the loops between different antibodies create both the specificity of antigen-binding sites and their diversity. The hypervariable loops are also called **complementarity-determining regions** (**CDRs**) because they provide a binding surface that is complementary to that of the antigen.

2-4 Antigen-binding sites vary in shape and physical properties

The function of antibodies is to bind to microorganisms and facilitate their destruction or ejection from the body. The antibodies that are most effective against infection are generally those that bind to the exposed and accessible molecules that make up the surface of a pathogen. The part of an antigen to which an antibody binds is called an **antigenic determinant** or an **epitope** (Figure 2.8). In nature, these structures are usually either carbohydrate or protein, or both, because the surface molecules of pathogens are commonly glycoproteins, polysaccharides, glycolipids, and peptidoglycans. Complex macromolecules such as these will usually contain several different epitopes, each of which can be bound by a different antibody. Individual epitopes are typically composed of a cluster of amino acids or part of a polysaccharide chain. Any antigen that contains more than one epitope, or more than one copy of the same epitope, is known as a **multivalent** antigen (Figure 2.9).

Antibodies can be made against a much wider range of chemical structures than proteins and carbohydrates. Such antibodies are, however, more commonly implicated in allergic reactions and autoimmune diseases than in

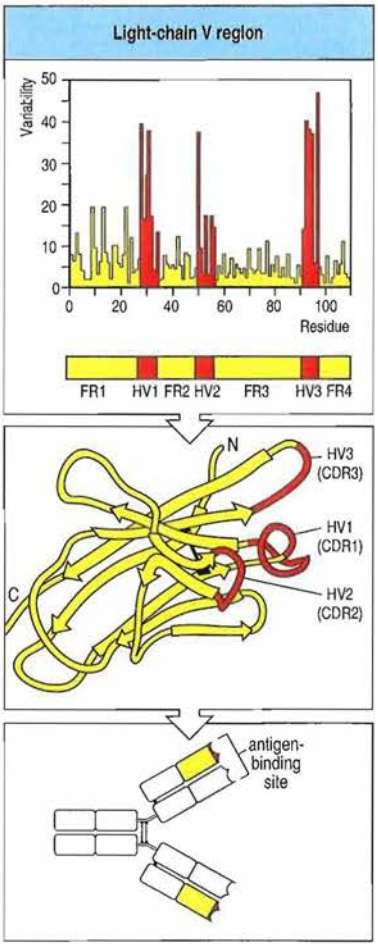


Figure 2.7 The hypervariable regions of antibody V domains lie in discrete loops at one end of the domain structure. The top panel shows the variability plot for the 110 positions within the amino-acid sequence of a light-chain V domain. It is obtained from comparison of many light-chain sequences. Variability is the ratio of the number of different amino acids found at a position and the frequency of the most common amino acid at that position. The maximum value possible for the variability is 400, the square of 20, the number of different amino acids found in antibodies. The minimum value for the variability is 1. Three hypervariable regions (HV1, HV2, and HV3) can be discerned (shown in red) flanked by four framework regions (FR1, FR2, FR3, and FR4) (shown in yellow). The center panel shows the correspondence of the hypervariable regions to three loops at the end of the V domain farthest from the C region. The location of hypervariable regions in the heavy-chain V domain is similar (not shown). The hypervariable loops contribute much of the antigen specificity of the antigen-binding site located at the tip of each arm of the antibody molecule. Hypervariable regions are also known as complementarity-determining regions: CDR1, CDR2, and CDR3.

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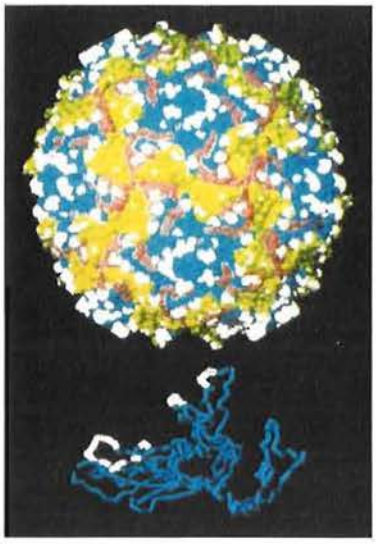


Figure 2.8 Epitopes for antibodies are exposed on the surface of pathogens. A spherical poliovirus particle is depcited. Its coat is an array of three different proteins (indicated in yellow, blue, and pink). In the lower part of the figure one of the viral proteins, VP1 (blue), is shown separately, but folded as in the virus particle. VP1 contains several epitopes (white). They are located at the surface of the protein and are exposed on the surface of the virus particle. Photograph courtesy of D. Filman and J.M. Hogle.

protective immunity against infection. Antibodies specific for DNA, for example, are characteristic of the autoimmune disease systemic lupus erythematosus (SLE), and allergies can be caused by antibodies against small organic molecules, such as antibiotics and other drugs.

The antigen-binding sites of antibodies vary according to the size and shape of the epitope. Antibodies that bind to the end of a polysaccharide chain, or to a small molecule such as vitamin K_1, use a deep pocket formed between the heavy- and light-chain V domains (Figure 2.10, left panel). In such cases not all the CDRs make contact with the antigen. By contrast, antibodies that bind to several of adjacent sugars within a polysaccharide, or amino acids within a polypeptide, use longer, shallower clefts formed by all the opposing CDRs of the V_H and V_L domains (see Figure 2.10, center panel). Epitopes of this kind, in which the antibody binds to parts of a molecule that are adjacent in the linear sequence, are called **linear epitopes** (Figure 2.11, left panel).

Another kind of epitope is formed by parts of a protein that are separated in the amino-acid sequence but are brought together in the folded protein. These are called **conformational** or **discontinuous epitopes** (see Figure 2.11, right panel). Antibodies that bind to complete proteins often make contact with a much larger area of the protein surface than can be accommodated within a groove between the CDRs of the V_H and V_L domains. These antibodies might have no discernible binding groove but interact with the antigen by using a surface (in area usually 700–900 $Å^2$) formed by the CDRs and which can even extend beyond them (see Figure 2.10, right panel).

The binding of antigens to antibodies is based solely on noncovalent forces—electrostatic forces, hydrogen bonds, van der Waals forces, and hydrophobic interactions. The antigen-binding sites of antibodies are unusually rich in aromatic amino acids, which can participate in many van der Waals and

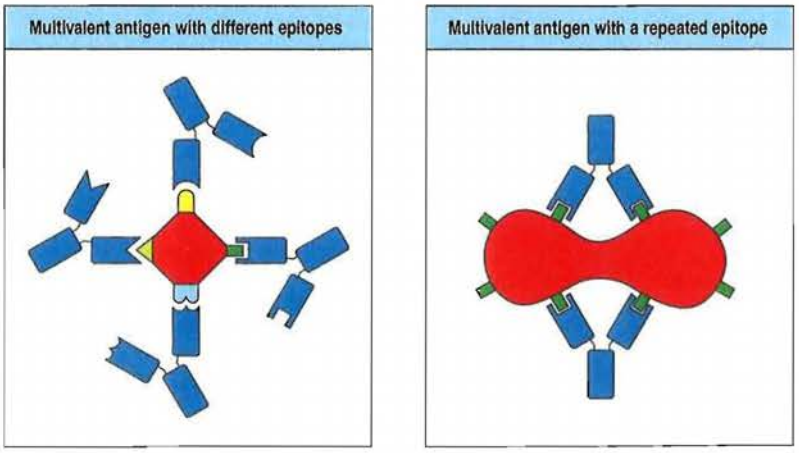


Figure 2.9 Two kinds of multivalent antigen.

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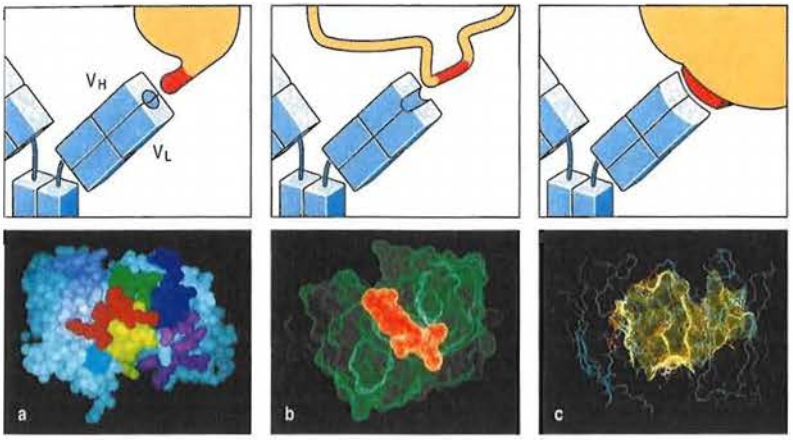


Figure 2.10 Epitopes can bind to pockets, grooves or extended surfaces in antibody-binding sites. The top row shows schematic representations of antibodies binding to three different types of epitope. The antibodies are shown in blue, the antigens in orange and the epitopes in red. On the left is a small compact epitope binding to a pocket, in the middle is an epitope consisting of a relatively unfolded part of a polypeptide chain binding to a shallow groove, and on the right is an epitope with extended surface binding to a similarly sized but complementary surface in an antibody-binding site. The crystallographic images in the bottom row are of Fab fragments binding to antigens in ways corresponding to those depicted in the top row. Panel a shows a peptide epitope (red) bound to a pocket formed by the CDR loops (colored), panel b shows a peptide epitope (red) bound to a groove formed between the two V domains (green), and panel c shows Fab binding to a surface epitope of the protein antigen, lysozyme. Here, the surface contour of the lysozyme (yellow dots) is superimposed on the antigen-binding site of the Fab. The peptide backbone of the Fab is shown in blue and the amino acids that contact lysozyme are shown in red. Photographs courtesy of I.A. Wilson, R.L. Stanfield, and S. Sheriff.

hydrophobic interactions. The better the general fit between the interacting surfaces of antigen and antibody, the stronger are the bonds formed by these short-range forces. Thus, small differences in shape and chemical properties of the binding site can give several antibodies specificity for the same epitope, but they bind to it with different binding strengths or **affinities**. Binding caused by van der Waals forces and hydrophobic interactions is complemented by the formation of electrostatic interactions (salt bridges) and hydrogen bonds between particular chemical groups on the antigen and particular amino-acid residues of the antibody.

In the immune response, the effective antibodies are those that bind tightly to an antigen and do not let go. This behavior contrasts with that of enzymes, which bind a substrate, chemically change the substrate's structure, and then release it. However, the same set of 20 amino acids is used to make enzymes and antibodies and, after intensive searching, some antibodies have been found that catalyze chemical reactions involving the antigen they bind. The application of such **catalytic antibodies** in medicine is being explored. A possible use for a catalytic antibody would be to convert a toxic chemical within the body into an innocuous product.

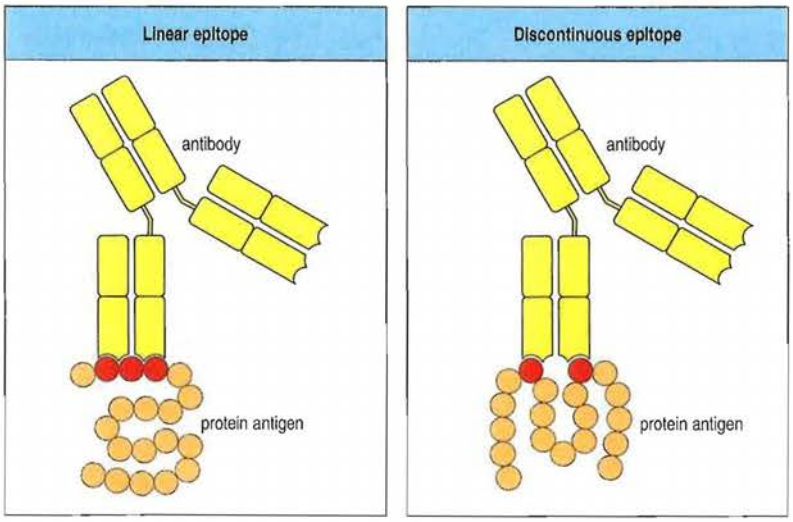


Figure 2.11 Linear and discontinuous epitopes. A linear epitope of a protein antigen is formed from contiguous amino acids. A discontinuous epitope is formed from amino acids from different parts of the polypeptide that are brought together when the chain folds.

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2-5 Monoclonal antibodies are produced from a clone of antibody-producing cells

The specificity and strength of an antibody for its antigen make antibodies useful reagents for detecting and quantifying a particular antigen. They are used in this way in a range of clinical and laboratory tests and in biological research generally. The traditional method for making antibodies of desired specificity is to immunize animals with the appropriate antigen and then prepare **antisera** from their blood. The specificity and quality of such antisera is highly dependent upon the purity of the immunizing antigen preparation because antibodies will be made against all the foreign components it contains. Consequently, antisera specific for a single protein or carbohydrate can only be made when the antigen is already available in a purified form.

A more modern method for making antibodies does not require a purified antigen. In this method, B cells are isolated from the immunized animals, usually mice, and immortalized by fusion with a tumor cell to form **hybridoma** cell lines that grow and produce antibodies indefinitely. The individual hybridoma cells are then separated from each other and the ones making antibodies of the desired specificity are identified and selected for further propagation. The antibodies made by a hybridoma cell line are all identical and are thus called **monoclonal antibodies** (Figure 2.12).

Since the invention of hybridomas in the 1970s, monoclonal antibodies have replaced antisera in many clinical assays. They also have been used to identify numerous previously unknown surface proteins of cells of the immune system. Monoclonal antibodies made in different laboratories are periodically compared at international workshops and assigned to different clusters of differentiation (CD), each of which represents a different molecule or a family of closely related molecules. More than 240 molecules have been numbered so far in the CD series. The reactions of monoclonal antibodies with different types of cell are measured by the technique of flow cytometry (Figure 2.13).

In medicine, flow cytometry is used to analyze the cell populations in peripheral blood and assess for perturbations caused by disease. For example, occasionally some children with an inherited genetic defect have no B cells and make no antibodies. For a period of around 1 year after birth and while nursing, these children are protected from infection by antibodies provided by their mothers, but this declines with age. They then become particularly susceptible to infections with encapsulated bacteria such as *Streptococcus pneumoniae* or *Haemophilus influenzae*, which, if left untreated with antibiotics, would eventually lead to death. Knowledge that a person is B-cell deficient allows infections to be prevented by regular injections of antibodies purified from the pooled sera of healthy blood donors.

Monoclonal antibodies are also used as therapeutic agents, for example, in preventing the imminent rejection of a transplanted kidney. Unfortunately,

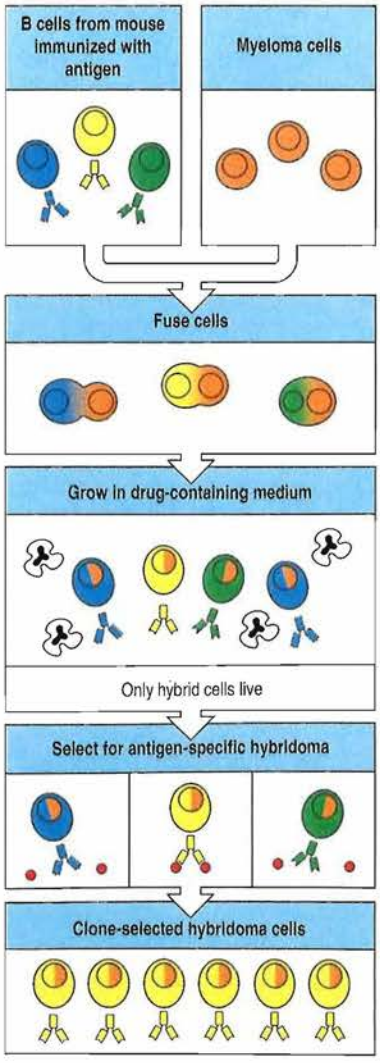


Figure 2.12 Production of a monoclonal antibody. Lymphocytes from a mouse immunized with the antigen are fused with myeloma cells using polyethyleneglycol. The cells are then grown in the presence of drugs that kill myeloma cells but permit the growth of hybridoma cells. Individual cultures of hybridomas are tested to determine if they make the desired antibody. The cells are then cloned to produce a homogeneous culture of cells making a monoclonal antibody. Myelomas are tumors of plasma cells; those used to make hybridomas were selected not to express heavy and light chains. Thus, hybridomas only express the antibody made by the B-cell fusion partner.

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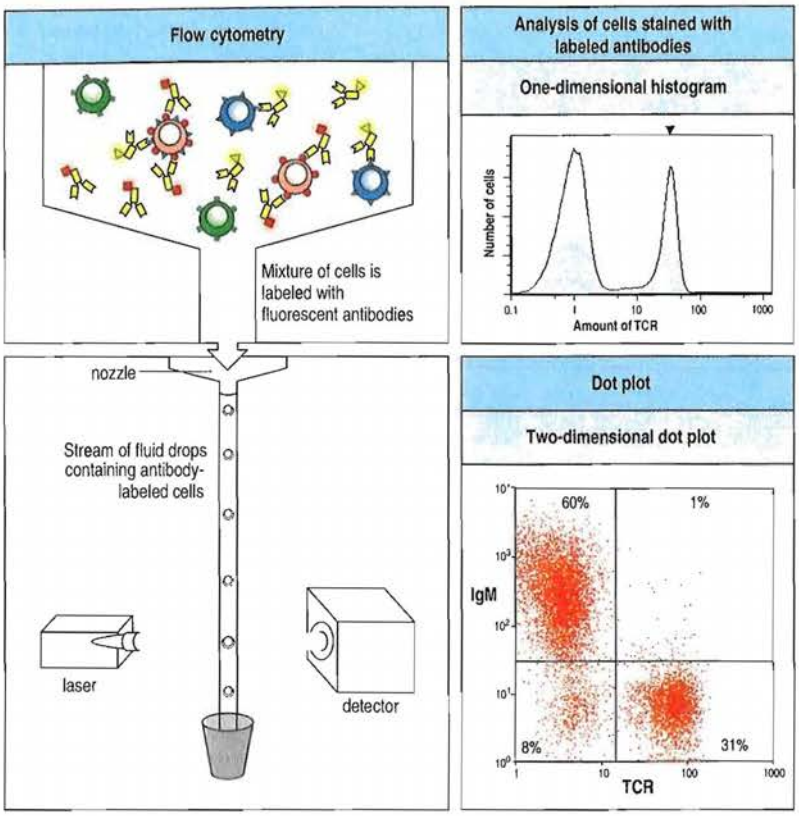


Figure 2.13 The flow cytometer allows individual cells to be identified by their cell-surface molecules. The left-hand panels illustrate the principles of flow cytometry. Human cells are labeled with mouse monoclonal antibodies specific for human cell-surface proteins, antibodies of different specificity being tagged with fluorescent dyes of different color. The labeled cells pass through a nozzle, forming a stream of droplets each containing one cell. The stream of cells passes through a laser beam, which causes the fluorescent dyes to emit light of different wavelengths. The emitted signals are analyzed by a detector: cells with particular characteristics are counted and the abundances of the labeled cell-surface molecules measured. The right-hand panels show how the data obtained can be represented, as exemplified by the presence of IgM and T-cell receptor (TCR) on lymphocytes from the human spleen. The expression of IgM and TCR distinguishes B cells and T cells, respectively. When the presence of one type of molecule is analyzed, the data is usually displayed as a histogram, as shown in the upper right panel for TCR. Two populations of cells are distinguished in the histogram. The larger peak to the left are lymphocytes (mostly B cells) that do not bind the anti-TCR monoclonal antibody and comprises 58% of the total cell number. The smaller peak to the right corresponds to the T cells that bind the anti-TCR antibody and comprises 32% of the total. Two-dimensional plots (lower right panel) are used to compare the expression of two cell-surface molecules. The horizontal axis represents the amount of fluorescent anti-TCR antibody bound by a cell, the vertical axis represents the amount of fluorescent anti-IgM antibody bound. Each dot represents the values obtained for a single cell. Many thousands of cells are usually analyzed, and the dots can blend into each other in those parts of the plot which are heavily populated. The dot plot is divided into four quadrants that roughly correspond to the four cell populations distinguished by analysis with two antibodies. In the top left quadrant are B cells; these bind anti-IgM antibody but not anti-TCR antibody and comprise 60% of the cells. In the bottom right quadrant are the T cells; these bind anti-TCR antibody but not anti-IgM antibody and comprise 31% of the cells. In the bottom left quadrant are cells that bind neither anti-IgM nor anti-TCR antibody; these comprise 8% of the cells and probably include NK cells and some contaminating non-lymphoid leukocytes. Theoretically, cells in the upper right quadrant would correspond to lymphocytes that bind both anti-IgM and anti-TCR. As no lymphocytes express both IgM and TCR at their surface, these double reactions (1% of the total) arise from imprecision in using quadrants to separate the cell population and from experimental artifact, for example the nonspecific sticking of antibody molecules to cells that do not express their specific antigen.

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many of these applications have limited use because after the initial administration the human patient makes antibodies specific for the C regions of the mouse antibodies used. Reaction of these antibodies with any subsequent doses of the mouse monoclonal antibodies diminishes their therapeutic effect and increases the likelihood of complications. To reduce this problem, genetic engineering can be used to **humanize** a useful mouse monoclonal antibody. In this procedure, sequences encoding the CDR loops of the mouse monoclonal antibody's heavy and light chains are used to replace the sequences encoding the corresponding CDR loops of a human immunoglobulin with irrelevant specificity. Thus, cells expressing the humanized heavy and light chains make an antibody in which only the CDR loops are of mouse origin and the rest is human.

Summary

IgG antibodies are made of four polypeptide chains—two identical heavy chains and two identical light chains. Each chain has a V region that contributes to the antigen-binding site and a C region that, in the heavy chain, determines the antibody isotype and its specialized effector functions. The IgG molecule is Y-shaped, in which the stem and the arms are of comparable sizes and can flex with respect to each other. Each arm contains an antigen-binding site, whereas the stem contains binding sites for cells and effector molecules that enable bound antigen to be cleared from the body. Immunoglobulin chains are built from a series of structurally related immunoglobulin domains. Within the V domain, sequence variability is localized to three hypervariable regions corresponding to the three loops that are clustered at one end of the domain. In the antibody molecule the hypervariable loops of the heavy and light chains form a variable surface that binds antigen. The type of antigen bound by an antibody depends on the shape of the antigen-binding site: antigens that are small molecules can be bound within deep pockets; linear epitopes from proteins or carbohydrates can be bound within clefts or grooves; and the binding of conformational epitopes of folded proteins takes place over an extended surface area. Monoclonal antibodies are antibodies of a single specificity that are derived from a clone of identical antibody-producing cells, which are used in diagnostic tests and as therapeutic agents.

Generation of immunoglobulin diversity in B cells before encounter with antigen

The number of different antibodies that can be produced by the human body seems to be virtually limitless. To achieve this, the immunoglobulin genes are organized differently from other genes. In all cells, except B cells, the immunoglobulin genes are in a fragmented form that cannot be expressed; instead of containing a single complete gene, immunoglobulin heavy-chain and light-chain loci consist of families of **gene segments**, sequentially arrayed along the chromosome, with each set of segments containing alternative versions of parts of the immunoglobulin V region. The immunoglobulin genes are inherited in this form through the germ line (egg and sperm); this arrangement is therefore called the **germline form** or **germline configuration**.

For an immunoglobulin gene to be expressed, individual gene segments must first be rearranged to assemble a functional gene, a process that occurs only in developing B cells. Immunoglobulin-gene rearrangements occur during the development of B cells from B-cell precursors in the bone marrow. When gene rearrangements are complete, heavy and light chains can be produced and membrane-bound immunoglobulin appears at the B-cell surface. The B

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cell can now recognize and respond to an antigen through this receptor. Much of the diversity within the mature antibody repertoire is generated during this process of gene rearrangement, as we shall see in this part of the chapter.

2-6 The DNA sequence encoding a V region is assembled from two or three gene segments

In humans, the immunoglobulin genes are found at three chromosomal locations: the heavy-chain locus on chromosome 14, the κ light-chain locus on chromosome 2 and the λ light-chain locus on chromosome 22. Different gene segments encode the leader peptide (L), the V region (V) and the constant region (C) of the heavy and light chains. Gene segments encoding C regions are commonly called C genes (Figure 2.14). Within the heavy-chain locus are C genes for all the different heavy-chain isotypes.

The gene segments encoding the leader peptide and the C region consist of exons and introns, like those found in other human genes, and they are ready to be transcribed. In contrast, the V regions are encoded by two (V_L) or three (V_H) gene segments that require rearrangement in order to produce an exon that can be transcribed. The two types of gene segment that encode the light-chain V region are called **variable (V)** and **joining (J) gene segments**. The heavy-chain locus includes an additional set of **diversity (D) gene segments** that lies between the arrays of V and J gene segments (see Figure 2.14).

The V region of a light chain is encoded by the combination of one V and one J segment, whereas the C region is encoded by a single C gene. The main differences between V gene segments are in the sequences that encode the first and second hypervariable regions of the V domain (see Section 2-3); the third hypervariable region is determined by the junction between the V and J segments. In an individual B cell, only one of the light-chain loci (κ or λ) gives rise to a functional light-chain gene. The V region of the heavy chain is encoded

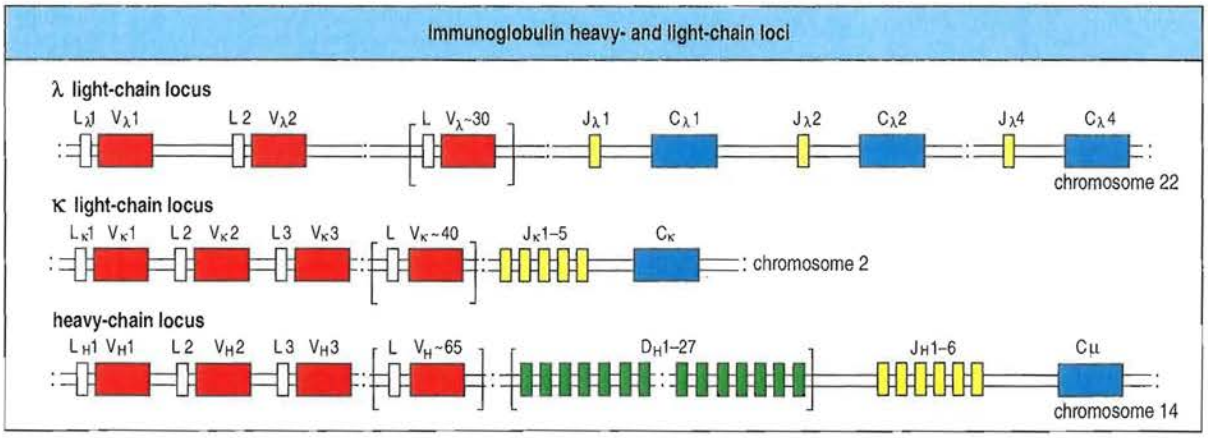
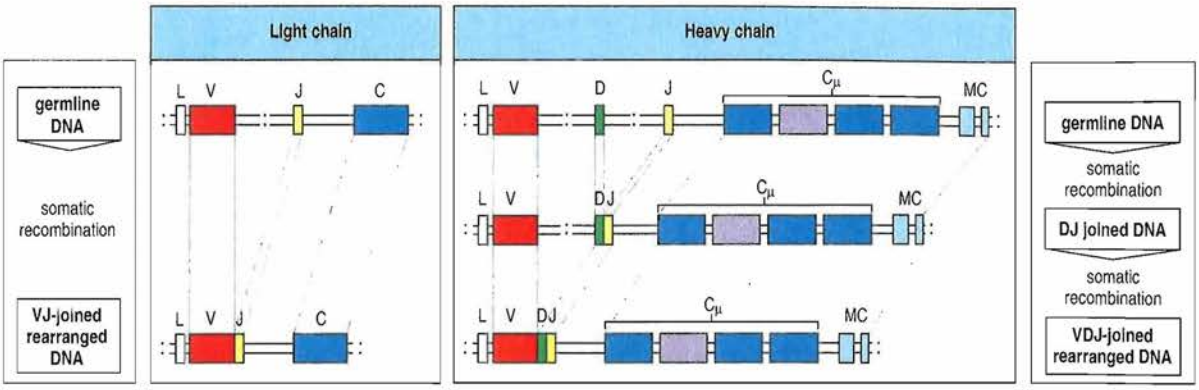


Figure 2.14 The germline organization of the human immunoglobulin heavy-chain and light-chain loci. The upper row shows the λ light-chain locus, which has about 30 functional V_λ gene segments and four pairs of functional J_λ gene segments and C_λ gene segments. The κ locus (center row) is organized in a similar way, with about 40 functional V_κ gene segments accompanied by a cluster of five J_κ gene segments but with a single C_κ gene segment. In approximately half of the human population, the entire cluster of V_κ gene segments is duplicated (not shown for simplicity). The heavy-chain locus (bottom row) has about 65 functional V_H gene segments, a cluster of about 27 D segments and six J_H gene segments. For simplicity, only a single C_H gene ($C\mu$) is shown in this diagram. This diagram is not to scale: the total length of the heavy-chain locus is over 2 megabases (2 million bases), whereas some of the D segments are only six bases long. L, leader sequence.

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by one V, one D, and one J segment. The first two hypervariable regions are caused by differences in the sequences of the heavy-chain V gene segments; the third hypervariable region is determined by differences in the D gene segments and the junctions they make with the V and J gene segments. The C region of a heavy chain is encoded by one of the C genes.

Figure 2.15 V-region sequences are constructed from gene segments. Light-chain V-region genes are constructed from two segments (left panel). A variable (V) and a joining (J) gene segment in the genomic DNA are joined to form a complete light-chain V-region (V_L) exon. After rearrangement the light-chain gene consists of three exons, encoding the leader (L) peptide, the V region and the C region, that are separated by introns. Heavy-chain V regions are constructed from three gene segments (right panels). First the diversity (D) and J gene segments join, then the V gene segment joins to the combined DJ sequence, forming a complete heavy-chain V-region (V_H) exon. For simplicity, only one of the heavy-chain genes, Cμ, is shown here. Each immunoglobulin domain is encoded by a separate exon and two additional membrane-coding exons (MC, colored light blue) specify the hydrophobic sequence that will anchor the heavy chain to the B-cell membrane.

2-7 Random recombination of gene segments produces diversity in the antigen-binding sites of immunoglobulins

During the development of B cells the arrays of V, D, and J segments are cut and spliced by DNA recombination. This process is called **somatic recombination** because it occurs in cells of the soma, a term that embraces all of the body's cells except the germ cells. A single gene segment of each type is brought together to form a DNA sequence encoding the V region of an immunoglobulin chain. For light chains, a single recombination occurs, between a V_L and a J_L segment (Figure 2.15, left panels), whereas for heavy chains, two recombinations are needed, the first to join a D and a J_H segment, and the second to join the combined DJ segment to a V_H segment (see Figure 2.15, right panels). In each case, the particular V, D, and J gene segments that are joined together are selected at random. Because of the multiple gene segments of each type, numerous different combinations of V, D, and J gene segments are possible. Thus, the gene rearrangement process generates many different V-region sequences in the B-cell population. This is one of the factors contributing to the diversity of immunoglobulin V regions.

For the human κ light chain, approximately 40 V_κ gene segments and 5 J_κ gene segments (Figure 2.16) can be recombined in 200 (40 × 5) different ways. Similarly, some 30 V_λ gene segments and 4 J_λ gene segments can be recombined in 120 (30 × 4) different ways. At the heavy-chain locus, 65 V_H segments, some 27 D segments and 6 J_H segments can produce 10,530 (65 × 27 × 6) combinations. If recombination between different gene segments were the only mechanism producing V-region diversity, then a maximum of 320 different light chains and 10,530 different heavy chains could be made.

Somatic recombination is carried out by enzymes that cut and rejoin the DNA, and it exploits some of the mechanisms more ubiquitously used by cells for DNA recombination and repair. The recombination of V, J, and D

	Number of gene segments		
Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Figure 2.16 The numbers of functional gene segments used to construct the variable regions of human immunoglobulin heavy chains and light chains.

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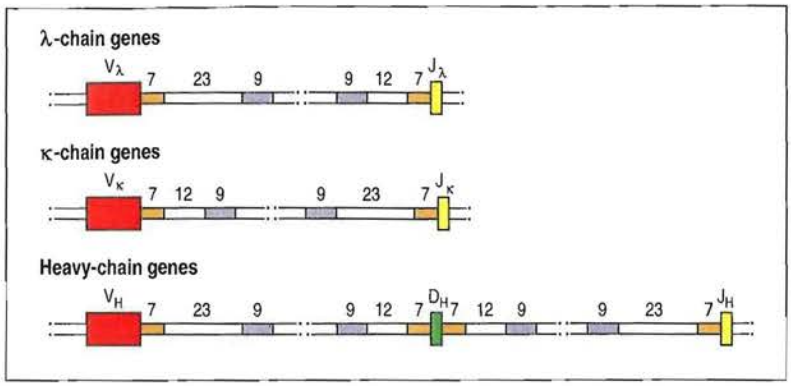


Figure 2.17 Each V, D, or J gene segment is flanked by recombination signal sequences (RSSs). Two types of RSS exist. One consists of a nonamer (9 bp, shown in purple) and a heptamer (7 bp, shown in orange) separated by a spacer of 12 bp (white). The other consists of the same 9- and 7-nucleotide sequences separated by a 23-bp spacer (white).

gene segments is directed by sequences called **recombination signal sequences** (**RSSs**), which flank the 3′ side of the V segment, both sides of the D segment, and the 5′ side of the J segment (Figure 2.17). There are two types of RSS and recombination can occur only between different types. One type of RSS comprises a defined heptamer [7 base pairs (bp); CACAGTG] sequence and a defined nonamer (9 bp; ACAAAAACC) separated by a 12-bp spacer, the other comprises the heptamer and the nonamer sequences separated by a 23-bp spacer. As well as providing recognition sites for the enzymes that cut and rejoin the DNA, RSSs ensure that the gene segments are joined in the correct order, as shown for light-chain VJ recombination in Figure 2.18. Because of

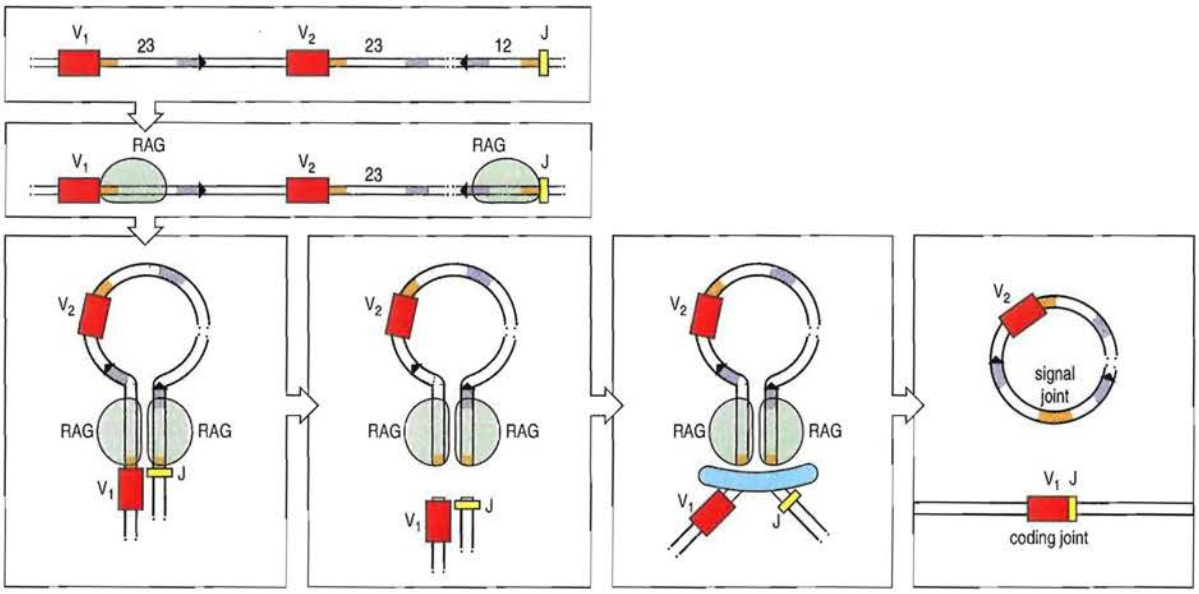


Figure 2.18 Gene segments encoding the variable region are joined by recombination at recombination signal sequences. The recombination between a V (red) and a J (yellow) segment of a light-chain gene is shown here. A RAG complex binds to the 23-bp spacer and another to the 12-bp spacer, so that a recombination signal sequence (RSS) containing a 12-bp spacer is brought together with that containing a 23-bp spacer. This is known as the 12/23 rule and ensures that gene segments are joined in the correct order. The DNA molecules are broken at the ends of the heptamer sequences (orange) and are then joined together with different topologies. The region of DNA that was originally between the V and J segments to be joined is excised as a small circle of DNA that has no function. The joint made in forming this circle is called the signal joint. Within the chromosomal DNA, the V and J segments are joined to form the coding joint. Formation of this joint involves opening up of hairpins that were formed at the original point of cleavage at one end of each V and J segment and then repairing the DNA in a way that introduces additional variability into the nucleotide sequence around the joint. The additional enzymes involved in these processes are represented in blue. Nonamers are shown in purple, heptamers in orange, spacers in white.

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this strict requirement, recombination in the heavy-chain DNA cannot join V_H directly to J_H without the involvement of D_H, because the V_H and J_H segments are flanked by the same type of RSS (see Figure 2.17).

2-8 Recombination enzymes produce additional diversity in the antigen-binding sites of immunoglobulins

The set of enzymes needed to recombine V, D, and J segments is called the **V(D)J recombinase**. Two of the component proteins are made only in lymphocytes; they are specified by the **recombination activating genes** (**RAG-1** and **RAG-2**). The other components are present in all nucleated cells and have activities that repair double-stranded DNA, bend DNA, or modify the ends of broken DNA strands. They include the enzymes DNA ligase IV, DNA-dependent protein kinase (DNA-PK), and the Ku protein associated with DNA-PK. The RAG-1 and RAG-2 proteins interact with each other and with other proteins, known as the high-mobility group of proteins, to form a RAG complex. The first step in recombination is when one RAG complex binds to one type of RSS and another binds the second type of RSS (see Figure 2.18). Interaction of the two RAG complexes aligns the two RSSs and then cleaves the DNA within the ends of the immunoglobulin gene segments. This process creates a single-stranded DNA "hairpin" at the end of each immunoglobulin segment and a clean break at the ends of the two heptamer sequences. The DNA molecules are held in place by the RAG complexes, whereas the broken ends are rejoined by DNA repair enzymes in a process called nonhomologous-end joining to form both the signal joint and the coding joint.

The enzymes that open the hairpins and form the coding joint introduce additional sequence diversity into the third hypervariable region (CDR3) of immunoglobulin heavy and light chains that is not determined by germline DNA (Figure 2.19). Firstly, the nick made to open the hairpin can occur at several different positions. When the nick occurs at a different position from where the hairpin was originally formed, nucleotides from one DNA strand are transferred to the other. This creates a new sequence that is palindromic because base pairs that were complementary in the two different DNA strands are now side-by-side on the same strand (a palindromic sequence is identical when read from either end). Because of this relationship, the added nucleotides are called **P nucleotides** (for palindromic nucleotides). The opened hairpins can then be variably modified by exonucleases that remove the germline-encoded nucleotides and by the enzyme **terminal deoxynucleotidyl transferase** (**TdT**), which randomly adds back nucleotides. The nucleotide sequence added by this enzyme need not correspond to the germline sequence in either sequence or length. When the single-stranded

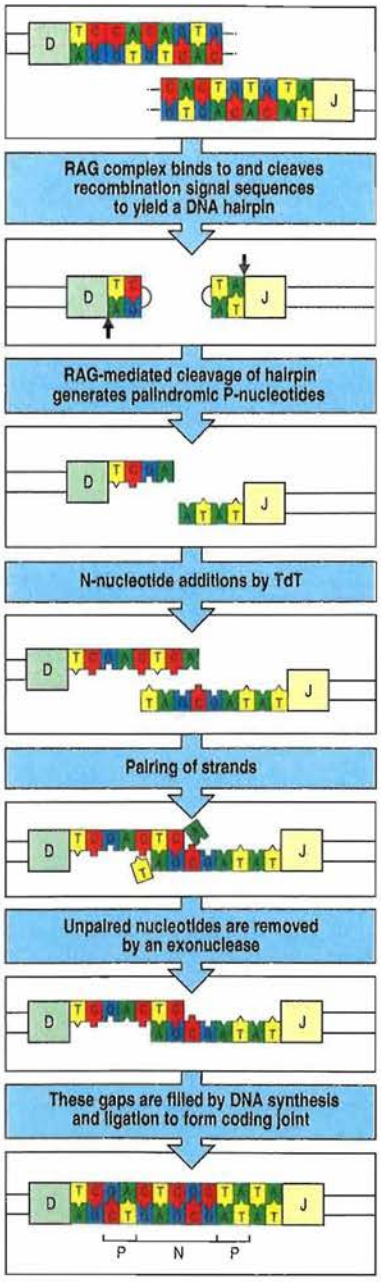


Figure 2.19 The generation of junctional diversity during gene rearrangement. The process is illustrated for a D to J rearrangement. The RSSs are brought together and endonuclease cleavage occurs within the heptamer sequences, as shown in the top panel. This leads to excision of the DNA that separates the D and J segments and modification of the D and J segments by extra nucleotides derived from the heptamers. The two DNA strands at the end of the modified D and J segments join together to form hairpins. Further cleavage on one DNA strand at the boundaries between the heptamers and the D and J segments releases the hairpins and generates short palindromic sequences. These extra nucleotides are known as P nucleotides. Terminal deoxynucleotidyl transferase (TdT) adds nucleotides randomly to the ends of the single DNA strands. These nucleotides, which are nongermline, are known as N nucleotides. The single strands pair and through the action of exonuclease, DNA polymerase, and DNA ligase, the double-stranded DNA molecule is repaired to give the coding joint.

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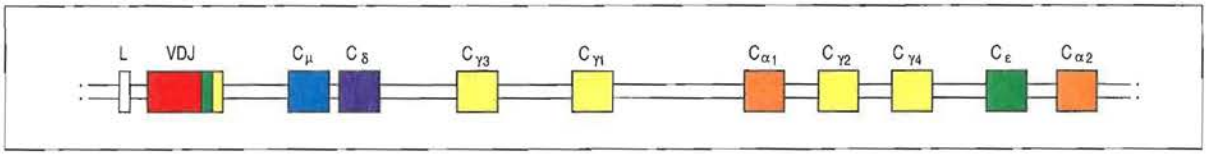


Figure 2.20 Rearrangement of V, D, and J segments produces a functional heavy-chain gene. The assembled VDJ sequence lies some distance from the cluster of C genes. Only functional C genes are shown here. The four different γ genes specify four different subtypes of the γ heavy chain, whereas the two α genes specify two subtypes of the α heavy chain. For simplicity, individual exons in the C genes are not shown. The diagram is not to scale.

tails of the two gene segments are able to pair, the sequences are filled in with complementary nucleotides to complete the coding joint. The new nucleotides inserted by TdT are called **N nucleotides** because they are non-templated (not encoded) in germline DNA.

The contribution of P nucleotides and N nucleotides to the resulting amino-acid sequence diversity in the third hypervariable region is called **junctional diversity**. This is an important source of immunoglobulin variability, adding a factor of 3×10^7 to the overall diversity. The third hypervariable region of the light-chain V domain is encoded by the junction formed between the V and J segments (see Figure 2.7), whereas the third hypervariable region of the heavy-chain V domain is formed by the D segment and its junctions with the rearranged V and J segments.

2-9 Naive B cells use alternative mRNA splicing to make both IgM and IgD

Rearrangement of the V, D, and J segments of the heavy-chain locus brings the gene's promoter and enhancer into a closer juxtaposition that enables transcription to proceed. The resulting mRNA is then spliced and translated to give a heavy-chain protein. The μ and δ heavy-chain genes are the first to be transcribed, so the first immunoglobulins that a B cell expresses on its surface are IgM and IgD. These are the only immunoglobulin isotypes that can be produced simultaneously by a B cell. They are also the only isotypes that B cells produce before they encounter antigen, which is at an early stage in their life when they are called **naive B cells**. Simultaneous expression of both μ and δ chains from the same heavy-chain locus is accomplished by differential splicing of the same primary RNA transcript, a process that involves no rearrangement of genomic DNA.

In the rearranged heavy-chain locus the exons encoding the leader peptide and the V region are on the 5′ side of the C genes, of which the μ and δ C genes are the nearest (Figure 2.20). In each C gene, separate exons encode each of the domains, as is shown for the μ and δ genes in Figure 2.21. Transcription starts upstream of the exons encoding the leader peptide and the V region, continues through the μ and δ C genes and terminates downstream of the δ gene, before the γ3 C gene. This long primary RNA transcript is then spliced and processed in two different ways: one that yields mRNA for the μ heavy chain (Figure 2.21, left panel) and one that yields mRNA for the δ heavy chain (Figure 2.21, right panel). In making μ chain mRNA from the primary transcript, the entire δ gene is removed as well as the introns from the μ gene. Conversely, in making δ chain mRNA the entire μ gene is removed as well as the introns from the δ gene.

2-10 Each B cell produces immunoglobulin of a single antigen specificity

In a developing B cell, the process of immunoglobulin-gene rearrangement is tightly controlled so that only one heavy chain and one light chain are finally expressed, a phenomenon known as **allelic exclusion**. This ensures that each B cell produces monoclonal immunoglobulin of a single antigen specificity.

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Thus, although every B cell has two copies, or alleles, of the heavy-chain locus and two copies of each light-chain locus, only one heavy-chain locus and one light-chain locus are rearranged to produce functional genes.

In a population of B cells the same functional light-chain gene rearrangement is found associated with different functional heavy-chain gene rearrangements. Conversely, the same functional heavy-chain gene rearrangement is found associated with different functional light-chain gene rearrangements. This combinatorial association of heavy and light chains within individual B cells, and in the immunoglobulins they produce, makes an important contribution to the overall diversity in the antigen-binding sites of immunoglobulins. B cells are free to produce any combination of light and heavy chains, and, thus, the potential number of antibodies of different specificities that can be made is the product of the total numbers of different heavy and light chains.

B-cell monospecificity has the consequence that an encounter with a given pathogen engages only the subset of B cells that make antibodies which bind to the pathogen. This ensures the specificity of the antibody response against an infection. For this reason, vaccination against diphtheria, for example, provides no protection against the influenza virus, neither does vaccination against the influenza virus protect against diphtheria.

The fact that the DNA sequence of the expressed immunoglobulin genes varies from one clone of B cells to the next can be used to detect the large clonal populations of cancer cells in patients with B-cell lymphoma or leukemia. Because the cancer cells are derived from a single clone of B cells, they can be readily distinguished from healthy B cells by comparing the immunoglobulin-gene rearrangements by DNA analysis (Figure 2.22). In the clinic, such analyses are used to determine the presence of cancer cells in blood samples or tissue biopsies and to monitor the response to therapy.

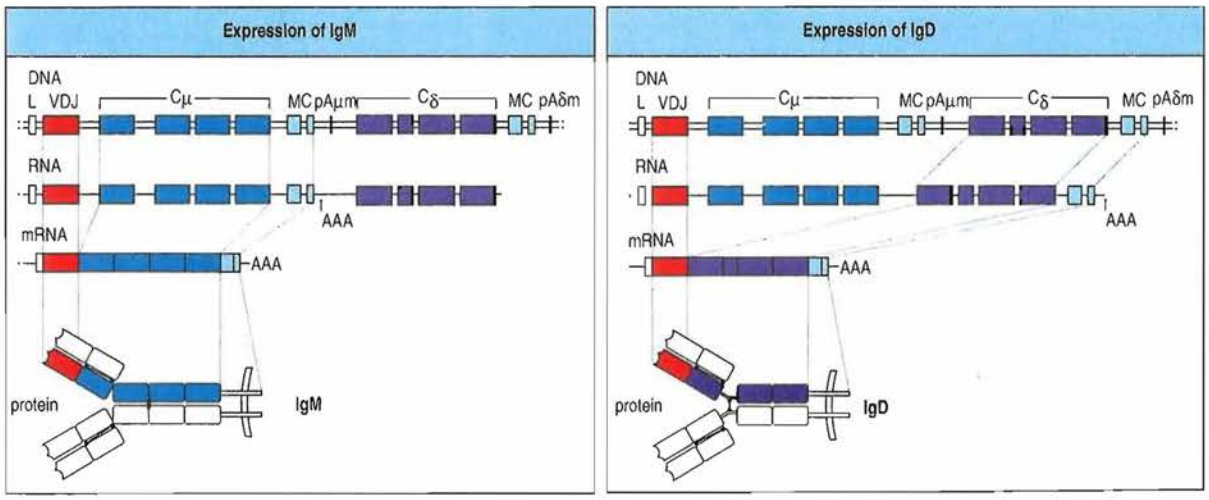


Figure 2.21 Co-expression of IgD and IgM is regulated by RNA processing. In mature B cells, transcription initiated at the V_H promoter extends through both the C_μ and C_δ genes. For simplicity we have not shown all the individual C-gene exons but only those of relevance to the differential production of IgM and IgD. The long primary transcript is then processed by cleavage, polyadenylation, and splicing. Cleavage and polyadenylation at the μ site (pAμ) and splicing between C_μ exons yields an mRNA encoding the μ heavy chain (left panel). Cleavage and polyadenylation at the δ site (pAδ) and a different pattern of splicing that removes the C_μ exons yields mRNA encoding the δ heavy chain (right panel). AAA designates the poly(A) tail. MC, membrane-coding.

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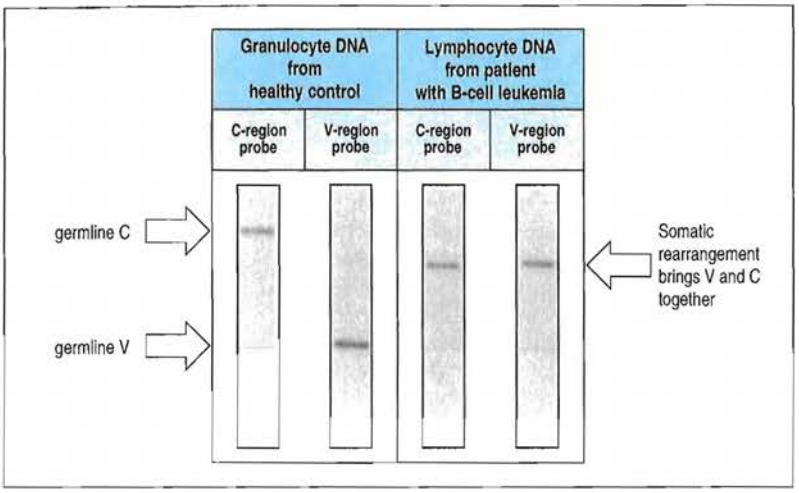


Figure 2.22 DNA analysis of immunoglobulin genes can help to diagnose B-cell tumors. The left two photographs (healthy control) show agarose gel electrophoresis of a restriction enzyme digest of DNA from peripheral blood granulocytes of a healthy person. In these cells, mostly neutrophils, the immunoglobulin genes are in the germline configuration. The state of immunoglobulin DNA sequences is examined by hybridization with V-region and C-region probes. The V and C regions are found in quite separate DNA fragments. The two right photographs (patient with B-cell leukemia) are of a similar restriction digest of DNA from peripheral blood lymphocytes from a patient with chronic lymphocytic leukemia, in which a particular clone of B cells is greatly expanded. For this reason, a unique rearrangement is visible. In this DNA, the V and C regions are found in the same fragment. Normal B lymphocytes in the patient's blood each have a different gene rearrangement, none of which is sufficiently well represented to be visible as a band. Photographs courtesy of S. Wagner and L. Luzzatto.

2-11 Immunoglobulin is first made in a membrane-bound form that is present on the B-cell surface

When a B cell first makes immunoglobulin the heavy chain (but not the light chain) has a hydrophobic sequence near the carboxy terminus by which it associates with cell membranes. Like all proteins destined for the cell surface, immunoglobulin chains enter the endoplasmic reticulum as soon as they are synthesized. There they associate with each other to form immunoglobulin molecules attached to the endoplasmic reticulum membrane. By themselves these immunoglobulin molecules cannot be transported to the cell surface. For that to happen they must associate with two additional transmembrane proteins called Igα and Igβ (Figure 2.23). These proteins are invariant in sequence, unlike the immunoglobulins, and travel to the B-cell surface with the immunoglobulins. At the cell surface the complex of immunoglobulin with Igα and Igβ forms the B-cell receptor for antigen.

In responding to specific antigen the immunoglobulin component of the B-cell receptor is responsible for binding to the specific antigen. However, this interaction alone cannot give the signal that tells the cell's interior when an antigen has bound. The cytoplasmic portions of immunoglobulin heavy chains are very short and do not interact with the intracellular proteins that signal B cells to divide and differentiate. That function is provided by the longer cytoplasmic tails of the Igα and Igβ components of the B-cell receptor.

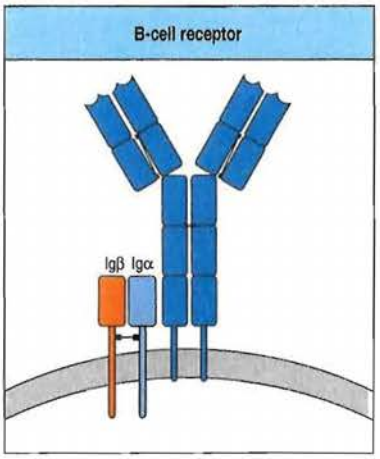


Figure 2.23 Membrane-bound immunoglobulins are associated with two other proteins, Igα and Igβ. Igα and Igβ are disulfide-linked. They have long cytoplasmic tails that can interact with intracellular signaling proteins. The immunoglobulin shown here is IgM, but all isotypes can serve as B-cell receptors.

Summary

In the human genome, the immunoglobulin heavy-chain and light-chain genes are in a form that is incapable of being expressed. In developing B cells, however, the immunoglobulin genes undergo structural rearrangements that permit their expression. The V domains of immunoglobulin light and heavy chains are encoded in two (V and J) or three (V, D, and J) different kinds of gene segment respectively that are brought into juxtaposition by recombination reactions. One mechanism contributing to the diversity in V-region sequences is the random combination of different V and J segments in light-chain genes and of different V, D, and J segments in rearranged heavy-chain genes. A second mechanism is the introduction of additional nucleotides (P and N nucleotides) at the junctions between gene segments during the process of recombination. A third mechanism that creates diversity in the antigen-binding sites of antibodies is the association of heavy and light

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chains in different combinations. Gene rearrangement in an individual B cell is strictly controlled so that only one type of heavy chain and one type of light chain are expressed, resulting in an individual B cell and its progeny expressing only immunoglobulin of a single antigen specificity. A naive B cell that has never encountered antigen expresses only membrane-bound immunoglobulin of the IgM and IgD classes. The μ and δ heavy chains are produced from a single transcriptional unit that undergoes alternative RNA processing and splicing.

Diversification of antibodies after B cells encounter antigen

A mature B cell's first encounter with antigen marks a watershed in its development. Binding of antigen to the surface immunoglobulin of a mature naive B cell triggers the cell's proliferation and differentiation, and ultimately the secretion of antibodies. As the immune response progresses, antibodies with different properties appear. In this part of the chapter we will look at the structural and functional changes that occur in immunoglobulins after antigen has been encountered and how antibodies with different functions are produced.

2-12 Secreted antibodies are produced by an alternative pattern of heavy-chain RNA processing

Gene rearrangement in an immature B cell leads to the expression of functional heavy and light chains and to the production of membrane-bound IgM and IgD on the mature B cell. After an encounter with antigen, these isotypes are produced as secreted antibodies. IgM antibodies are produced in large amounts and are important in protective immunity, whereas IgD antibodies are produced only in small amounts and have no known effector function.

All the isotypes, or classes, of immunoglobulin (IgA, IgD, IgE, IgG, and IgM) can be made in two forms: one that is bound to the cell membrane and serves as the B-cell receptor for antigen, and one, the antibody, that is secreted in order to bind to antigen and aid its destruction. During differentiation to antibody-secreting plasma cells, B cells change from making the membrane-bound form to making the secreted form; plasma cells only make secreted antibody. The difference between membrane-bound and secreted immunoglobulin lies at the carboxy terminus of the heavy chain: here, membrane-associated immunoglobulin has a hydrophobic anchor sequence that is inserted into the membrane, whereas antibody has a hydrophilic sequence. This difference is determined by different patterns of RNA splicing and processing of the same primary RNA transcript, and involves no rearrangement of the underlying genomic DNA. The alternative patterns of splicing for IgM are compared in Figure 2.24.

The hydrophilic carboxy terminus of the secreted μ chain is encoded at the 3′ end of the exon encoding the fourth C-region domain, whereas the hydrophobic membrane anchor of the membrane-associated μ chain is encoded by two small, separate exons downstream. The splicing to give secreted μ chain is the simpler pattern: the sequence encoding the hydrophilic carboxy terminus is retained and the sequences 3′ of that, including the exons encoding the hydrophobic membrane anchor, are discarded (Figure 2.24, right panel). To produce mRNA encoding membrane-associated μ chain, alternative splicing in the exon encoding the fourth C-region domain removes the sequence encoding the hydrophilic anchor, whereas the exons

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encoding the hydrophobic carboxy terminus are retained and incorporated into the mRNA when the introns are spliced out (Figure 2.24, left panel).

2-13 Rearranged V-region sequences are further diversified by somatic hypermutation

The diversity generated during gene rearrangement is concentrated in the third CDR of the V_H and V_L domains. Once a B cell has been activated by antigen, however, further diversification of the whole of the V-domain coding sequences occurs through a process of **somatic hypermutation**. This almost randomly introduces single-nucleotide substitutions (point mutations) at a high rate throughout the rearranged V regions of heavy- and light-chain genes (Figure 2.25). The immunoglobulin constant regions are not affected, and neither are other B-cell genes. The mutations occur at a rate of about one mutation per V-region sequence per cell division, which is more than a million times greater than the ordinary mutation rate for a gene. Mutation occurs at both copies of each immunoglobulin locus, even though one copy is not being expressed. Hypermutation appears to act selectively at certain types of DNA sequence motif that are more common than usual within the sequences encoding the hypervariable CDRs. The mechanism involves activation-induced cytidine deaminase (AID), an enzyme that converts cytidine residues in the DNA to uracil. In turn, the uracil residues are excised by the enzyme uracil-DNA glycosylase (UNG) and, on subsequent replication of the B-cell DNA, nontemplated nucleotides are introduced at these sites.

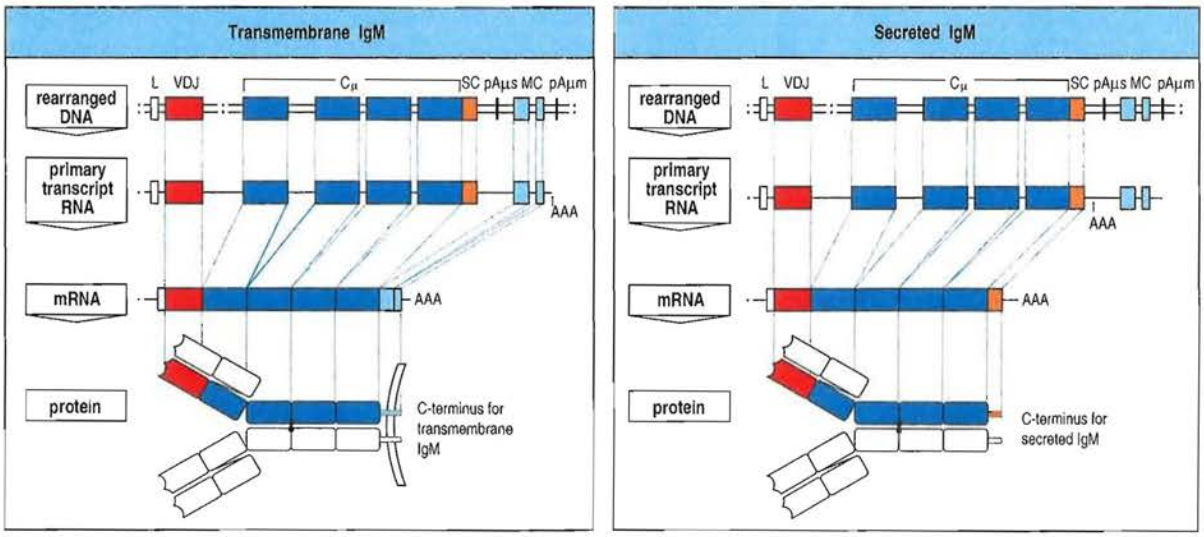


Figure 2.24 The surface and secreted forms of an immunoglobulin are derived from the same heavy-chain gene by alternative RNA processing. Each heavy-chain C gene has two exons [membrane-coding (MC), light blue] encoding the transmembrane region and cytoplasmic tail of the surface form of that isotype, and a secretion-coding (SC) sequence (orange) encoding the carboxy terminus of the secreted form. The events that dictate whether a heavy-chain RNA will result in a secreted or transmembrane immunoglobulin occur during processing of the initial transcript and are shown here for IgM. Each heavy-chain C gene has two potential polyadenylation sites (shown as pAμs and pAμm). In the left panel, the transcript is cleaved and polyadenylated at the second site (pAμm). Splicing between a site located between the fourth C_μ exon and the SC sequence, and a second site at the 5′ end of the MC exons, removes the SC sequence and joins the MC exons to the fourth C_μ exon. This generates the transmembrane form of the heavy chain. In the right panel, the primary transcript is cleaved and polyadenylated at the first site (pAμs), eliminating the MC exons and giving rise to the secreted form of the heavy chain. AAA designates the poly(A) tail.

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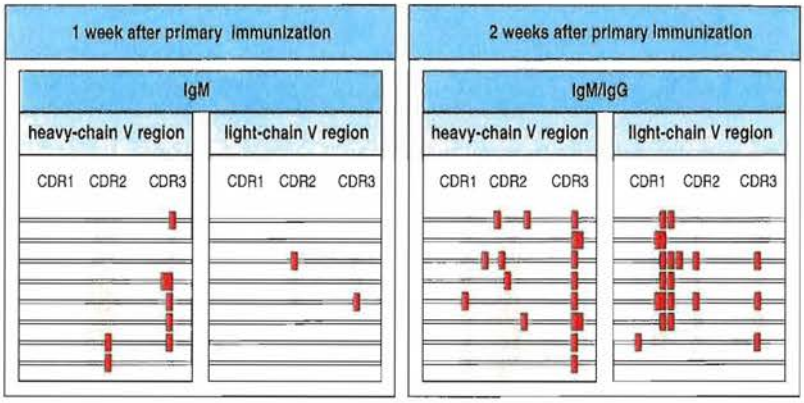


Figure 2.25 Somatic hypermutation introduces diversity into expressed immunoglobulin genes. Antibodies raised by immunization with the same antigen were collected 1 and 2 weeks after immunization and their amino-acid sequences were determined. Each line represents one antibody and the red bars represent amino-acid positions that differ from the prototypic sequence. One week after primary immunization, most of the antibodies were IgM and showed very little sequence variation in the V region. Two weeks after immunization, both IgG and IgM were present and all six CDRs were affected.

Somatic hypermutation gives rise to B cells bearing mutant immunoglobulin molecules on their surface. Some of these mutant immunoglobulin molecules have a higher affinity for the antigen; they will, therefore, be more likely to bind the antigen, and so B cells bearing them will be preferentially selected to mature into antibody-secreting cells. Therefore, as the immune response proceeds antibodies of progressively higher affinity for the immunizing antigen are produced. This phenomenon is called **affinity maturation**.

2-14 Isotype switching produces immunoglobulins with different C regions but identical antigen specificities

IgM is the first antibody produced in an immune response. Unlike IgG, IgM is secreted as a circular pentamer of Y-shaped immunoglobulin monomers (Figure 2.26). Because of its 10 antigen-binding sites, IgM binds strongly to the surface of pathogens with multiple repetitive epitopes, but it is limited in the effector mechanisms that it uses to clear antigen from the body. Antibodies with other effector functions are produced by the process of **isotype switching** or **class switching**, in which a further DNA recombination event enables the rearranged V-region coding sequence to be used with other heavy-chain C genes.

Isotype switching is accomplished by a recombination within the cluster of C genes that excises the previously expressed C gene and brings a different one into juxtaposition with the assembled V-region sequence. Thus, the antigen

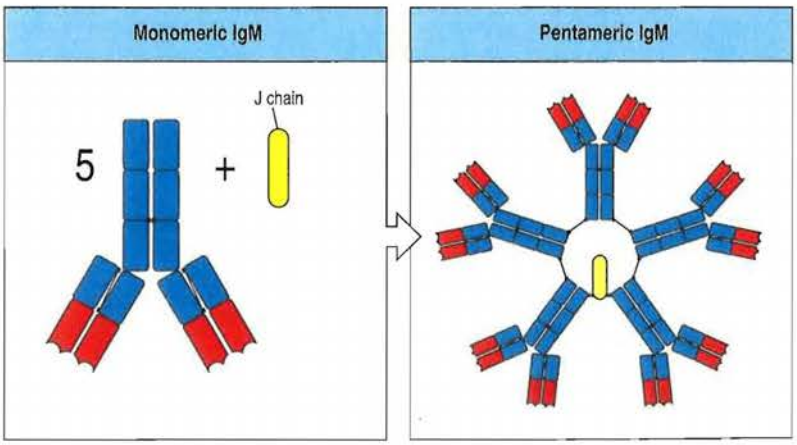
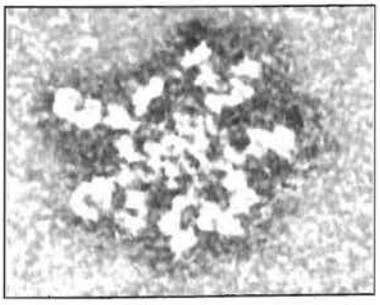


Figure 2.26 IgM is secreted as a pentamer of immunoglobulin monomers. The left two panels show schematic diagrams of the IgM monomer and pentamer. The IgM pentamer is held together by a polypeptide called the J chain, for joining chain (not to be confused with a J segment). The monomers are cross-linked by disulfide bonds to each other and to the J chain. The right panel shows an electron micrograph of an IgM pentamer, showing the arrangement of the monomers in a flat disc. The lack of a hinge region in the IgM monomer makes the molecule less flexible than, say, IgG, but this is compensated for by the large number of binding sites. Given a pathogen with multiple identical epitopes on its surface, IgM is likely to be able to bind to it with several of its binding sites simultaneously. Photograph (× 900,000) courtesy of K.H. Roux and J.M. Schiff.



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specificity of the antibody remains unchanged, even though its isotype changes. Flanking the 5′ side of each C gene, with the exception of the δ gene, are highly repetitive sequences that mediate recombination. They are called **switch sequences** or **switch regions** (Figure 2.27). In switching the heavy-chain isotype, the switch region that flanks the μ gene interacts with a switch region flanking one of the other C genes. This interaction permits recombination in which the μ, δ, and any other intervening C genes are excised as a circular DNA molecule, bringing the V region into juxtaposition with the new C gene. The mRNA for the new immunoglobulin is then produced as described in Section 2-9. Recombination can take place between the μ switch region and that of any other isotype. Sequential switching can also occur, for example from μ to γ_3 to α_1. Isotype switching only occurs during an active immune response and the patterns of isotype switching are regulated by cytokines secreted by antigen-activated T cells. The enzyme AID is important for class switch recombination as well as for somatic hypermutation, suggesting that the initial stages of these rather different reactions may be similar.

2-15 Antibodies with different C regions have different effector functions

In humans and other mammals the five classes of immunoglobulin are IgA, IgD, IgE, IgG, and IgM (see Figure 2.4). Certain classes are divided further into subclasses, which differ in both nomenclature and properties between species. In humans, IgA is divided into two subclasses (IgA1 and IgA2), whereas IgG is divided into four subclasses (IgG1, IgG2, IgG3, and IgG4), which are numbered according to their relative abundance in plasma, IgG1 being the most abundant. The heavy chains of the human IgA subclasses are designated α1 and α2 and the heavy chains of the human IgG subclasses by γ1, γ2, γ3, and γ4. The α, δ, and γ heavy-chain C regions are made up of three C domains, whereas the μ and ε heavy chains have four (see Figure 2.4). Each C domain is encoded by a separate exon in the relevant C gene. Additional

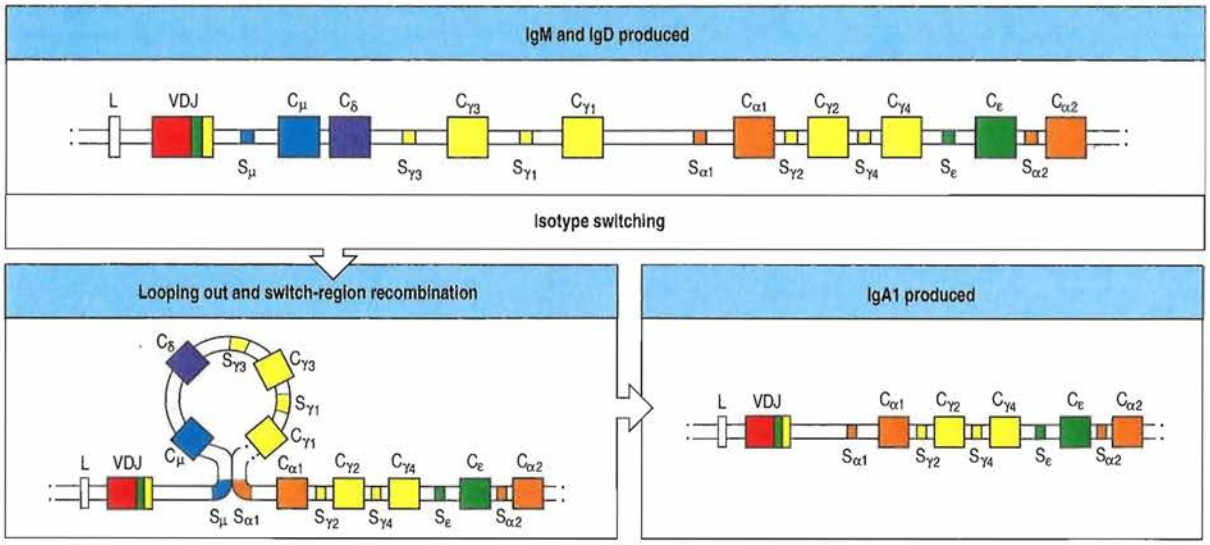


Figure 2.27 Isotype switching involves recombination between specific switch regions. Repetitive DNA sequences are found to the 5′ side of each of the heavy-chain C genes, with the exception of the δ gene. Switching occurs by recombination between these switch regions (S), with deletion of the intervening DNA. The initial switching event takes place from the μ switch region; switching from other isotypes can take place subsequently.

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	Immunoglobulin class or subclass								
	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgE
Heavy chain	μ	δ	γ_1	γ_2	γ_3	γ_4	α_1	α_2	ε
Molecular weight (kDa)	970	184	146	146	165	146	160	160	188
Serum level (mean adult mg ml^{-1})	1.5	0.03	9	3	1	0.5	2.0	0.5	$5x10^{-5}$
Half-life in serum (days)	10	3	21	20	7	21	6	6	2

Figure 2.28 The physical properties of the human immunoglobulin isotypes. The molecular weight given for IgM is that of the pentamer (see Figure 2.26) the predominant form in serum. The molecular weight given for IgA is that of the monomer. Large amounts of IgA are also produced in the form of dimers, which is the form found in secretions.

exons are used to encode the hinge region and the carboxy terminus, depending on the isotype. The physical properties of the human immunoglobulin isotypes are shown in Figure 2.28.

Antibodies aid the clearance of pathogens from the body in various ways. **Neutralizing** antibodies directly inactivate a pathogen or a toxin and prevent it from interacting with human cells. Neutralizing antibodies against viruses, for example, bind to a site on the virus that is normally used to gain entry to cells. Another function of antibodies is **opsonization**, a term used to describe the coating of pathogens with an immune-system protein (see Figure 1.29, p. 27). The common **opsonins** are antibodies and complement proteins. Opsonized pathogens are more efficiently ingested by phagocytes, which have receptors for the Fc region of some antibodies and for certain complement proteins. Complement activation by antibodies bound to a pathogen's surface can also lead to the direct lysis of the bacterium by complement.

IgM is the first antibody produced in an immune response against a pathogen. It is made principally by plasma cells resident in lymph nodes, spleen, and bone marrow and circulates in blood and lymph. On initiation of an immune response, most of the antibodies that bind the antigen will be of low affinity and the multiple antigen-binding sites of IgM are needed if enough antibody is to bind sufficiently strongly to a microorganism to be of any use. When bound to antigen, sites exposed in the constant region of IgM initiate reactions with complement, which can kill microorganisms directly or facilitate their phagocytosis. Because somatic hypermutation leads to antibodies of increased affinity for the antigen, two antigen-binding sites are then sufficient to produce strong binding; by switching isotype, different effector functions can be brought into play while preserving antigen specificity. Synthesis of IgM then gives way to synthesis of IgG.

IgG is the most abundant antibody in the internal body fluids, including blood and lymph. Like IgM, it is made principally in the lymph nodes, spleen, and bone marrow and circulates in lymph and blood. IgG is smaller and more flexible than IgM, properties that give it easier access to antigens in the extracellular spaces of damaged and infected tissues. The flexibility of the hinge region in IgG enables the two Fab arms to move relative to each other. This enables both the antigen-binding sites to bind to repeated epitopes on the surfaces of pathogens. IgG antibodies can also implement more effector functions than IgM (Figure 2.29). Once they have bound an antigen, the IgG1 and IgG3 subclasses can directly recruit phagocytic cells to ingest the antigen:antibody complex, as well as activating the complement system. During pregnancy, IgG antibodies can be transferred across the placenta,

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Function	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activation of complement system	+++	–	++	+	+++	–	+	–

Property	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.03	9	3	1	0.5	2.1	$5x10^{-5}$

Figure 2.29 Each human immunoglobulin isotype has specialized functions and distinct properties. The major effector functions of each isotype (+++) are shaded in dark red; lesser functions (++) are shown in dark pink, and very minor functions (+) in pale pink. Other properties are similarly marked, with mean serum concentrations shown in the bottom row. Opsonization refers to the ability of the antibody itself to facilitate phagocytosis. Antibodies that activate the complement system indirectly cause opsonization via complement. *IgG2 acts as an opsonin in the presence of a genetic variant of its phagocyte Fc receptor, which is found in about 50% of Caucasians. NK cells, natural killer cells.

providing the fetus with protective antibodies from the mother in advance of possible infection.

Monomeric IgA is made by plasma cells in lymph nodes, spleen, and bone marrow and is secreted into the bloodstream. Dimeric IgA is made in the lymphoid tissues underlying mucosal surfaces and is the antibody that is secreted into the lumen of the gut; it is also the principal antibody of other secretions, including milk, saliva, sweat, and tears. The mucosal surface of the gastrointestinal tract provides an extensive surface of contact between the human body and the environment, and the transport processes involved in the uptake of food make it vulnerable to infection. More IgA is made than any other isotype. Some of this is against the resident microorganisms that colonize mucosal surfaces, keeping their population in check. In contrast to monomeric IgA, dimeric IgA has a J chain identical to that present in pentameric IgM (Figure 2.30).

The IgE class of antibodies is highly specialized towards the activation of mast cells, which are present in epithelial tissues. IgE bound tightly to mast cells triggers strong inflammatory reactions in the presence of its antigen, and is thought to be involved in the expulsion of worms and other parasites. In medical practice, however, the major impact of IgE is in the allergies that result when it is produced against otherwise harmless antigens.

IgE binds tightly to a high-affinity IgE receptor carried by tissue mast cells and the basophils of the blood. Even though IgE is present in the body at minute

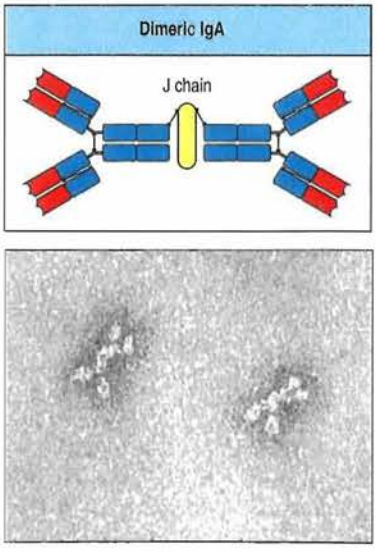


Figure 2.30 IgA molecules can form dimers. In mucosal lymphoid tissue IgA is synthesized as a dimer in association with the same J chain found in pentameric IgM. In dimeric IgA, the monomers have disulfide bonds to the J chain but not to each other. The bottom panel shows an electron micrograph of dimeric IgA. Photograph (× 900,000) courtesy of K.H. Roux and J.M. Schiff.

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concentrations in both normal and allergic individuals, the affinity of this receptor for IgE is so high that it is constantly occupied with IgE. Binding of antigen to receptor-bound IgE induces mast cells to release stored histamine and other activators, which recruit cells and molecules of the immune system to local sites of trauma, causing inflammation.

Summary

The C region of the heavy chain defines classes of immunoglobulin that serve different functions in immunity. On binding of antigen to the B-cell receptor, the cell receives signals that cause both cell division and proliferation and the initiation of mechanisms that diversify the antigen specificity and effector function of the antibody. Antigen specificity is diversified by somatic hypermutation, which introduces single-nucleotide substitutions randomly throughout the V-region sequence. This leads to the selection of B cells that make higher-affinity antibodies. With higher-affinity antibodies, the dependence on pentameric IgM for strong binding to antigen is relaxed and the switching of immunoglobulin isotype from IgM/IgD to IgG, IgE, or IgA by further somatic recombination events produces antibodies of different isotype, tissue distribution and effector function while preserving antigen specificity. B cells control the extent to which its immunoglobulin is a cell-surface receptor or a secreted effector antibody by alternative splicing of the primary RNA transcript made from the same rearranged heavy-chain gene.

Summary to Chapter 2

The principal function of B lymphocytes is to produce antibodies, which are secreted immunoglobulins that bind tightly to infectious agents and tag them for destruction or elimination. Each antibody is highly specific for its corresponding antigen; the antibody repertoire of each person is enormous because it is composed of many millions of different antibodies that can bind a wide variety of different antigens. Antibodies can also be divided into five different effector classes—IgM, IgG, IgD, IgA, and IgE—that have different functions in the immune response. This chapter has provided an overview of the structure and function of the antibody molecule and of the unusual genetic mechanisms that create this diversity in specificity and effector function. Within an antibody molecule, the V regions that bind antigen are physically separated from the C region that interacts with effector molecules and cells of the immune system, such as complement, phagocytes, and other leukocytes. Antigen binding is the property of the paired V domains of the heavy and light chains, which can form an almost unlimited number of different binding sites with structural complementarity to a vast range of molecules. The immunoglobulin genes (heavy chain, κ light chain, and λ light chain) are expressed only in B cells, and their expression involves an unusual process of DNA rearrangement in which somatic DNA recombination assembles a V-region coding sequence from sets of gene segments that are present in the unrearranged gene. The random selection of gene segments for assembly creates much of the collective diversity of antigen-binding sites. The lymphocyte-specific proteins and ubiquitous enzymes of DNA repair and recombination are involved in the recombination machinery. Imprecision is inherent in some of their reactions, which creates additional diversity at the junctions between gene segments. In an individual B cell, only one rearranged heavy-chain gene and one rearranged light-chain gene become functional, ensuring that each B cell expresses immunoglobulin of a single specificity. The series of gene rearrangements that result in production of membrane-bound IgM, the first immunoglobulin produced, is summarized in Figure 2.31.

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On mature B cells, the membrane-bound immunoglobulin functions as the specific receptor for antigen; when encountering antigen the B cell is stimulated to proliferate and differentiate into plasma cells that secrete antibody of the same specificity as the membrane-bound immunoglobulin. This ensures that an immune response is directed only against the invading pathogen or immunizing antigen. On stimulation of a B cell with its specific antigen, a mutational mechanism is also switched on that introduces point mutations into the rearranged V-region DNA. Thus, the diversity of antibodies is in part due to inherited variation that is encoded in the genome and in part to non-inherited diversity that develops in B cells during an individual's lifetime. The first antibody produced after an encounter with antigen is always IgM. As the immune response proceeds, the process of isotype switching then transfers the antigen specificity onto different C regions to produce antibody molecules with different effector functions—IgG, IgA, and IgE. The changes in the immunoglobulin genes that occur throughout the life of a B cell are summarized in Figure 2.32.

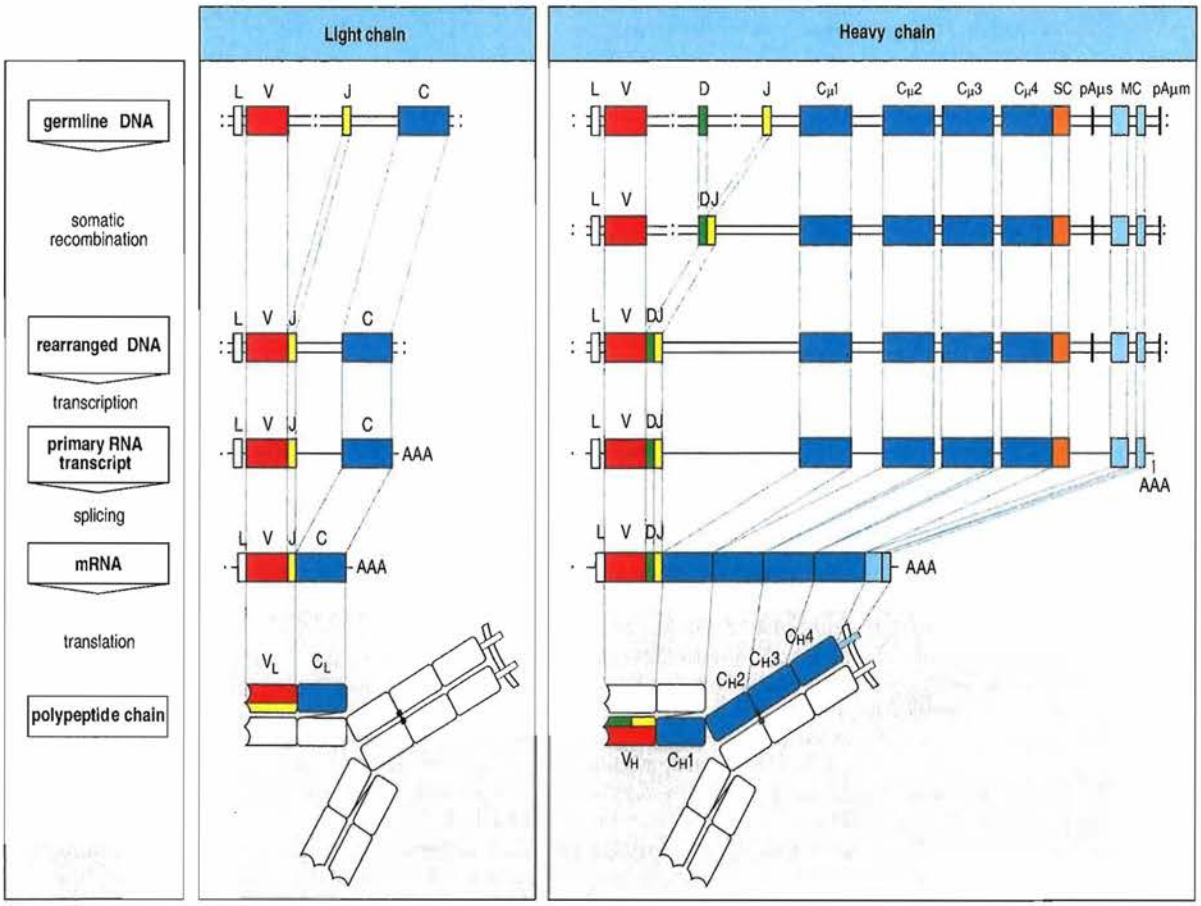


Figure 2.31 Biosynthesis of cell-surface IgM in B cells. Before immunoglobulin light- (center panel) and heavy- (right panel) chain genes can be expressed, rearrangements of gene segments are needed to produce exons encoding the V regions. Once this has been achieved the genes are transcribed to give primary transcripts containing both exons and introns. The latter are spliced out to produce mRNAs that are translated to give κ or λ light chains and μ heavy chains that assemble inside the cell and are expressed as membrane IgM at the cell surface. The main stages in the biosynthesis of the heavy and light chains are shown in the panel on the left.

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Changes in immunoglobulin genes during a B cell's life			
	Event	Mechanism	Permanence of change to the B cell's genome
1	V-region assembly from gene fragments	Somatic recombination of genomic DNA	Irreversible
2	Generation of junctional diversity	Imprecision in joining rearranged DNA segments adds nongermline nucleotides (P and N) and deletes germline nucleotides	Irreversible
3	Assembly of transcriptional controlling elements	Promoter and enhancer are brought closer together by V-region assembly	Irreversible
4	Transcription activated with coexpression of surface IgM and IgD	Two patterns of splicing and processing RNA are used	Reversible and regulated
5	Synthesis changes from membrane Ig to secreted antibody	Two patterns of splicing and processing RNA are used	Reversible and regulated
6	Somatic hypermutation	Point mutation of genomic DNA	Irreversible
7	Isotype switch	Somatic recombination of genomic DNA	Irreversible

Figure 2.32 The changes in the immunoglobulin genes that occur over a B cell's lifetime.

Questions

Question 2–1

A. What is the difference between antibodies and immunoglobulins?

B. Which cell types produce each type?

Question 2–2

Describe the structure of an antibody molecule and how this structure enables it to bind to a specific antigen. Include the following terms in your description: heavy chain (H chain), light chain (L chain), variable region, constant region, Fab, Fc, antigen-binding site, hypervariable region, and framework region. (Refer to Figures 2.2, 2.3, and 2.7.)

Question 2–3

A. What is an epitope?

B. Define the term multivalent antigen.

C. How does a linear epitope differ from a conformational epitope?

D. Do antibodies bind their antigens via noncovalent or covalent bonding? (Refer to Figures 2.9–2.11.)

Question 2–4

A. What is the difference between polyclonal antibodies and monoclonal antibodies?

B. How is each produced? (Refer to Figure 2.12.)

Question 2–5

A. Explain briefly how a vast number of immunoglobulins of different antigen specificities can be produced from the relatively small number of immunoglobulin genes present in the genome. Include the following terms in your explanation: somatic recombination, germline configuration, V, D, and J segments. (Refer to Figures 2.14 and 2.15.)

B. What is the final arrangement of gene segments in the rearranged immunoglobulin heavy-chain gene V region, and in what order do these gene segment rearrangements occur?

C. In what order do the various immunoglobulin loci rearrange?

Question 2–6

How do recombination signal sequences ensure that gene segment rearrangement occurs in the right order?

Question 2–7

How is additional diversity introduced into the variable region by the molecular mechanism of somatic recombination? Include the following terms in your answer: junctional diversity, P nucleotides, N nucleotides, terminal deoxynucleotidyl transferase (TdT). (Refer to Figures 2.17–2.19.)

Question 2–8

Explain how mature, naive B cells co-express IgM and IgD. (Refer to Figure 2.21.)

Question 2–9

Describe the process responsible for altering the expression of membrane-bound immunoglobulin to secreted antibody. (Refer to Figures 2.24 and 2.31.)

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Question 2–10

A. What is affinity maturation and what molecular process enables it to occur?
B. Describe this process and its consequences. (Refer to Figure 2.25.)

Question 2–11

A. What is isotype switching?
B. Explain the molecular mechanism of isotype switching.
C. Why is isotype switching important? (Refer to Figure 2.27.)

Question 2–12

Which immunoglobulin isotypes (out of IgM, IgG1, IgG2, IgG3, IgG4, IgA, IgE, and IgD) participate in (a) neutralization; (b) opsonization; (c) sensitization for killing by NK cells; (d) sensitization of mast cells; (e) activation of complement? Which isotypes (f) are transported across epithelium; (g) are transported across the placenta; (h) diffuse into extravascular sites? (Refer to Figure 2.29.)

Question 2–13

Isotype switching and immunoglobulin gene rearrangement by somatic recombination are both recombinational processes but have very different outcomes. Give five ways in which they differ from each other.

Question 2–14

Monoclonal antibodies are used for a wide range of applications including serological assays and diagnostics probes in the laboratory, and as therapeutic reagents in the clinic. Discuss why "humanizing" monoclonal antibodies is necessary for use as therapeutic reagents but not necessary when monoclonal antibodies are used as serological or diagnostic reagents.

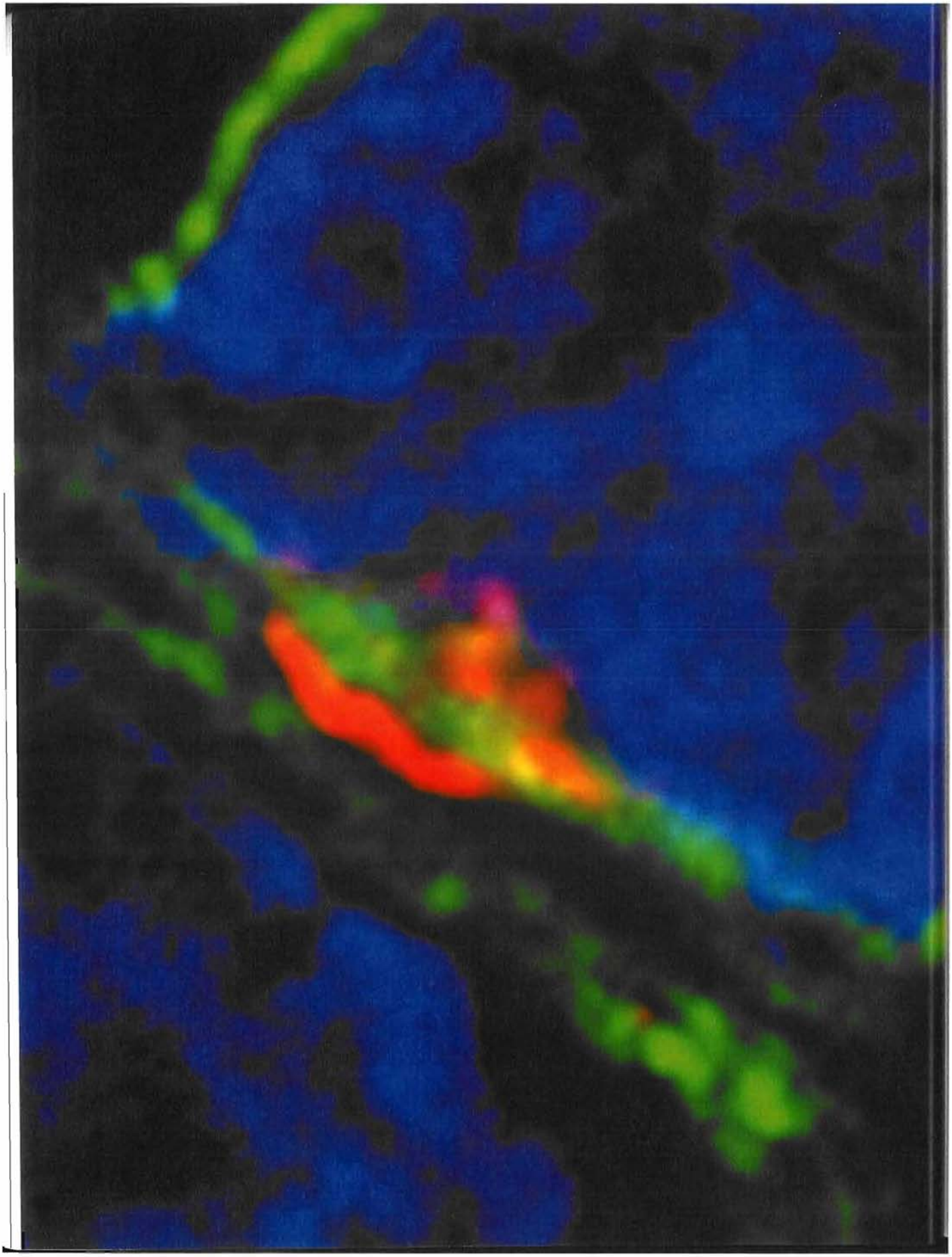
Question 2–15

What would be the effect of a genetic defect that resulted in a lack of recombination between the switch regions in the immunoglobulin C-region genes?

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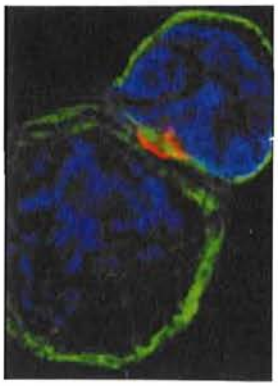


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Chapter 3

Antigen Recognition by T Lymphocytes



In Chapter 2 we saw that the function of B lymphocytes is to make immunoglobulins, a family of proteins with variable binding sites for antigen. T lymphocytes are a related lineage of antigen-specific cells that perform complementary functions to those of B cells in the adaptive immune response (see Chapter 1). Whereas the sole function of B cells is to produce secreted antibodies, T cells have more diverse roles, all of which involve interactions with other cells. B cells and T cells use similar means of recognizing antigen, but because of the distinct functions of B and T cells there are important differences between them, particularly in the type of antigen that they recognize.

In this chapter we shall consider the antigens that stimulate T cells and the receptors that T cells use to recognize them. In the first part of the chapter we will describe the structure of the T-cell antigen receptor and the mechanisms that generate its antigen specificity and diversity. The antigen receptor on T cells is commonly referred to simply as the **T-cell receptor**. T-cell receptors have much in common with the immunoglobulins. They have a similar structure, are produced as a result of gene rearrangement, and are highly variable and diverse in their antigen specificity. Like B cells, each clone of T cells expresses a single species of antigen receptor and thus different clones possess different and unique antigen specificities. This clonal distribution of diverse T-cell receptors is produced by genetic mechanisms that are similar to those that generate immunoglobulins during B-cell development.

Despite the fundamental similarity in immunoglobulin and T-cell receptor structures, the antigens recognized by T cells are quite distinct from those recognized by immunoglobulins. Immunoglobulins bind epitopes on a wide range of intact molecules, such as proteins, carbohydrates, and lipids. These kinds of molecule are present on the surfaces of bacteria, viruses, and parasites and also on soluble toxins. T-cell receptors, in contrast, recognize and bind to mainly one type of antigen, which must be presented to them on the surface of another human cell.

T-cell receptors recognize peptide antigens that are bound to specialized antigen-presenting glycoproteins called **MHC molecules**, which are expressed on almost all the cells of the body. There are an exceptionally large number of genetically determined variants of the MHC molecules in the human population, and differences between individuals in the MHC molecules that they possess are the primary cause of graft rejection and graft-versus-host disease

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in clinical transplantation. Because of this clinical effect, these antigen-presenting molecules were first discovered as determinants of tissue incompatibility in transplantation long before their function in antigen presentation was known. The genes that encode them are genetically linked in a chromosomal region called the **major histocompatibility complex** or **MHC**; the glycoproteins have, therefore, become known as MHC molecules.

In the second part of the chapter we shall consider the complex of peptide antigen and MHC molecule that is recognized by the T-cell receptor and we will trace the pathways by which peptides are derived from the proteins of infecting microorganisms and become bound to MHC molecules. The third part of the chapter will deal with the MHC itself and the immunological implications of the exceptional genetic polymorphism of the MHC genes.

T-cell receptor diversity

The T-cell receptor is a membrane-bound glycoprotein that closely resembles a single antigen-binding arm of an immunoglobulin molecule. It is composed of two different polypeptide chains and has one antigen-binding site. T-cell receptors are always membrane bound and there is no secreted form as there is for immunoglobulins. Like immunoglobulins, each chain has a variable region, which binds antigen, and a constant region. During T-cell development, gene rearrangement produces sequence variability in the variable regions of the T-cell receptor by the same mechanisms that are used by B cells to produce the variable regions of immunoglobulins. However, after the T cell is stimulated with antigen, there is no further mutation in the antigen-binding site and there is no switching of constant-region isotype as occurs for immunoglobulins. These differences correlate with the fact that T-cell receptors are used only as receptors to recognize antigen, whereas immunoglobulins serve as both recognition and effector molecules.

3-1 The T-cell receptor resembles a membrane-associated Fab fragment of immunoglobulin

A T-cell receptor consists of two different polypeptide chains, termed the **T-cell receptor α chain** (**TCRα**) and the **T-cell receptor β chain** (**TCRβ**). The genes encoding the α and β chains have similar germline organization to the genes encoding immunoglobulin heavy- and light-chain genes in that they are made up of sets of gene segments that must be rearranged to form a functional gene. As a consequence of gene rearrangements that occur as part of T-cell development, each mature T cell expresses one functional α chain and one functional β chain, which together define a unique T-cell receptor molecule. Within the population of T cells possessed by a healthy human being there are many millions of different T-cell receptors, each of which defines a clone of T cells and a single antigen-binding specificity.

Comparison of the amino-acid sequences of the T-cell receptor α and β chains from different T-cell clones shows that they are organized into variable regions (V regions) and constant regions (C regions), like those found in immunoglobulin chains. The α and β chains are folded into discrete protein domains resembling those in immunoglobulin chains. Each chain consists of an amino-terminal V domain, followed by a C domain, and then a membrane-anchoring domain. The antigen-recognition site of T-cell receptors is formed from the V_α and V_β domains and is the most variable part of the molecule, as in the immunoglobulins. The three-dimensional structure of the four extracellular domains of the T-cell receptor is very similar to that of the antigen-binding Fab fragment of IgG (Figure 3.1).

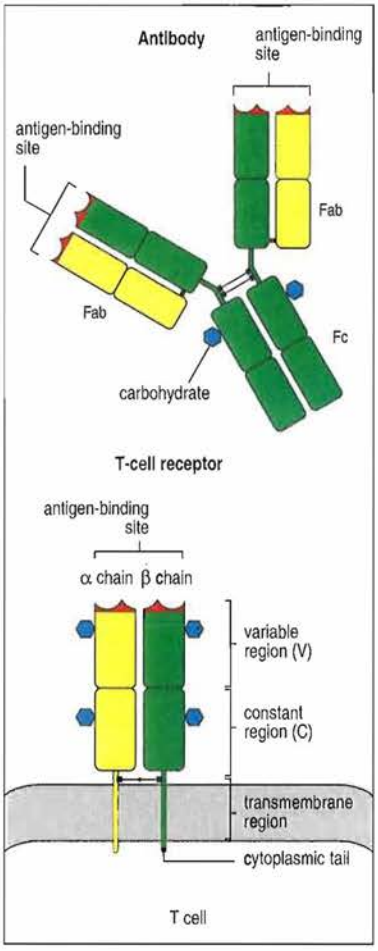


Figure 3.1 The T-cell receptor resembles a membrane-bound Fab fragment. Comparison of the T-cell receptor and an IgG antibody molecule. The T-cell receptor is a heterodimer composed of an α chain of 40–50 kDa and a β chain of 35–46 kDa in size. The extracellular portion of each chain consists of two immunoglobulin-like domains: the domain nearest to the membrane is a C region and the domain farthest from the membrane is a V region. Both the α and β chains span the cell membrane and have very short cytoplasmic tails. The three-dimensional structure formed by the four immunoglobulin-like domains of the T-cell receptor resembles that of the antigen-binding Fab fragment of antibody.

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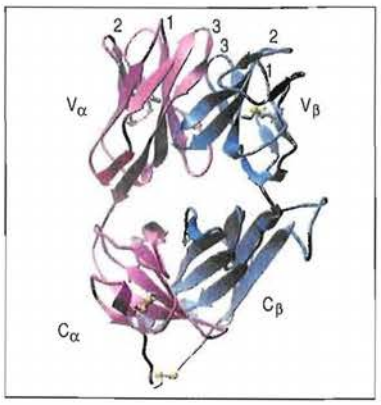


Figure 3.2 Three-dimensional structure of the T-cell receptor showing the antigen-binding CDR loops. The ribbon diagram shows the α chain (in magenta) and the β chain (in blue). The receptor is viewed from the side as it would sit on a cell surface with the highly variable CDR loops, which bind the peptide:MHC molecule ligand, arrayed across its relatively flat top surface. The CDR loops are numbered 1–3 for each chain. Courtesy of I.A. Wilson.

Comparison of the amino-acid sequences of V domains from different clones of T cells shows that sequence variation in the α and β chains is clustered into regions of hypervariability, which correspond to loops of the polypeptide chain at the end of the domain farthest from the T-cell membrane. These loops form the binding site for antigen and are termed complementarity-determining regions (CDRs), as in the immunoglobulins. The T-cell receptor α- and β-chain V domains each have three CDR loops, called CDR1, CDR2, and CDR3 (Figure 3.2).

Immunoglobulins possess two or more binding sites for antigen; this strengthens the interactions of soluble antibody with the repetitive antigens found on the surfaces of microorganisms. T-cell receptors possess a single binding site for antigen and are used only as cell-surface receptors for antigen, never as soluble antigen-binding molecules. Antigen binding to T-cell receptors occurs always in the context of two opposing cell surfaces, where multiple copies of the T-cell receptor bind to multiple copies of the antigen:MHC complex on the opposing cell, thus achieving multipoint attachment.

3-2 T-cell receptor diversity is generated by gene rearrangement

In Chapter 2 we divided the mechanisms that generate immunoglobulin diversity into two categories: those operating before the B cell is stimulated with specific antigen and those operating afterwards. In the first category were the gene rearrangements that generate the V-region sequence, whereas in the second category were changes in mRNA splicing that produce a secreted immunoglobulin, C-region DNA rearrangements that switch the heavy-chain isotype, and somatic hypermutation of the V-region gene to produce antibodies of higher affinity. In T cells the mechanisms that generate diversity before antigen stimulation are essentially the same as those in B cells, but after antigen stimulation the picture is quite different: whereas immunoglobulin genes continue to diversify, the genes encoding T-cell receptors remain unchanged.

This fundamental difference reflects the fact that the T-cell receptor is used only for the recognition of antigen and not for the generation of effector functions, which is handled by other T-cell molecules. In contrast, the effector functions of B cells are solely dependent on secreted antibodies, whose different C-region isotypes trigger different effector mechanisms. The diversification of an antibody that occurs subsequently to antigen stimulation is directed towards optimizing both its antigen-binding and effector functions.

The human T-cell α-chain locus is on chromosome 14 and the β-chain locus is on chromosome 7. The organization of the gene segments encoding T-cell receptor α and β chains is essentially like that of the immunoglobulin gene segments (Figure 3.3). The main difference is the simplicity of the T-cell receptor C region: there is only one C_α gene, and, although there are two C_β genes, no functional distinction between them is known. The T-cell receptor

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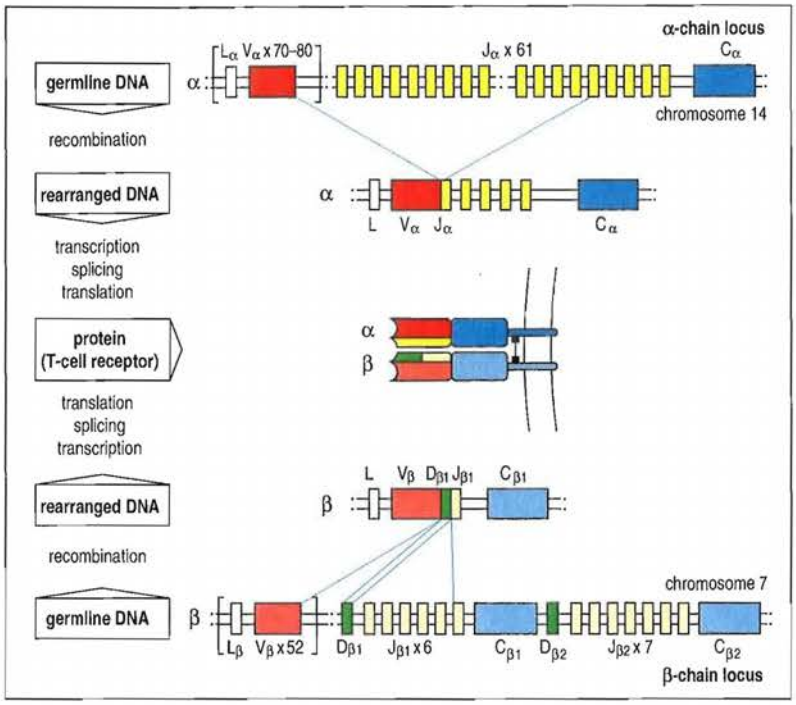


Figure 3.3 Organization and rearrangement of the T-cell receptor genes. The top and bottom rows of the figure show the germline arrangement of the variable (V), diversity (D), joining (J), and constant (C) gene segments at the T-cell receptor α- and β-chain loci. During T-cell development, a V-region sequence for each chain is assembled by DNA recombination. For the α chain (top), a V_α gene segment rearranges to a J_α gene segment to create a functional exon encoding the V domain. For the β chain (bottom), rearrangement of a V_β, a D_β, and a J_β gene segment creates the functional V-domain exon. The assembled genes are transcribed and spliced to produce mRNA (not shown) encoding the α and β chains. Exons encoding the membrane-spanning regions are not shown. L, leader sequence.

α-chain locus is otherwise more similar to an immunoglobulin light-chain locus, containing sets of V and J gene segments only; the β-chain locus is similar to an immunoglobulin heavy-chain locus, containing D gene segments in addition to V and J gene segments. The V domain of the T-cell receptor α chain is thus encoded by a V gene segment and a J gene segment; that of the β chain is encoded by a diversity (D) gene segment in addition to a V and a J gene segment.

T-cell receptor gene rearrangement occurs during T-cell development in the thymus, and the mechanisms involved are similar to those outlined in Chapter 2 for the immunoglobulin genes. In the α-chain gene, a V gene segment is joined to a J gene segment by somatic DNA recombination to make the V-region sequence; in the β-chain gene, recombination first joins a D and a J gene segment, which are then joined to a V gene segment (see Figure 3.3). The T-cell receptor gene segments are flanked by recombination signal sequences similar to those found in immunoglobulin genes and the same RAG complex and other DNA-modifying enzymes are involved in the recombination process in both cell types (see Section 2-8, p. 51). During recombination, additional, nontemplated P and N nucleotides (see Section 2-8, p. 51) are inserted in the junctions between the V, D, and J gene segments of the T-cell receptor β-chain coding sequence and between the V and J gene segments of the α-chain sequence. These mechanisms contribute junctional diversity in the CDR3 to T-cell receptor α and β chains.

Rare genetic defects that cause one or other of the RAG genes not to work are one of the causes of a syndrome called severe combined immunodeficiency disease (SCID). The disease is called combined because functional B and T lymphocytes are equally absent and the disease is severe compared to immunodeficiencies in which only B cells are lacking. Without a bone marrow transplant or other medical intervention, children with SCID die in infancy

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from common infections (Figure 3.4). In the case of the RAG genes, missense mutations that produce RAG proteins with partial enzymatic activity have also been found; these cause a rapidly fatal immunodeficiency that differs from SCID in some of its symptoms and is known as Omenn syndrome (Figure 3.5).

After gene rearrangement functional α- and β-chain genes consist of exons encoding the leader peptide, V region, and C region, as well as the membrane-spanning region. The exons are separated by introns, which in the case of the intron between the V-domain and the C-domain exon may contain unrearranged gene segments (see the α-chain gene in Figure 3.3). Upon transcription, the primary RNA transcript is spliced to remove the introns and is processed to give mRNA. Translation of the α-chain and β-chain mRNA produces α and β chains, respectively. Like all proteins destined for the cell membrane, newly synthesized α and β chains enter the endoplasmic reticulum. There they pair to form the **α:β T-cell receptor** (see Figure 3.3).

3-3 Expression of the T-cell receptor on the cell surface requires association with additional proteins

T-cell receptors are diverse and specific receptors for antigen. By themselves, however, heterodimers of α and β chains are unable to leave the endoplasmic reticulum and be expressed on the T-cell surface. In this respect the T-cell receptor resembles immunoglobulin. Before leaving the endoplasmic reticulum, an α:β heterodimer associates with four invariant membrane proteins. Three of the proteins are encoded by closely linked genes of human chromosome 11 and are homologous to each other: these proteins are collectively termed the **CD3 complex** and individually called CD3γ, CD3δ, and CD3ε. The fourth protein is known as the ζ chain and is encoded by a gene on human chromosome 1.

At the cell surface the CD3 proteins and the ζ chain remain in stable association with the T-cell receptor and form the functional **T-cell receptor complex** (Figure 3.6). In this complex the CD3 proteins and the ζ chain transduce signals to the cell's interior after antigen has been recognized by the α and β chain heterodimer. Correlating with these functional differences the cytoplasmic domains of the CD3 proteins and the ζ chain contain sequences that associate with intracellular signaling molecules. In contrast, the T-cell receptor α and β chains have very short cytoplasmic tails that lack signaling function, as is also true for immunoglobulin chains. In people lacking functional CD3δ or CD3ε chains, transport of T-cell receptors to the cell surface is inefficient and, thus, their T cells express abnormally low numbers of receptors. As a consequence of both low T-cell receptor expression and impaired signal transduction these people suffer from immunodeficiency.

3-4 γ and δ chains form a second class of T-cell receptor expressed by a distinct population of T cells

There is a second type of T-cell receptor that is similar in overall structure to the α:β receptor but is formed of two different protein chains termed γ (not to be confused with CD3γ) and δ. The γ chain resembles the α chain, and the δ chain resembles the β chain (Figure 3.7). T cells express either α:β receptors or γ:δ receptors but never both. Those expressing α:β T-cell receptors are called **α:β T cells**, whereas those expressing γ:δ receptors are called **γ:δ T cells**. Cells with γ:δ receptors form a small subset of all T cells. Much more is known of the functions of α:β T cells than of γ:δ T cells. Consequently, in the rest of this book, T cells will generally refer to α:β T cells and T-cell receptor will refer to the α:β T-cell receptor, unless specified otherwise.



Figure 3.4 An infant with SCID suffering from *Candida albicans* infection in the mouth. Courtesy of Fred Rosen.



Figure 3.5 Bright red rash on the face and shoulders of an infant with Omenn syndrome. Photograph kindly provided by Luigi Notarangelo.

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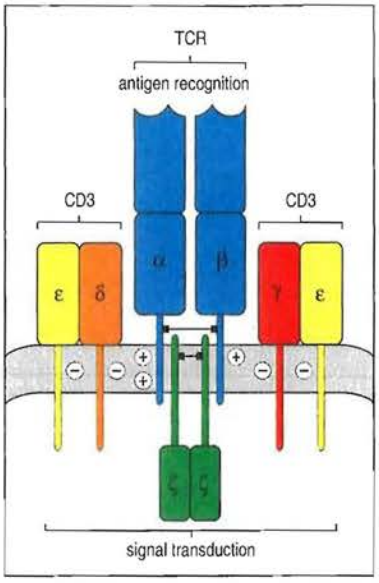


Figure 3.6 Polypeptide composition of the T-cell receptor complex. The functional antigen receptor on the surface of T cells is composed of eight polypeptides and is called the T-cell receptor complex. The α and β chains bind antigen and form the core T-cell receptor (TCR). They associate with one copy each of CD3γ and CD3δ and two copies each of CD3ε and the ζ chain. These associated invariant polypeptides are necessary for transport of newly synthesized TCR to the cell surface and for transduction of signals to the cell's interior after the TCR has bound antigen. The transmembrane domains of the α and β chains contain positively charged amino acids (+), which form strong electrostatic interactions with negatively charged amino acids (–) in the transmembrane regions of the CD3γ, δ, and ε chains.

The organization of the γ and δ loci resembles that of the β and α loci, but there are some important differences (Figure 3.8). The δ gene segments are situated within the α-chain locus on chromosome 14, between the V_α and J_α gene segments. This location means that DNA rearrangement within the α-chain locus inevitably results in the deletion and inactivation of the δ-chain locus. The human γ-chain locus is on chromosome 7. The γ- and δ-chain loci contain fewer V gene segments than the α- or β-chain loci, and so, in theory, might produce less diverse receptors, but for the γ chain this limitation is compensated for by an increase in junctional diversity.

Rearrangement at the γ and δ loci proceeds as for the α and β loci, with the exception that during δ-gene rearrangement two D segments can be incorporated into the final gene sequence. This increases the variability of the δ chain in two ways. First, the potential number of combinations of gene segments is increased. Second, extra N nucleotides can be added at the junction between the two D segments, as well as at the VD and DJ junctions.

T cells bearing γ:δ receptors comprise about 1–5% of the T cells found in the circulation, but they can be the dominant T-cell population in epithelial tissue. The immune function of γ:δ T cells is less well defined than that of α:β T cells, as are the antigens to which these cells respond and the ligands that their receptors engage. Unlike α:β T cells, the γ:δ T cells are not restricted to the recognition of antigens associated with MHC molecules.

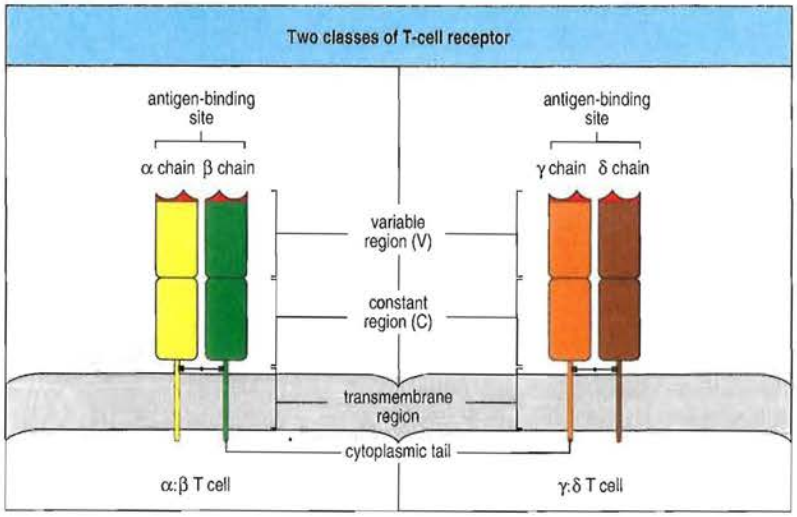


Figure 3.7 There are two classes of T-cell receptors. The α:β T-cell receptor (left panel) and the γ:δ T-cell receptor (right panel) have similar structures, but they are encoded by different sets of rearranging gene segments and have different functions.

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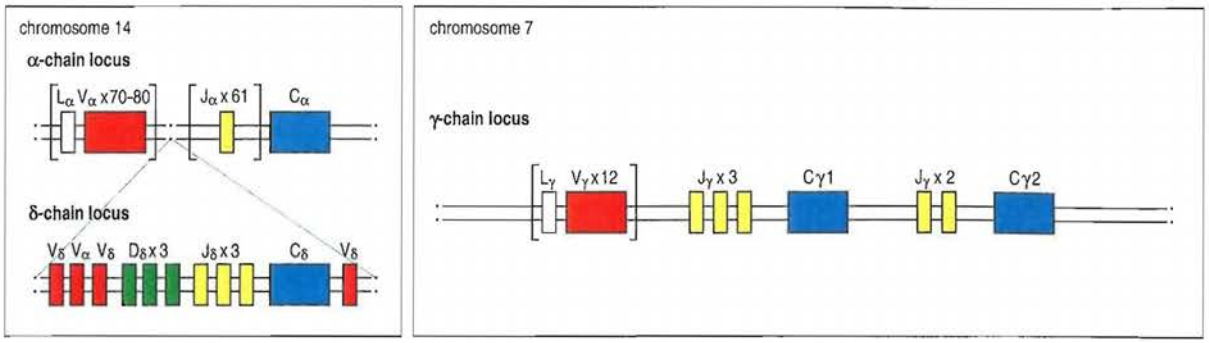


Figure 3.8 The organization of the human T-cell receptor γ- and δ-chain loci. The γ and δ loci, like the α and β loci, contain sets of variable (V), diversity (D), joining (J), and constant (C) gene segments. The δ locus is located within the α-chain locus on chromosome 14, lying between the clusters of V_α and J_α gene segments. There are at least three V_δ gene segments, three D_δ gene segments, three J_δ gene segments, and a single C_δ gene segment. V_δ segments are interspersed amongst V_α and other gene segments. The γ locus, on chromosome 7, resembles the β locus, with two C gene segments each with its own set of J segments.

Summary

T cells recognize antigen through a cell-surface receptor known as the T-cell receptor, which has structural and functional similarities to the membrane-bound immunoglobulin that serves as the B-cell receptor for antigen. T-cell receptors are heterodimeric glycoproteins in which each polypeptide chain consists of a V domain and a C domain, similar to those found in immunoglobulin chains, and a membrane-spanning region. The three-dimensional structure of the extracellular domains of the T-cell receptor resembles that of the antigen-binding Fab fragment of IgG. There are two types of T-cell receptor: one made up of α and β chains and expressed by T cells whose function is understood, and another made up of γ and δ chains and carried by T cells whose function remains elusive. All four types of T-cell receptor chain are encoded by genes that resemble the immunoglobulin genes and require similar DNA rearrangements in order to be expressed. The critical difference between T-cell receptors and immunoglobulins is that T-cell receptors serve only as cell-surface receptors and are not secreted as soluble proteins with effector function; T cells use other molecules for effector function. This explains why the T-cell receptor has only a single binding site for antigen that does not change its affinity on encountering antigen and a simple constant region that does not switch isotype. Expression of the T-cell receptor at the T-cell surface requires association with proteins of the CD3 complex. These proteins transmit signals to the interior of the cell when the T-cell receptor binds antigen.

Antigen processing and presentation

Unlike the immunoglobulins of B cells, which can recognize a wide range of molecules in their native form, a T-cell receptor can recognize antigen only in the form of a peptide bound to an MHC molecule on the surface of a human cell. This means that pathogen-derived proteins must be degraded into peptides to be recognized by T cells. This **antigen processing** occurs inside the body's own cells; the peptides are then assembled into peptide:MHC molecule complexes for display on cell surfaces, where they are recognized by T cells. The binding of a peptide antigen by an MHC molecule and its display at the cell surface is termed **antigen presentation** (Figure 3.9).

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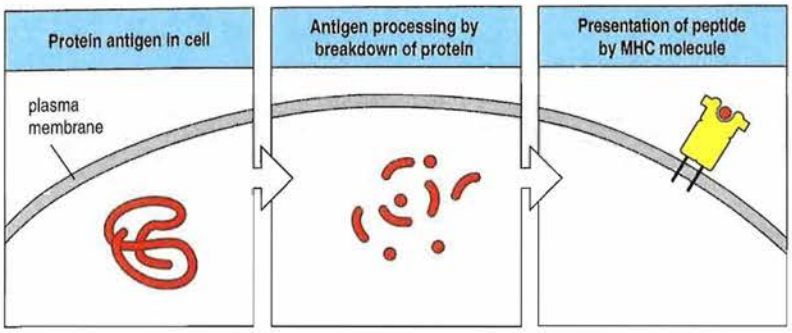


Figure 3.9 Antigen processing and presentation. The antigens recognized by T cells are peptides that arise from the breakdown of macromolecular structures, the unfolding of individual proteins, and their cleavage into short fragments. These events constitute antigen processing. For a T-cell receptor to recognize a peptide antigen, the peptide must be bound by an MHC molecule and displayed at the cell surface, a process called antigen presentation.

The microorganisms that infect the human body can be broadly divided into those that propagate within cells, such as viruses, and those, such as most bacteria, that live in the extracellular spaces. The T-cell population that fights infection is composed of two subpopulations: one specialized to fight intracellular infections, the other to fight extracellular sources of infection. The latter include bacterial species that live in extracellular spaces and virus particles present in the extracellular fluid after release from infected cells. In this part of the chapter we shall consider how these two sources of antigen are distinguished by the immune system and processed by intracellular pathways, and how the appropriate type of T cell is activated.

3-5 Two classes of T cell are specialized to respond to intracellular and extracellular sources of infection

Circulating α:β T cells fall into one of two mutually exclusive classes: one is defined by expression of the **CD4** glycoprotein on the cell surface and the other by expression of the **CD8** glycoprotein (Figure 3.10). These two classes of T cell have different functions and deal with different types of pathogen. **CD8 T cells** are cytotoxic and their main function is to kill cells that have become infected with a virus or some other intracellular pathogen. This response prevents the multiplication of the pathogen and further infection of healthy cells. The general function of **CD4 T cells** is to help other cells of the immune system to respond to extracellular sources of infection. Different aspects of this response are carried out by two subclasses of CD4 T cells—T_H1 and T_H2. The H in their names stands for 'helper' and CD4 T cells are often also known as **helper T cells**. **T_H2 cells** are involved mainly in stimulating B cells to make antibodies, which bind to extracellular bacteria and virus particles, whereas a function of **T_H1 cells** is to activate tissue macrophages to phagocytose and kill extracellular pathogens, and to secrete cytokines and other biologically active molecules that affect the course of the immune response (Figure 3.11).

The human immunodeficiency virus (HIV), which causes acquired immunodeficiency syndrome (AIDS), selectively infects CD4 T cells by exploiting the CD4 molecule as its receptor. On binding to CD4 on a T-cell surface the virus gains entry to the cell.

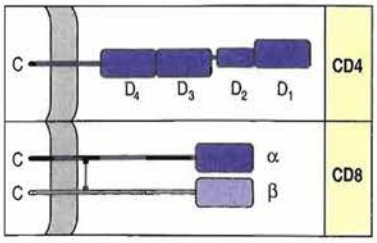


Figure 3.10 The structures of the CD4 and CD8 glycoproteins. CD4 has four extracellular immunoglobulin-like domains (D_1–D_4) with a hinge between the D_2 and D_3 domains. CD8 consists of an α and a β chain, which both have an immunoglobulin-like domain that is connected to the membrane-spanning region by an extended stalk. C denotes the carboxy terminus.

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3-6 Two classes of MHC molecule present antigen to CD8 and CD4 T cells respectively

The MHC molecules are crucial in ensuring that the appropriate class of T cells is activated in response to a particular source of infection. There are two different classes of MHC molecule—**MHC class I** and **MHC class II**—and each presents peptides from one kind of antigen source to one type of T cell. MHC class I molecules present antigens of intracellular origin to CD8 T cells, whereas MHC class II molecules present antigens of extracellular origin to CD4 T cells. The basis for this correlation is specific molecular interactions between the CD8 glycoprotein and MHC class I molecules and between CD4 and MHC class II molecules (Figure 3.12). These occur when a T-cell receptor recognizes its specific peptide:MHC molecule ligand. Because of their close involvement in antigen recognition, the CD4 and CD8 molecules are called T-cell **co-receptors**.

Before we consider how the appropriate MHC molecules become associated with peptides from different sources, we shall look at the structure and general peptide-binding properties of MHC molecules.

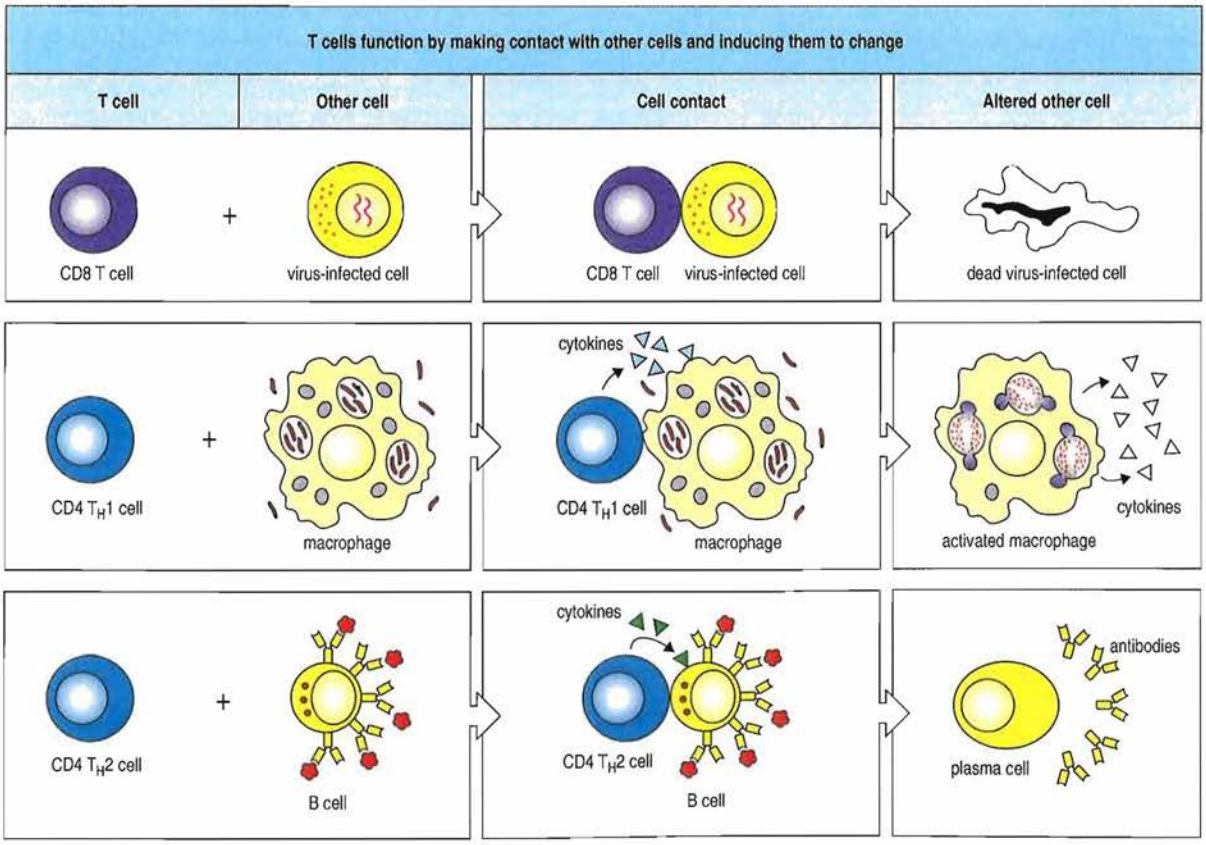


Figure 3.11 T cells function by making contact with other cells. The functions of three types of T cell are shown. Top panels: the cytotoxic CD8 T cell makes contact with a virus-infected cell, recognizes that it is infected, and kills it. Middle panels: the CD4 T_H1 helper cell contacts a macrophage that is engaged in the phagocytosis of bacteria and secretes cytokines that increase the microbicidal powers of the macrophage and its secretion of inflammatory cytokines. Bottom panels: the CD4 T_H2 helper cell contacts a B cell that is binding its specific antigen and secretes cytokines that cause the B cell to differentiate into an antibody-secreting plasma cell.

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3-7 The two classes of MHC molecule have similar three-dimensional structures

MHC class I and class II molecules are membrane glycoproteins whose function is to bind peptide antigens and present them to T cells. Underlying this common function is a similar three-dimensional structure, which is formed in different ways in the two types of molecule.

The MHC class I molecule is made up of a transmembrane heavy chain, or α chain, which is noncovalently complexed with the small protein **β_2-microglobulin** (Figure 3.13). The heavy chain has three extracellular domains (α_1, α_2, and α_3). The peptide-binding site is formed by the folding of α_1 and α_2, the domains farthest from the membrane, and is supported by the α_3 domain and the β_2-microglobulin. The MHC class I heavy chain is encoded by a gene in the MHC, whereas β_2-microglobulin is not.

In contrast, the MHC class II molecule consists of two transmembrane chains (α and β), each of which contributes one domain to the peptide-binding site and one immunoglobulin-like supporting domain (see Figure 3.13). Both of these chains are encoded by genes in the MHC. Thus, both classes of MHC molecule have similar three-dimensional structures consisting of two pairs of

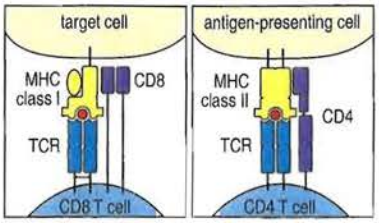


Figure 3.12 MHC class I and II molecules bind to different T-cell co-receptors. Left panel: the CD8 co-receptor of cytotoxic T cells binds to the MHC class I molecule on an infected target cell. Right panel: the CD4 co-receptor of helper T cells binds to the MHC class II molecule on an antigen-presenting cell. TCR, T-cell receptor.

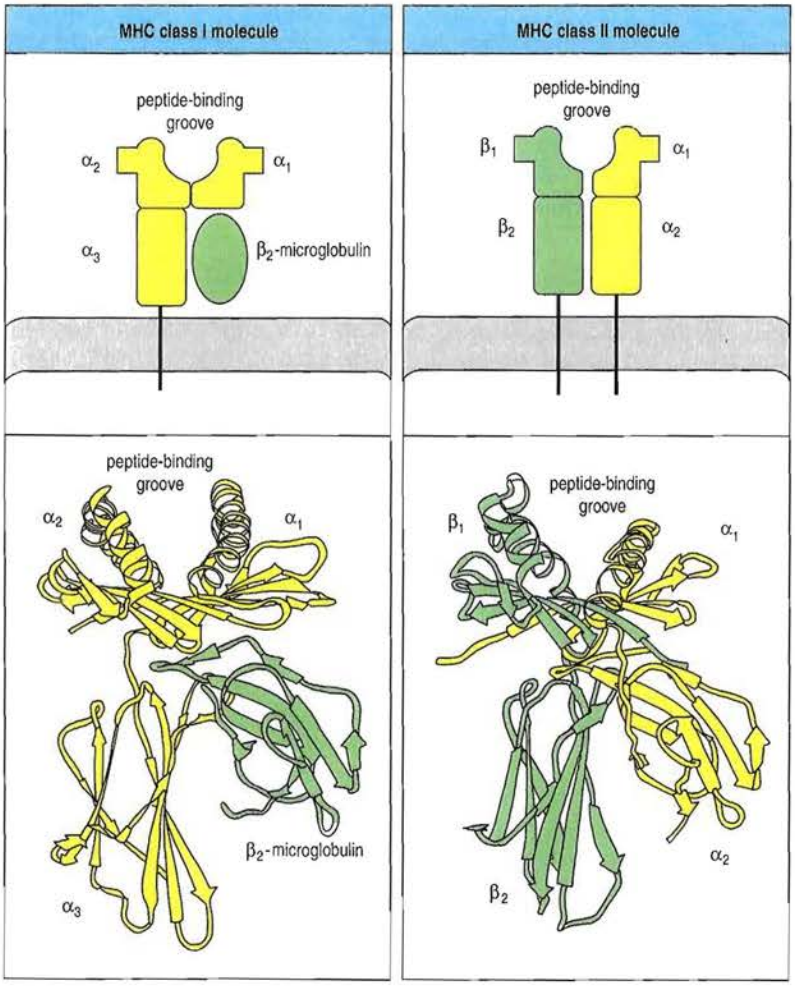


Figure 3.13 The structures of MHC class I and MHC class II molecules are variations on a theme. An MHC class I molecule (left panels) is composed of one membrane-bound heavy (or α) chain and noncovalently bonded β_2-microglobulin. The heavy chain has three extracellular domains, of which the amino-terminal α_1 and α_2 domains resemble each other in structure and form the peptide-binding site. An MHC class II molecule (right panels) is composed of two membrane-bound chains, an α chain (which is a different protein from MHC class I α) and a β chain. These have two extracellular domains each, the amino-terminal two (α_1 and β_1) resembling each other in structure and forming the peptide-binding site. The β_2 domain of MHC class II molecules should not be confused with the β_2-microglobulin of MHC class I molecules. The ribbon diagrams in the lower panels trace the paths of the polypeptide backbone chains.

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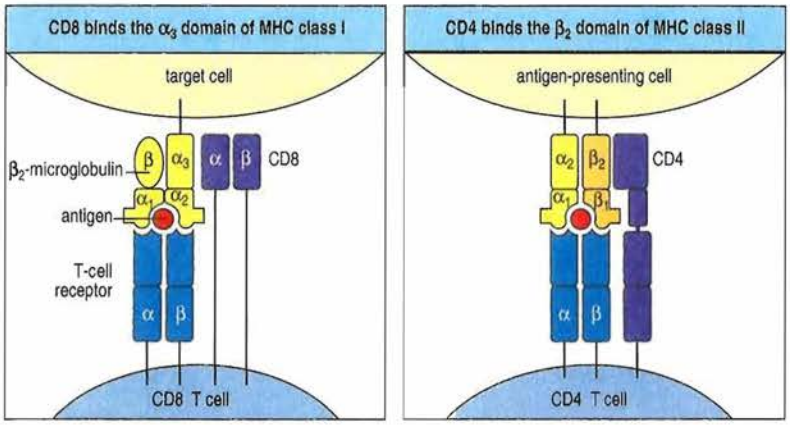


Figure 3.14 MHC class I molecules bind to CD8, and MHC class II molecules bind to CD4. The CD8 co-receptor binds to the α_3 domain of the MHC class I heavy chain, ensuring that MHC class I molecules present peptides only to CD8 T cells (left panel). In a complementary fashion, the CD4 co-receptor binds to the β_2 domain of MHC class II molecules, ensuring that peptides bound by MHC class II stimulate only CD4 T cells (right panel).

extracellular domains, with the paired domains farthest from the membrane resembling each other and forming the peptide-binding site. In both types of MHC molecule, the domains supporting the peptide-binding domains are immunoglobulin-like domains: α_3 and β_2-microglobulin in MHC class I molecules, and α_2 and β_2 in MHC class II molecules.

The immunoglobulin-like domains of MHC class I and II molecules are not just a support for the peptide-binding site; they also provide binding sites for the CD4 and CD8 co-receptors. Thus, the sites on the MHC molecule that interact with the T-cell receptor and the co-receptor are separated, allowing the simultaneous engagement of both T-cell receptor and co-receptor by an MHC molecule (Figure 3.14).

3-8 MHC molecules bind a variety of peptides

MHC molecules have peptide-binding sites that are capable of binding peptides of many different amino-acid sequences. This **degenerate binding specificity** contrasts with the specificity of an immunoglobulin or a T-cell receptor for a single epitope. The peptide-binding site is a deep groove on the surface of the MHC molecule (Figure 3.15), within which a single peptide is held tightly by noncovalent bonds.

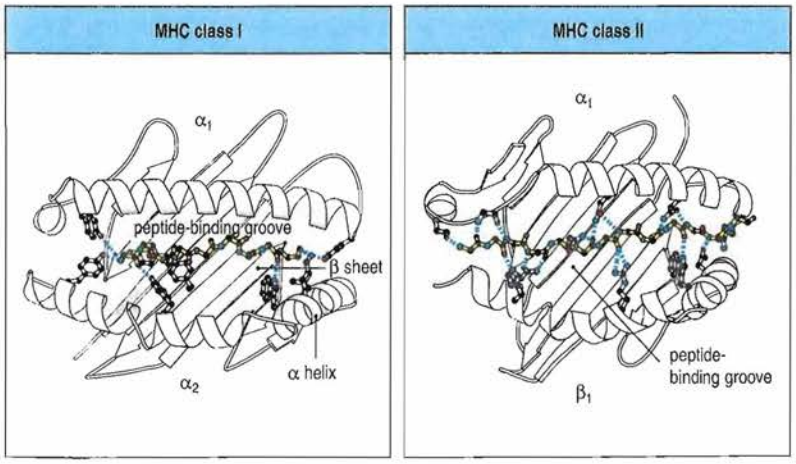


Figure 3.15 The peptide-binding groove of MHC class I and MHC class II molecules. The T-cell receptor's view of the peptide-binding groove, with a peptide bound, is shown. In the MHC class I molecule (left panel) the groove is formed by the α_1 and α_2 domains of the MHC class I heavy chain; in the MHC class II molecule (right panel) it is formed by the α_1 domain of the class II α chain and the β_1 domain of the class II β chain. Amino-acid side-chains on the MHC molecule that are important for making interactions with the bound peptide are shown. The dotted blue lines indicate hydrogen bonds and ionic interactions made between the peptide and the MHC molecule. Peptides bind to MHC class I molecules by their ends (left panel), whereas in MHC class II molecules, the peptide extends beyond the peptide-binding groove and is held by interactions along its length (right panel).

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There are, however, constraints on the length and amino-acid sequence of peptides that are bound by MHC molecules. These are dictated by the structure of the peptide-binding groove, which differs between MHC class I and MHC class II molecules. The length of the peptides bound by MHC class I molecules is limited because the two ends of the peptide are grasped by pockets situated at the ends of the peptide-binding groove (see Figure 3.15, left panel). The vast majority of peptides that bind MHC class I molecules are eight, nine, or ten amino acids in length; most are nine amino acids. The differences in peptide length are accommodated by a slight kinking of the extended conformation of the bound peptide. Features that are common to all peptides—the amino terminus, the carboxy terminus, and the peptide backbone—interact with binding pockets present in all MHC class I molecules, and these form the basis for all peptide–MHC class I interactions. Most peptides bound by MHC class I molecules also have a hydrophobic or basic residue at the carboxyl terminus, which corresponds to a complementary pocket present in the binding groove of MHC class I molecules.

In MHC class II molecules, the two ends of the peptide are not pinned down into pockets at each end of the peptide-binding groove (see Figure 3.15, right panel). As a consequence, they can extend out at each end of the groove and so peptides bound by MHC class II molecules are both longer and more variable in length than peptides bound by MHC class I. Peptides that bind to MHC class II molecules are usually 13–25 amino acids in length and some are much longer.

3-9 Peptides generated in the cytosol are transported into the endoplasmic reticulum where they bind MHC class I molecules

The peptide antigens that are bound and presented by MHC molecules are generated inside cells of the body by the breakdown of larger protein antigens. Proteins derived from "intracellular" and "extracellular" antigens are present in different intracellular compartments (Figure 3.16). They are processed into peptides by two intracellular pathways of degradation, and bind to the two classes of MHC molecule in separate intracellular compartments. Peptides derived from the degradation of intracellular pathogens are formed in the cytosol and delivered to the endoplasmic reticulum. This is where MHC class I molecules bind peptides. In contrast, extracellular microorganisms and proteins are taken up by cells via phagocytosis and endocytosis and are degraded in the lysosomes and other vesicles of the endocytic pathways. It is in these cellular compartments that MHC class II molecules bind peptides. In this way, the class of the MHC molecule labels the peptide as being extracellular or intracellular in origin. In this section we shall look at the processing pathway for intracellular antigens.

When viruses infect human cells they exploit the cell's ribosomes to synthesize viral proteins, which are therefore present in the cytosol before being assembled into viral particles. In response, the infected cell uses its normal processes of breakdown and turnover of cellular proteins to degrade some of the viral proteins into peptides that can be bound by MHC class I molecules and presented to CD8 T cells.

Proteins in the cytosol are degraded by a large barrel-shaped protein complex called the **proteasome**, which has several different protease activities. It consists of 28 polypeptide subunits, each of 20–30 kDa molecular weight. Once formed, the antigenic peptides are transported out of the cytosol and into the endoplasmic reticulum (Figure 3.17). Transport of peptides across the endoplasmic reticulum membrane is accomplished by a protein, called the **transporter associated with antigen processing** (**TAP**), embedded in the membrane.

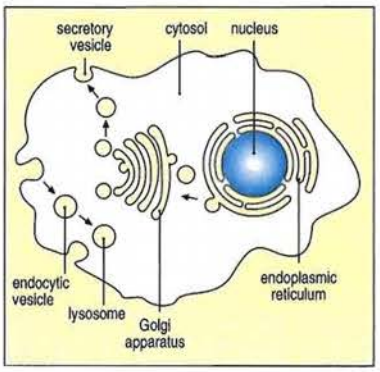


Figure 3.16 There are two major compartments within cells, separated by membranes. One compartment is the cytosol, which is contiguous with the nucleus via the pores in the nuclear membrane. The other compartment is the vesicular system, which consists of the endoplasmic reticulum, the Golgi apparatus, endocytic vesicles, lysosomes, and other intracellular vesicles. The vesicular system is effectively contiguous with the extracellular fluid. Secretory vesicles bud off from the endoplasmic reticulum and by successive fusion and budding with the Golgi membranes move vesicular contents out of the cell. In contrast, endocytic vesicles take up extracellular material into the vesicular system.

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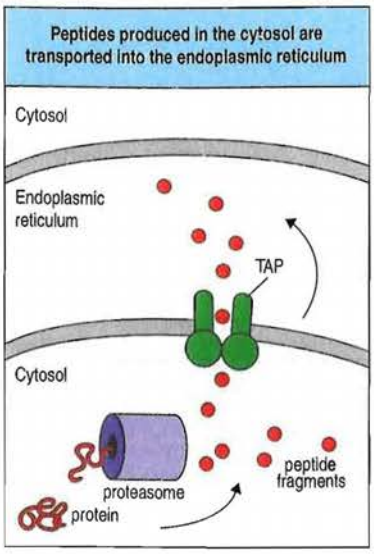


Figure 3.17 Formation and transport of peptides that bind to MHC class I molecules. In all cells, proteasomes degrade cellular proteins that are poorly folded, damaged, or unwanted. When a cell becomes infected, pathogen-derived proteins in the cytosol are also degraded by the proteasome. Peptides are transported from the cytosol into the lumen of the endoplasmic reticulum by the protein called transporter associated with antigen processing (TAP), which is in the endoplasmic reticulum membrane.

TAP is a heterodimer consisting of two structurally related polypeptide chains, TAP-1 and TAP-2. Peptide transport by TAP is dependent on the binding and hydrolysis of ATP, properties shared with the other transporters in this family. The types of peptide that are preferentially transported by TAP are similar to those that bind MHC class I molecules: they are eight or more amino acids long and have either hydrophobic or basic residues at the carboxy terminus.

Newly synthesized MHC class I heavy chains and β_2-microglobulin are also translocated into the endoplasmic reticulum where they partially complete their folding, then associate together, and finally bind peptide, at which point folding is completed. The correct folding and peptide loading of MHC class I molecules within the endoplasmic reticulum is aided by proteins known as chaperones. These are proteins that help the correct folding and subunit assembly of other proteins while keeping them out of harm's way until they are ready to enter cellular pathways and carry out their functions.

When MHC class I heavy chains first enter the endoplasmic reticulum they bind the membrane protein known as **calnexin**, which retains the partly folded heavy chain in the endoplasmic reticulum (Figure 3.18). Calnexin is a

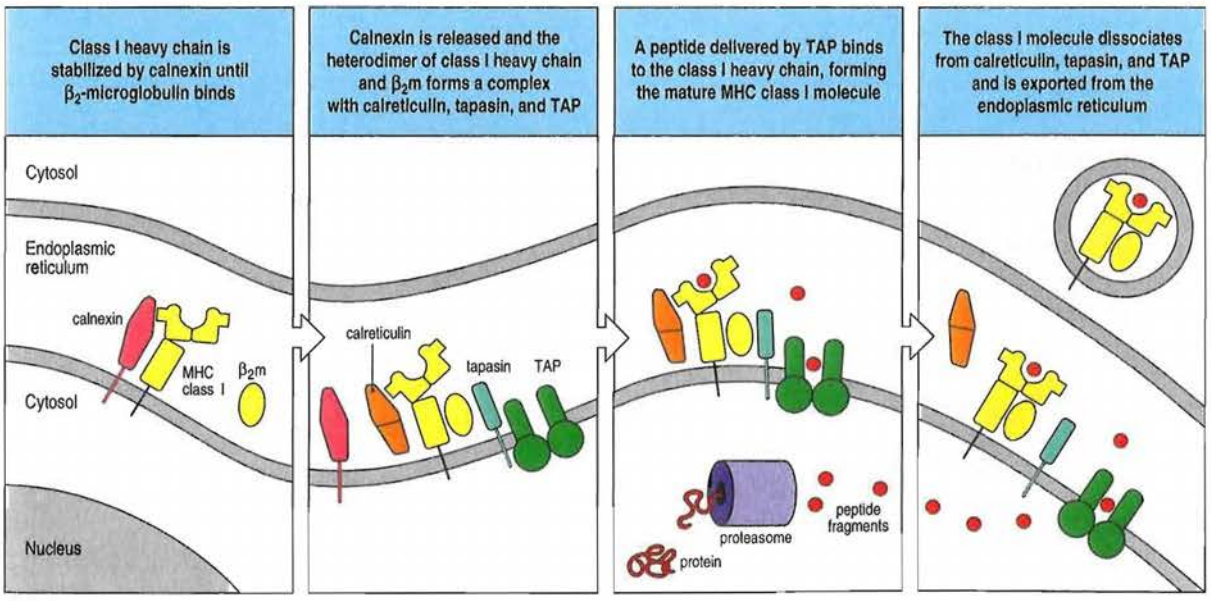


Figure 3.18 Chaperone proteins aid the assembly and peptide loading of MHC class I molecules in the endoplasmic reticulum. MHC class I heavy chains assemble in the endoplasmic reticulum with the membrane-bound protein calnexin. When this complex binds β_2-microglobulin (β_2m) the partly folded MHC class I molecule is released from calnexin and then associates with the TAP-1 subunit of TAP by interacting with the TAP-associated protein tapasin and the chaperone protein calreticulin. The MHC class I molecule is retained in the endoplasmic reticulum until it binds a peptide, which completes the folding of the molecule. The peptide:MHC class I molecule complex is then released from tapasin and calreticulin, leaves the endoplasmic reticulum, and is transported to the cell surface.

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calcium-dependent lectin, a carbohydrate-binding protein that retains many multisubunit glycoproteins, including T-cell receptors and immunoglobulins, in the endoplasmic reticulum until they have folded correctly. When the MHC class I heavy chain has bound β_2-microglobulin, calnexin is released from the α:β_2-microglobulin heterodimer to be replaced by a complex of proteins, one of which, **calreticulin**, resembles a soluble form of calnexin and probably has a similar chaperone function. A second component of the complex, called **tapasin**, binds to the TAP-1 subunit of the peptide transporter, positioning the partly folded heterodimer of heavy chain and β_2-microglobulin to await receipt of a suitable peptide from the cytosol.

On binding a peptide, the now completely assembled MHC class I molecule is released from all chaperones and leaves the endoplasmic reticulum in a membrane-bound vesicle. It then makes its way through the Golgi stacks to the plasma membrane. Most of the peptides transported by TAP are not successful in binding to a MHC class I molecule and are cleared out of the endoplasmic reticulum.

MHC class I molecules cannot leave the endoplasmic reticulum unless they have bound a peptide. In one form of a rare disease called **bare lymphocyte syndrome**, the TAP protein is nonfunctional and so no peptides enter the endoplasmic reticulum. Cells of patients with this defect have less than 1% of the normal level of MHC class I molecules on their surface. As a consequence of this deficiency, patients develop very poor CD8 T-cell responses to viruses and suffer from chronic respiratory infections from a young age.

Protein degradation and peptide transport occur continuously, not only when cells are infected. In the absence of infection, MHC class I molecules carry peptides derived from normal human self-proteins, as also do MHC class II molecules. These do not normally provoke an immune response because of mechanisms that act during T-cell development which eliminate or inactivate T cells reactive to these **self-peptides**. Occasionally, however, this state of T-cell tolerance to self-peptides breaks down, resulting in autoimmunity.

3-10 Peptides presented by MHC class II molecules are generated in acidified intracellular vesicles

Proteins of extracellular bacteria, extracellular virus particles, and soluble protein antigens are processed by a different intracellular pathway from that followed by cytosolic proteins, and their peptide fragments end up bound to MHC class II molecules. Most cells are continually internalizing extracellular fluid and material bound at their surface by the process of **endocytosis**. In addition, cells specialized for **phagocytosis**, the neutrophils and macrophages, engulf larger objects such as dead cells. These uptake mechanisms produce intracellular vesicles known as **endocytic vesicles** or **phagosomes** (in the case of phagocytosis), in which the vesicular membrane is derived from the plasma membrane and the lumen contains extracellular material.

These membrane-bound vesicles become part of an interconnected vesicle system that carries materials to and from the cell surface. As vesicles travel inwards from the plasma membrane, their interiors become acidified by the action of proton pumps in the vesicle membrane and they fuse with other vesicles, such as lysosomes, that contain proteases and hydrolases that are active in acid conditions. Within the **phagolysosomes** formed by this fusion, enzymes degrade the vesicle contents to produce, among other things, peptides from proteins and glycoproteins.

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Microorganisms present in the extracellular environment are taken up by phagocytosis, for example by macrophages, and are then degraded within phagolysosomes. B cells also bind specific antigens via their surface immunoglobulin, then internalize these antigens by receptor-mediated endocytosis. These antigens are similarly degraded within the vesicular system. Peptides produced within phagolysosomes become bound to MHC class II molecules within the vesicular system, and the peptide:MHC class II complexes are carried to the cell surface by outward-going vesicles. Thus, the MHC class II pathway samples the extracellular environment, complementing the MHC class I pathway, which samples the intracellular environment (Figure 3.19).

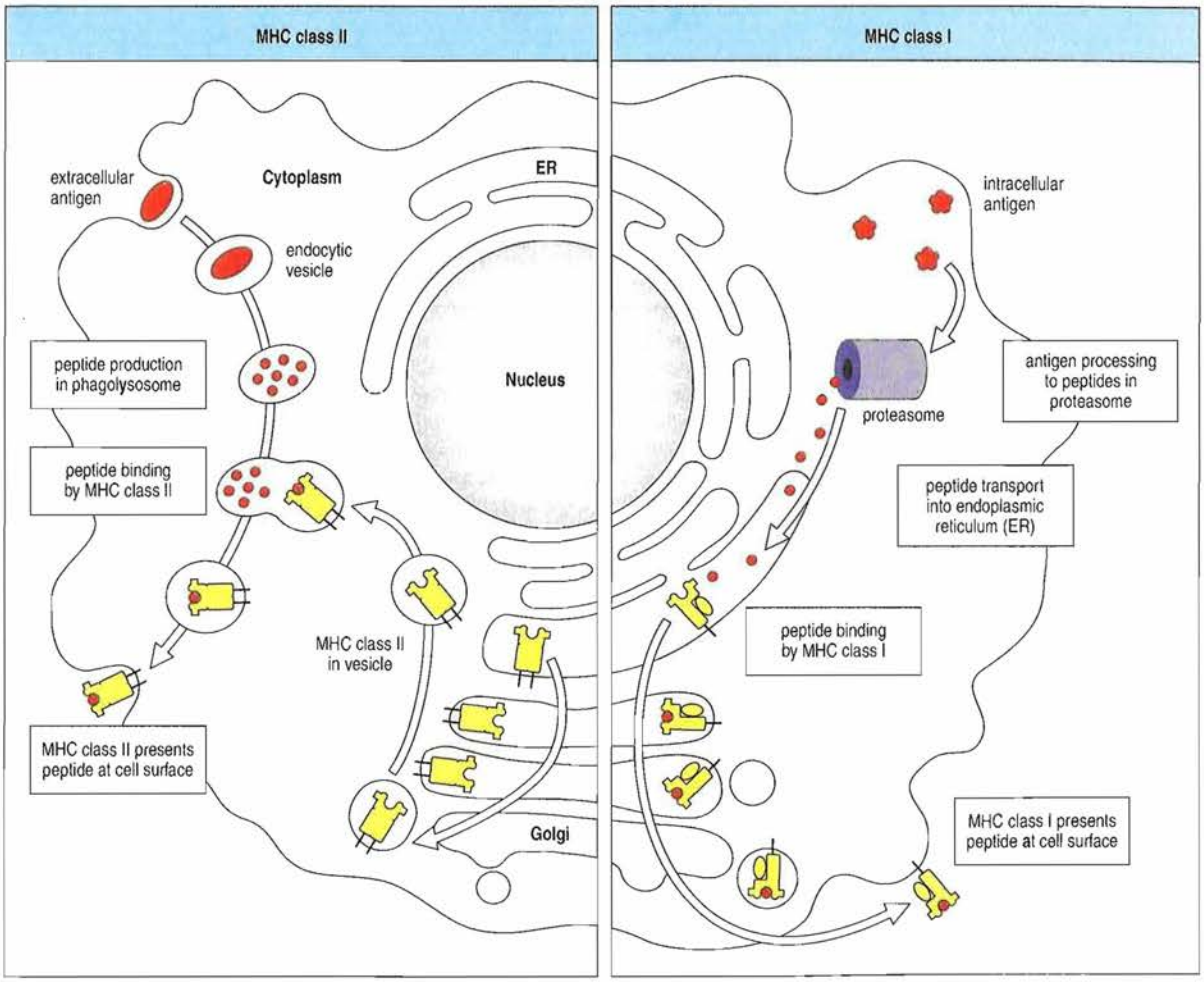


Figure 3.19 Processing of antigens presented by MHC class II and MHC class I molecules occurs in different cellular compartments. The left half of the figure shows the fate of peptides derived from extracellular antigens and pathogens. Extracellular material is taken up by endocytosis and phagocytosis into the vesicular system of the cell, in this case a macrophage. Proteases in these vesicles break down proteins to produce peptides that are bound by MHC class II molecules, which have been transported to the vesicles via the endoplasmic reticulum (ER) and the Golgi apparatus. The peptide:MHC class II complex is transported to the cell surface in outgoing vesicles. The right half of the figure shows the fate of peptides generated in the cytosol as a result of infection with viruses or intracytosolic bacteria. Proteins from such pathogens are broken down in the cytosol by the proteasome to peptides, which enter the ER. There the peptides are bound by MHC class I molecules. The peptide:MHC class I complex is transported to the cell surface via the Golgi apparatus.

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Certain pathogens, for example the species of mycobacteria that cause leprosy and tuberculosis, actually exploit the vesicular system as a protected site for intracellular growth and replication. As their proteins do not enter the cytosol, they are not processed and presented to cytotoxic CD8 T cells by MHC class I molecules. They protect themselves from degradation by lysosomal enzymes by preventing fusion of the phagosome with a lysosome. This also prevents presentation of mycobacterial antigens by MHC class II molecules.

3-11 MHC class II molecules are prevented from binding peptides in the endoplasmic reticulum by the invariant chain

Newly synthesized MHC class II α and β chains are translocated from the ribosomes into the membranes of the endoplasmic reticulum. There, an α chain and a β chain associate with a third chain, called the **invariant chain** (Figure 3.20). This is so called because it is identical in all individuals, whereas the α and β chains vary from one person to another. One function of the invariant chain is to prevent the peptide-binding site formed by association of an α and a β chain from binding peptides present in the endoplasmic reticulum; these peptides are therefore targeted to MHC class I molecules only.

A second function of the invariant chain is to deliver MHC class II molecules to endocytic vesicles, where they bind peptide. These vesicles, which have been called MIIC, for MHC class II compartment, contain proteases, for example cathepsin S, that selectively attack the invariant chain. A series of cleavages leaves just a small fragment of the invariant chain that covers up the MHC class II peptide-binding site. This fragment is called **class II-associated invariant-chain peptide (CLIP)**. Removal of CLIP and binding of peptide is aided by the interaction of the MHC class II molecule with a glycoprotein in the vesicle membrane called **HLA-DM** (see Figure 3.20). HLA-DM resembles an MHC class II molecule in structure but does not bind peptides or appear on the cell surface. HLA-DM catalyzes the removal of CLIP and then allows

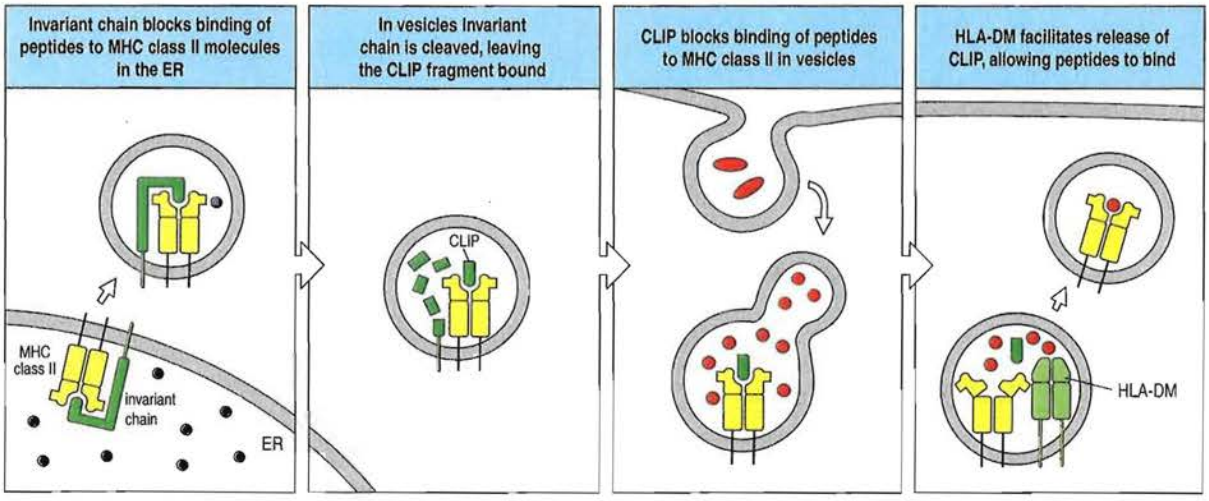


Figure 3.20 The invariant chain prevents peptides from binding to a MHC class II molecule until it reaches the site of extracellular protein breakdown. MHC class II α and β chains are assembled with an invariant chain in the endoplasmic reticulum (ER); this complex is transported to the acidified vesicles of the endocytic system. The invariant chain is broken down, leaving just a small fragment called class II-associated invariant-chain peptide (CLIP) attached in the peptide-binding site. The vesicle membrane protein HLA-DM catalyzes the release of the CLIP fragment and its replacement by a peptide derived from endocytosed antigen that has been degraded within the acidic interior of the vesicles.

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the MHC class II molecule to sample other peptides until it finds one that binds strongly. Once the MHC class II molecule has lost its invariant chain and has bound peptide it is carried to the cell surface by outward-going vesicles.

3-12 The T-cell receptor specifically recognizes both peptide and MHC molecule

Once a peptide:MHC complex appears on the cell surface it can be recognized by its corresponding T-cell receptor. When the T-cell receptor binds to a peptide:MHC molecule complex it makes contacts with both the peptide and the surrounding surface of the MHC molecule. Thus, each peptide:MHC complex forms a unique ligand for its T-cell receptor.

The floor of the peptide-binding groove of both classes of MHC molecule is formed by eight strands of antiparallel β-pleated sheet, on which lie two antiparallel α helices (see Figure 3.15). The peptide lies between the helices and parallel to them, such that the top surfaces of the helices and peptide form a roughly planar surface to which the T-cell receptor binds. The residues of the peptide that bind to the MHC molecule lie deep within the peptide-binding groove and are inaccessible to the T-cell receptor; side chains of other peptide amino acids stick out of the binding site and bind to the T-cell receptor.

In its overall organization the T-cell receptor antigen-binding site resembles that of an antibody (see Figure 3.2). The interaction between T-cell receptors and ligands comprising peptides bound to MHC molecules has been visualized by X-ray crystallography. Analysis of several complexes has revealed similar interactions for peptides bound to either MHC class I or class II molecules, and the interaction is principally illustrated here for MHC class I (Figure 3.21). The T-cell receptor binds to the MHC class I:peptide complex with the long axis of its binding site oriented diagonally across the peptide-binding groove of the MHC class I molecule (Figure 3.21, panel d). The T-cell receptor binds to an MHC class II:peptide complex in a similar orientation (Figure 3.21, panel e). The CDR3 loops of the T-cell receptor α and β chains form the central part of the binding site and they grasp the side chain of one of the amino acids in the middle of the peptide. In contrast, the CDR1 and CDR2 loops form the periphery of the binding site and contact the α helices of the MHC molecule. The CDR3 loops directly contact peptide antigen and they are also the most variable part of the T-cell receptor antigen-recognition site; the α-chain CDR3 includes the joint between the V and J sequences, and the β chain CDR3 includes the joints between V and D, the whole of the D segment, and the joint between D and J.

The T-cell receptor does not interact symmetrically with the face formed by the peptide and the two α helices of the MHC molecule. Consequently, the CDR1 and CDR2 loops of the α chain make stronger contacts with the peptide:MHC complex than do the CDR1 and CDR2 loops of the β chain.

3-13 The two classes of MHC molecule are expressed differentially on cells

T-cell responses are guided in appropriate directions by differential expression of the two classes of MHC molecules on human cells (Figure 3.22). Virtually all the cells of the body express MHC class I molecules constitutively, and as all cell types are susceptible to infection by viral pathogens, this enables comprehensive surveillance by CD8 T cells. The erythrocyte is one cell type that lacks MHC class I, a property that might facilitate its persistent infection by malaria parasites.

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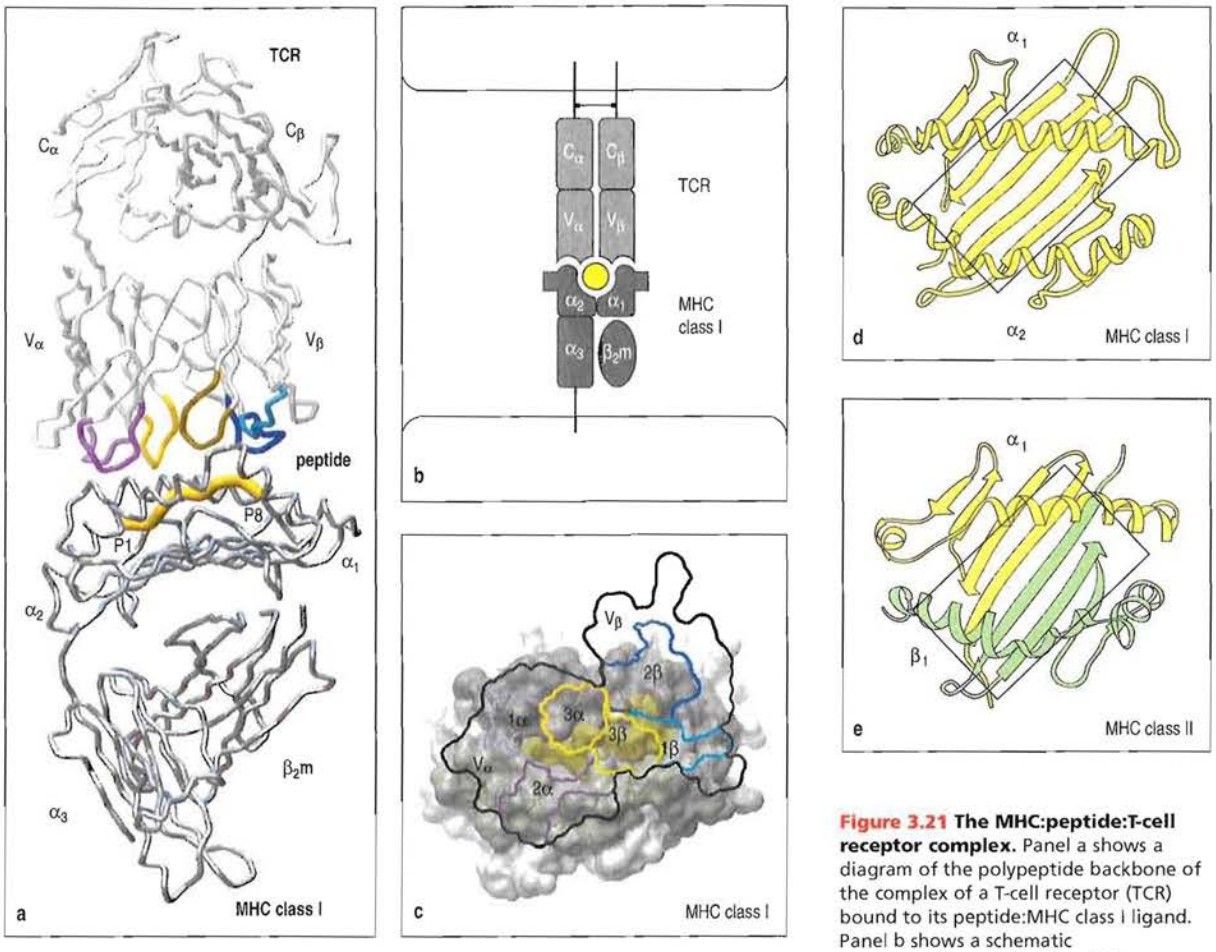


Figure 3.21 The MHC:peptide:T-cell receptor complex. Panel a shows a diagram of the polypeptide backbone of the complex of a T-cell receptor (TCR) bound to its peptide:MHC class I ligand. Panel b shows a schematic representation of this view of the receptor:ligand complex. In panel a the T-cell receptor's CDRs are colored: the α-chain CDR1 and CDR2 are light and dark blue respectively, while the β-chain CDR1 and CDR2 are light and dark purple respectively. The α chain CDR3 is yellow and the β-chain CDR3 is dark yellow. The eight amino-acid peptide is colored yellow and the positions of the first (P1) and last (P8) amino acids are indicated. Panel c is a view rotated 90° from that of panel a and shows the surface of the peptide:MHC class I ligand and the footprint made upon it by the T-cell receptor (outlined in black). Within this footprint the contributions of the CDRs are outlined in different colors and labeled. In panels d and e the diagonal orientation of the T-cell receptor with respect to the peptide-binding grooves of MHC class I and class II molecules, respectively, is shown in schematic diagrams. The T-cell receptor is represented by the black rectangle superimposed on the ribbon diagram (yellow) of the peptide-binding domains of the MHC molecules. Panels a and c courtesy of I.A. Wilson.

MHC class II molecules are, in contrast, constitutively expressed on only a few cell types, which are cells of the immune system specialized for the uptake, processing, and presentation of antigens from the extracellular environment. This distribution is consistent with MHC class II function, alerting CD4 T cells to the presence of extracellular infections. To be effective, this function need not be performed by every cell within a tissue or organ, but requires only a sufficient number of cells equipped to guard the extracellular territory. These **professional antigen-presenting cells** are: dendritic cells, which are supremely specialized for antigen presentation and the activation of T cells; macrophages, which take up antigens by phagocytosis and endocytosis; and B cells, which efficiently internalize specific antigens bound to their surface immunoglobulin.

During the course of an immune response, cells can increase the synthesis and cell-surface expression of MHC molecules beyond constitutive levels. This upregulation, which enhances antigen presentation and T-cell activation, is induced by several cytokines produced by activated immune system cells, in particular the interferons. In addition to increasing the levels of constitutively expressed MHC molecules, interferon-γ (IFN-γ) can induce the expression of MHC class II molecules on some cell types that do not normally produce them. In this manner, presentation of antigen to CD4 T cells by MHC class II molecules can be induced and increased in infected or inflamed tissues.

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Tissue	MHC class I	MHC class II
Lymphoid tissues		
T cells	+++	+*
B cells	+++	+++
Macrophages	+++	++
Other antigen-presenting cells (e.g., dendritic cells)	+++	+++
Epithelial cells of the thymus	+	+++
Other nucleated cells		
Neutrophils	+++	–
Hepatocytes	+	–
Kidney	+	–
Brain	+	–†
Non-nucleated cells		
Red blood cells	–	–

Figure 3.22 Most human cells express MHC class I, whereas only a few cell types express MHC class II. MHC class I molecules are expressed on almost 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 stromal cells in the thymus, although they can be produced by other cell types on exposure to the cytokine interferon-γ. *In humans, activated T cells express MHC class II molecules, whereas resting T cells do not. †In the brain, most cell types are MHC class II-negative, but microglia, which are related to macrophages, are MHC class II-positive.

Summary

T cells expressing α:β T-cell receptors recognize peptides presented at cell surfaces by MHC molecules, the third type of antigen-binding molecule in the adaptive immune system. Unlike immunoglobulins and T-cell receptors, however, MHC molecules have degenerate binding sites and each MHC molecule can bind peptides of many different amino-acid sequences. The peptides are produced by the intracellular degradation of proteins of infectious agents and of self-proteins. An α:β T-cell either expresses the CD8 co-receptor and recognizes peptides presented by MHC class I molecules, or expresses the CD4 co-receptor and recognizes peptides presented by MHC class II molecules. The CD8 co-receptor interacts specifically with MHC class I molecules, and the CD4 co-receptor interacts specifically with MHC class II molecules. Protein antigens from intracellular and extracellular sources are processed into peptides by two different pathways. Peptides generated in the cytosol from viruses and other intracytosolic pathogens enter the endoplasmic reticulum, where they are bound by MHC class I molecules. Thus, these peptides are recognized by CD8 T cells, which are specialized to fight intracellular infections. As all human cells are susceptible to infection, MHC class I molecules are expressed by most cell types. Extracellular material that has been taken up by endocytosis is degraded into peptides in endocytic vesicles and these peptides are bound by MHC class II molecules within the vesicular system. The resulting complex is recognized by CD4 T cells that are specialized to fight extracellular sources of infection by mobilizing other cells of the immune system, such as B cells and macrophages. MHC class II molecules are expressed by a few cell types of the immune system that are specialized to take up extracellular antigens efficiently and activate CD4 T cells.

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The major histocompatibility complex

MHC molecules and other proteins involved in antigen processing and presentation are encoded in a cluster of closely linked genes, which in humans is located on chromosome 6. This region is called the major histocompatibility complex (MHC) because it causes T cells to reject tissues transplanted from unrelated donors to recipients. For some MHC class I and class II molecules, numerous genetic variants are present in the human population. Each variant functions differently in the peptides it binds and the T cells it triggers, differences that, individually and collectively, have helped the human species survive predation by diverse and numerous pathogens. Although the magnitude of MHC diversity is much smaller than that of immunoglobulins or T-cell receptors, it has a major impact on the immune response, disease susceptibility, and the practice of medicine. In this part of the chapter we will consider the immunological and medical consequences of MHC diversity within the human population.

3-14 The diversity of MHC molecules in the human population is due to multigene families and genetic polymorphism

The human MHC is called the **human leukocyte antigen (HLA) complex** because the antibodies used to identify human MHC molecules react with the white cells of the blood—the leukocytes—but not with the red cells, which lack MHC molecules. This observation distinguished the MHC from the other known systems of cell-surface antigens, for example the ABO system matched in blood transfusion, which all involve antigens on the surface of red blood cells. Human MHC class I and II molecules are also called **HLA class I molecules** and **HLA class II molecules**, respectively.

In contrast to immunoglobulins and T-cell receptors, the MHC class I and II molecules are encoded by conventionally stable genes that neither rearrange nor undergo any other developmental or somatic process of structural change. The inherited diversity of MHC molecules has two components. The first component is provided by **gene families**, consisting of multiple similar genes encoding the MHC class I heavy chains, MHC class II α chains and MHC class II β chains. The second component is **genetic polymorphism**, which is the presence within the population of multiple alternative forms of a gene.

The products of the different molecules in a MHC class I or class II family are called isotypes. The different forms of any given gene are called **alleles** and their encoded proteins are called **allotypes**. When considering the diversity of MHC class I or II molecules that arises from the combination of multiple genes and multiple alleles, the term **isoform** can be useful to denote any particular MHC protein. The numerous alleles of certain MHC class I and II genes, and the many differences that distinguish them, make these MHC genes stand out from other polymorphic genes, and they are therefore said to be **highly polymorphic**. MHC class I and II genes that have no polymorphism are described as **monomorphic** and genes having a few alleles are described as **oligomorphic.** An important consequence of genetic polymorphism is that an individual can inherit different forms of the gene from their two parents, in which case they are said to be **heterozygous**; when they inherit the same form of the gene from both parents they are said to be **homozygous**.

In humans there are six MHC class I isotypes: **HLA-A**, **HLA-B**, **HLA-C**, **HLA-E**, **HLA-F**, and **HLA-G**, and five MHC class II isotypes: **HLA-DM**, **HLA-DO**, **HLA-DP**, **HLA-DQ**, and **HLA-DR** (Figure 3.23). Of the class I isotypes, HLA-A, HLA-B, and HLA-C are highly polymorphic and their function is to present antigens

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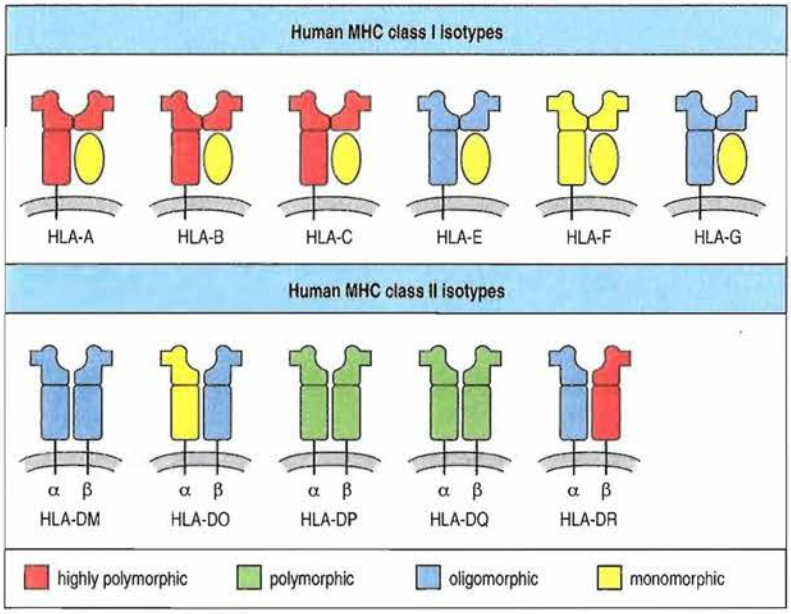


Figure 3.23 Human MHC class I and II isotypes differ in function and the extent of their polymorphism. Of the human MHC class I isotypes, HLA-A, HLA-B and HLA-C present peptide antigens to CD8 T cells and also interact with NK-cell receptors. HLA-E and HLA-G are oligomorphic and interact with NK-cell receptors. HLA-F is intracellular and of unknown function. Of the human MHC class II isotypes, HLA-DP, HLA-DQ and HLA-DR present peptide antigens to CD4 T cells, whereas HLA-DM and HLA-DO are intracellular and regulate peptide loading of HLA-DP, HLA-DQ and HLA-DR.

to CD8 T cells and to form ligands for receptors on natural killer (NK) cells. HLA-E and HLA-G are oligomorphic and form ligands for NK-cell receptors. HLA-F appears to be monomorphic and remains intracellular; its function is unknown. The human MHC class II isotypes also display a range of properties. The three highly polymorphic molecules, **HLA-DP**, **HLA-DQ**, and **HLA-DR**, are those that directly present peptide antigens to CD4 T cells, whereas the oligomorphic **HLA-DM** and **HLA-DO** molecules have functions that regulate peptide loading of HLA-DP, HLA-DQ, and HLA-DR. The numbers of alleles currently known for each HLA locus are listed in Figure 3.24.

The polymorphism of the HLA-A, B, and C molecules is the property of the heavy chain, β_2-microglobulin being monomorphic. By contrast, the polymorphic HLA class II isotypes differ in the diversity contributed by their α and β chains. In HLA-DR the α chain contributes almost no diversity and the β chain is highly polymorphic, whereas for HLA-DP and HLA-DQ class II molecules, both the α and the β chains are polymorphic. Overall there is greater diversity in HLA class I molecules than in HLA class II molecules.

3-15 The MHC class I and class II genes occupy different regions of the MHC

The HLA complex consists of about 4 million base pairs of DNA on the short arm of chromosome 6 and is divided into three regions (Figure 3.25). The **class I region**, at the end of the complex farthest from the chromosome's centromere, contains the six expressed HLA class I genes as well as several nonfunctional class I genes and gene fragments. At the other end of the complex is the **class II region**, which contains all the expressed class II genes and several nonfunctional class II genes. Separating the class I and II regions is a 1 megabase region of DNA called the **class III region** or the **central MHC**; although dense with other types of gene, it contains no class I or II genes. Notably absent from the HLA complex is the gene encoding β_2-microglobulin, the invariant light chain of HLA class I molecules, which is located on human chromosome 15.

HLA polymorphism		
MHC class	HLA locus	Number of allotypes
MHC class I	A	218
	B	439
	C	96
	E	4
	F	1
	G	6
MHC class II	DMA	4
	DMB	6
	DOA	1
	DOB	2
	DPA1	12
	DPB1	88
	DQA1	17
	DQB1	42
	DRA	2
	DRB1	269
	DRB3	30
	DRB4	7
	DRB5	12

Figure 3.24 The polymorphism of HLA class I and class II genes. The number of known functional alleles in the human population for each gene is shown.

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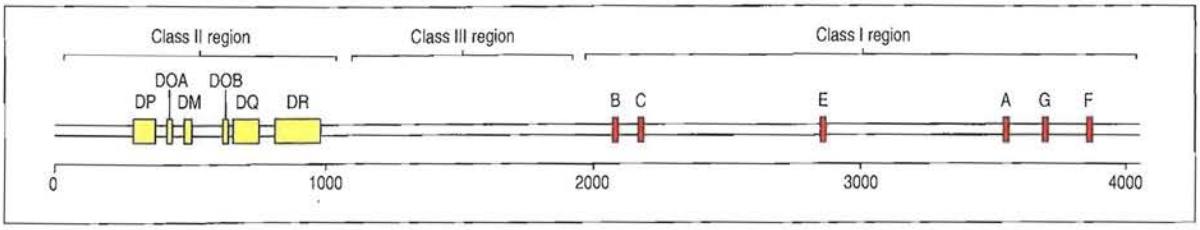


Figure 3.25 The MHC is divided into three regions containing different types of genes. Shown are the positions within the HLA complex (the human MHC) of the HLA class I and II genes. The class I genes (shown in red) are all contained in the class I region and the class II genes (shown in yellow) are all contained in the class II region. Separating the class I and class II regions is the class III region, which contains a variety of genes (not shown), none of which contributes to antigen processing and presentation. For HLA-DM, HLA-DP, HLA-DQ and HLA-DR, the α- and β-chain genes are close together and are shown as a single yellow block; for HLA-DO the α and β genes (DOA and DOB, respectively) are separated by the DM genes and are, therefore, shown separately. Approximate distances are given in thousands of base pairs (kb).

For each HLA class II isotype, the genes encoding the α and β genes are called A and B respectively, for example HLA-DMA and HLA-DMB. When there is more than one gene, including nonfunctional genes, a number in series is added, for example HLA-DQA1 and HLA-DQA2. The genes for the α and β chains of the HLA-DM, HLA-DP, HLA-DQ and HLA-DR class II isotypes cluster together in different subregions within the class II region of the MHC (see Figure 3.25). An exception is HLA-DO, for which HLA-DOA and DOB genes are separated by HLA-DM and other genes. For both HLA-DP and HLA-DQ there are two pairs of genes, one being functional (HLA-DPA1, DPB1 and HLA-DQA1,DQB1) and the other nonfunctional (HLA-DPA2, DPB2 and HLA-DQA2, DQB2). For HLA-DR there is a single HLA-DRA gene, but four different genes encoding HLA-DR β chains (DRB1, DRB3, DRB4, and DRB5) and several nonfunctional genes (DRB2, DRB6, DRB7, DRB8, and DRB9). Only the DRB1 gene is present on all chromosomes 6 and for some people this is the only DRB gene expressed. Three other types of chromosome 6 carry either DRB3, DRB4, or DRB5 in addition to DRB1 (Figure 3.26).

The particular combination of HLA alleles found on a given chromosome 6 is known as the **haplotype**. Within the HLA complex, meiotic recombination occurs at a frequency of about 2%. In the population this mechanism reassorts alleles of polymorphic HLA genes into new haplotypes, although in most families the parental HLA haplotypes are inherited intact. Although there are no more than a few hundred alleles for any one HLA gene (see Figure 3.24), in the course of human history they have been recombined into many thousands of different haplotypes. The further combination of two HLA haplotypes in each individual means that millions of different HLA isoform combinations are represented in the human population. Consequently, individuals who are homozygous for the HLA complex are rare but usually healthy. Minimally they express three class I (HLA-A, B, and C) and three class II (HLA-DP, DQ, and DR) isoforms that present antigens to their T cells. HLA heterozygous individuals can express up to six class I and eight class II isoforms, the maximum number requiring each HLA haplotypes to contain two functional DRB genes and to contribute a different allele for all of the polymorphic HLA class I and II genes.

3-16 Other proteins involved in antigen processing and presentation are encoded in the MHC class II region

The HLA complex contains a total of more than 200 genes of which the HLA class I and II genes constitute a minority. The other genes embrace a variety of functions including several that are important for the immune system. Particularly striking is the fact that the class II region of the MHC is almost entirely dedicated to genes involved in the processing of antigens and their

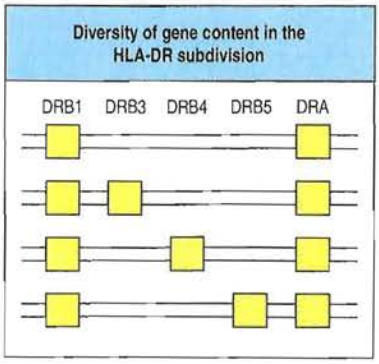


Figure 3.26 Human MHCs differ in the number of DR genes. The MHC on every human chromosome 6 carries one gene (DRA) for the HLA class II DR α chain and one gene (DRB1) for the DR β chain. In addition, some MHCs carry either DRB3 or DRB4 or DRB5. Any DR β chain can pair with the DR α chain to form a class II molecule.

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presentation to T cells. In addition to genes encoding the α and β chains of the five HLA class II isotypes, the class II region contains genes encoding the two polypeptides of the TAP peptide transporter, the gene for tapasin, and genes encoding two subunits of the proteasome called LMP2 and LMP7 (Figure 3.27). Notably absent from the class II region and the HLA complex is the gene encoding the invariant chain; it is on chromosome 5.

Genes encoding proteins that work together in antigen processing and presentation are coordinately regulated by the cytokines IFN-α, -β, and -γ, which are produced at sites of infection at an early stage in the immune response. These cytokines stimulate cells in the vicinity to increase their expression of HLA class I heavy chains, β_2-microglobulin, TAP, and the LMP2 and LMP7 proteasome subunits. LMP2 and LMP7 are not constitutive components of the proteasome in healthy cells, but are specifically made in response to interferon. When they replace the corresponding constitutive proteasome subunits they bias the proteasome towards production of peptides compatible with MHC class I binding requirements.

Expression of the HLA-DM, HLA-DP, HLA-DQ, HLA-DR, and invariant-chain genes is coordinated by the cytokine IFN-γ. These genes are turned on by a transcriptional activator known as **MHC class II transactivator (CIITA)**, which is itself induced by IFN-γ. Inherited impaired CIITA function leads to a form of bare lymphocyte syndrome in which HLA class II molecules are not made and CD4 T cells cannot function.

The majority of genes in the class I region are not involved in the immune system, neither do the class I genes form as compact a cluster as the class II genes (see Figure 3.25). Whereas genes encoding class II molecules are only present in the class II region of the MHC, genes encoding class I molecules and related class I-like molecules are found on several different chromosomes. A further difference is that the class II molecules all function in antigen presentation to T cells, whereas class I molecules encompass a broader range of functions, including uptake of IgG in the gut, regulation of iron metabolism, and regulation of NK cell function.

3-17 MHC polymorphism affects the binding and presentation of peptide antigens to T cells

The alleles of highly polymorphic MHC genes encode proteins that differ by 1–50 amino-acid substitutions. The substitutions are not randomly distributed within the sequence but are mainly in the domains that bind peptide

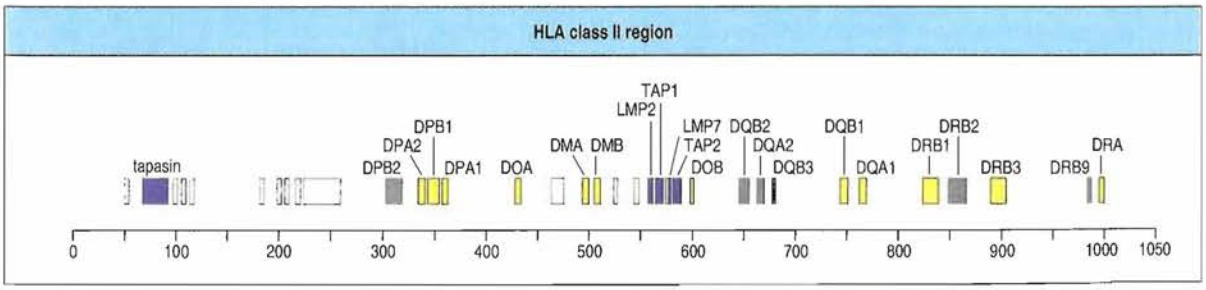


Figure 3.27 Almost all of the genes in the HLA class II region are involved in processing and presentation of antigens to T cells. A detailed map of the HLA class II region is shown. Genes shown in dark gray are pseudogenes that are related to functional genes but are not expressed. Unnamed genes in light gray are not involved in immune system function. In addition to genes encoding the MHC class II isoforms the class II region includes genes for the peptide transporter (TAP), proteasome components (LMP) and tapasin. Approximate distances are given in thousands of base pairs (kb).

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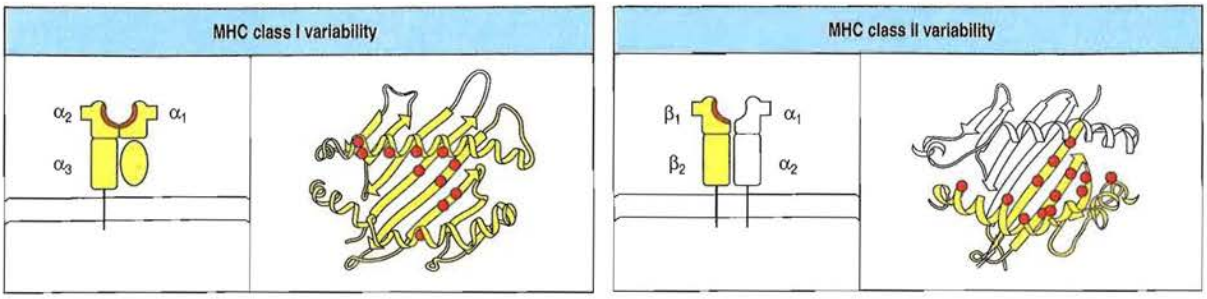


Figure 3.28 Variation between MHC allotypes is concentrated in the sites that bind peptide and T-cell receptor. In the HLA class I molecule (left), allotype variability is clustered in specific sites (shown in red) within the α_1 and α_2 domains. These sites line the peptide-binding groove, lying either in the floor of the groove, where they influence peptide binding, or in the α helices that form the walls, which are also involved in binding the T-cell receptors. In the HLA class II molecule illustrated (right), which is a DR molecule, variability is found only in the β_1 domain because the α chain is monomorphic.

and interact with the T-cell receptor: the α_1 and α_2 domains of MHC class I and the α_1 and β_1 domains of MHC class II. More specifically, the substitutions focus at positions within those domains that contact either bound peptide or the T-cell receptor (Figure 3.28). Not all the contact residues vary as is apparent from the HLA-DR molecule in which the α_1 domain is invariant. In contrast, variability occurs in both the α_1 and β_1 domains of HLA-DP and HLA-DQ molecules.

Variation in the peptide-contact residues on the floor and sides of the peptide-binding groove determines the types of peptides that each isoform binds. At certain positions within the peptide sequence, most of the peptides that bind an MHC isoform have the same amino acid, or one of a few chemically similar amino acids. The preferences arise because the side chains of the amino acids at these positions are bound by complementary pockets within the binding groove. These amino acids are called **anchor residues** because they anchor the peptide to the MHC molecule. The combination of anchor residues that binds to a particular MHC isoform is called its **peptide-binding motif**. For MHC class I molecules, which bind mostly nonamer peptides, positions 2 and 9 are the usual anchor residues. For MHC class II the anchor residues are less clearly defined, in part because of the heterogeneity in length of the bound peptides (Figure 3.29).

The number of peptide-binding motifs is limited and so MHC allotypes that differ by only a few amino acids often bind overlapping populations of peptides. To a rough approximation, the greater the difference in sequence between two MHC allotypes the more disparate will be the populations of peptides they bind.

In the complex of peptide bound to an MHC molecule the anchor residues are buried and inaccessible to the T-cell receptor. In contrast, the peptide's other residues, which have a much greater diversity of amino acids, are available for contact with T-cell receptors. These form part of the planar surface that interacts with the T-cell receptor and includes variable residues on the upper surfaces of the MHC molecule's α helices. Any given T-cell receptor is specific for the complex of particular peptide bound to a particular MHC molecule, a requirement known as **MHC restriction** because the antigen-specific T-cell response is restricted by the MHC type. Consequently, a T cell that responds to a peptide presented by one MHC allotype will neither respond to another peptide bound by that same MHC allotype nor to the same peptide when bound to another MHC allotype (Figure 3.30).

3-18 MHC diversity results from selection by infectious disease

In the previous section we saw that the amino-acid substitutions that distinguish MHC isoforms are not randomly distributed but are concentrated at sites that affect peptide binding and presentation. Further nonrandomness is

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	MHC molecule	Amino-acid sequence of peptide-binding motifs and bound peptides	Source of bound peptide
		Position in peptide sequence: N–1 2 3 4 5 6 7 8 9–C	
Class I	HLA-A*0201	Peptide-binding motif: (L/M) V (V/L) Bound peptide: I L K E P V H G V	HIV reverse transcriptase
	HLA-B*2705	Peptide-binding motif: R (L/F) Bound peptide: R R Y P D A V Y L	Measles virus F protein
Class II	HLA-DRB1*0401	Self peptide: G V Y F Y L Q W G R S T L V S V S	Igκ light chain
	HLA-DQA1*0501 HLA-DQB1*0301	Self peptide: I P E L N K V A R A A A	Transferrin receptor

seen in the gene sequences, where the frequency of nucleotide substitutions that change an amino acid is much greater than would be generated at random. The inescapable conclusion is that MHC diversity is due to natural selection, and because of the immunological functions of MHC molecules the likely source of the selection are the infections caused by pathogens.

For an individual the advantage to having multiple MHC class I and class II genes is that they contribute different peptide-binding specificities, allowing a greater number of pathogen-derived peptides to be presented during any infection. This improves the strength of the immune response against the pathogen by increasing the number of activated pathogen-specific T cells. The same argument applies to polymorphism at any given MHC locus, the advantage to the heterozygote being that two different peptide-binding

Figure 3.29 Peptide-binding motifs and the sequences of peptides bound for some MHC isoforms. For the HLA-A and HLA-B isoforms, both the peptide-binding motif for the isoform and the complete amino-acid sequence of one peptide presented by that isoform are given. Blank boxes in the peptide-binding motifs are positions at which the identity of the amino acid can vary. For the HLA-DR and HLA-DQ isoforms, only the sequence of a self-peptide that is bound by the isoform is shown. Anchor residues are in green circles. Peptide-binding motifs for MHC class II molecules are not readily defined. The one-letter code for amino acids is used.

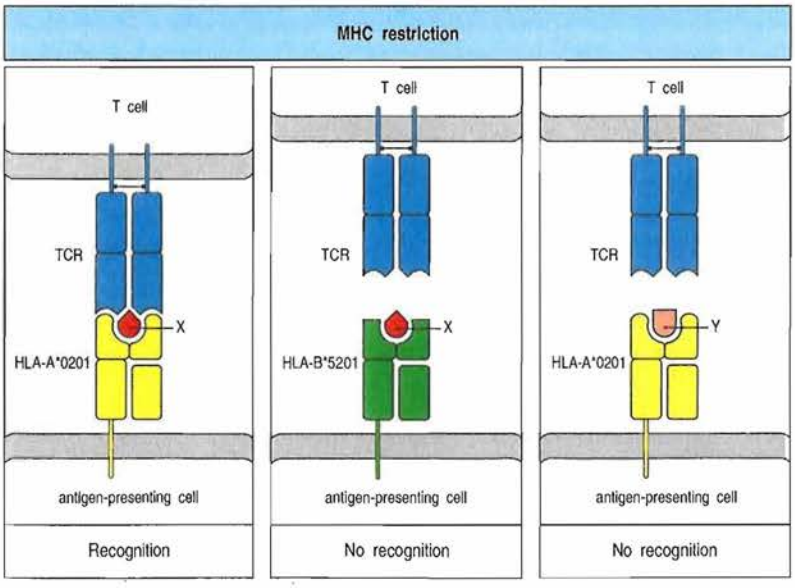


Figure 3.30 T-cell recognition of antigens is MHC restricted. The receptor of the CD8 T cell shown in the left panel is specific for the complex of peptide X with the class I molecule HLA-A*0201. Because of this co-recognition, which is called MHC restriction, the T-cell receptor (TCR) does not recognize the same peptide when it is bound to a different class I molecule, HLA-B*5201 (middle panel). Nor does the T-cell receptor recognize the complex of HLA-A*0201 with a different peptide, Y (right panel). X is HIV-1 Nef residues 190–198 AFHHVAR. Y is influenza A matrix protein residues 58–68 GILGFVFTL.

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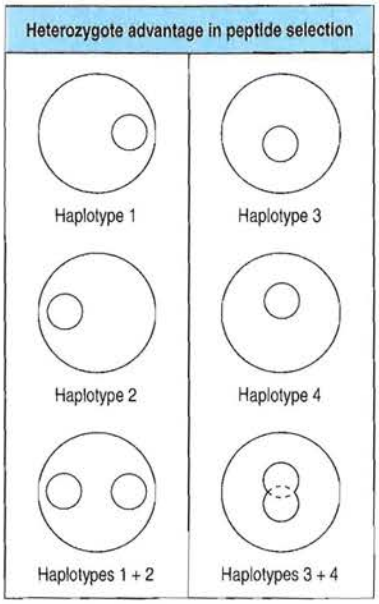


Figure 3.31 The advantage of being heterozygous for the MHC. The large circles represent the total number of antigenic peptides derived from a pathogen that can be presented by human MHC class I and II molecules. The small circles represent the subpopulation of peptides that can be presented by the MHC class I and II molecules encoded by the genes of particular MHC haplotypes. These subpopulations differ between the haplotypes. In general, heterozygous individuals will have a set of MHC class I and II molecules able to present a wider range of pathogen-derived peptides than a homozygote. However, the extent of this benefit varies. The person who has the divergent haplotypes 1 and 2 will, on average, present more peptides from any pathogen than a person who has the more related haplotypes 3 and 4.

specificities can be brought into play as compared to one for the homozygote. Moreover, the high degree of polymorphism of the antigen-presenting HLA isotypes ensures that most members of the population are heterozygotes. The extent of the heterozygote advantage will vary, however, depending upon the difference in the peptide-binding specificities of the two allotypes. This type of advantage can be considered as arising from **balancing selection** because it acts to maintain a variety of MHC isoforms in the population (Figure 3.31).

A different mode of selection favors certain MHC alleles, or combinations of alleles, at the expense of others and is imposed by specific, epidemic disease. Here, presentation of particular pathogen-derived peptides by particular MHC allotypes is advantageous and in the extreme case makes the difference between life and death. As a consequence of the selection the selected alleles are driven to higher frequency while other alleles decrease in frequency and some may even be lost. Because pathogens adapt to the MHC of their host populations, it has been argued that rare, recently formed MHC alleles to which the pathogen has not adapted will more probably confer advantage to the host and be selected during disease epidemics. Instead of maintaining variety this type of selection replaces older alleles with newer variants and its characteristic outcome is change, not balance. It is therefore called **directional selection** (Figure 3.32). The numerous HLA differences between

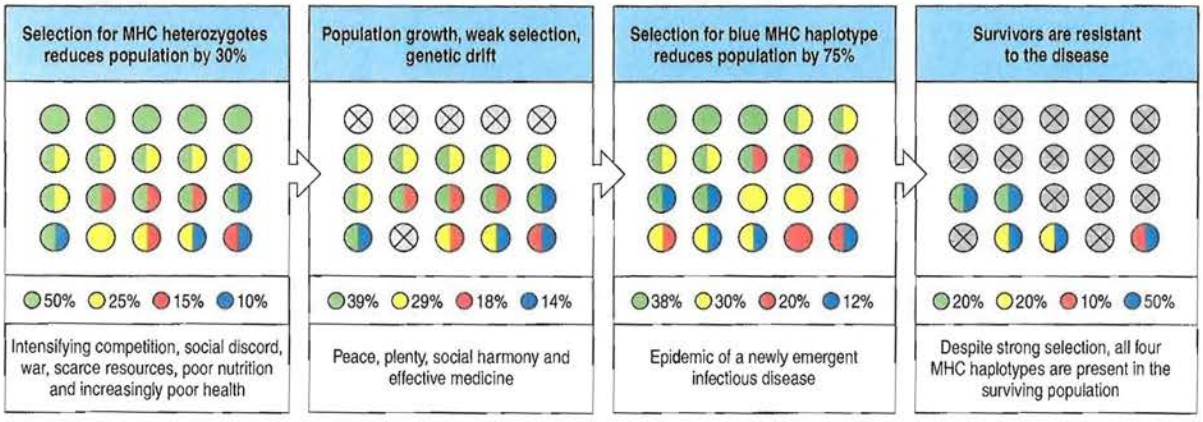


Figure 3.32 Pathogen selection on MHC polymorphism. A population is considered in which there are four different MHC haplotypes, each represented by a different color. The frequencies of different genotypes are represented by the 20 circles in each panel, the four haplotype frequencies being given in the panel below. The population first experiences a period characterized by balancing selection arising from successive epidemic infections, after which only heterozygotes survive and in which 30% of the population die, as indicated by circles containing an X. After recovery of the population during a period of relative calm and health it becomes subject to directional selection by a new and particularly nasty infection. Only individuals with the blue MHC haplotype survive and 75% of the population dies. As a result of these selections the frequencies of the MHC haplotypes change considerably, but all four MHC haplotypes are retained within the population.

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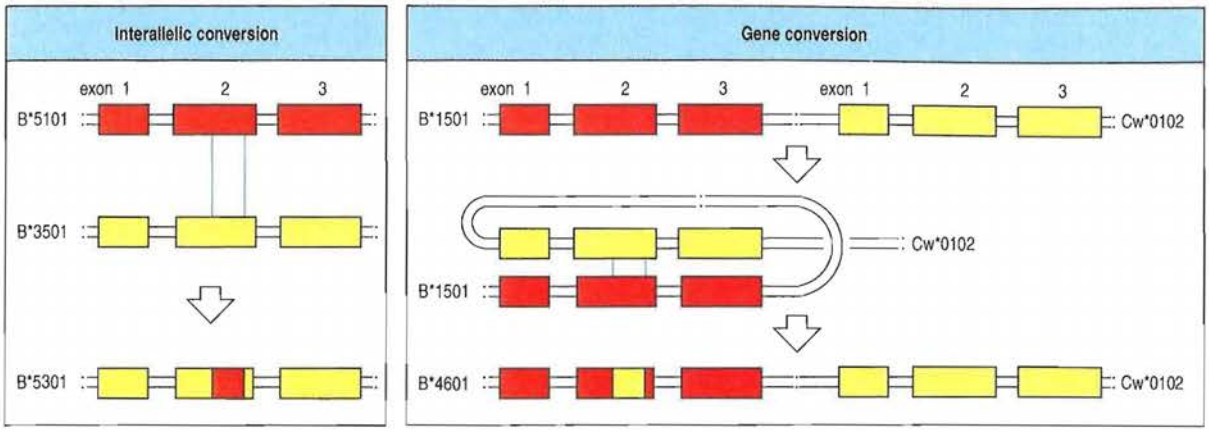


Figure 3.33 New MHC alleles are generated by interallelic conversion or gene conversion. Recombination between alleles of the same gene (HLA-B*5101 and HLA-B*3501), as shown on the left, and between alleles of different genes (HLA-B*1501 and HLA-Cw*0102), as shown on the right, can both result in the formation of a new allele in which a small block of DNA sequence has been replaced. Whereas B*5301 is characteristic of African populations and has been associated with resistance to severe malaria, B*4601 is found in southeast Asian populations and has been associated with susceptibility to nasopharyngeal carcinoma.

human populations of different ethnicity and geographical origins are evidence for directional selection. Only a minority of the HLA alleles listed in Figure 3.24 are common to all human populations, the majority being of recent origin and specific to particular ethnic groups.

New variants of HLA class I and II alleles arise through point mutation and several types of recombination, which can involve alleles of the same gene or alleles of two different genes in the same family (Figure 3.33). Particularly favored appear to be new alleles in which a small segment of one HLA allele has been replaced by the homologous section of another allele, with the introduction of several substitutions that change contact residues in the peptide-binding groove (see Figure 3.28). The recombination mechanism that produces such variants has been termed **interallelic conversion** or **segmental exchange** (Figure 3.33, left panel). Selection for new HLA-B alleles of this type has been particularly strong upon the HLA-B locus in native populations of South and Central America, because today a majority of their HLA-B alleles are 'new variants' specific to these populations.

In industrialized countries the current epidemic of HIV infection provides a unique opportunity to study the effects of HLA polymorphism on an infectious disease. In addition to a general advantage of HLA heterozygosity (Figure 3.34), certain families of alleles (HLA-B14, B27, B57, HLA-C8, C14) are associated with slow progression of the disease, whereas others are associated with rapid progression (HLA-A29, HLA-B22, 35, HLA-C16, and HLA-DR11). Almost all the correlations are with HLA class I; this is consistent with killing of virus-infected cells by cytotoxic CD8 T cells being the principal mechanism for controlling the infection.

3-19 MHC polymorphism triggers T-cell reactions that can reject transplanted organs

During T-cell development any cells having T-cell receptors that respond to complexes of peptide and MHC class I and II molecules at healthy cell surfaces are eliminated. This quality-control mechanism, which prevents a person's T cells from attacking their own healthy tissue and causing disease, only encompasses the MHC isoforms expressed by the person and not other MHC isoforms. In this context the self-MHC isoforms are described as **autologous**, and all other MHC isoforms are described as **allogeneic**. Accordingly, in every person's circulation there are T cells that respond to complexes of peptide and allogeneic MHC class I and II molecules that are present on healthy cells of

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other individuals. T cells with this property are called **alloreactive T cells**, and those reactive against any given allogeneic cell constitute between 1–10% of circulating T cells.

When allogeneic kidneys are transplanted to patients with renal failure a major danger is that the kidney graft will be rejected by the patient's immune system. One way this happens is when alloreactive T cells in the patient's circulation are activated by allogeneic HLA molecules expressed by the graft, leading to an **alloreaction**, a potent T-cell response that attacks the graft. To reduce the probability of kidney graft rejection, donors are selected who have identical or similar combinations of HLA alleles to the patient. The combination of HLA alleles a person has is called their **HLA type**. Immunosuppressive drugs are also used to preempt the alloreactive T-cell response and to treat rejection when it occurs.

A natural situation in which alloreactions occur is pregnancy, when the mother's immune system can be stimulated by the HLA molecules of the fetus that derive from the father but are not expressed on the mother's cells. This response leads to **alloantibodies** in the mother's circulation with specificity for paternal MHC molecules. Alloantibody is the name given to any antibody raised in one member of a species against an allotypic protein from another member of the same species. Although of no harm to the fetus, which is protected, the alloantibodies produced by pregnancy can have disastrous effects should the mother needs a kidney transplant at some future time. If the alloantibodies react with the allogeneic MHC class I of the transplanted kidney they cause a type of graft rejection that is almost impossible to treat. To avoid this outcome a patient's serum is tested for reactivity with a donor's leukocytes and transplantation is only undertaken when the reaction is acceptably low. Before molecular genetic methods were available, the HLA types of transplant recipients and donors were determined using the anti-HLA class I and class II alloantibodies present in sera obtained from multiparous women.

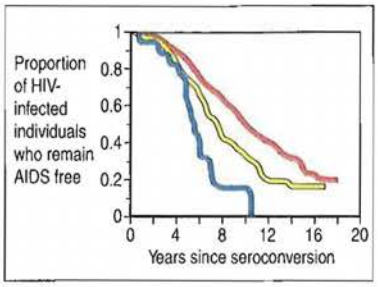


Figure 3.34 MHC heterozygosity delays the progression to AIDS in people infected with HIV-1. When people who have been infected with HIV-1 start to make detectable antibodies to the virus they are said to have undergone seroconversion. The onset of overt symptoms of AIDS occurs years after seroconversion. The rate of progress to AIDS decreases with the extent of HLA heterozygosity as compared here for individuals who are heterozygous for all the highly polymorphic HLA class I and II loci (red), to those who are homozygous for one locus (yellow) or for two or three loci (blue).

Summary

In humans, the highly polymorphic MHC class I and II genes are closely linked in the HLA region on chromosome 6, which comprises the human MHC. In contrast to immunoglobulin and T-cell receptor genes, MHC class I and II genes have a conventional organization and do not rearrange. In humans, the MHC class I genes encode the heavy (α) chains of three different class I molecules—HLA-A, HLA-B, and HLA-C—whereas the MHC class II genes encode the α and β chains of three different MHC class II molecules—HLA-DP, HLA-DQ, and HLA-DR. β_2-Microglobulin, the light chain of MHC class I molecules, is encoded outside the MHC, on chromosome 15. Certain MHC class I and class II genes are highly polymorphic; some have several hundred alleles. Genes encoding other proteins involved in antigen presentation are located in the MHC and, like the MHC class I and II genes, their expression is regulated by the interferons produced during an immune response. The strategy used by MHC molecules to bind diverse antigens contrasts with that of the T-cell receptors. MHC molecules have highly degenerate binding sites for peptides; an MHC molecule is, therefore, usually able to present a diversity of peptide antigens to a large number of T-cell receptors with highly specific binding sites. Polymorphism in families of MHC class I heavy-chain genes and MHC class II α- and β-chain genes is a secondary strategy that serves to increase the breadth and strength of T-cell immunity. It also diversifies T-cell immunity within human populations, a strategy that helps them survive epidemic disease but has also created the main immunological barrier to clinical transplantation.

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Summary to Chapter 3

The general structure of T-cell antigen receptors resembles that of the membrane-bound immunoglobulins of B cells and they are encoded by similarly organized genes that undergo gene rearrangement before they are expressed. As with B cells, this gives rise to a population of T cells each expressing a unique receptor. Differences between immunoglobulins and T-cell receptors reflect the fact that the T-cell receptor is used only as a membrane-bound receptor, whereas immunoglobulins are also used as secreted effector molecules.

T-cell receptors are more limited than immunoglobulins in the antigens that they bind, recognizing only short peptides bound to MHC molecules on a cell surface. The two main classes of T cell—CD8 and CD4—are specialized to respond to intracellular and extracellular pathogens, respectively. They are activated in response to an antigen from the appropriate source by specific interactions between the MHC molecules displaying the peptide antigen and the CD4 or CD8 glycoproteins on the T-cell surface. Processing of extracellular and intracellular pathogen antigens into peptides, and their binding to MHC molecules, occurs inside the cells of the infected host. Cytotoxic CD8 T cells are activated by peptides presented by MHC class I molecules and are directed to destroy the antigen-presenting cell. Most cells express MHC class I molecules and so can present pathogen-derived peptides to CD8 T cells if infected with a virus or other pathogen that penetrates the cytosol. The function of CD4 T cells is the activation of other types of effector cell and their recruitment to sites of infection through cell–cell interactions and the production of cytokines. They are activated by peptides presented by MHC class II molecules, which are usually expressed only on specialized antigen-presenting cells that take up and process material from the extracellular environment and can activate CD4 T cells.

MHC molecules have degenerate peptide-binding sites, which enables the relatively small number of different MHC molecules present in each individual to bind peptides of many different sequences. The diversity of peptides that can be presented by the human population as a whole is further increased by the highly polymorphic nature of MHC class I and II genes. Each person differs from almost all other individuals in some or all of the MHC alleles that they possess. Thus, the population is able to respond to the pathogens it encounters with a wide diversity of individual immune responses. Because of the polymorphism of the MHC, organs or tissues transplanted between individuals of different MHC type provoke strong T-cell responses directed against the "foreign" MHC molecules.

Questions

Question 3–1

Describe (a) four ways in which T-cell receptors are similar to immunoglobulins, and (b) four ways in which they are different. (Refer to Figures 3.1–3.3, and 3.6.)

Question 3–2

Discuss several ways in which T-cell receptors differ from immunoglobulins in the way that they recognize antigen. Use the following terms in your answer: peptides, antigen-presenting cells, MHC molecules, and antigen-binding sites. (Refer to Figures 3.9 and 3.13.)

Question 3–3

Compare the organization of T-cell receptor α and β genes (the TCRα and TCRβ loci) to the organization of immunoglobulin heavy- and light-chain genes. (Refer to Figures 2.14 and 3.3.)

Question 3–4

There are considerably more J segments in the T-cell receptor loci than in the immunoglobulin gene loci. Why might this have arisen?

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Question 3–5

Why do antibodies but not T-cell receptors undergo isotype switching?

Question 3–6

What is meant by the terms (a) antigen processing and (b) antigen presentation? (c) Why are these processes required before T cells can be activated? (Refer to Figures 3.9, 3.19, and 3.21.)

Question 3–7

Pathogens that infect the human body replicate either inside cells (such as viruses) or extracellularly, in the blood or in the extracellular spaces in tissues.

A. Identify (i) the class of T cells that are stimulated by intracellular pathogens, (ii) their co-receptor, (iii) the MHC molecule used for recognition of antigen and (iv) the T-cell effector function.

B. Repeat this for the classes of T cells that are stimulated by extracellular pathogens. For the purposes of this question, count those pathogens (such as mycobacteria) that can survive and live inside intracellular vesicles after being taken up by macrophages as extracellular pathogens. (Refer to Figures 3.10, 3.12, and 3.19.)

Question 3–8

A. (i) Describe the structure of an MHC class I molecule, identifying the different polypeptide chains and domains. (ii) What are the names of the MHC class I molecules produced by humans? Which part of the molecule is encoded within the MHC region of the genome? (iii) Which domains or parts of domains participate in: antigen binding; binding the T-cell receptor; and binding the T-cell co-receptor? (iv) Which domains are the most polymorphic?

B. Repeat this for an MHC class II molecule. (Refer to Figures 3.13, 3.14, 3.21, and 3.28.)

Question 3–9

A. What is the maximum number of MHC molecules that a heterozygous individual could theoretically express? Explain your answer. (Ignore the possibility of MHC class II molecules composed of chains from different isoforms.)

B. How does this relatively small number of MHC molecules have the potential to bind the huge number of antigenic peptides encountered in the environment, and what features of a peptide determine whether it will be bound by a given MHC molecule? (Refer to Figures 3.23, 3.25, and 3.26.)

Question 3–10

A. What is the difference between MHC polygeny and MHC polymorphism?

B. How do (i) polygeny and (ii) polymorphism in the MHC genes influence the antigens a person's T cells can recognize? (Refer to Figures 3.23, 3.24, and 3.29.)

Question 3–11

A. Describe in chronological order the steps of the endogenous antigen-processing pathway for intracellular, cytosolic pathogens.

B. (i) What would be the outcome if a mutant MHC class I α chain could not associate with β_2-microglobulin, and (ii) what would happen if the TAP transporter was lacking as a result of mutation? Explain your answers. (Refer to Figures 3.17–3.19.)

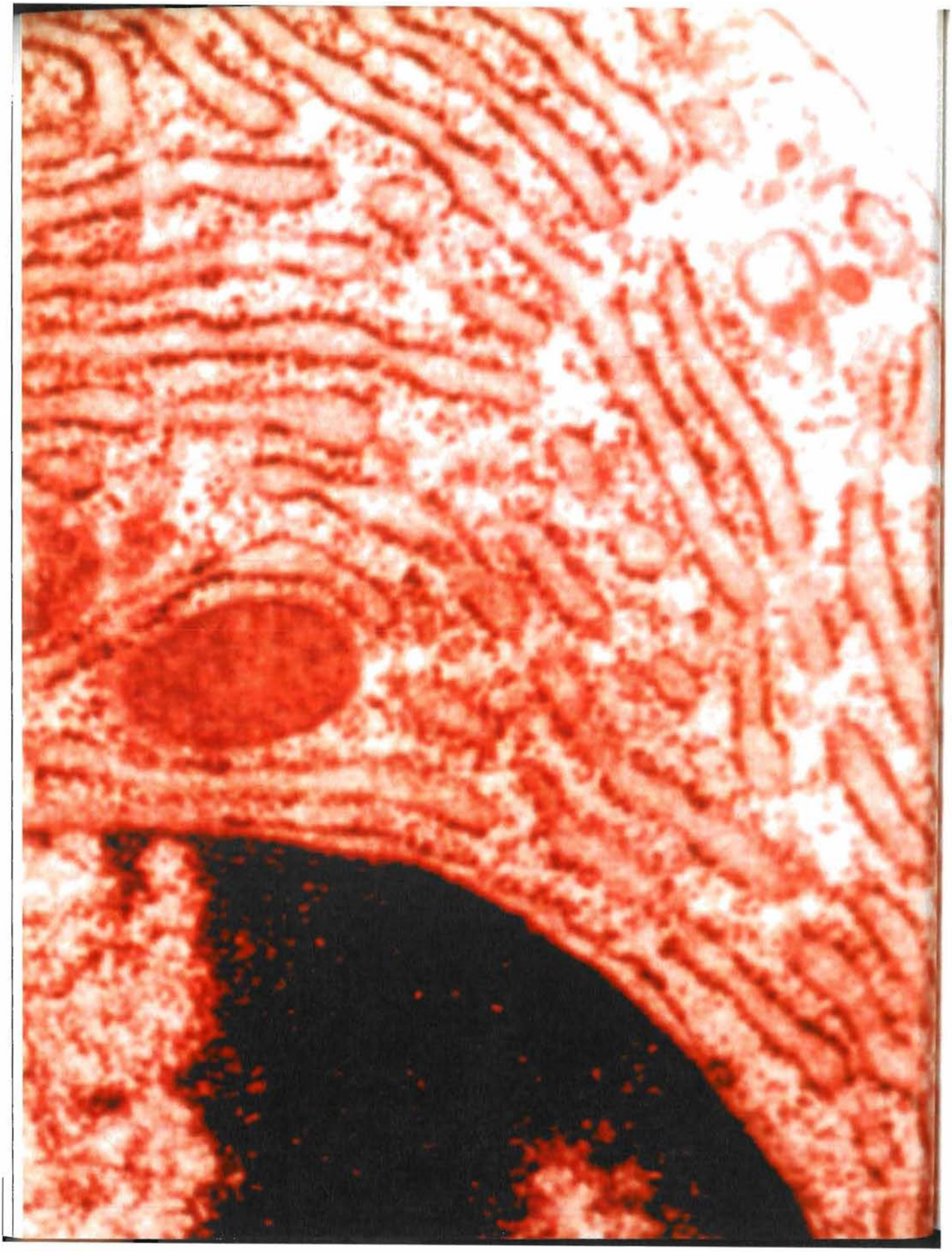
Question 3–12

A. Describe in chronological order the steps of the antigen-processing pathway for extracellular pathogens.

B. What would be the outcome (i) if invariant chain were defective or missing, or (ii) if HLA-DM were not expressed? (Refer to Figures 3.19–3.20.)

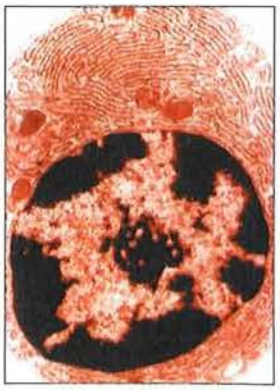
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Chapter 4

The Development of B Lymphocytes



The B cells of the human immune system have the capacity to make immunoglobulins (Ig) specific for almost every nuance of chemical structure, which gives each person the potential to make antibodies against all the infectious microorganisms that could possibly be encountered in a lifetime. However, the body does not stockpile all the B cells needed to make an optimal response against all possible pathogens. That would probably mean devoting the vast majority of the body's resources to the immune system, leaving little left for the immune system to protect. Instead, the body carries a less complete inventory of B cells, but one that expands and contracts its individual clones according to need and circumstance. Fueling this system are stem cells in the bone marrow, which generate tens of billions of new B cells every day of your life.

The life cycle of B cells can be divided into four broad stages (Figure 4.1). The first stage is one of maturation and takes place in the bone marrow, which is, therefore, designated as a **primary lymphoid tissue**. During this stage, which will be described in the first part of this chapter, developing B cells acquire functional B-cell receptors through the ordered rearrangements of the immunoglobulin genes described in Chapter 2. The second stage involves testing whether a B cell's immunoglobulin receptor will bind to normal constituents of the body and, therefore, has the potential to produce autoreactivity and autoimmune disease. In the third stage, mature, naive B cells that survive this selection process leave the bone marrow, enter the blood, and from there move into **secondary lymphoid tissues** (Figure 4.2). If they do not encounter their specific antigens in the lymphoid tissues, the B cells continue to recirculate. If contact is made with antigen in a secondary lymphoid tissue, the fourth stage of a B cell's life begins. The B cell proliferates and its progeny differentiate either into plasma cells, which synthesize large quantities of antibody, or into long-lived memory B cells, which will respond more quickly than naive B cells on a subsequent encounter with the same antigen. The last three stages of the B-cell life cycle will be dealt with in the second part of this chapter.

The development of B cells in the bone marrow

The stages of B-cell development in the bone marrow are marked by successive steps in the rearrangement and expression of the immunoglobulin genes.

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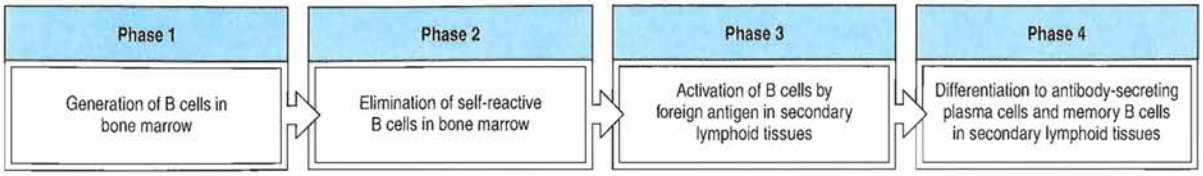


Figure 4.1 The development of B cells can be divided into four broad phases.

In this part of the chapter we shall see how gene rearrangement is controlled at each step to produce a mature but naive B cell that makes only one heavy chain and one light chain, and thus expresses immunoglobulin of a single antigen specificity on its surface.

4-1 B-cell development in the bone marrow proceeds through several stages

B cells derive from pluripotential hematopoietic stem cells in the bone marrow. The earliest identifiable cells of the B-cell lineage are called **pro-B cells**. These progenitor cells retain a limited capacity for self-renewal, dividing to produce both more pro-B cells and cells that will go on to develop further. Rearrangement of heavy-chain genes takes place first and this occurs in pro-B cells: D_H to J_H joining occurs at the **early pro-B cell** stage, followed by V_H to DJ_H joining at the **late pro-B cell** stage. A μ heavy chain is the first type of heavy chain to be produced. Once a B cell expresses a μ chain it is known as a **pre-B cell** (Figure 4.3). Pre-B cells represent two stages in B-cell development: the less mature **large pre-B cells** and the more mature **small pre-B cells**. Large pre-B cells are distinguished by a protein complex called the **pre-B-cell receptor**, which consists of μ heavy chains, surrogate light chains—made only in pre-B cells—and the Igα and Igβ polypeptides (Figure 4.4). Although some molecules of the pre-B-cell receptor are present at the cell surface, most are retained in the endoplasmic reticulum.

The presence of B-cell receptor molecules leads to intracellular signals that halt rearrangement at the immunoglobulin heavy-chain locus and synthesis of surrogate light chains. The large pre-B cell then proliferates to yield many small pre-B cells, in which the pre-B-cell receptor is no longer present, μ chains are restricted to the cytoplasm, and rearrangement of the immunoglobulin light-chain loci proceeds. Once light chains have been made, they assemble with μ chains to form IgM molecules, which are transported to the cell surface in the form of a functional B-cell receptor complex (see Section 2-11, p. 54). At this stage the B cell expresses IgM only and is defined as an **immature B cell**.

Up to this stage, the development of B cells occurs in the bone marrow and does not require interaction with specific antigen. The randomness of the gene rearrangement process leads to a proportion of the B-cell receptors being reactive against constituents of the body. Unless removed from the repertoire, B cells bearing these receptors have the potential to produce an autoimmune response, leading to autoimmune disease. The immature B cells now begin to be selected for tolerance of the normal constituents of the body, and at about this time they also start to enter the peripheral circulation. At this point in development, alternative mRNA splicing of heavy-chain gene transcripts produces IgD, as well as IgM, as membrane-bound immunoglobulin. The cell is now considered to be a **mature B cell**. Such cells are also called **naive B cells** because they have yet to be exposed to their specific antigen.

B-cell development in the bone marrow is dependent on a network of non-lymphoid **stromal cells**, which provide specialized microenvironments for

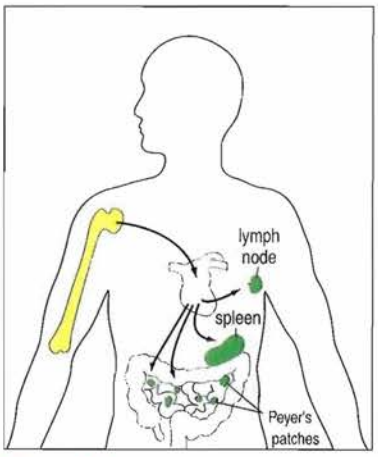


Figure 4.2 B cells develop in bone marrow and then migrate to secondary lymphoid tissues. B cells leaving the bone marrow (yellow) are carried in the blood to lymph nodes, the spleen, Peyer's patches (all shown in green), and other secondary lymphoid tissues such as those lining the respiratory tract (not shown).

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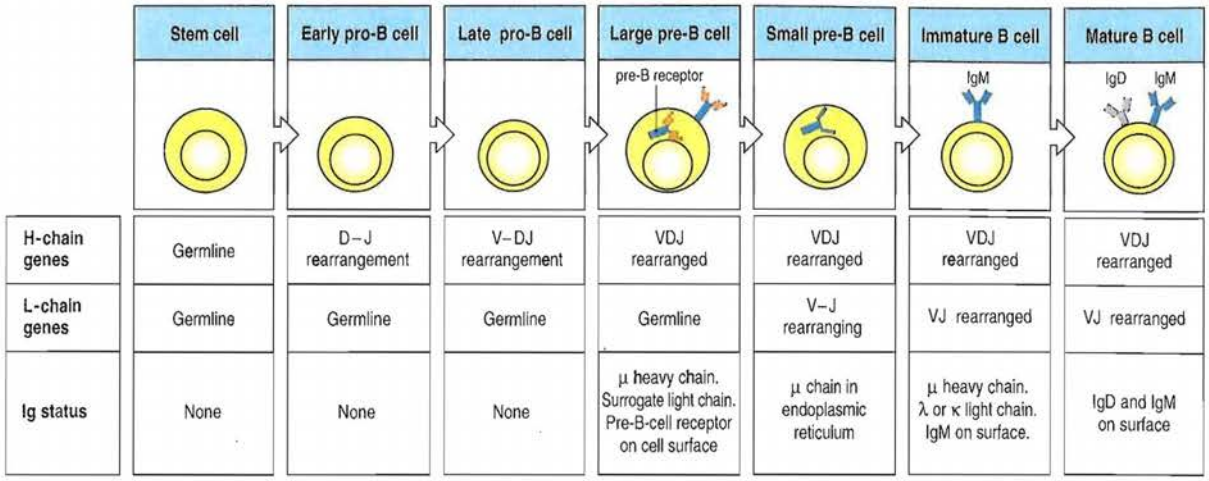


Figure 4.3 The development of B cells proceeds through stages defined by the rearrangement and expression of the immunoglobulin genes. In the stem cell, the immunoglobulin (Ig) genes are in the germline configuration. The first rearrangements are of the heavy-chain (H-chain) genes. Joining D_H to J_H defines the early pro-B cell, which becomes a late pro-B cell on joining V_H to DJ_H. Expression of a functional μ chain and its expression at the cell surface as part of the pre-B receptor defines the large pre-B cell. Large pre-B cells proliferate, producing small pre-B cells in which rearrangement of the light-chain (L-chain) gene occurs. Successful light-chain gene rearrangement and expression of IgM on the cell surface define the immature B cell. The mature B cell is defined by the use of alternative splicing of heavy-chain mRNA to place IgD on the cell surface as well as IgM.

B cells at various stages of maturation (Figure 4.5). The stromal cells perform two distinct functions. First, they make specific cell-surface contacts with the B cells through the interaction of adhesion molecules and their ligands. Second, they produce growth factors that act on the bound B cells, for example the membrane-bound, stem-cell factor (SCF), which is recognized by a receptor called Kit on immature B cells. Another important growth factor for B-cell development is interleukin-7 (IL-7), a cytokine secreted by stromal cells that acts on late pro-B and pre-B cells.

The most immature stem cells lie in a region of the bone marrow called the subendosteum, which is adjacent to the bone's interior surface. As the B cells mature, they move, while maintaining contact with stromal cells, to the central axis of the marrow cavity. Later stages of maturation are less dependent on contact with stromal cells, allowing the B cells to leave the bone marrow eventually. The final stage of development, when immature B cells become mature B cells, can occur either in the bone marrow or in secondary lymphoid organs such as the spleen.

Although B-cell development in humans and mice is quite similar, there are considerable differences in other species. For example, in chickens, immunoglobulin-gene rearrangement in the bone marrow provides little diversity. After completing heavy- and light-chain gene rearrangement, chicken B cells migrate to the bursa of Fabricius, a lymphoid organ near the cloaca of the gut. B cells were, in fact, originally named after this organ because the distinction between B cells and T cells was discovered from studying the chicken's immune response. In the bursa, B cells are signaled to

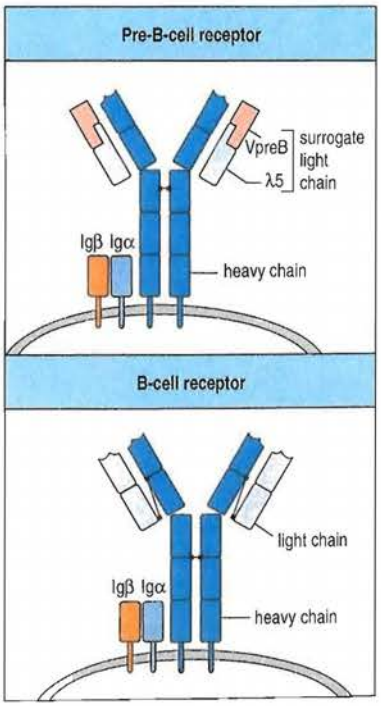
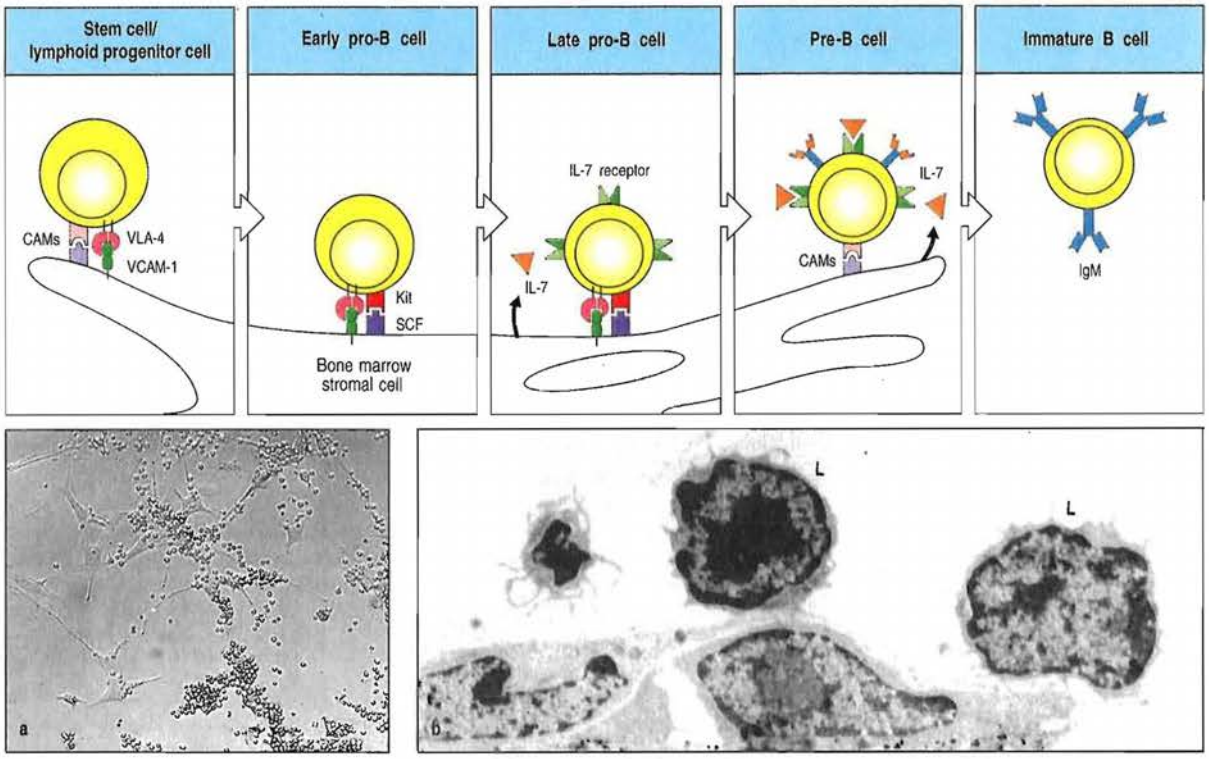


Figure 4.4 The pre-B-cell receptor resembles the B-cell receptor except for the surrogate light chain.

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proliferate and their rearranged heavy- and light-chain genes then diversify through gene conversion (Figure 4.6).

4-2 The survival of a developing B cell depends on the productive rearrangement of a heavy- and a light-chain gene

Because of imprecision in the gene rearrangement process, including the random addition of N and P nucleotides at joints between gene segments (see Section 2-8, p. 51), not all DNA rearrangements result in a sequence with a reading frame that can be translated into an immunoglobulin chain. Gene rearrangements that cannot be translated into a protein are called **unproductive rearrangements**. Those rearrangements that preserve a correct reading frame and give rise to a complete and functional immunoglobulin chain are known as **productive rearrangements**. At each rearrangement event, there is a one in three chance of the correct reading frame being maintained.

Every B cell has two copies of each of the immunoglobulin loci—the heavy-chain locus, the κ light-chain locus, and the λ light-chain locus. The two copies of each locus are on homologous chromosomes; one is inherited from the mother and the other from the father. In the developing B cell, gene rearrangements can be made on both homologous chromosomes. Therefore, a B cell that has made an unproductive rearrangement on one chromosome still has a chance of producing an immunoglobulin chain if it makes a productive rearrangement at the locus on the other homologous chromosome. Developing B cells are allowed to proceed to the next stage only when a productive rearrangement has been made. If all rearrangements are unproductive, the B cell does not produce immunoglobulin and dies in the bone marrow.

Figure 4.5 The early stages of B-cell development are dependent on bone marrow stromal cells. The top panels show the interactions of developing B cells with bone marrow stromal cells. Stem cells and early pro-B cells use the integrin VLA-4 to bind to the adhesion molecule VCAM-1 on stromal cells. This and interactions between other cell adhesion molecules (CAMs) promote the binding of the receptor Kit on the B cell to stem-cell factor (SCF) on the stromal cell. Activation of Kit causes the B cell to proliferate. B cells at a later stage of maturation require interleukin-7 (IL-7) to stimulate their growth and proliferation. Panel a is a light micrograph of a tissue culture showing small round B-cell progenitors in intimate contact with stromal cells, which have extended processes fastening them to the plastic dish on which they are grown. Panel b is a high-magnification electron micrograph showing two lymphoid cells (L) adhering to a flattened stromal cell. Photographs courtesy of A. Rolink (a); Paul Kincade and P.L. Witte (b).

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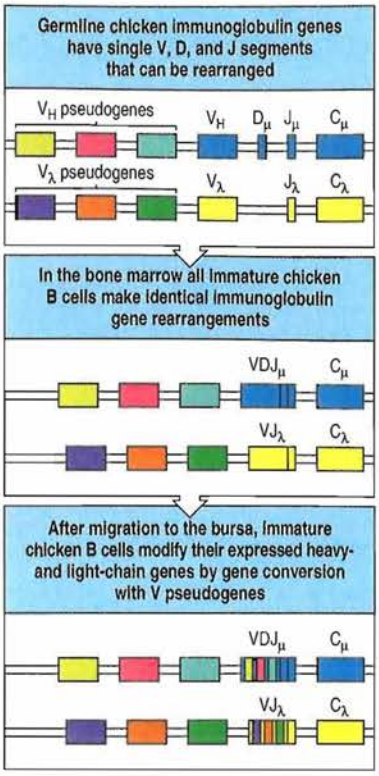


Figure 4.6 Diversification of the immunoglobulin genes in chickens is achieved by gene conversion. In chickens the development of mature B cells involves successive modifications of the immunoglobulin genes in two anatomical sites. In the bone marrow, the heavy- and light-chain genes are rearranged. This process produces little immunoglobulin diversity. In the bursa, diversity is introduced by gene conversion between the rearranged genes and a series of V_H and V_λ pseudogenes.

The order of gene rearrangements is outlined in Figure 4.7. The immunoglobulin heavy-chain locus rearranges before the light-chain loci. Recombination is heralded by expression of the **recombination activation genes RAG-1** and **RAG-2**. The first rearrangement event is the joining of a D_H gene segment to a J_H gene segment, which can occur concurrently in the two copies of the heavy-chain locus. B cells with unproductive D_H to J_H rearrangements on both chromosomes are prevented from developing further because, although they will undergo V_H to DJ_H rearrangement, they cannot produce a heavy chain. In humans, few B cells are lost from unproductive rearrangements at this stage because most human D segments can be translated in all three reading frames. In contrast, mouse D segments have a single productive reading frame.

The second event in heavy-chain gene rearrangement is the joining of a V_H gene segment to the rearranged DJ_H sequence. This event first occurs at only one of the heavy-chain loci. When V_H to DJ_H rearrangement on the first chromosome is unproductive, rearrangement then proceeds on the second chromosome. The two-thirds failure rate in maintaining the reading frame, and the independent chances for success offered by having two chromosomes, mean that just over half the total number of pro-B cells make a functional heavy-chain gene. They become large, dividing pre-B cells producing

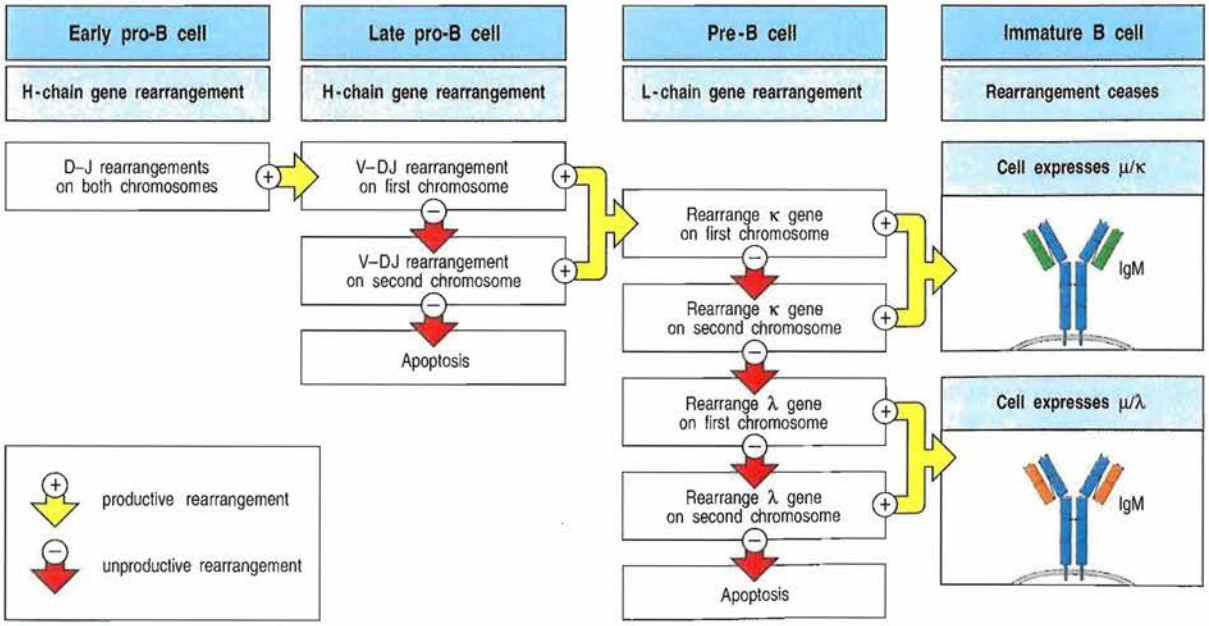


Figure 4.7 The order of gene rearrangements leading to the expression of cell-surface immunoglobulin. The heavy-chain genes are rearranged before the light-chain genes. Developing B cells are allowed to proceed to the next stage only when a productive rearrangement has been made. If an unproductive rearrangement is made on one chromosome of a homologous pair, then rearrangement is attempted on the second chromosome.

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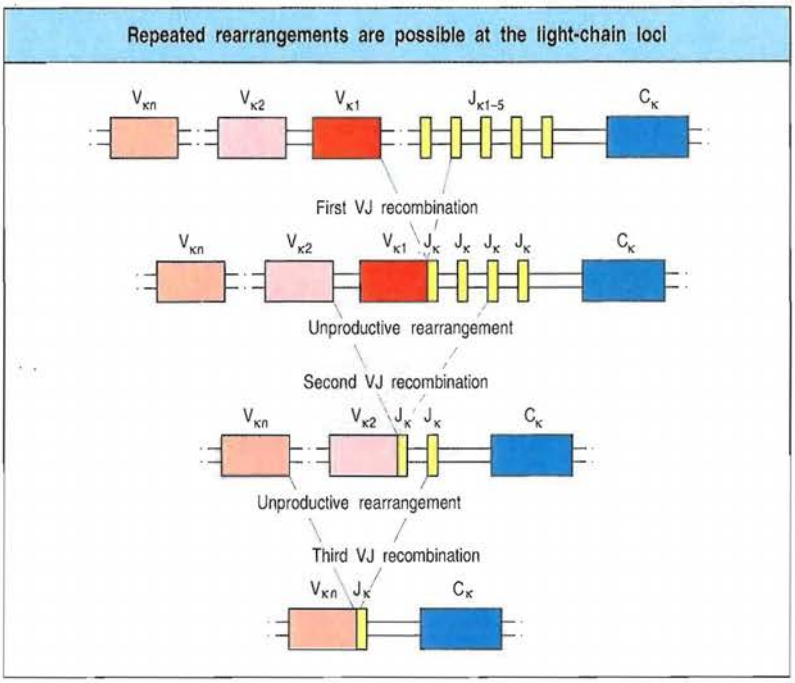


Figure 4.8 Unproductive light-chain gene rearrangements can be replaced by further gene rearrangement. The organization of the light-chain loci allows unproductive rearrangements to be followed by further rearrangement that can result in a functional gene. This type of rescue is shown for the κ light-chain gene. After an unproductive rearrangement of V_κ to a J_κ, in which the translational reading frame has been lost, a second rearrangement can be made by $V_{\kappa 2}$, or any other V_κ that is on the 5′ side of the first joint, with a J_κ that is on the 3′ side of the first joint. When the second joint is made, the intervening DNA containing the first joint will be excised. There are five J_κ gene segments and many more V_κ gene segments, so that as many as five successive attempts at productive rearrangement of the κ light-chain gene can be made on a single chromosome.

μ chains. Those pro-B cells that fail to make μ chains are programmed to die, a process called **apoptosis**. A general feature of lymphocyte development is for apoptosis to be the default pathway that is followed unless a positive signal for further differentiation is received.

The large pre-B cells go through five or six rounds of cell division, after which they become small, resting pre-B cells. At this stage, the light-chain genes begin to rearrange. Rearrangements take place at one light-chain locus at a time, with the κ locus tending to be rearranged before the λ locus. In contrast to the heavy-chain locus, several attempts to rearrange the same light-chain gene can be made by using V and J gene segments not involved in previous rearrangements (Figure 4.8). A number of attempts to rearrange the κ light-chain locus on one or other chromosome can, therefore, be made before commencing rearrangement of a λ-chain locus on a different chromosome. The possession of four light-chain loci, and the opportunity for making several attempts to rearrange each locus, means that about 85% of the pre-B cell population makes a successful rearrangement of a light-chain gene. Overall, less than half of the B-lineage cells end up producing functional immunoglobulin heavy and light chains.

Before each rearrangement there is a small amount of transcription from the individual gene segments that are about to be joined. This opens up the chromatin structure and makes the DNA accessible to the enzymes that carry out somatic recombination. It is likely that this low-level transcription is initiated by transcription factors expressed only in B cells, and is the mechanism that directs the recombination enzymes common to both B cells and T cells to the immunoglobulin genes rather than to the T-cell receptor genes (and vice versa in T cells). In addition, the gene rearrangement process brings promoter and enhancer within the locus into closer juxtaposition with each other so that the rearranged immunoglobulin gene can now be transcribed at a high rate.

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4-3 Cell-surface expression of the products of rearranged immunoglobulin genes prevents further gene rearrangement

Given that a B cell can rearrange up to two heavy-chain loci and four light-chain loci, there are mechanisms for ensuring that it ends up making only one type of heavy chain and one type of light chain. The success of a gene rearrangement is signaled by the appearance of the protein product of the gene at the cell surface; once that has occurred, a signal is sent back to the interior of the cell to shut down the processes of DNA recombination and repair that are needed for gene rearrangement. The RAG genes are turned off and no further gene rearrangement is possible at this time. Once one heavy-chain gene has been successfully rearranged, all further rearrangement of heavy-chain genes is shut down, and the same happens with the light-chain genes (Figure 4.9).

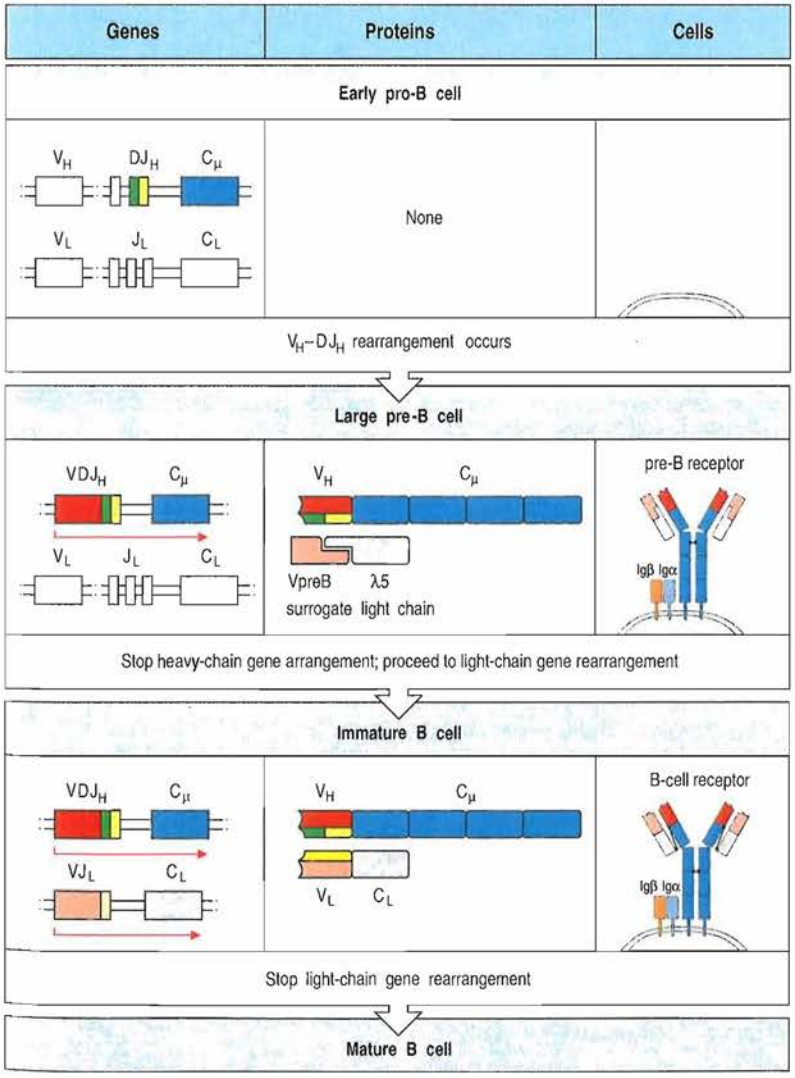


Figure 4.9 Signaling via the functional protein product is used to terminate immunoglobulin-gene rearrangement. First panel: in early pro-B cells, no functional μ protein is expressed and the biochemical machinery that performs gene rearrangement is active. Second panel: on completion of a productive heavy-chain gene rearrangement, μ heavy chains are synthesized and assembled into a complex with the λ5 and VpreB polypeptides that make up the surrogate light chain, and with Igα and Igβ. This complex, the pre-B-cell receptor, is transported to the cell surface, where it interacts with an unknown ligand that signals the cell to halt heavy-chain gene rearrangement and to proceed to the large pre-B-cell stage. Proliferation of the large pre-B cell yields small pre-B cells that commence immunoglobulin light-chain rearrangement. Note that λ5 and VpreB are coded by genes that are distinct from those in the immunoglobulin loci. Third panel: on completion of a productive light-chain gene rearrangement a light chain is made and assembles with μ to form IgM. This associates with Igα and Igβ and is transported to the cell surface. Its presence tells the cell to halt light-chain gene rearrangements.

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Once it has formed a functional heavy-chain gene, a pro-B cell begins to synthesize μ chains. The μ chains assemble into dimers in the endoplasmic reticulum of the cell. These cannot, however, assemble with κ or λ light chains to form a regular IgM receptor because the B cell has not yet rearranged its light-chain genes. The receptor that reaches the surface at this stage contains a μ dimer, in which each μ chain is bound to an invariant **surrogate light chain**. Each surrogate light chain is formed from two proteins coded by genes away from the immunoglobulin loci; **λ5** substitutes for a light-chain constant region and **VpreB** substitutes for a variable region.

The tetramer of two μ chains and two surrogate light chains associates with the Igα and Igβ polypeptides (see Section 2-11, p. 54) to form a complex that moves to the cell surface to become the pre-B-cell receptor. The pre-B-cell receptor is present at the cell surface for only a short period of time, which represents a checkpoint in B-cell development. This checkpoint allows the B cell to confirm whether a productive rearrangement has occurred and a functional heavy chain has been made. Expression of the pre-B-cell receptor generates the positive signal that allows the B cell to avoid apoptosis and proceed to the next stage of maturation. After the checkpoint, the surrogate light chain ceases to be synthesized and the pre-B-cell receptor gradually disappears from the cell surface. The μ, Igα, and Igβ chains continue to be synthesized but are now retained in the endoplasmic reticulum. The actual mechanism by which the pre-B-cell receptor signals the pre-B cell to shut down the machinery of somatic recombination and gear up the biosynthetic machinery for DNA replication and cell division is still unknown.

The RAG genes, which were turned off in the dividing pre-B cells, are now turned on again and light-chain gene rearrangement proceeds. When a functional light-chain gene has been formed, light chains are made and assemble with μ to form IgM. This associates with Igα and Igβ to form the mature B-cell receptor, which moves to the cell surface. At this second checkpoint in B-cell maturation, the appearance of a functional B-cell receptor leads to the shutting down of light-chain gene rearrangement.

Cell division initiated by signaling through the pre-B-cell receptor results in a clone of 30–70 small pre-B cells. These all have the same rearranged heavy-chain gene but each has the potential to end up with a different light chain. The combinations of different heavy and light chains generated at this stage contribute to the diversity of the B-cell repertoire.

4-4 The proteins involved in immunoglobulin-gene rearrangement are controlled developmentally

The successive steps in immunoglobulin-gene rearrangement are accompanied by changes in the expression of the other proteins involved in the recombination reactions (Figure 4.10). The RAG-1 and RAG-2 proteins are essential components of the recombination machinery (see Section 4-2, p. 102), and their genes are specifically turned on at the stages in B-cell development when heavy- or light-chain gene rearrangement occurs. Conversely, at the large pre-B cell stage, after rearrangement of a heavy-chain gene and before rearrangement of a light-chain gene, RAG proteins are absent. Terminal deoxynucleotidyl transferase (TdT), the enzyme that adds N nucleotides at the junctions between rearranging gene segments, is expressed in pro-B cells when the heavy-chain genes begin to rearrange, and turned off in small pre-B cells when the light-chain genes begin to rearrange; this explains why N nucleotides are found in all VD and DJ joints of rearranged human heavy-chain genes but in only about half of the VJ joints of rearranged human light-chain genes.

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Several proteins contribute to B-cell development by transducing signals from cell-surface receptors. Synthesis of the Igα and Igβ polypeptides is turned on at the pro-B cell stage and continues throughout the life of a B cell, which is consistent with these polypeptides being necessary for the cell-surface expression of immunoglobulin and for the transduction of signals from pre-B cell and B-cell receptors to the interior of the cell. Only when antigen-stimulated B cells differentiate into antibody-secreting plasma cells, and their antigen receptors are no longer required, are the Igα and Igβ genes turned off and immunoglobulin ceases to appear on the cell surface.

Another signal transduction molecule is the Bruton's tyrosine kinase (Btk), which is encoded by a gene on the X chromosome and is essential for B-cell maturation. Patients who lack a functional Btk gene have almost no circulating antibodies because their B cells are blocked at the pre-B-cell stage. The immune deficiency suffered by these patients is called **X-linked agammaglobulinemia** and leads to recurrent infections from common extracellular bacteria such as *Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes,* and *Staphylococcus aureus.* The infections respond to treatment with antibiotics and can be prevented by regular intravenous infusions of immunoglobulin from pooled blood from healthy donors. Because the Btk gene is located on the X chromosome and the defective gene is recessive, X-linked agammaglobulinemia is seen mostly in boys.

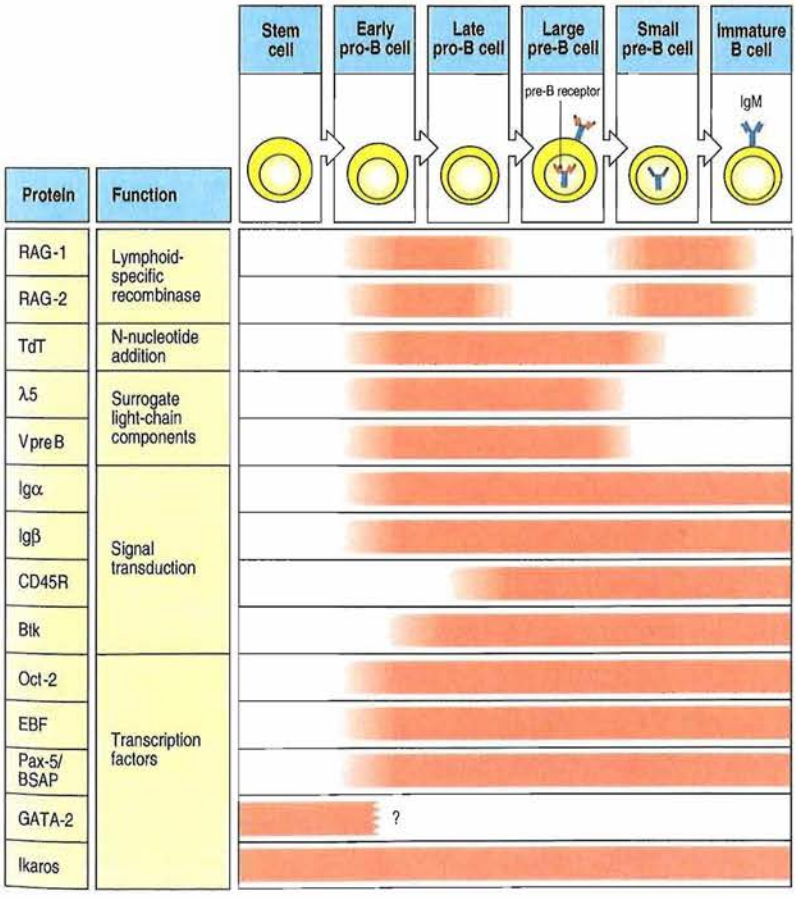


Figure 4.10 The expression of proteins involved in the rearrangement and expression of immunoglobulin genes changes during B-cell development. The rearrangement of immunoglobulin genes and the expression of the pre-B-cell receptor and IgM on the cell surface requires several categories of specialized proteins at different times during B-cell development. Examples of such proteins are listed here, with their expression during B-cell development shown with red shading. Of the proteins not discussed in the text, EBF regulates the transcription of the gene for Igα, and the B-lineage specific-activator protein (BSAP) regulates the expression of several of the other proteins listed. Oct-2 acts at the heavy-chain promoter and GATA-2 is a transcription factor active in several types of hematopoietic cells. CD45 is a cell-surface phosphotyrosine phosphatase that is restricted to hematopoietic cells. It is involved in regulating signal transduction from the pre-B-cell and B-cell receptors.

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A variety of transcription factors control the genes involved in immunoglobulin-gene rearrangement and B-cell development, only some of which are shown in Figure 4.10. Their coordinated activity ensures that the enzymatic machinery for gene rearrangement common to B and T cells is used in B cells to rearrange and express the immunoglobulin genes, and not the T-cell receptor genes.

4-5 Many B-cell tumors carry chromosomal translocations that join immunoglobulin genes to genes regulating cell growth

As B cells cut, splice, and mutate their immunoglobulin genes in the normal course of events, it is hardly surprising that this process sometimes goes awry to produce a mutation that helps convert a B cell into a tumor cell. Transformation of a normal cell into a tumor cell involves a series of mutations that release the cell from the normal restraints on its growth. In B-cell tumors, the disruption of regulated growth is often associated with an aberrant immunoglobulin-gene rearrangement that has joined an immunoglobulin gene to a gene on a different chromosome. Events that fuse part of a chromosome with another are called **translocations** and, in B-cell tumors, the immunoglobulin gene has often become joined to a gene involved in the control of cellular growth. Genes that cause cancer when their function or expression is perturbed are collectively called **proto-oncogenes**. Many were discovered in the study of RNA tumor viruses that can transform cells directly. The viral genes responsible for transformation were named **oncogenes**, and it was only later realized that they had evolved from cellular genes that control cell growth, division, and differentiation.

The translocations between immunoglobulin genes and proto-oncogenes in B-cell tumors can be seen in metaphase chromosomes examined in the light microscope. Certain translocations define particular types of tumor and are valuable in diagnosis. In Burkitt's lymphoma, for example, the MYC proto-oncogene on chromosome 8 is joined by translocation to either an immunoglobulin heavy-chain gene on chromosome 14, a κ light-chain gene on chromosome 2, or a λ light-chain gene on chromosome 22 (Figure 4.11). The Myc protein is normally involved in regulating the cell cycle, but in B cells that carry these translocations its expression is abnormal, which removes some of the restraints on cell division. Single genetic changes are rarely sufficient to transform cells malignantly. To produce Burkitt's lymphoma, mutations elsewhere in the genome are required in addition to the translocation between MYC and an immunoglobulin gene.

Another translocation found in B-cell tumors is the fusion of an immunoglobulin gene to the proto-oncogene BCL2. Normally, the function of the Bcl-2 protein is to prevent premature apoptosis, or programmed cell death, in B-lineage cells. Production of Bcl-2 in the tumors enables B cells to live longer than is normal, during which time they accumulate additional mutations that can lead to malignant transformation. In addition to their value in immunological research, B-cell tumors have provided fundamental advances in knowledge of the proteins involved in the mechanism and regulation of cell division.

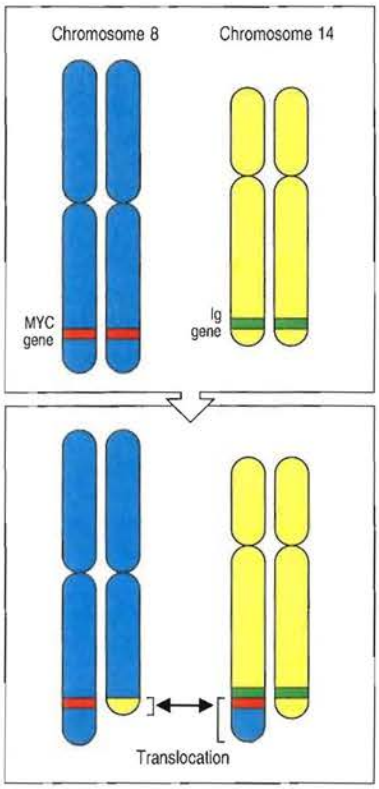


Figure 4.11 Chromosomal rearrangements in Burkitt's lymphoma. In this example from a Burkitt's lymphoma, parts of chromosome 8 and chromosome 14 have been exchanged. The sites of breakage and rejoining are in the proto-oncogene MYC on chromosome 8 and the immunoglobulin heavy-chain gene on chromosome 14. In these tumors it is usual for the second immunoglobulin gene to be productively rearranged and for the tumor to express cell-surface immunoglobulin. The translocation probably occurred during the first attempt to rearrange a heavy-chain gene. This counted as an unproductive rearrangement and so the other gene was rearranged. In cases where the second rearrangement is also unproductive, the cell dies and thus cannot give rise to a tumor.

4-6 B cells expressing the glycoprotein CD5 express a distinctive repertoire of receptors

Not all B cells conform exactly to the developmental pathway described above. A subset of human B cells that arises early in embryonic development is distinguished from the other B cells by the expression of CD5, a cell-surface glycoprotein that is otherwise considered a marker for the human T-cell lineage. This minority subset of B cells is termed **B-1 cells** because their

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development precedes that of the majority subset, whose development we have traced in previous sections and, in this context, are sometimes called **B-2 cells**. B-1 cells are also characterized by little or no IgD on the surface and a distinctive repertoire of antigen receptors. The B-1 cells are also known as **CD5 B cells**, although the CD5 molecule is not essential for their function: B-1 cells develop normally in mice lacking CD5 glycoprotein and rat B-1 cells never express CD5. B-1 cells are the dominant B cells in the pleural and peritoneal cavities. Although comprising only around 5% of the B cells in humans and mice, B-1 cells are the main type of B cells in rabbits.

B-1 cells arise from a stem cell that is most active in the prenatal period. Their immunoglobulin heavy-chain gene rearrangements are dominated by the use of the V_H gene segments that lie closest to the D gene segments in the germline. As TdT is not expressed early in the prenatal period, the rearranged heavy-chain genes of B-1 cells are characterized by a lack of N nucleotides, and their VDJ junctions are less diverse than those in rearranged heavy-chain genes of B-2 cells. Consequently, the antibodies secreted by B-1 cells tend to be of low affinity and each binds to many different antigens, which is known as **polyspecificity**. B-1 cells contribute to the antibodies made against common bacterial polysaccharides and other carbohydrate antigens but are of little importance in making antibodies against protein antigens.

Those B-1 cells that develop postnatally use a more diverse repertoire of V gene segments and their rearranged immunoglobulin genes have abundant N nucleotides. With time, B-1 cells are no longer produced by the bone marrow and, in adults, the population of B-1 cells is maintained by the division of existing B-1 cells at sites in the peripheral circulation. This self-renewal is dependent on the cytokine IL-10. The properties of B-1 and B-2 cells are compared in Figure 4.12. In the remainder of this chapter, and in the rest of

Property	B-1 cells	Conventional B-2 cells
When first produced	Fetus	After birth
N-regions in VDJ junctions	Few	Extensive
V-region repertoire	Restricted	Diverse
Primary location	Peritoneal and pleural cavities	Secondary lymphoid organs
Mode of renewal	Self-renewing	Replaced from bone marrow
Spontaneous production of immunoglobulin	High	Low
Isotypes secreted	IgM >> IgG	IgG > IgM
Response to carbohydrate antigen	Yes	Maybe
Response to protein antigen	Maybe	Yes
Requirement for T-cell help	No	Yes
Somatic hypermutation	Low–none	High
Memory development	Little or none	Yes

Figure 4.12 Comparison of the properties of B-1 cells and B-2 cells. B-1 cells develop in the omentum as well as in the liver in the fetus, and are produced by the bone marrow for only a short period around the time of birth. A pool of self-renewing B-1 cells is then established, which does not require the microenvironment of the bone marrow for its survival. The limited diversity of the antibodies made by B-1 cells and their tendency to be polyreactive and of low affinity suggests that B-1 cells produce a simpler, less adaptive, immune response than that involving B-2 cells.

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