chapter 4:

THE IMMUNE SYSTEM

OBJECTIVES

After studying this chapter you should be able to:

- give an account of the role of the immune system in maintaining health;
- explain the difference between nonspecific and specific immune defenses;
- describe the cells of the immune system and outline their roles in immune defense;
- explain the roles of major proteins involved in immune defense;
- describe the major features of inflammation and the acute phase response;
- describe the major features of humoral and cell-mediated specific immunity;
- outline the use of antibodies for detecting and quantifying molecules of biomedical significance.

4.1 INTRODUCTION

The **immune system** is a set of organs, tissues, cells and molecules that protect the body from disease caused by microorganisms and multicellular parasites (*Chapter 2*). **Immunology** is the study of the immune system and how it works. Unraveling of the mechanisms of immune defense and the use of products of the immune system in investigating and treating disease has revolutionized the biomedical sciences in a way that is comparable to that of molecular biology in recent years. This chapter describes the major components of the immune system and explains how a coordinated immune response is produced against infectious agents. In addition, the use of immunological techniques in the biomedical sciences will be outlined.

4.2 TYPES OF IMMUNE DEFENSE

The immune system has to distinguish between the cells and macromolecules that make up the body that is, **self**, and those that are foreign or **nonself**. Immunological defenses are usually classified as **nonspecific** and **specific**. Nonspecific defenses constitute a first line of defense and are available immediately any foreign material, including substances such as wood splinters as well as microorganisms, enters the body. Nonspecific defenses include responses such as **inflammation**, a rapid immediate response to tissue damage, and the **acute phase response**, a relatively rapid response to infection. In specific immunological defense, cells of the immune system recognize not just individual microorganisms, but also the particular proteins or glycoproteins found on that microorganism. This type of defense may take several days to become effective, depending on whether the immune system was previously exposed to that specific microorganism but, once activated, results in a long-lasting immunity to it. This immunity can be **humoral**, in that it involves the production of antibodies and/or **cell-mediated**, which involves the production of cells that kill or recruit other cells to kill the infected cells. Humoral immunity is effective against microorganisms that do not invade cells and this includes most bacteria and multicellular parasites (*Chapter 2*). Cell-mediated immunity is effective against intracellular parasites, including viruses and some bacteria. However, these two types of specific immunity are not mutually exclusive and usually both types are activated on exposure to the infectious agent.

4.3 NONSPECIFIC DEFENSES

Microorganisms are prevented from entering the body by structural barriers, such as the skin and the mucosal membranes. These barriers are further protected by chemical secretions, for example lactic acid in sebum and hydrochloric acid secreted in the stomach. Furthermore, mucus, secreted by the mucosal membranes that line the urogenital, respiratory and gastrointestinal tracts, contains lysozyme, an antibacterial enzyme that catalyzes the hydrolysis of the peptidoglycan wall of some types of bacteria. Should microorganisms breach these barriers, other antimicrobial proteins such as complement and interferons may be activated and/or produced. Finally, there are a number of cells that have a role in the elimination of microorganisms. These cells may be found in the blood, although a number are also found in the solid tissues. Some blood cells move between the blood and the extravascular tissues, particularly during an infection.

INTERFERONS

Interferons (IFNs) are families of inducible, secretory proteins produced by eukaryotic cells in response to viral infections and other stimuli. They disrupt viral replication in neighboring healthy cells by inducing those cells to produce enzymes that inhibit replication of viral nucleic acid and the production of viral proteins. There are three major families of interferons: IFNs α, β and γ. Interferons α and β are produced by cells that have been infected with a virus. They are the predominant forms produced by leukocytes and fibroblasts respectively, although both types are produced by other viral infected cells in the body. Interferon γ is produced by cells of the specific immune system in response to any agent, whether bacterium, virus or foreign protein, which stimulates that system.

Interferons belong to a large family of proteins called **cytokines**, a general name given to proteins secreted by cells that stimulate activities in other cells after binding to receptors on their surfaces. Cytokines act at low concentrations and may stimulate different activities depending on the type of target cell. Many

different cytokines are involved in the immune response and some are now used therapeutically. Interferon α has been used to treat hairy cell leukemia and Kaposi's sarcoma (*Chapter 5*), while IFN β is used in the treatment of multiple sclerosis and IFN γ has been used to treat several immune deficiency diseases (*Chapter 5*). Cytokines are also involved in the pathology of a number of diseases, particularly inflammatory disorders, such as rheumatoid arthritis (*Chapter 5*).

COMPLEMENT

Complement is the name given to a set of about 30 plasma proteins, some of which are listed in *Table 4.1* and which, when activated, can combat an infection by lysing the invading microorganisms, stimulating inflammation and promoting the uptake of the microbe by phagocytic cells. Activation of complement can be achieved in one of several ways. The **classical pathway** is initiated when antibody binds to the microorganism. This pathway may therefore take several days to become effective if specific antibody is not already present. The classical pathway for activation initially involves complement proteins C1–C4 (*Table 4.1*). An **alternative pathway** can be activated in the absence of antibody by cell wall components of bacteria, such as lipopolysaccharide. This first line of defense against microorganisms uses the complement proteins C3, and Factors B and D. Both pathways feed into a common pathway involving complement proteins C5–C9 that results in lysis of the target cell (*Figure 4.1*). Both pathways also result in the production of small peptides that induce phagocytosis and inflammation. The classical and alternative pathways are described in more detail in *Chapter 6*. Complement is also activated by C-reactive protein (CRP), and mannose-binding lectin (MBL), both of which are plasma proteins produced by the liver during the early or acute stage of an infection. Mannose-binding lectin is, as its name implies, a protein that binds to mannose residues on bacteria. This activates an associated protease that, in turn, activates proteins of the classical pathway. Thus, it provides a means of entering the classical pathway in the absence of antibody.

Complement is essential for immunological defense. Indeed, a deficiency of just a single complement protein can lead to increased susceptibility to bacterial infections. Complement is also involved in the pathology of a number of immunological disorders, including autoimmune diseases, such as rheumatoid arthritis and autoimmune hemolytic anemias (*Chapter 5*).

Protein	M_r
C1q	410 000
C1r	190 000
C1s	87 000
C2	115 000
C3	180 000
C4	210 000
C5	190 000
C6	128 000
C7	121 000
C8	163 000
C9	79 000
MBL (Mannose Binding Lectin)	200 000–700 000
MASP-II (MBP-associated serine protease II)	76 000
Factor B	93 000
Factor D	24 000
Factor H	150 000
Factor I	88 000
Factor P	220 000

Table 4.1 Complement proteins

NONSPECIFIC CELLS

Leukocytes are the white blood cells (*Chapter 13*) produced from precursor stem cells present in the bone marrow. All have immunological roles. About 80% of them are involved in nonspecific immune defense but the small lymphocytes (*Section 4.5*) are the cells of the specific immune system whose products control the numbers and activities of the nonspecific cells and so the two systems are interlinked.

All leukocytes can be classified into one of two groups, the **polymorphonuclear leukocytes** (**PMN**), which have lobed nuclei and granular cytoplasm, and **mononuclear leukocytes** (**MN**) that have a more rounded nucleus (*Figure 4.2*). Polymorphonuclear leukocytes form approximately 65% of all blood leukocytes. They are classified into three groups: **neutrophils**, **basophils** and **eosinophils**.

Neutrophils, which make up around 60% of the blood leukocytes, are phagocytic cells that ingest and kill bacteria. These cells have receptors for antibodies and for the activated complement protein, C3b. Thus, neutrophils will bind readily to bacteria coated with any of these proteins, and phagocytosis is promoted (*Figure 4.3*). This phenomenon is known as **opsonization**. The killing of the

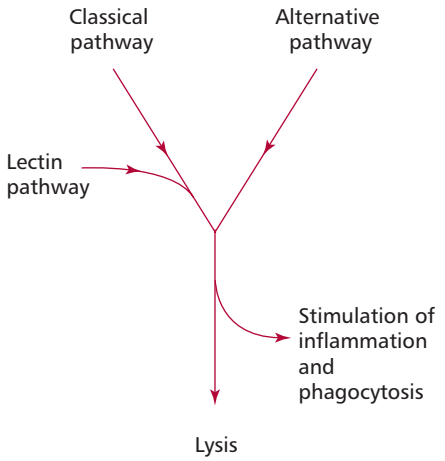


Figure 4.1 Activation of complement. See text for details.

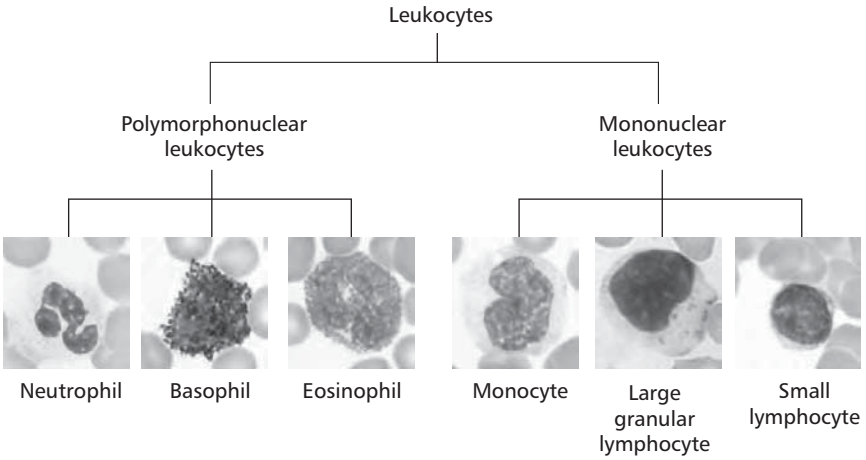


Figure 4.2 Photomicrographs of blood leukocytes. Courtesy of Drs L. Seal and S.J. Richards, School of Biology, Chemistry and Health Science, Manchester Metropolitan University, UK.

ingested bacteria is achieved through several different mechanisms, including the use of lysosomal enzymes (*Chapter 16*), production of antibacterial chemicals, such as hydrogen peroxide, hypochlorite and nitric oxide and the use of cytoplasmic proteins known as defensins that attack the membranes of the ingested microbe.

Basophils (*Figure 4.2*) are found in low numbers in the blood and usually form less than 1% of the leukocytes present. Basophils promote inflammation. They have prominent cytoplasmic granules which take up basic stains such as toluidine blue, and contain an abundance of pharmacologically active agents, such as histamine and heparin, and factors that are **chemotactic** for other PMNs (*Table 4.2*). In addition, basophils have the capacity to synthesize and secrete other mediators when appropriately stimulated. The primary, that is granular, and secondary induced mediators are necessary to promote and maintain inflammation and their inappropriate release can result in immunological disorders such as hay fever and allergic asthma (*Chapter 5*). Despite their relatively low numbers in the blood, basophils are essential for initiating inflammation. However, a similar type of cell known as a **mast cell**, which is found in solid tissues rather than blood, is of greater clinical significance. Mast cells are found throughout the body but especially in the skin, mucosal membranes and epithelia of the respiratory and gastrointestinal tracts and in the connective tissue of a variety of internal organs. Mast cells are highly granular and contain similar mediators to the basophil although the granular contents may vary according to location. Mast cells also secrete a number of cytokines, many of which are **proinflammatory**, that is they

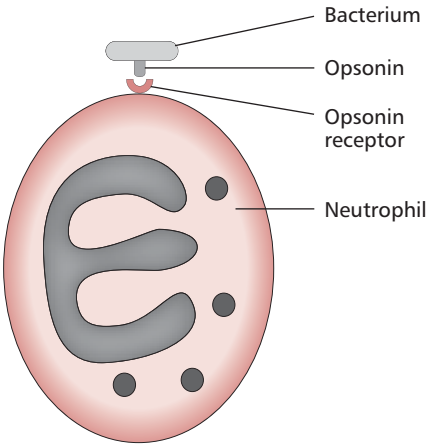


Figure 4.3 The role of opsonin (from the Greek word *opsonion*, meaning victual (food)) in triggering phagocytosis following the binding of an opsonized bacterium to a neutrophil. See text for details.

Chemical	Activity promoted
Histamine	dilates blood vessels, increases vascular permeability, promotes contraction of smooth muscle cells
Heparin	inhibits clotting of blood
Chemotactic factor for neutrophils (NCF-A)	attracts neutrophils into inflammatory area
Chemotactic factor for eosinophils (ECF-A)	attracts eosinophils into inflammatory area
Proteases	degrades basement membrane of blood vessels, promoting migration of cells associated with inflammation into that area
Serotonin	increases vascular permeability and promotes contraction of smooth muscle

Table 4.2 Some pharmacologically active chemicals in basophil granules

promote inflammation. Both mast cells and basophils have receptors for a particular class of antibody, known as immunoglobulin E (*Section 4.4*) and this has significance in the pathology of some of the most common immunological disorders, namely **allergies** (*Chapter 5*).

Like basophils, eosinophils are found in low numbers in the blood and usually constitute less than 2% of white cells. They also have prominent cytoplasmic granules but in this case they take up acidic stains, for example eosin, because they contain highly basic proteins. Though capable of phagocytosis, the major role of eosinophils is to assist in the elimination of multicellular parasites such as tapeworms and nematodes (*Chapter 2*). Eosinophils first bind to the surface of the helminth, often using antibodies and then secrete their toxic granular proteins onto its surface. Eosinophils can be attracted to areas of inflammation by chemotactic factors secreted by basophils and mast cells.

MONONUCLEAR LEUKOCYTES

The mononuclear leukocytes comprise three distinct groups of cells: **monocytes** and **large granular lymphocytes (LGLs)**, which are nonspecific cells, and **small lymphocytes** that are responsible for the specific immune response.

Monocytes (*Figure 4.2*) make up approximately 5% of the blood leukocytes. They have a characteristic indented, often horseshoe-shaped nucleus and a granular cytoplasm. Monocytes are immature cells that circulate for only a matter of hours before they enter the solid tissues and develop into **macrophages.** The spleen, lungs, liver, lymph nodes and tonsils contain especially high numbers of macrophages. Monocytes and macrophages are phagocytic cells, clearing the blood and solid tissues of microbes as well as dead or dying host cells, including neutrophils and erythrocytes. Their numbers and generalized distribution ensure they effectively clear foreign material. Macrophages have killing mechanisms similar to those found in neutrophils and can be stimulated by target cells coated with antibodies and/or complement proteins. Following phagocytosis of microorganisms, especially bacteria, they secrete a range of cytokines, including interleukins 1 (IL-1), 6 (IL-6) and 8 (IL-8) and tumor necrosis factor alpha (TNF α). Interleukin 1, IL-6 and TNF-α are proinflammatory and are responsible for initiating events in the early or acute phase of infection, while IL-8 is a chemokine that attracts neutrophils to its source.

Monocytes and macrophages also play significant roles in the specific immune response since they are able to 'process' foreign material and 'present' it to certain types of small lymphocyte in a form they can recognize. As such, they are known as **antigen presenting cells (APC)**, although they are not the only cells to carry out this activity.

Large granular lymphocytes (LGL) make up 5–10% of the blood leukocytes. These cells have rounded nuclei and a granular cytoplasm (*Figure 4.2*). Functionally, LGL represent a mixed population of cells: some are **natural killer (NK)** cells that kill virus-infected cells and some tumor cells nonspecifically. Natural killer cells bind to the target cell and release proteins, some of which perforate the target cell membrane while others induce a genetically programmed cell death called **apoptosis**. Natural killer cells are the first line of defense against viruses since they prevent their replication and spread. They may also form a defense against potential tumors by destroying some cancerous cells as they arise in the body (*Chapter 17*).

INFLAMMATION AND THE ACUTE PHASE RESPONSE

The term **inflammation** is sometimes used to describe a whole array of responses to infection and tissue damage. Acute inflammation will be used

Margin Note 4.1 Chemokines

Chemokines are cytokines with common structural and functional features. They are all small polypeptides that are chemotactic for and/or activate different types of leukocytes. Their structure consists of 70–90 amino acid residues with a M_r between 8000 and 10 000. Nearly all belong to one of two families with four conserved cysteine residues. These families differ in the presence or absence of an amino acid between the first two cysteine residues.

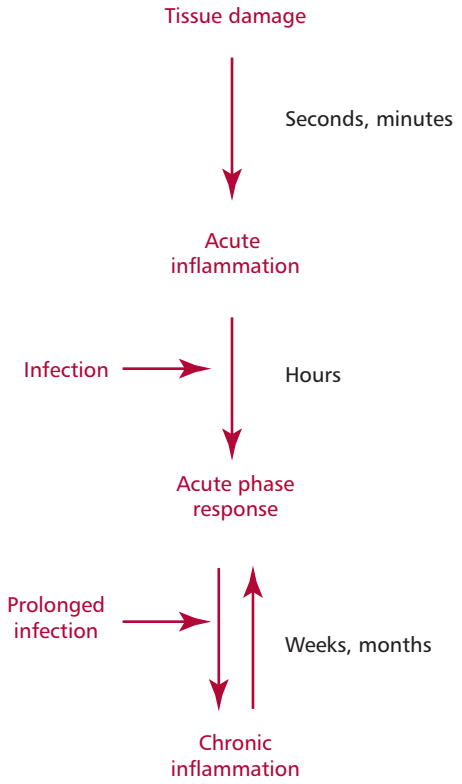


Figure 4.4 The relationship between inflammation and the acute phase response. See text for details.

here to describe the body's immediate and localized response to tissue damage. However, should an infection arise as a result of damage to tissue, acute inflammation can merge with the acute phase response, which is a whole body response that occurs within a few hours of an infection entering the body. The acute phase response involves the production of a number of chemical mediators that promote and prolong local inflammation. In addition, chronic infection can lead to chronic inflammation together with a prolonged acute phase response. This potentially confusing series of overlapping events is clarified in *Figure 4.4*.

Inflammation is a rapid local response to tissue damage, as would happen, for example, if the skin is scratched by a thorn. The response is characterized by reddening and swelling of the skin as well as sensations of heat and pain at the damaged site. Inflammation is initiated by the release of mediators, especially histamine, from mast cells in the damaged area (*Figure 4.5*). Histamine causes blood vessels to dilate and become leaky so that plasma escapes into the damaged tissue where it dilutes any noxious agents that entered at the damaged site, and helps to wash them away into the lymph (*Section 4.5*). If bacteria enter the damaged area they activate complement to release proteins that are chemotactic to neutrophils, encouraging them to move out of the blood between the lining endothelial cells.

The acute phase response occurs within hours of exposure to microorganisms. This is a systemic response involving the whole body and several organ systems. The response is brought about by cytokines, including IL-1, IL-6 and TNF α released by monocytes and macrophages. An acute phase response results in changes in the composition of the blood, including an increased neutrophil count and the appearance of, or increase in, a number of defense proteins called **acute phase proteins**. The appearance in the blood of a particular protein, C-reactive protein (CRP), which binds to bacteria and activates complement, is often used as a marker of infection. The plasma concentration of CRP prior to an acute phase response is so low as to be barely measurable; thereafter its synthesis increases 100–1000 fold.

The acute phase response is a primary defense against infection, with multiple beneficial effects. However, it has the potential to cause harm if an infection is prolonged. For example, acute phase proteins are synthesized in the liver from amino acids released by the enzyme catalyzed proteolysis of muscle tissues. Following an acute infection, this muscle protein is rapidly replaced. However, a chronic infection, such as tuberculosis, can result in severe and prolonged muscle wastage, a condition known as **cachexia** (*Chapters 10* and *17*). High body temperature, or fever, is another symptom of the acute phase response. While elevated body temperatures may inhibit the replication of bacteria, fever can be dangerous, especially in children, if the temperature becomes too high.

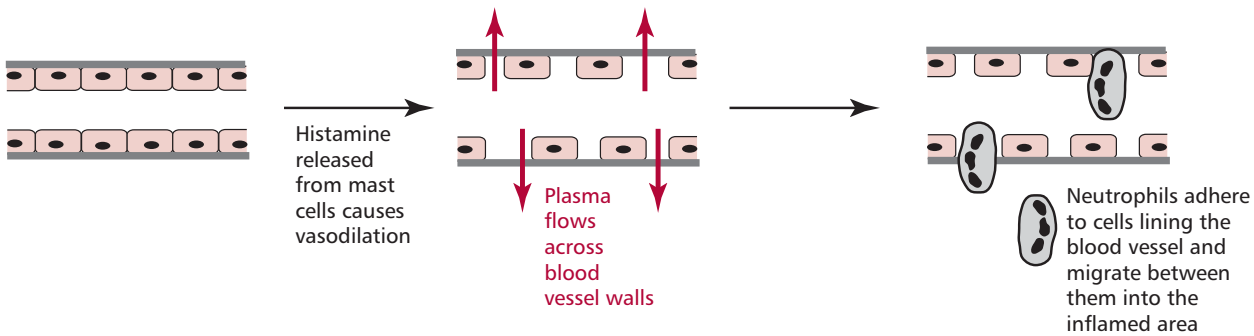


Figure 4.5 Acute inflammation as triggered by histamine release from mast cells. See text for details.

4.4 SPECIFIC IMMUNE RESPONSES

The specific immune response allows the development of true immunity to an infectious agent. Since true immunity can only develop after exposure to the virulent microorganism or a harmless vaccine derived from it, the response is often called **acquired immunity**. For example, an individual who has had measles is unlikely to suffer that disease again, even though exposure to the virus may occur subsequently even after many years. Two major features define a specific immune response. First, specific immunity is only induced towards the agent, called the **immunogen**, which stimulated it and

BOX 4.1 Vaccination

The process of protecting people from infection by deliberately exposing them to microbial components was initiated in modern times by Jenner (1749–1823) in 1796, when he showed that immunity to smallpox, which is caused by the Variola virus, was induced by the introduction into the skin of material from the crusts of cowpox lesions. This process became known as **vaccination**, from the Latin *vacca,* a cow, a term which is still used today. In this case, immunization with the cowpox virus induced immunity that cross-reacted with the variola virus, because the viruses share some molecular similarities. Cross-reactions between vaccines are quite rare and most vaccines are usually specific for only one type of microorganism.

Since Jenner's time, vaccination has been widely applied to protect people from many serious infections. Vaccination against smallpox became compulsory in several developed countries from the 1900s to the 1940s and the WHO embarked on an eradication program, using the cross-reacting Vaccinia virus (*Figure 4.6*) as a vaccine. This program was deemed successful in 1980, the last case of smallpox having been reported in 1977. With this success, the WHO also embarked on a program to eradicate polio by world-wide vaccination but this has not yet been achieved.

Traditional vaccines to induce immunity to microorganisms include the use of attenuated viruses, such as polio, measles, mumps, killed bacteria, for example whooping cough, toxoids, which are derived from bacterial toxins, for example those of tetanus and diphtheria and bacterial cell wall polysaccharides from, for example, *Neisseria meningitidis* serogroup A (*Chapter 2*). More recent developments include recombinant subunit vaccines. Here the vaccine is a microbial protein that has been produced by genetically engineered eukaryotic cells. An example of this is the vaccine for hepatitis B, which consists of a viral surface protein produced by the recombinant yeast, *Saccharomyces cerevisiae*. Other recent developments in vaccine production include polysaccharide conjugate vaccines in which a bacterial polysaccharide is conjugated to a protein, in order to stimulate a more potent immune response. Examples of polysaccharide conjugate vaccines include those for *Haemophilus influenzae* and *Neisseria meningitidis* serogroup C. Where several strains of a microorganism are known to cause disease, multivalent vaccines containing components from a number of strains may be used. The newest development in vaccine production is the DNA vaccine, which consists of plasmid DNA containing a gene coding for the microbial protein in question. This DNA is injected intramuscularly and is taken up by the muscle cells. For a limited period the gene is transcribed and translated to form the foreign protein, which stimulates the immune response ***in situ***. Several DNA vaccines are currently in clinical trial.

At present there are no successful vaccines in routine use against protozoa, including the malarial parasites (*Chapter 2*) that infect 300–500 million annually and kill two to three million people worldwide each year. Such organisms have very complex life cycles, often with secondary animal hosts and often accompanied by distinct antigenic changes, which divert the immune system. In 2005, a new vaccine against malaria was tested on a group of over 2000 children in Mozambique. This vaccine is aimed at the sporozoite form of the parasite, which is the form injected into humans by mosquitoes, and has been shown to cut the risk of developing severe malaria by 58%.

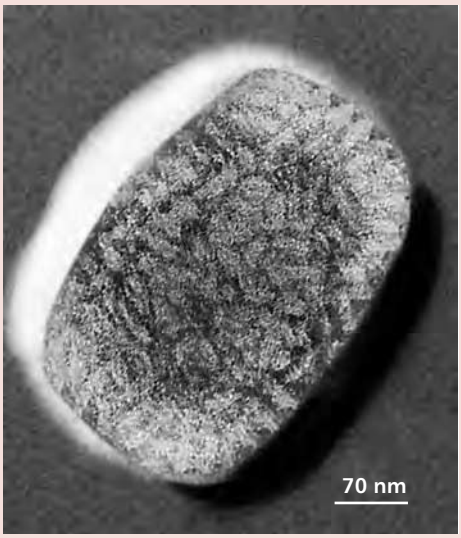


Figure 4.6 Electron micrograph of Vaccinia virus. Courtesy of North West Regional Virus Laboratory, Booth Hall Hospital, Manchester, UK.

Margin Note 4.2 Haptens

Some molecules, such as steroid hormones, are too small to stimulate a specific immune response. However, if such molecules are attached covalently to an immunogenic protein they can be recognized by the immune system and an immune response will then be mounted against them. In this case, the small molecule is called a hapten and the protein to which it is attached the carrier. The ability of the immune system to recognize small molecules when they are presented in the appropriate context has proved extremely useful as it is possible to produce antibodies to a wide range of smaller molecules. The antibodies can then be used in specific assays to determine the presence and concentration of the hapten in biological samples (*Box 4.2*). For example, assays for steroid hormones use antibodies that have been produced in animals by immunizing them with the steroid linked to a protein.

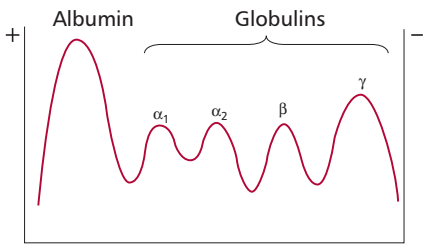


Figure 4.7 The order of separation of serum proteins (albumin and globulins) by electrophoresis (densitometric scan).

secondly, the specific immune response is more rapid following subsequent contacts with that same immunogen. It is the rapid secondary response that prevents the development of disease in the immune individual. The ability to produce a quicker response on second contact with an immunogen is known as **immunological memory**. This process is mimicked by the use of vaccines, where an attenuated or less virulent strain of the microorganism is administered to induce immunity without causing the disease.

The body's specific response to an infectious agent such as rubella virus, the cause of German measles, is to respond with two types of immunity, that is, humoral immunity and cell-mediated immunity. A specific immune response is only stimulated by an **immunogen**, which may be the whole microorganism but could merely be a protein, glycoprotein or lipoprotein. As a general rule, proteins with a M_r greater than 5000–10 000 are immunogenic. Polysaccharides are usually only weakly immunogenic, although they generally become more immunogenic when conjugated to a protein.

Cells of the specific immune system do not recognize the whole immunogen but, rather, small regions of immunogenic macromolecules known as **epitopes.** Usually, epitopes are sequences of 5–7 amino acid residues on immunogenic proteins, but they may also be short sequences of sugar residues in a polysaccharide, lipopolysaccharide or glycoprotein. A large macromolecule may have many epitopes that are recognized by the immune system. However, a protein that has some similarities with our own proteins, for example bovine serum albumin that has a similar size and structure to human albumin, will have fewer nonself regions recognizable as epitopes.

The term **antigen** is often confused with an immunogen and, indeed, some textbooks use the terms interchangeably. Here, an antigen is defined as something that will bind to the product of an immune response, such as an antibody. Nowadays the term is most commonly used when discussing immunotechniques, where an antibody is used to detect an antigen or quantify how much of it is present in a sample (*Box 4.2*).

ROLES OF HUMORAL AND CELL-MEDIATED IMMUNITY

Most immunogens stimulate both humoral and cell-mediated immunity. Antibodies are found in body fluids, including blood and lymph, and have access to extracellular organisms. They are therefore most effective at eliminating microorganisms that live outside the cells of the host. Cell-mediated immunity, on the other hand, is effective against intracellular parasites, which includes all viruses and a number of intracellular bacteria, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*. However, antibodies are useful in protecting cells in the initial stages of a viral infection and in preventing the spread of viruses from one cell to another.

HUMORAL IMMUNITY

In a humoral response, the immune system produces specific glycoproteins called **antibodies** that are found in the blood plasma, mostly in the γ globulin fraction (*Figure 4.7*), in lymph and in body secretions such as saliva, tears, mucus and milk. Antibodies have binding sites that are complementary to the shape of an epitope. These sites allow antibodies to bind to the epitopes of the immunogen and initiate its destruction by a variety of other agents, such as complement, phagocytic cells and LGLs. Collectively, antibodies are known as **immunoglobulins (Igs)** which are heterogeneous molecules indeed because each antibody is specific for an individual epitope. Despite this heterogeneity, they can be divided into one of five major classes or isotypes, IgG, IgM, IgA, IgE and IgD, based on differences in their structures, and particularly in the amino acid sequences of their largest polypeptide subunits which are known as heavy chains (*see below*). Some of their properties are listed in *Table 4.3*.

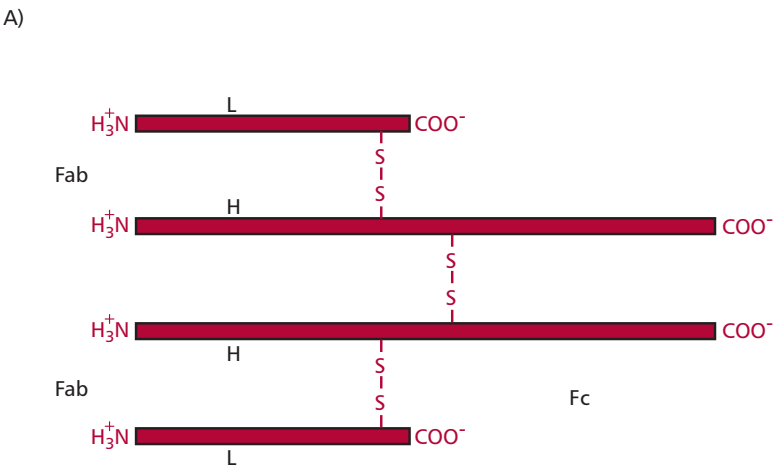
Class	Subclasses	M_r	Heavy chains	Activates complement	Crosses placenta	Opsonization	Triggers inflammation
IgM	none	900 000	μ	yes	no	no	indirectly via complement
IgG	IgG_1 IgG_2 IgG_3 IgG_4	146 000 145 000 170 000 146 000	γ_1 γ_2 γ_3 γ_4	yes yes yes –	yes yes yes yes	yes – yes yes	indirectly via complement
IgA	IgA_1 IgA_2	160 000 to 380 000	α_1 α_2	no no	no no	yes yes	no no
IgE	none	188 000	ε	no	no	no	directly by binding to ε receptors on mast cells
IgD	none	184 000	δ	no	no	no	no

Table 4.3 Properties of immunoglobulin classes. Light chains are always either κ or λ.

Immunoglobulin G

Immunoglobulin G (IgG) is the most abundant antibody in the blood where it is found at concentrations of approximately 13.5 mg cm^{-3}. It is evenly distributed between the vascular and extravascular compartments. In humans, IgG occurs as four subclasses, all with slightly different properties and roles in the body. For example, all subclasses except IgG_4 can activate complement (*Table 4.3*). Immunoglobulin G is produced after IgM on exposure to an immunogen and is the predominant antibody produced on a second exposure, that is in the secondary response. It is also the only antibody in humans that can cross the placenta to allow maternal antibodies to protect the developing fetus from infection. On some occasions, this may cause problems as, for example, when the mother has become sensitized to fetal antigens or has an autoimmune disease (*Chapter 5*).

Immunoglobulin G is a symmetrical molecule consisting of four polypeptide chains joined by disulfide bonds as shown in *Figure 4.8(A)*. The four chains



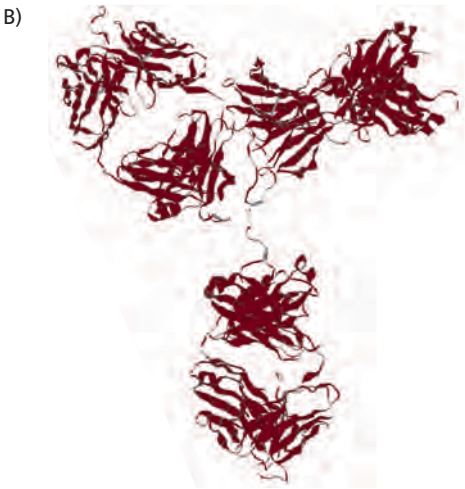


Figure 4.8 The structure of IgG shown (A) diagrammatically, (B) molecular model PDB file 1HZH and (C) showing a bound antigen (black). (C) Courtesy of Dr R.S.H. Pumphrey, St Mary's Hospital, Manchester, UK.

Margin Note 4.3 Domains

The domain substructure of polypeptides is found in proteins throughout the immune system, indicating that these proteins share a common evolutionary relationship. Proteins with this domain structure are members of the immunoglobulin superfamily and include T cell receptors, MHC molecules and cell surface proteins such as Cluster of Differentiation CD4 and CD8 (*Section 4.5*).

consist of two identical heavy chains each with a M_r of 50 000 and two identical light chains each with a M_r of 25 000. Both heavy and light chains have a domain structure. A domain is a globular region made up of approximately 110 amino acid residues and stabilized by an intrachain disulfide bond (*Figure 4.9*). The heavy chain of IgG has four domains while the light chain has only two.

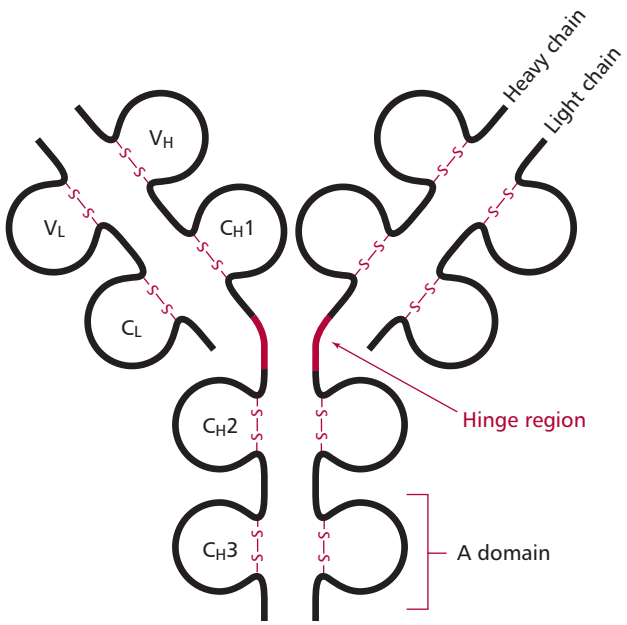


Figure 4.9 A schematic showing the domains of an immunoglobulin molecule. The interchain disulfide bonds have been omitted for clarity.

The interchain disulfide links between the two heavy chains and between the light and the heavy chains (*Figure 4.8 (A)*) produce two units, called the Fab, or fragment antigen binding, part of which can recognize and bind to an epitope (*Figure 4.8 (C)*). The remainder of the molecule, consisting only of the carboxyl terminal halves of the heavy chains is known as the Fc, or fragment crystallizable, portion. The Fc region is concerned with complement activation, placental transfer and binding to LGL and phagocytes.

Immunoglobulin M

Immunoglobulin M (IgM) is the largest of the antibodies with a M_r of about 900 000. Its concentration in the plasma is roughly 1.5 mg cm^{-3}, which is approximately 10% of the plasma immunoglobulins. Most IgM is vascular, with little present in lymph or secretions. Immunoglobulin M is always the first antibody to be produced during an immune response and is also the predominant one formed when an immunogen is encountered for the first time, that is, during a primary response (*Figure 4.10*). The structure of IgM is shown in *Figure 4.11 (A)*. Four polypeptide chains form a structure somewhat similar to that of IgG but this four-chain structure is repeated five times. The five units are joined by their Fc portions by a J chain. Thus, each molecule of IgM has ten binding sites, the largest number of any of the antibody classes. Immunoglobulin M is efficient at **agglutinating** cells or clumping them together, and is an effective activator of complement.

Immunoglobulin A

Immunoglobulin A (IgA) is found in plasma at concentrations between 0.5–3.0 mg cm^{-3} but is also the major antibody found in body secretions, including mucus, saliva and tears. Thus, it protects mucosal surfaces. In humans, most plasma IgA occurs in the familiar four chain structure, similar to IgG

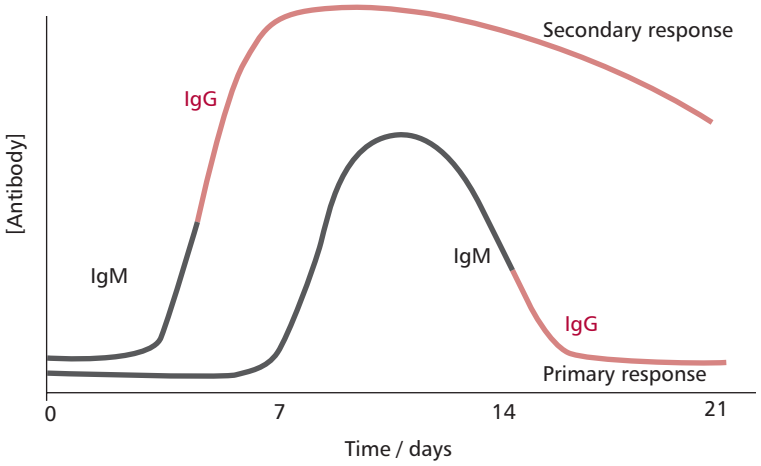


Figure 4.10 The primary and secondary antibody responses to an immunogen.

and with a M_r of 160 000. However, a proportion of plasma IgA also exists as a dimer, in which IgA molecules are joined together by a protein called the joining chain(s) (*Figure 4.11 (B)*). Secretory IgA exists in this dimeric form but is protected from enzymic attack by an additional protein known as the secretory component.

Immunoglobulin E

Immunoglobulin E (IgE) is found in plasma at a concentration of about 5×10^{-5} mg cm^{-3}. Most IgE is found bound to the surface of blood basophils and tissue mast cells, which possess a high affinity receptor for the heavy chain of this immunoglobulin. Binding to these cells prolongs the half-life of IgE from two

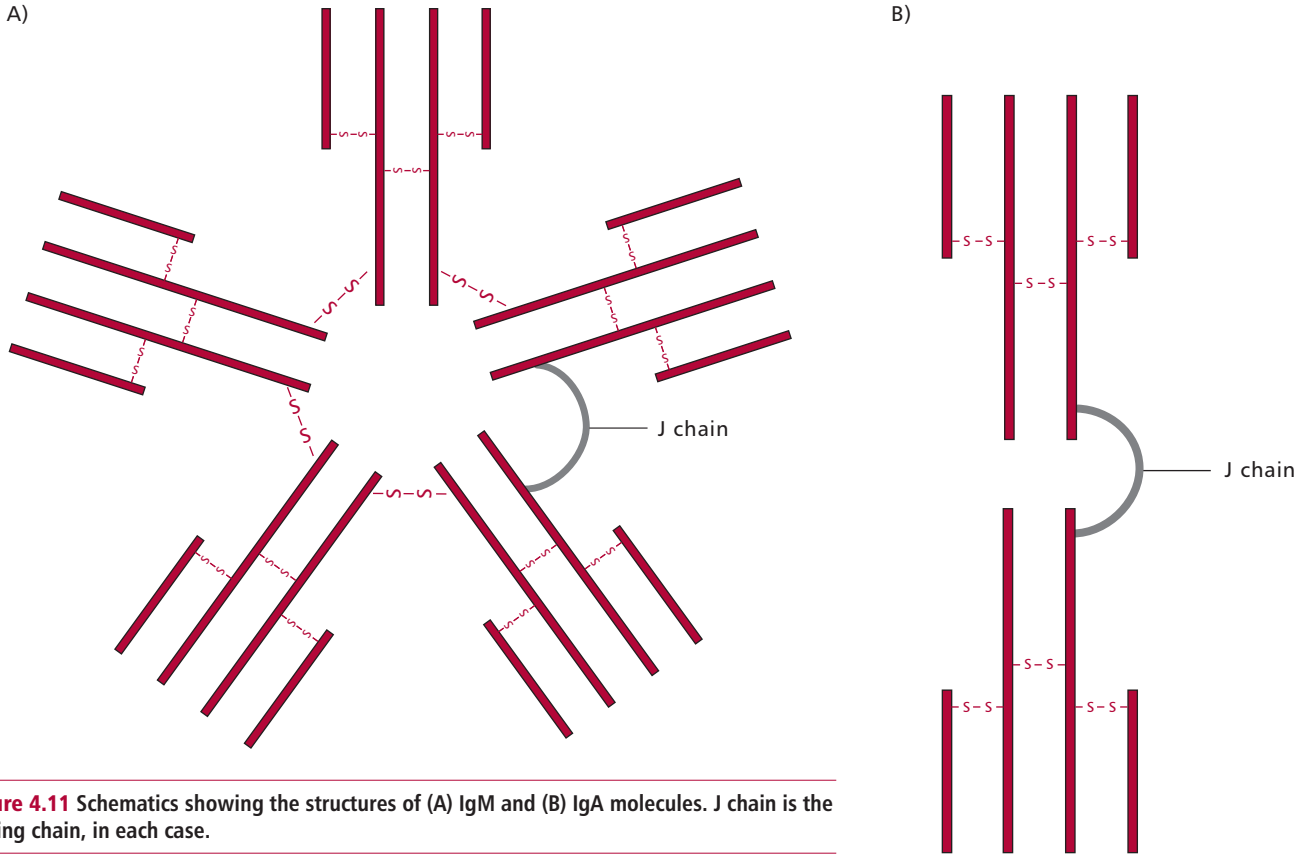


Figure 4.11 Schematics showing the structures of (A) IgM and (B) IgA molecules. J chain is the joining chain, in each case.

days to several weeks. Despite its low plasma concentrations, IgE is a potent stimulator of inflammation, since binding of epitopes to mast-cell bound antibody can trigger degranulation of these cells. The proinflammatory nature of IgE is seen in the elimination of multicellular parasites, such as tapeworms and nematodes. However, in susceptible individuals, this antibody can also trigger an inflammatory response to otherwise harmless immunogens, such as pollen, resulting in allergic reactions (Chapter 5).

Immunoglobulin D

Immunoglobulin D (IgD) is found in plasma at a concentration of about 30 μg cm^{-3}. Its role as a secreted antibody is uncertain. Hyperimmunoglobulin D syndrome (HIDS), in which IgD levels are increased, is associated with periodic fever and joint disease. However, IgD does have a role in the recognition of epitopes by cells of the specific immune system (*Section 4.6*).

The concentration of immunoglobulins shown above for all the immunoglobulins represents their mean plasma concentrations. During

BOX 4.2 Immunoassay

As well as being molecules of major significance in protecting the body from infection, antibodies are also powerful reagents for detecting and quantifying antigens. The specificity of antibodies allows biomedical scientists to measure the level of an analyte, such as a steroid hormone, in biological fluids such as plasma, which contain hundreds of other biomolecules, some of which may be very similar to the analyte being measured. The use of antibodies to quantify antigens is called **immunoassay** and immunoassays are used in all branches of the biomedical sciences. Some immunoassays are among the most sensitive assays known, detecting antigens in the range of mg cm^{-3} to pg cm^{-3}.

One of the earliest immunoassays developed is known as radial immunodiffusion (RID). This method relies on the ability of antibodies to precipitate soluble protein antigens. Antibody is incorporated evenly into an agar gel and a measured volume of antigen solution is added to wells cut into the agar. As the antigen diffuses into the agar the reaction with antibody forms a circle of precipitation (*Figure 4.12*). After allowing all the antigen to diffuse, which takes about 72 h, the diameter of this precipitin ring is measured. The antigen concentration is proportional to the square of the diameter so that the concentration of the unknown can be determined from a standard curve produced using known antigen concentrations.

Radial immunodiffusion is a simple and reliable method, which can be used, for example, to measure the concentrations of a number of serum proteins. However, it is not a sensitive method, suitable for determining concentrations in the μg cm^{-3} to mg cm^{-3} range, and takes several days before the result can be read. Nephelometry is another technique which detects precipitation,

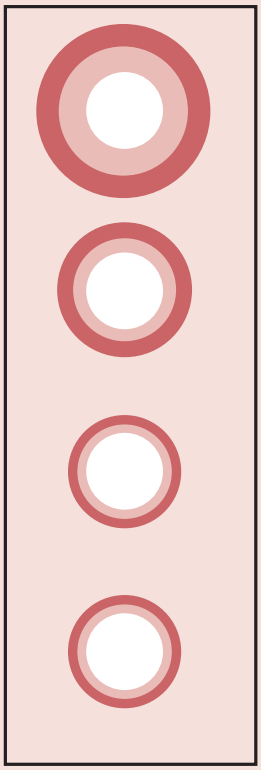


Figure 4.12 An outline of a radial immunodiffusion assay as described in the text. Note that the diameters of the precipitin rings increase with larger concentrations of antigen.

infection, concentrations increase as humoral immunity is activated. Levels significantly above the normal range for the appropriate age group are associated with myeloma, a cancer of antibody-producing cells or with infection with Epstein Barr virus. Concentrations significantly below the normal range are associated with immunodeficiency disorders (*Chapter 5*).

CELL-MEDIATED IMMUNITY

Cell-mediated immunity (CMI) involves the direct and indirect destruction of host cells infected by viruses or other intracellular parasitic microorganisms, such as rickettsias (*Chapter 2*). The direct destruction of infected cells is brought about by the production of specific **cytotoxic cells** that are capable of killing any such infected cell that induced their formation. Indirect destruction is brought about by the release of cytokines that promote destruction by macrophages and LGLs. This type of immunity forms the major defense against viral infections since the destruction of virus-infected host cells prevents the replication and spread of the virus.

Margin Note 4.4 Immunity and self damage

Both humoral and specific immunity protect the body from infection but, sometimes, these responses can lead to tissue damage as, for example, when IgE causes allergic reactions (*Chapter 5*) or when both forms of immunity help to bring about the destruction and rejection of a transplant (*Chapter 6*).

but in solution rather than in agar. It relies on the ability of precipitates formed by an antibody reacting with a protein antigen to scatter a beam of light passed through it. Scattered light is detected at right angles to the original light source. This method is much more rapid than RID, although optically clear antibodies must be used. Other methods, which use 'labeled' reagents, are much more sensitive and can be used to measure nonprotein antigens. In addition, results from these assays can be obtained within hours rather than days. The first of these labeled reagent techniques to be developed was radioimmunoassay (RIA), which was devised in 1960, but is still used extensively. Radioimmunoassay relies on the competition between radiolabeled and unlabeled antigen for a limited amount of antibody. The more unlabeled antigen (standard or unknown) there is in a sample, the less radioactive antigen will bind to the antibody. Radioimmunoassays are extremely sensitive, measuring routinely in the pg cm^{-3} to µg cm^{-3} range.

Enzyme immunoassays (EIA) rely on the use of an enzyme-labeled antibody to measure an antigen. The enzyme used is one that will convert a colorless substrate into a colored soluble product that can be measured spectrophotometrically. The most frequently used enzyme labels are horseradish peroxidase and alkaline phosphatase and the most common EIA is the enzyme-linked immunosorbent assay (ELISA). The simplest ELISA format is to allow a protein antigen to adsorb onto the wells of a plastic microtiter plate (*Figure 4.13*). An enzyme labeled antibody is then added which binds to the antigen in the wells. Wells containing a large amount of antigen will contain more antibody, and therefore more enzyme. The substrate for the enzyme is then added and, after a limited period, the reaction is stopped and the absorbance measured. There are many different adaptations of ELISA which allow the measurement of nonprotein antigens, or which allow the sensitivity to be increased to that approaching RIA.

Other labels that can be used in immunoassays include fluorescent labels and bio- and chemiluminescent labels.

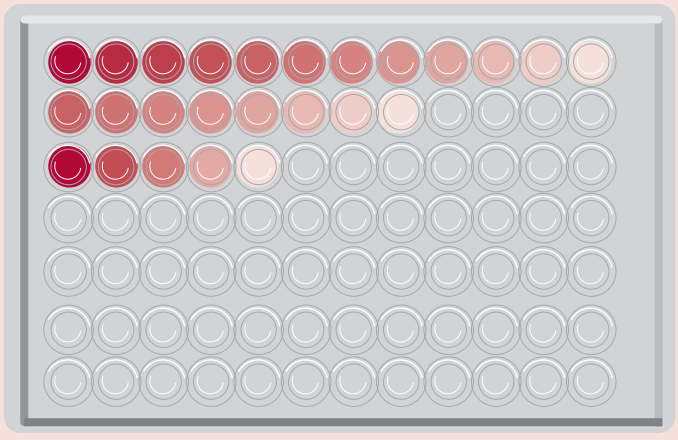


Figure 4.13 The outcome of a typical ELISA assay using three sources of antigen which have each been serially diluted across the rows of wells.

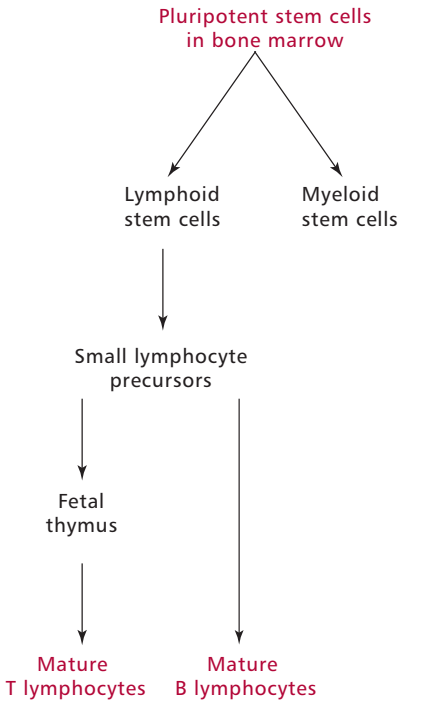


Figure 4.14 Schematic showing the development of small (B and T) lymphocytes.

4.5 SMALL LYMPHOCYTES

Small lymphocytes are the cells responsible for specific immunity (*Figure 4.2*). They make up approximately 20% of the blood leukocytes. There are two populations of small lymphocytes that mature at different sites in the body and have distinct functions (*Figure 4.14*).

The precursors of small lymphocytes originate in the bone marrow by division of **lymphoid stem cells**. Some small lymphocytes remain in the bone marrow where they mature into **B lymphocytes**. When maturation is complete, B lymphocytes have antibodies on their surface that are receptors for an individual epitope. Thus a single B lymphocyte is specific for an epitope and is capable of clonal division and of making antibody to it when stimulated appropriately by the immunogen. Thus they are responsible for humoral immunity. The second population of small lymphocytes, known as **T lymphocytes**, leave the bone marrow when immature and complete their maturation in the fetal thymus, a bilobed organ situated just above the heart. During their development in the thymus, T lymphocytes first acquire specificity for an epitope, by producing a cell surface receptor. They then mature into one of two T cell subsets. Cells of the first subset are known as **cytotoxic precursors** or **T_C cells**. When appropriately stimulated, T_C cells develop into **cytotoxic T lymphocytes (CTL)** that are capable of killing virus-infected cells. Cells of the second subset of T cells are the **helper T lymphocytes** or **T_H cells**. When stimulated by an immunogen, T_H cells develop into cytokine-secreting T_H cells that produce an array of cytokines that control the activities of both specific and nonspecific cells of the immune system. Thus T_H cells have a central role in the regulation of all immune responses.

When mature, both B and T lymphocytes are released into the circulation. However, small lymphocytes are not confined to the blood and many move into the lymphoid tissues: the spleen, lymph nodes, tonsils and the mucosa-associated lymphoid tissues found in the respiratory, gastrointestinal and urogenital tracts. Small lymphocytes constantly move between the blood and the lymphoid systems, a phenomenon known as recirculation. The route of this recirculatory process is shown in *Figure 4.15*. Lymph is the fluid that drains from the tissues into small lymphatic vessels. These merge with larger lymphatic vessels, the largest of which, the thoracic duct, delivers the lymph to the blood at its junction with the left subclavian vein. *En route* to the thoracic duct, lymph is filtered through many lymph nodes. Small lymphocytes circulating in the blood are able to move between the

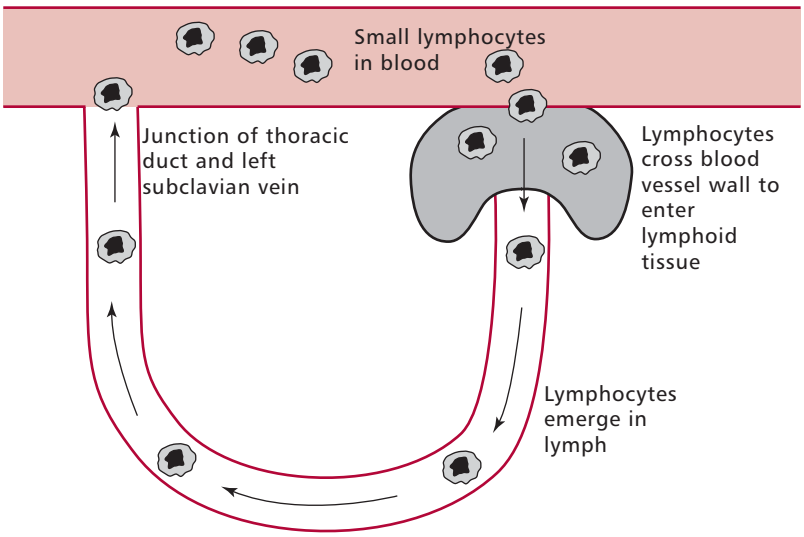


Figure 4.15 Schematic showing the recirculation of small lymphocytes between the blood and lymph systems.

endothelial cells lining the blood vessels that supply the lymph nodes. These blood vessels have a specialized endothelium which aids this process. By crossing the blood vessel wall, the small lymphocytes enter the lymph node and from this they enter the lymphatics and, eventually, return to the blood.

4.6 PRODUCTION OF A SPECIFIC IMMUNE RESPONSE

All small lymphocytes are specific for a single epitope on an individual immunogen. This means that they will respond only to that epitope and no other. The basis of this specificity depends on lymphocyte membrane proteins that act as receptors for individual epitopes. The receptors on B lymphocytes are surface immunoglobulins that have the same specificity as the antibody that the cells will subsequently secrete following appropriate stimulation. The surface antibodies on a single cell may belong to more than one class, so, for example, B lymphocytes may express both IgG and monomeric IgM, but these will have identical specificities. The T cell receptor (TCR) is composed of two polypeptide chains that form a single binding site. All the receptors on a single T cell have the same specificity.

Although T and B cells have different types of receptor and are involved in different aspects of the immune response, there are some similarities in the way they are activated and respond to a specific epitope. For example when both B and T lymphocytes are exposed to an appropriate epitope they enter a series of cell divisions that result in the production of a clone of cells of identical specificity (*Figure 4.16*). These cell divisions require cytokines that are produced and secreted by T_H lymphocytes once they have been appropriately stimulated. Most of the cells in the clone of small lymphocytes then differentiate into effector cells, the nature of which depends on the type of small lymphocyte that was stimulated. Not all the cells in the clone differentiate at this stage. Some remain as memory cells, awaiting the next exposure to the same immunogen when a faster and quantitatively greater response is produced.

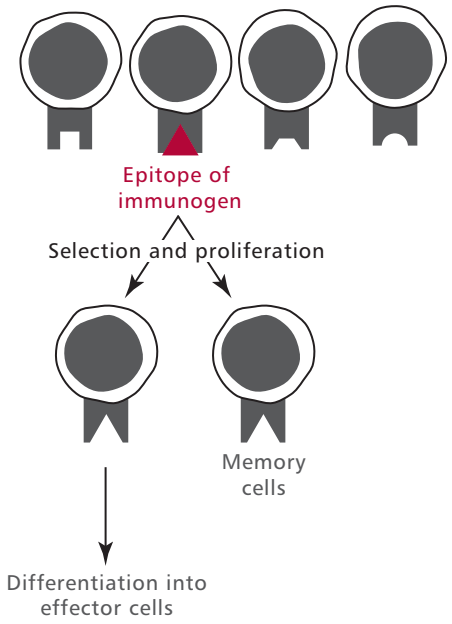


Figure 4.16 Schematic showing clonal selection in lymphocyte activation. See text for details.

BOX 4.3 Distinguishing T and B lymphocytes

In a standard stained blood smear, all small lymphocytes appear similar and it is not possible to distinguish between B and T lymphocytes, or between the T_C and T_H cells. However, these cells can be distinguished by staining for marker proteins in the membranes of these cells. Different markers that can be used to distinguish T and B lymphocytes are shown in *Table 4.4.*

The marker molecules can be distinguished by **immunohistochemistry**, in which a labeled antibody is used to stain the cells. One of the commonest labels used is a fluorescent molecule, such as fluorescein, which produces an apple-green colored fluorescence when irradiated with light of short wavelengths.

B lymphocytes can be stained with an anti-immunoglobulin carrying a conjugated fluorescein label. This binds to the cell surface immunoglobulin, so that the B lymphocyte fluoresces when examined with a fluorescence microscope. All mature T lymphocytes can be identified by the CD3 protein carried on their surface. Staining usually involves an indirect method that is a two-stage process. This involves incubating the lymphocytes with an unconjugated anti-CD3 antibody, which is a monoclonal antibody originating in mice (*Box 4.4*). This is then followed by the second stage involving incubation with a fluorescein-conjugated antibody to the mouse immunoglobulin bound to the CD3 proteins on the T lymphocyte surface (*Figure 4.17*). The cells can then be distinguished by their fluorescence. Similarly, T_H and T_C cells can be stained and identified using anti-CD4 and anti-CD8 antibodies respectively.

Fluorescent labeled antibody techniques, known as **immunofluorescence**, have been in use since the 1950s. They are easy to perform and reliable, although the preparations are not permanent as the fluorescent label tends to bleach, and they need to be examined soon after the test has been performed. In addition, they require the use of fluorescence microscopes although lymphocyte samples can also be quantified using a flow cytometer, which is usually available in major laboratories that deal with multiple samples on a regular basis.

BOX 4.3 Distinguishing T and B lymphocytes

Labels other than fluorochromes are available, which allow the production of permanent preparations and the use of an ordinary light microscope. For example, antibodies labeled with an enzyme, such as horseradish peroxidase or alkaline phosphatase can be located by their ability to convert a colorless substrate into an insoluble colored compound that can be seen when the cells are examined microscopically. This technique is called **enzyme immunohistochemistry** (*Figure 4.18*).

Protein	Role *in vivo*	Found on
Surface immunoglobulin	epitope receptor	B lymphocytes
CD3	signal transduction following binding of T cell receptor to epitope	all mature T lymphocytes
CD4	coreceptor molecule	helper T lymphocytes (T_H)
CD8	coreceptor molecule	cytotoxic T cell precursors (T_C) and cytotoxic T lymphocytes (CTL)

Table 4.4 Marker proteins for T and B lymphocytes

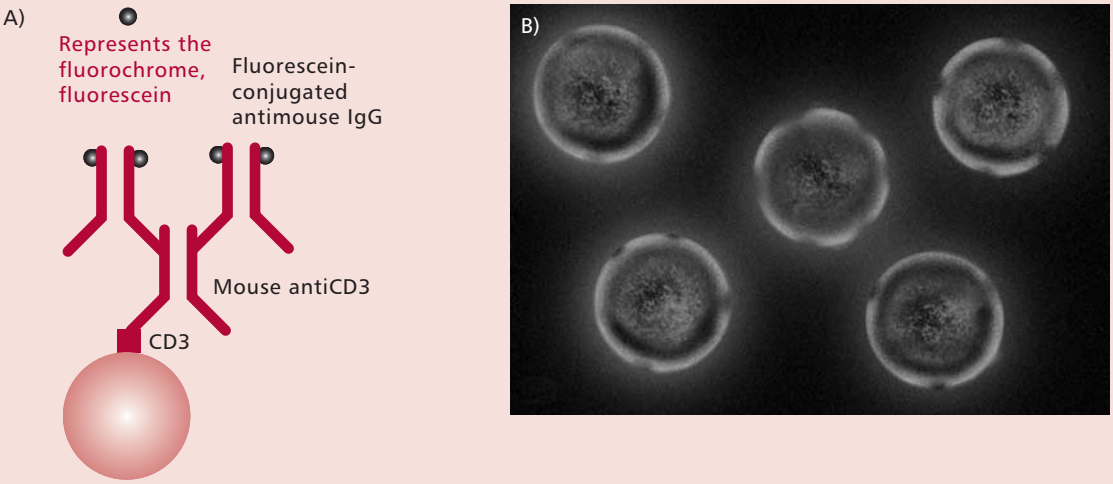


Figure 4.17 (A) Schematic showing the detection of cellular antigens, in this case CD3, by immunofluorescence as described in the text. (B) Computer generated image showing the distribution of membrane antigens as detected by immunofluorescence.

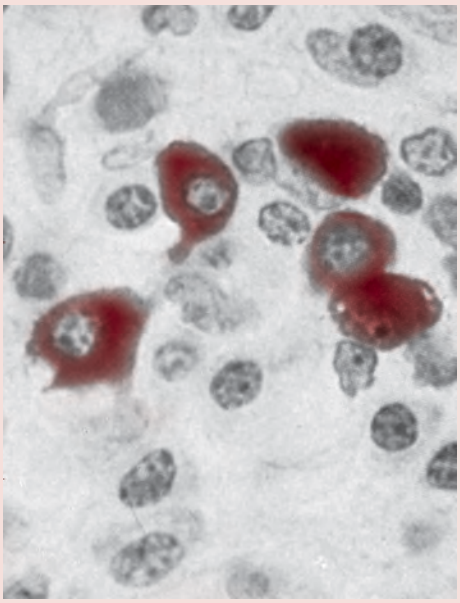


Figure 4.18 Photomicrograph showing the detection of cytoplasmic antigens following the production of a colored marker using enzyme immunohistochemistry.

HUMORAL IMMUNITY

The response of B lymphocytes to an immunogen is outlined in *Figure 4.19*. Once the receptors on B lymphocyte bind to an epitope on the immunogen, the lymphocyte is stimulated to divide and differentiate under the influence of cytokines released from T_H lymphocytes, to form a clone of antibody-secreting cells called **plasma cells** (*Figure 4.20*). Plasma cells are not found in the blood of healthy individuals. Instead, they are present in the lymph nodes and spleen, where they secrete antibodies until they die after a lifespan of a few days to several months. Antibody secreted by plasma cells in lymph nodes first appears in the lymph and then the blood, while antibody produced in the spleen moves directly to the blood.

The class of antibody that is secreted depends, in part, on which B lymphocyte was stimulated and on the cytokines that influenced its differentiation. Certain cytokines are known to favor the production of antibodies of a particular class. For example IL-4 promotes the production of IgE, and favors a response to multicellular parasites. However, a predisposition to produce IgE may also make that individual more susceptible to allergic reactions, such as hay fever and allergic asthma, as will be discussed in *Chapter 5*.

Although *Figure 4.19* illustrates a single B lymphocyte responding to a single epitope on an immunogen to give rise to a single clone of plasma cells, in reality an immunogen, such as a bacterium, contains numerous proteins each of which may have hundreds of epitopes. Thus, a humoral immune response involves the stimulation of many B lymphocytes, each of which can proliferate into a clone. Although each clone produces only a single type of antibody, called a **monoclonal antibody**, hundreds of clones are formed so that the response of the system is **polyclonal**, resulting in a heterogeneous array of antibodies appearing in the blood.

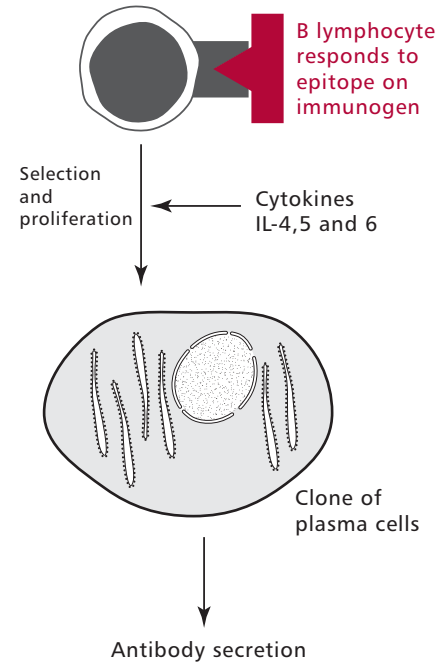


Figure 4.19 Schematic illustrating the activation of a B lymphocyte and its differentiation into a plasma cell. Note that the plasma cell has more cytoplasm, with extensive rough endoplasmic reticulum synthesizing immunoglobulins.

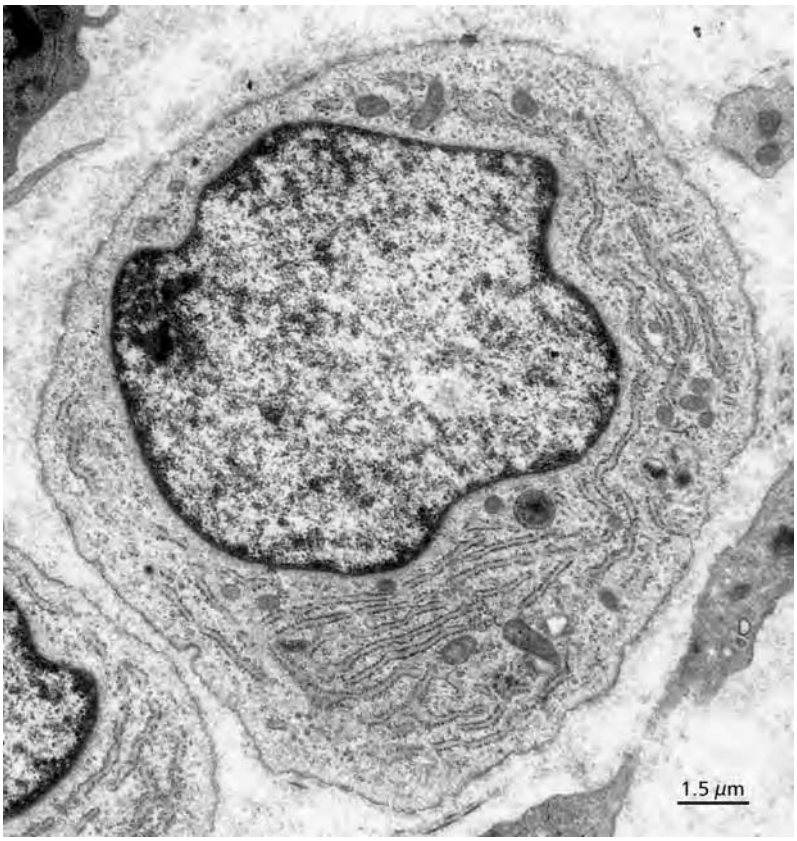


Figure 4.20 An electron micrograph of a plasma cell showing extensive rough endoplasmic reticulum.

BOX 4.4 Monoclonal antibodies

Each clone of plasma cells produces homogeneous or monoclonal antibody that is specific for a single epitope. In 1975, Kohler and Milstein, working at Cambridge, developed a technique whereby plasma cells could be immortalized producing a specified monoclonal antibody so that they could be cultured indefinitely. The technique, outlined in *Figure 4.21*, involves immunizing mice with the immunogen in question. After the required immunization protocol, the mouse spleen containing antibody-secreting plasma cells is removed and gently homogenized to form a suspension of single cells. The plasma cells are then fused with cultured cells derived from a mouse myeloma, that is a plasma cell tumor, the cells of which are immortal. The fusing agent, or fusogen, is polyethylene glycol (PEG). The resulting cells are known as hybrid myelomas, or, more frequently **hybridomas** and are, like their myeloma progenitor, essentially immortal when grown in suspension. Since the fusogen is indiscriminate in its actions, the cell suspension also contains hybrids consisting of plasma cell–plasma cell and myeloma–myeloma cell fusions. While the plasma–plasma cell fusions die within a short time in culture, the myeloma–myeloma fusions and any unfused myeloma cells have to be selectively removed, as they would quickly outgrow the hybridomas. The commonest selection procedure involves using a myeloma cell lacking hypoxanthine guanine phosphoribosyl transferase (HGPRT) activity and growing the resulting hybridomas in medium containing hypoxanthine, aminopterin and thymidine (HAT medium) for a period after hybridization. Aminopterin inhibits dihydrofolate reductase (DHFR), which is essential for the synthesis of DNA. Cells that possess HGPRT can overcome this block by using HGPRT and thymidine kinase (TK), as long as they are supplied with hypoxanthine and thymidine. Thus, hybridoma cells, which have HGPRT, supplied by the plasma cells, and TK activities survive in HAT medium but myeloma cells do not.

A suspension of fused cells produced from a single mouse spleen may contain millions of hybridoma cells derived from different clones. To produce monoclonal antibodies, individual hybridoma cells need to be isolated and grown individually. Isolation is achieved by diluting the cell suspension to such a degree that there is a high degree of certainty that aliquots will contain only a single hybridoma cell. Such aliquots can then be grown on in culture to produce a clone, which will secrete a monoclonal antibody.

Monoclonal antibodies have widespread uses in the diagnosis and treatment of disease. They can be used in immunoassays (*Box 4.2*) to measure the concentrations of biomolecules in clinical samples. For example, commercial monoclonal antibodies to hormones such as thyroxine, estrogen, and testosterone can be used to confirm suspected hormonal deficiencies (*Chapter 7*). In addition, monoclonal antibodies to cancer associated antigens can be used to screen for cancers or to monitor the treatment of cancers. An example of such an antigen is the prostate-specific antigen (PSA) which is elevated in the blood of patients with benign hyperplasia of the prostate gland, prostatitis and tumors of the prostate gland (*Chapter 17*).

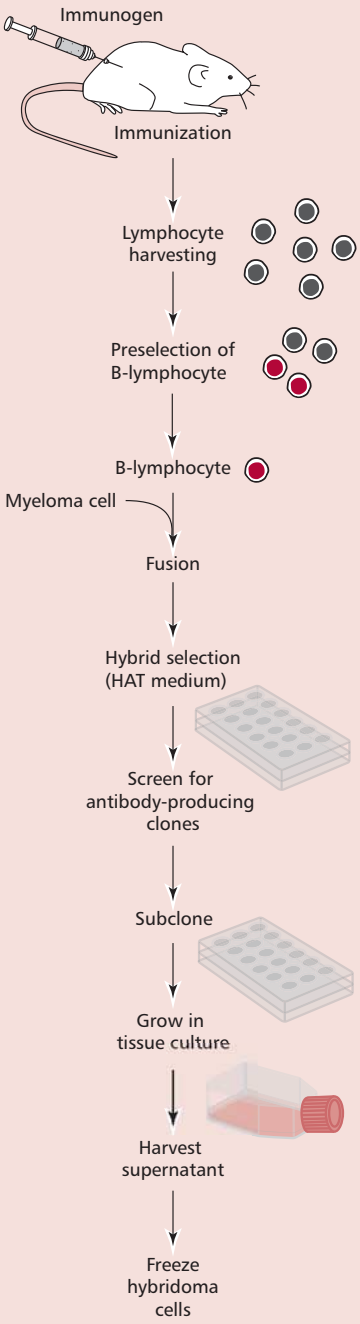


Figure 4.21 Schematic showing the production of monoclonal antibodies from the immunization of a mouse to the culture and storage of the hybridoma cells for future use.

Monoclonal antibodies have been used in clinical trials for treating autoimmune diseases and a variety of malignancies. Since most monoclonal antibodies are mouse immunoglobulins, they need to be 'humanized' by linking the mouse Fab region to a human Fc portion so that they are less likely to be recognized as foreign protein by the immune system when injected. The monoclonal antibody, MRA, is a humanized antibody to the human IL-6 receptor, which began clinical trials in 2005 for the treatment of systemic lupus erythematosus (SLE), an autoimmune disease (*Chapter 5*).

Activation of T lymphocytes

The manner in which T lymphocytes recognize foreign epitopes is rather more complex than that used by their B lymphocyte counterparts, as the receptors on T cells are unable to bind to epitopes on 'native' proteins. Instead, peptides derived from the foreign protein are 'presented' to them on the surfaces of other host cells, bound to membrane proteins encoded by a genetic region called the **Major Histocompatibility Complex** (**MHC**). In humans, this genetic region is found on chromosome 6. The proteins encoded by the MHC include two classes of membrane proteins. Class I proteins are found on all nucleated cells in the body. They consist of a single polypeptide, the α chain, which is associated with a smaller protein called β_2 microglobulin (β_2M) that is not encoded within the MHC. Class II proteins are found on only a few cell types and are made up of two polypeptides, α and β, both encoded within the MHC. Both Class I and II proteins have grooves that can bind a foreign peptide in an extended form (*Figures 4.22* and *4.23*). The peptide-binding groove is formed by the α_1 and α_2 domains of the Class I molecule and the α_1 and β_1 domains of the Class II molecule, while these structures are supported, in the Class I molecule by the α_3 domain and the β_2M, and in the Class II molecules by the α_2 and β_2 domains.

The requirement to have foreign peptides presented by different MHC molecules to T_C and T_H cells can be explained by looking at their roles *in vivo*.

Activation of T_C cells

The role of T_C cells is, ultimately, to destroy virus-infected cells. Since all nucleated cells are susceptible to such infections, MHC encoded Class I molecules are required by these cells to present endogenously produced viral peptides to T_C cells. Thus, viral protein produced within the cytoplasm of an infected cell may be hydrolyzed to produce short peptides, about 8–12 amino acid residues in length. These peptides are then transported across the endoplasmic reticulum where they become attached to Class I proteins and the complex transported to the surface of the cell membrane in Golgi vesicles.

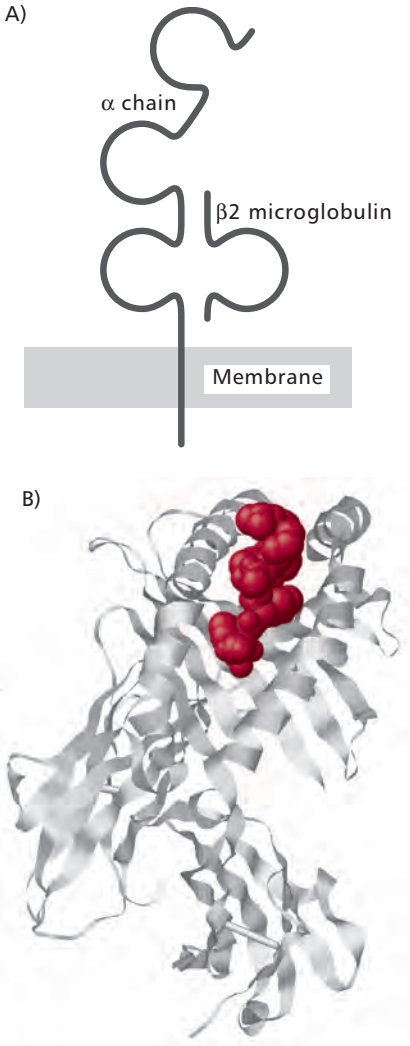


Figure 4.22 Structure of an MHC Class I molecule shown (A) diagrammatically and (B) molecular model showing a bound peptide (red) PDB file 1KJV.

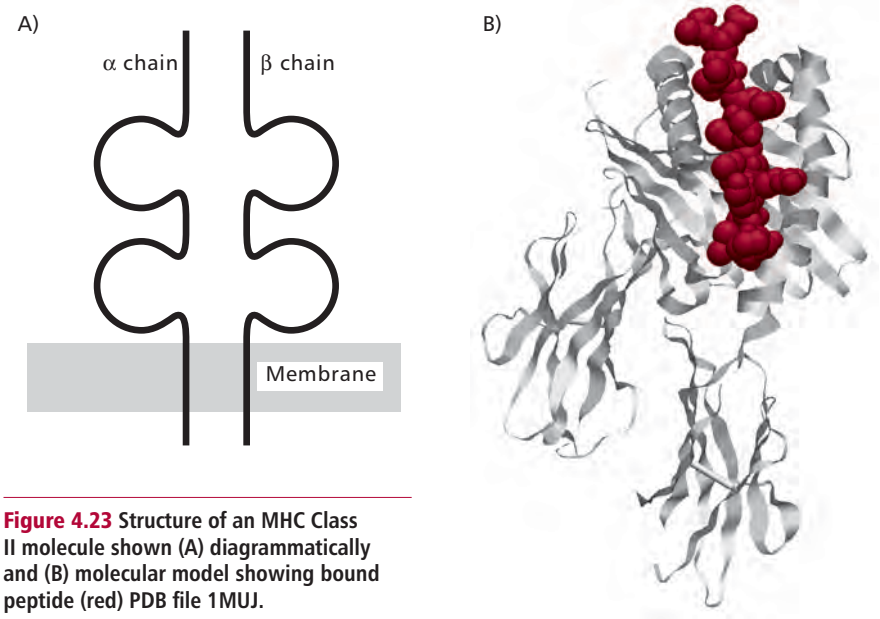


Figure 4.23 Structure of an MHC Class II molecule shown (A) diagrammatically and (B) molecular model showing bound peptide (red) PDB file 1MUJ.

The T_C cell is activated when the T cell receptor recognizes the complex of MHC Class I protein with its bound virus derived peptide and binds to it (*Figure 4.24 (A)* and *(B)*). An accessory protein, CD8, is also involved in this interaction as it also binds to the MHC protein. Once bound, the T_C cell is stimulated into a series of mitotic divisions to form a clone of cells, which then differentiate into cytotoxic T lymphocytes (CTL). These cells resemble T_C cells but their cytoplasm is more granular, owing to the presence of vesicles containing cytotoxic proteins called granzymes and perforins. The CTL binds to a virus infected cell using the same mechanism as the T_c cell, and then releases these cytotoxic proteins which destroy the target cell. Cytotoxic T lymphocytes also release IL-2 and IFN γ which stimulate NK cells and macrophages to kill virus infected cells.

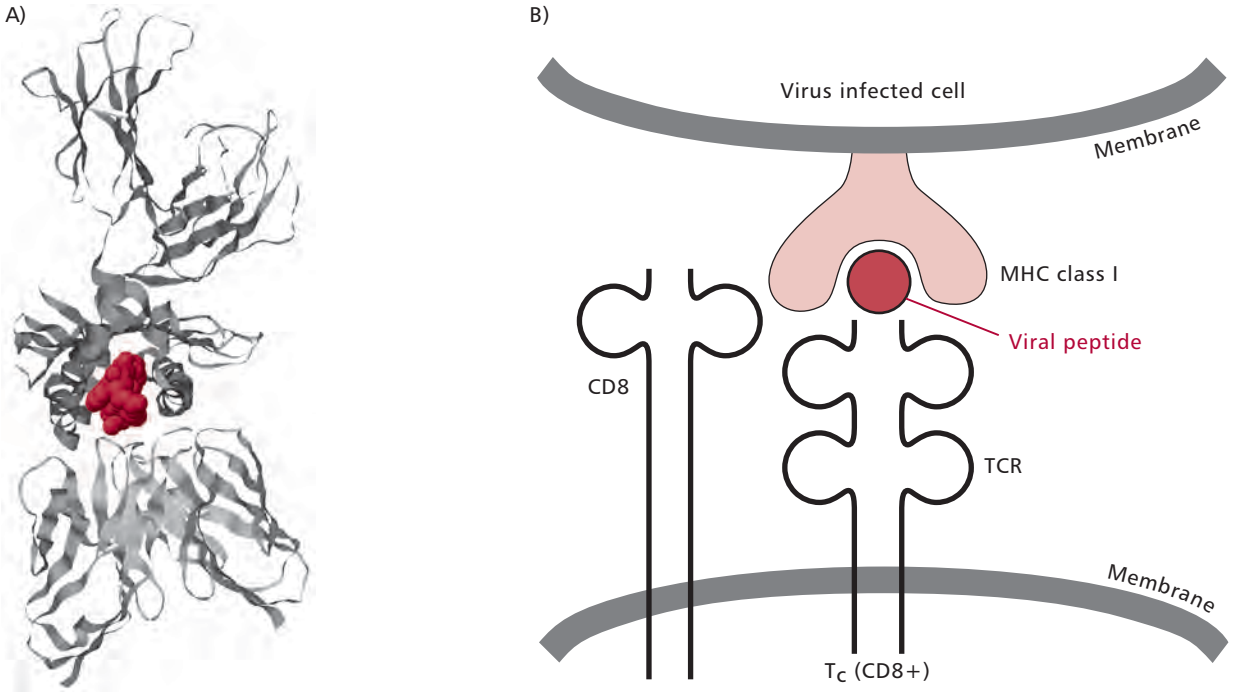


Figure 4.24 (A) Molecular model showing the recognition of a MHC Class I-peptide complex by a T_c cell receptor. PDB file 1NAM. (B) A schematic showing the interactions between a virus-infected cell and a T_c, as described in the text. TCR, T cell receptor.

Activation of T_H cells

When stimulated by an immunogen, T_H cells secrete cytokines required for specific and nonspecific immune responses. Thus they are required for an effective immune response to the whole range of infectious agents, from viruses to multicellular parasites. In order to activate a T_H cell, an immunogen has to be taken up by an **antigen presenting cell** (**APC**). Here, its proteins are hydrolyzed within endocytic vesicles and the resulting peptides of 12–19 amino acid residues are attached to MHC encoded Class II proteins and transported

to the cell membrane. The MHC Class II protein and foreign peptide complex is recognized and bound by a TCR (*Figure 4.25*). The coreceptor protein, CD4, also binds to the MHC protein. Several different types of cells can act as APCs, including monocytes, macrophages, dendritic cells in the lymphoid tissues and Langerhans cells of skin. However, under certain conditions, other cells, including epithelial cells, can be induced to express MHC Class II proteins and it has been proposed that such cells may initiate autoimmune reactions (*Chapter 5*).

When a T_H cell is stimulated by the MHC class II protein–peptide complex on the surface of an APC, it is stimulated to proliferate and differentiate into cells that actively secrete cytokines. The cytokines include growth and differentiation factors that stimulate B and T cells, hemopoietic factors, as well as factors that stimulate mast cells, macrophages and eosinophils.

There are two subsets of T_H cells, called T_H1 and T_H2. They differ in the profile of cytokines they secrete as shown in *Table 4.5*. Cytokines produced by T_H1 favor the development of cell-mediated immunity, which is used to destroy intracellular parasites such as viruses. Those produced by T_H2 cells stimulate humoral immunity to extracellular parasites, including most bacteria and helminth worms and flukes. In addition, cytokines produced by T_H1 inhibit T_H2 cells and *vice versa*.

Margin Note 4.5 Dendritic cells

Dendritic cells are cells with characteristic membrane processes that resemble the dendrites of nerve cells. The processes give the cells a large surface area for presenting antigens to T_H cells. Dendritic cells are found in the lymphoid tissues and are highly efficient at antigen presentation. Langerhans cells in skin resemble dendritic cells in having multiple processes. They are involved in taking up antigens that have entered the skin before migrating to lymph nodes where they present the antigen to T lymphocytes.

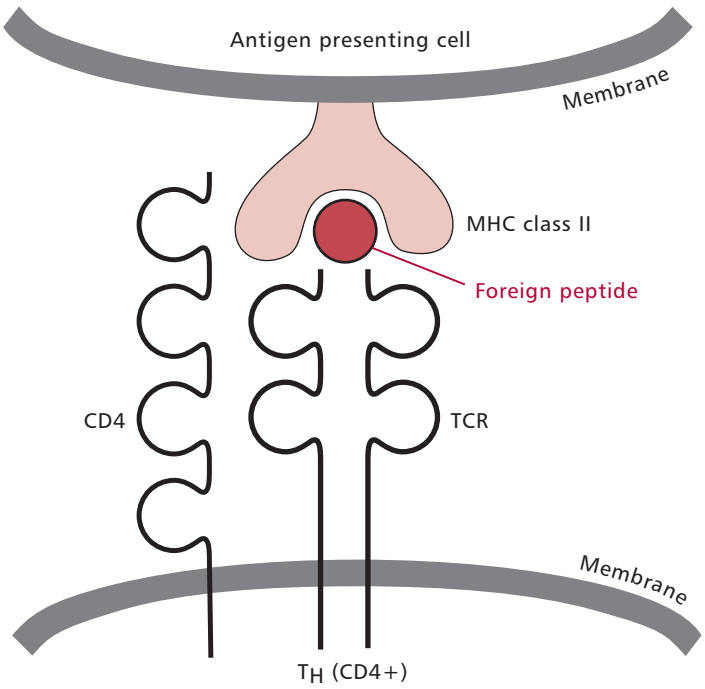


Figure 4.25 Schematic showing the activation of a T_H cell by an antigen presenting cell as described in text. TCR, T cell receptor.

Cytokine	Roles *in vivo*	T_H1 or T_H2 cytokine
IL-2	stimulates growth of T lymphocytes; increases activity of NK cells	T_H1
IL-3	stimulates hemopoiesis	T_H1 and T_H2
IL-4	promotes growth and differentiation of B lymphocytes; promotes growth of T_H2 cells; growth factor for mast cells	T_H2
IL-5	promotes growth and differentiation of B lymphocytes and eosinophils	T_H2
IL-6	promotes acute phase response; stimulates differentiation of B lymphocytes	T_H2
IL-9	activates mast cells	T_H2
IL-10	inhibits production of T_H1 cells	T_H2
IL-13	growth and differentiation of B lymphocytes	T_H2
IL-14	B cell growth factor	
Interferon γ	activates macrophages and NK cells; inhibits T_H2 cells	T_H1
TNF-β	promotes acute phase response; cytotoxin	T_H1
Granulocyte-Monocyte Colony Stimulating Factor (GM-CSF)	stimulates hemopoiesis	T_H1 and T_H2

Table 4.5 A range of T_H cytokines

Margin Note 4.6 Superantigens

Superantigens such as Toxic Shock Syndrome Toxin cause massive stimulation of T_H cells by linking the T cell receptor to the MHC Class II protein outside the peptide binding groove (*Figure 4.26*). Since this does not depend on the TCR being specific to the antigen, many more T cells are stimulated than usual. The high level of cytokine release leads to shock with severe clinical consequences (*Chapter 3*).

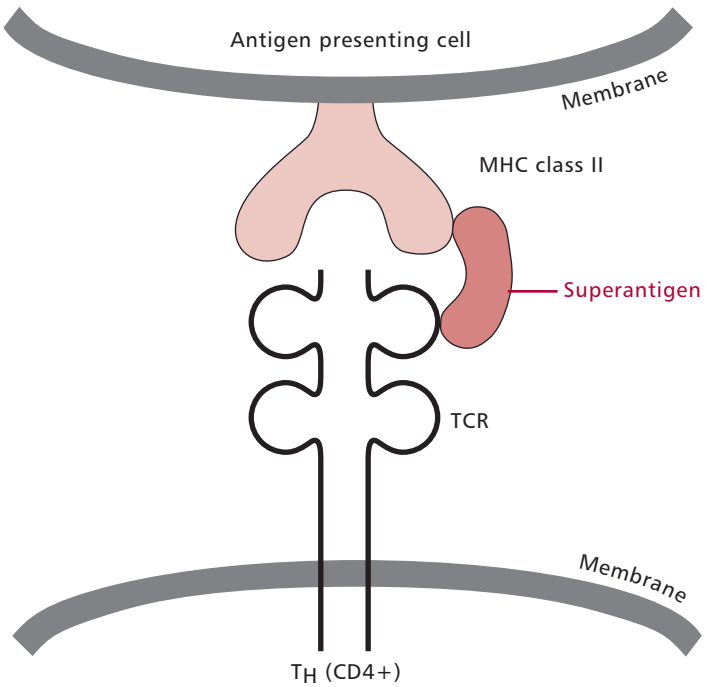


Figure 4.26 A schematic showing the linking of an MHC Class II molecule to a TCR by a superantigen as described in text.

CASE STUDY 4.1

Maria is a 28-year-old biology teacher who suspected she might be pregnant as it was now eight weeks since the start of her last period. Her pregnancy was confirmed when she carried out a home pregnancy test. Maria and her partner were delighted and began to make plans for the future. However, Maria became concerned when, during the eighth week of her pregnancy, several children in one of her classes developed German measles (rubella). Maria knew that, if she developed rubella, there was a high risk that the baby would be harmed. Unfortunately, she could not remember if and when she had ever been immunized against rubella. She consulted her family doctor who took a blood sample and had it tested for antibodies. The test revealed that Maria's blood was positive for IgM antibodies specific for rubella but no IgG antibodies specific for rubella were detected.

Questions

(a) What are the consequences of this result for the unborn baby?

(b) Why would the consequences be different if IgG antibodies specific for rubella had been detected?

(c) What counseling and/or advice would you recommend to Maria?

CASE STUDY 4.2

Alfred is a 70-year-old man who is suspected of having a myeloma, that is a plasma cell tumor. Suggest tests which could be carried out to confirm the diagnosis. Assuming the plasma cell was producing IgG, suggest an assay which could be used to measure the level of IgG in his blood.

4.7 SUMMARY

The basis of the actions of the immune system is its ability to distinguish self from nonself. It defends the body in a variety of nonspecific and specific ways. Nonspecific defenses include structural barriers and complement. Specific defenses are the development of immune responses against infectious agents. An effective immune response results from the complex interaction of nonspecific and specific cells. The nonspecific cells include the monocytes, large granular lymphocytes and polymorphonuclear leukocytes found in the blood. The specific cells are the small lymphocytes found in the blood and lymphoid tissues. Nonspecific responses include inflammation and the acute phase response, while specific responses include the production of antibodies, known as humoral immunity, and the production of cytotoxic cells, in the process known as cell-mediated immunity. Humoral immunity is effective at dealing with extracellular bacteria and multicellular parasites, while cell-mediated immunity is effective at killing cells infected with a virus. Small lymphocytes are highly specific since they bear cell surface receptors for epitopes found on foreign proteins. Small lymphocytes belong to one of two major subsets, the B lymphocytes responsible for humoral immunity and the T lymphocytes, some of which, the T_C cells, can develop into cytotoxic cells, while others, the T_H cells, regulate immune responses through the secretion of cytokines.

Antibodies are glycoproteins which are highly specific for epitopes. This specificity has enabled them to be used in the detection and quantitation of antigens, in highly sensitive techniques such as ELISA.

QUESTIONS

1. Which of the following is **NOT** part of the nonspecific defense against invading microorganisms?
   a) skin;
   b) mucus;
   c) antibodies;
   d) lysozyme;
   e) lactic acid.

2. Which of the following is **NOT** found in the blood?
   a) macrophage;
   b) monocyte;
   c) neutrophil;
   d) small lymphocyte;
   e) large granular lymphocyte.

3. Which of the following statements best describes complement?
   a) a defense protein found in sweat;
   b) a group of proteins which results in the lysis of bacteria;
   c) a cytokine produced by macrophages;
   d) an antibody which lyses bacteria;
   e) a protein found in the cell wall of bacteria.

4. Pair up the cells in column one with the appropriate cell surface molecule in column two.

Column 1	Column 2
a) All mature T lymphocytes	1) CD4
b) All B lymphocytes	2) CD8
c) Macrophages	3) CD3
d) Helper T lymphocytes	4) Surface immunoglobulin
e) Cytotoxic T lymphocytes	5) Receptors for complement

5. Which immunoglobulin class in each case best fits the following description?
   a) the only antibody that crosses the placenta;
   b) the most abundant antibody in the blood;
   c) also known as the secretory antibody;
   d) produced in response to parasitic worms;
   e) the antibody with the largest M_r.

6. Why is it unnecessary for erythrocytes to have MHC encoded Class I proteins?

FURTHER READING

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