

IMMUNOLOGY



1. Principles of immunology 3
2. The functioning immune system 31

Principles of immunology

1



Objectives

You should be able to:

- Have a general understanding of the immune system and its interactions
- Know the components of the innate and adaptive immune systems
- Understand how phagocytes kill pathogens
- Outline the complement cascade
- Know the functions of the differing classes of immunoglobulins
- Draw the structure and understand the functions of immunoglobulins and T cell receptors
- Understand the structure and function of MHC
- Understand the principle of genetic recombination, and how this leads to receptor diversity
- Understand the differences in the types of T helper cells and the immune responses they evoke.

AN OVERVIEW OF IMMUNOLOGY

Learning immunology is challenging, and one of those subjects that begins to make sense after the very last lecture in the module. This is because the information received in the last lecture helps you understand the first and subsequent lectures. To help with this phenomenon I start with an overview, with the purpose of providing an understanding of immunology as a whole and building a framework on which information learned later can be stored.

The function of the immune system is to protect the body from overwhelming infection. The invaders (pathogens) range from large parasitic worms living in body cavities to small viruses that can only survive inside their host cells, with bacteria, fungi and protozoans in between these two extremes of size.

These pathogens and their hosts have evolved side by side, with pathogens' increasing sophistication resulting in greater evolutionary pressure for more complex immune responses.

The first way the immune system protects against pathogens is to deny them entry through various physical barriers. These are the skin and mucous membranes that line the respiratory, gastrointestinal and reproductive tracts.

Once a pathogen has penetrated into the body, it is greeted by the human immune system. This system is divided into two forces, both of which get to work straight away: one responds quickly in a non-specific manner and the other occurs slowly and is specific to infecting organisms. These are the innate and adaptive immune systems, respectively.

The innate immune system is composed of both cellular and chemical components, the most important cellular component being the phagocyte. Phagocytes are cells that engulf foreign cells and debris (phage coming from the Greek 'to eat').

There are two types of phagocyte: the macrophage and the neutrophil. The macrophage is long lived and stationed within tissues, patrolling for the presence of trespassers. Upon contact with a pathogen (e.g. a bacterium), the macrophage engulfs it, a process known as phagocytosis. The bacterium is contained inside the macrophage, within a 'phagosome' which then fuses with a lysosome that contains enzymes and chemicals that destroy the bacterium.

Macrophages secrete chemicals called cytokines, the functions of which include attracting other cells (including short-lived neutrophils), increasing the permeability of the vascular endothelium and even increasing the production of neutrophils in the bone marrow.

Neutrophils act as reinforcements for the sentinel macrophages, following the trail of cytokines to the site of infection, a process called chemotaxis. Neutrophils are professional phagocytes extremely effective at killing pathogens; however, they are short lived (a failsafe to avoid excessive destruction of normal tissue) and, if the infection persists, continued secretion of cytokines will result in further mobilization of neutrophils.

Neutrophils can be recognized histologically as they have multilobed nuclei and cytoplasmic granules.



The chemical section of the innate immune system is complement. Complement comprises approximately 20 proteins that are activated through various pathways and can destroy pathogens directly through the formation of the membrane attack complex (MAC) or prepare (opsonize) them for destruction by other parts of the immune system.

The combination of phagocytes and complement systems is sufficient for dealing with most bacteria and fungi. However, certain pathogens have evolved to hide inside a host's cells where phagocytes and complement cannot reach them. Another problem with this early immune system is that a host can be infected with the same pathogen over and over again and the response has to start afresh each time.

The adaptive immune system, which evolved at about the same time as the vertebrates, has developed to combat both these flaws of the innate system. This development has led to the adaptive

immune system having many more receptors for pathogenic molecules, since such receptors are formed through genetic recombination. These receptors are expressed on specialized lymphocytes called T and B cells.

T cells are able to recognize intracellular infections. This is possible as our cells evolved a method whereby the complete range of proteins within the cell is expressed as short peptides on its surface. The molecules that bind the small peptides to be expressed on the cell surface are called the major histocompatibility complex (MHC), also known as human leucocyte antigen (HLA).

B cells can release their receptors into blood and bodily secretions. These free receptors are called antibodies or immunoglobulin (Ig). The function of antibody is to flag up foreign antigens for destruction by other parts of the immune system.

Essential differences between the innate and adaptive immune systems are outlined in Fig. 1.1.

Immunity in more detail

Both the innate and adaptive immune systems comprise cellular and humoral components (Fig. 1.2).

Cytokines

Cytokines are small, secreted proteins. They act locally, via specific cell-surface receptors, as part of both the innate and adaptive immune response. Cytokines have many effects, but in general they stimulate the immune response through:

- Growth, activation and survival of various cells
- Increased production of surface molecules such as MHC.

Some important cytokines and their main actions are shown in Fig. 1.3.

Fig. 1.1 Essential differences between the innate and adaptive immune systems

Innate immune system	Adaptive immune system
Provides a rapid response It is not antigen specific The response does not improve with repeated exposure	The response takes time to develop, because: <ul style="list-style-type: none"> • It is specific for each different antigen • Initial exposure to an antigen leaves memory cells; subsequent infections with the same antigen are therefore dealt with more quickly

Fig. 1.2 Components of the innate and adaptive immune systems

	Innate system	Adaptive system
Cellular components	Monocytes/macrophages Neutrophils Eosinophils Basophils Mast cells Natural killer cells	B cells/plasma cells T cells
Secreted components	Complement Cytokines Lysozyme Acute phase proteins Interferons	Antibody Cytokines

Fig. 1.3 Important cytokines and their actions

Cytokine	Main sources	Main actions
IL-1	Macrophages	Fever T-cell and macrophage activation
IL-2	T helper 1 cells	Growth of T cells Stimulates growth of B cells and NK cells
IL-3	T helper cells	Growth factor for progenitor haemopoietic cells
IL-4	T helper 2 cells	Activation and growth of B cells IgG1, IgE and MHC class II induction of B cells Growth and survival of T cells
IL-6	Macrophages	Lymphocyte activation Increased antibody production Fever, induces acute phase proteins
IL-8	Macrophages	Chemotactic factor for neutrophils Activates neutrophils
IL-10	T helper 2 cells Macrophages	Inhibits immune function
IL-12	Macrophages	Activates NK cells Causes CD4 T cells to differentiate into T helper 1 cells
IFN- γ	T helper 1 cells NK cells	Activation of macrophages and NK cells Produces antiviral state in neighbouring cells Increases expression of MHC class I and II molecules Inhibits T helper 2 cells
TNF- α	T helper cells Macrophages	Activates macrophages and induces nitric oxide production Proinflammatory Fever and shock
TNF- β	T helper 1 cells	Activates macrophages and neutrophils Induces nitric oxide production Kills T cells, fibroblasts and tumour cells

IL, interleukin; IFN- γ , interferon- γ ; MHC, major histocompatibility complex; NK, natural killer; TNF, tumour necrosis factor.

THE INNATE IMMUNE SYSTEM

Innate defences can be classified into three main groups:

1. Barriers to infection
2. Cells
3. Serum proteins and the complement system.

Barriers to infection

Physical and mechanical

Skin and mucosal membranes act as physical barriers to the entry of pathogens. Tight junctions between cells prevent the majority of pathogens from entering the body. The flushing actions of tears, saliva and urine protect epithelial surfaces from colonization. High oxygen tension in the lungs, and body temperature, can also inhibit microbial growth.

In the respiratory tract, mucus is secreted to trap microorganisms. They are then mechanically expelled by:

- Beating cilia (mucociliary escalator)
- Coughing
- Sneezing.

Chemical

The growth of microorganisms is inhibited at acidic pH (e.g. in the stomach and vagina). Lactic acid and fatty acids in sebum (produced by sebaceous glands) maintain the skin pH between 3 and 5. Enzymes such as lysozyme (found in saliva, sweat and tears) and pepsin (present in the gut) destroy microorganisms.

Biological (normal flora)

A person's normal flora is formed when non-pathogenic bacteria colonize epithelial surfaces. Normal flora protects the host by:

- Competing with pathogenic bacteria for nutrients and attachment sites
- Production of antibacterial substances.

The use of antibiotics disrupts the normal flora and pathogenic bacteria are then more likely to cause disease.

Cells of innate immunity

The cells of the innate immune system consist of:

- Phagocytes
- Degranulating cells
- Natural killer cells.

Phagocytes

Phagocytes (macrophages and neutrophils) engulf and then destroy pathogens. Macrophages are long-lived sentinel cells stationed at likely sites of infection; upon infection they release cytokines that recruit the shorter-lived but more actively phagocytic neutrophils.

Neutrophils (for structure, see p. ••; for production, see p. ••)

Neutrophils comprise 50–70% of circulating white cells. Neutrophils arrive quickly at the site of inflammation and in the act of killing pathogens they die; in fact, dead neutrophils are the major constituent of pus. In response to tissue damage, chemicals released by macrophages and complement proteins, neutrophils migrate from the bloodstream to the site of the insult (see Chapter 2). They are phagocytes and have an important role in engulfing and killing extracellular pathogens. The process of phagocytosis and the mechanisms of killing are shown on page ••.



Neutropenic individuals are at an increased risk of serious bacterial infections. These patients should be treated early and aggressively to reduce the chance of sepsis.

Mononuclear phagocyte system (for structure and production, see p. ••)

Mononuclear phagocytes comprise the other major group of phagocytic cells. Monocytes account for 5–10% of the white cell count and circulate in the blood for approximately 8 hours before migrating into the tissues, where they differentiate into macrophages; these macrophages can live for decades. Some macrophages become adapted for specific functions in particular tissues, e.g. Kupffer cells in the liver and glial cells in the brain. Monocytes also differentiate into osteoclasts and microglial cells.

In comparison to monocytes, macrophages:

- Are larger and longer-lived
- Have greater phagocytic ability

- Have a larger repertoire of lytic enzymes and secretory products.

Macrophages phagocytose and destroy their targets using similar mechanisms to neutrophils. The rate of phagocytosis can be greatly increased by opsonins such as IgG and C3b (neutrophils and macrophages have receptors for these molecules, which may be bound to the antigenic surface). Intracellular pathogens, e.g. *Mycobacterium*, can prove difficult for macrophages to kill. They are either resistant to destruction inside the phagosome or can enter the macrophage cytoplasm. For the immune system to act against these pathogens, T cell help is required.

In addition to phagocytosis, macrophages can secrete a number of compounds into the extracellular space, including cytokines (TNF and IL-1), complement components and hydrolytic enzymes. Macrophages are also able to process and present antigen in association with class II MHC molecules.

Macrophages express a wide array of surface molecules including:

- Fc- γ RI–III (receptors for the Fc portion of IgG, types I–III) and complement receptors
- Receptors for bacterial constituents
- Cytokine receptors, e.g. TNF- α and interferon- γ (IFN- γ)
- MHC and B7 molecules (to activate the adaptive immune response).

Macrophages can be activated by:

- Cytokines such as IFN- γ
- Contact with complement or products of blood coagulation
- Direct contact with the target.

Following activation, macrophages become more efficient phagocytes and have increased secretory and microbicidal activity. They also stimulate the adaptive immune system by expressing higher levels of MHC class II molecules and secreting cytokines.

In comparison to neutrophils, macrophages:

- Are longer-lived (they do not die after dealing with pathogens)
- Are larger (diameter 25–50 μ m), enabling phagocytosis of larger targets
- Move and phagocytose more slowly
- Exhibit a less pronounced respiratory burst
- Retain Golgi apparatus and rough endoplasmic reticulum and can therefore synthesize new proteins, including lysosomal enzymes and secretory products

- Secrete a variety of substances
- Can act as antigen-presenting cells (APCs).

Killing by phagocytes

The process of phagocytosis allows cells to engulf matter that needs to be destroyed. The cell can then digest the material in a controlled fashion before releasing the contents. The process of phagocytosis is shown in Fig. 1.4.

Microbial degradation within the phagolysosome occurs along two pathways; one requires oxygen, the other is oxygen independent.

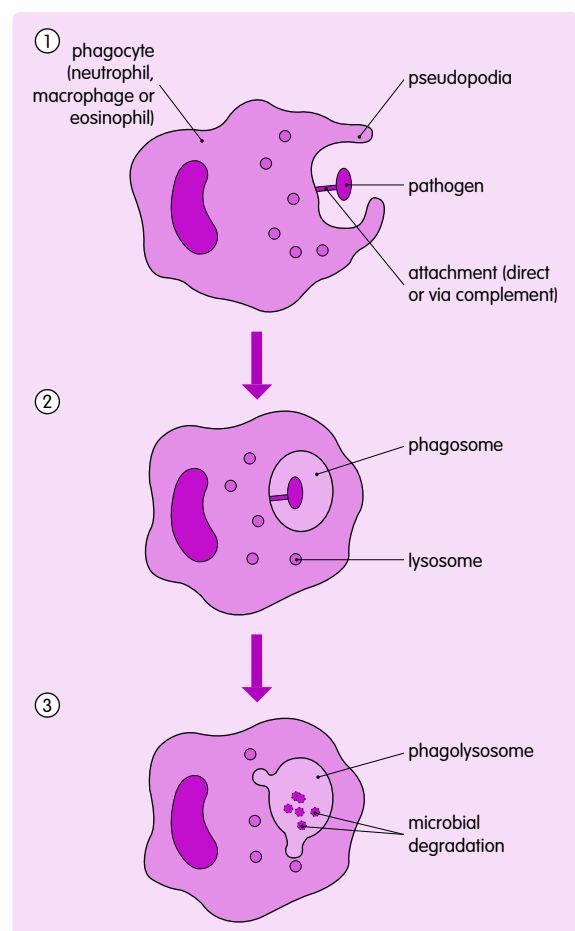
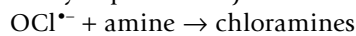
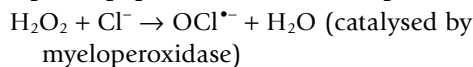
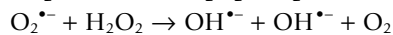
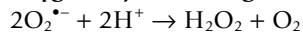


Fig. 1.4 Phagocytosis. Phagocytes sense an organism and bind it via non-specific receptors or via complement or antibody. Pseudopodia extend from the surface of the cell to surround the pathogen (1). The pseudopodia fuse around the organism, producing a vesicle known as a phagosome (2). Lysosomes fuse with the phagosome to form phagolysosomes (3). Chemicals within the lysosome, and other granules that fuse with the phagolysosome, lead to degradation of the organism. The microbial products are then released.

Oxygen-independent degradation Neutrophil granules contain several antimicrobial agents including:

- Lysozyme (splits peptidoglycan)
- Lactoferrin and reactive nitrogen intermediates (which complex with, and deprive pathogens of, iron)
- Proteolytic enzymes (degrade dead microbes)
- Defensins, cathepsin G and cationic proteins (damage microbial membranes).

Oxygen-dependent degradation



Hypochlorous acid (HOCl) and chloramines are longer-lived than the other oxidizing agents and are probably the most important target killing compounds in vivo. If a target cannot be easily phagocytosed, there may be extracellular release of granule contents, causing tissue damage.

Natural killer (NK) cells

NK cells do not require T cell help to kill pathogens, although they are more effective when T helper cells secrete IFN- γ . NK cells utilize cell-surface receptors to identify virally modified or cancerous cells. One set

of receptors activates NK cells, initiating killing; others inhibit the cells:

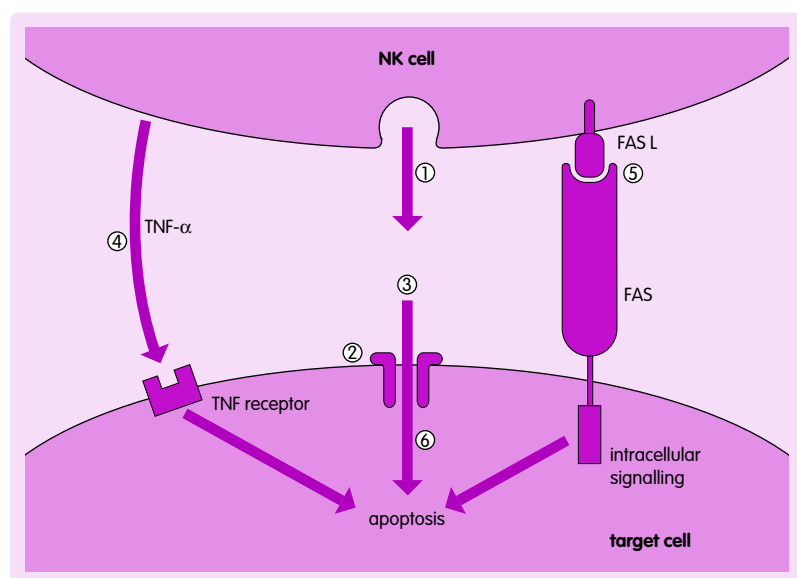
- Activating receptors include calcium-binding C-lectins, which recognize certain cell-surface carbohydrates. Because these carbohydrates are present on the surface of normal host cells, a system of inhibitory receptors acts to prevent killing
- Killer inhibitor receptors (KIRs), members of the immunoglobulin gene superfamily, are specific for class I MHC molecules. Human NK cells also express an inhibitory receptor (a heterodimer CD94: NKG2) that detects non-classical class I molecules.

NK cells can also destroy antibody-coated target cells irrespective of the presence of MHC molecules, a process known as antibody-dependent cell-mediated cytotoxicity. This occurs because killing is initiated by cross-linking of receptors for the Fc portion of IgG1 and IgG3.

NK cells are not clonally restricted, have no memory and are not very specific in their action. They induce apoptosis in target cells (Fig. 1.5) by:

- Ligation of FAS or TNF receptors on the target cells (NK cells produce TNF and exhibit FASL). This initiates a sequence of caspase recruitment and activation, resulting in apoptosis
- Degranulation by NK cells, which releases perforins and granzymes. Perforin molecules

Fig. 1.5 Mechanism of killing by natural killer (NK) cells (1). Activation of NK cells in the absence of an inhibitory signal results in degranulation (2). Perforins form a pore in the target cell, allowing entry of granzymes (3). TNF produced by NK cells acts on the target's cell receptors (4). FASL interacts with target cell FAS (5). Intracellular signalling from FAS, TNF receptors and granzymes results in apoptosis (6).



insert into and polymerize within the target cell membrane. This forms a pore through which granzymes can pass. Granzyme B then initiates apoptosis from within the target cell cytoplasm.

Mast cells and basophils (for structure, see p. ••)

Mast cells and basophils have similar functions but are found in different locations; basophils comprise <1% of circulating white cells, whereas mast cells are resident in the tissues. This has led to the theory that mast cells are a population of differentiated basophils.

High concentrations of mast cells are found close to blood vessels in connective tissue, skin and mucosal membranes. The two types of mast cell—mucosal and connective tissue—differ in their tissue distribution, protease content and secretory profiles.

Mast cells function by discharging their granule contents. Degranulation is triggered by cross-linking of high-affinity receptors for the Fc portion of IgE (Fig. 1.6). Cross-linkage results in an influx of calcium ions into the cell, which induces release of pharmacologically active mediators from granules (Fig. 1.7). Mast cell activation releases leukotrienes, which attract eosinophils to the site of

worm infection. This plays an important role in the development of an episode of a type I hypersensitivity, with the mast cells and basophils providing the early phase response and the eosinophils mediating the late phase response. This is important in allergic responses (type I hypersensitivity reactions, see p. ••).

Eosinophils (for structure, see p. ••)

Eosinophils comprise 1–3% of circulating white cells and are found principally in tissues. They are derived from the colony-forming unit for granulocytes, erythrocytes, monocytes and megakaryocytes (CFU-GEMM) haematopoietic precursor and their maturation is similar to that of the neutrophil (see p. ••). They are important in the defence against parasites and cause damage by extracellular degranulation. Their granules contain major basic protein, cationic protein, peroxidase and perforin-like molecules. The peroxidase generates hypochlorous acid, major basic protein damages the parasite's outer surface (as well as host tissues) and cationic protein acts as a neurotoxin, damaging the parasite's nervous tissue.

Soluble proteins

The soluble proteins that contribute to innate immunity (Fig. 1.8) can be divided into antimicrobial serum agents and proteins produced by cells of the immune system.

Acute phase proteins

The acute phase response is a systemic reaction to infection or tissue injury, where macrophages release cytokines IL-1, IL-6 and TNF; these cytokines reach the liver through the circulation. The liver responds by increasing its production of certain plasma proteins. These so-named acute phase proteins (APPs) are:

- C-reactive protein
- Serum amyloid A
- Complement components
- Fibrinogen
- α_1 -Antitrypsin
- Caeruloplasmin
- Haptoglobin.

The change in plasma concentration is accompanied by fever, leucocytosis, thrombocytosis, catabolism of muscle proteins and fat deposits. Synthesis of

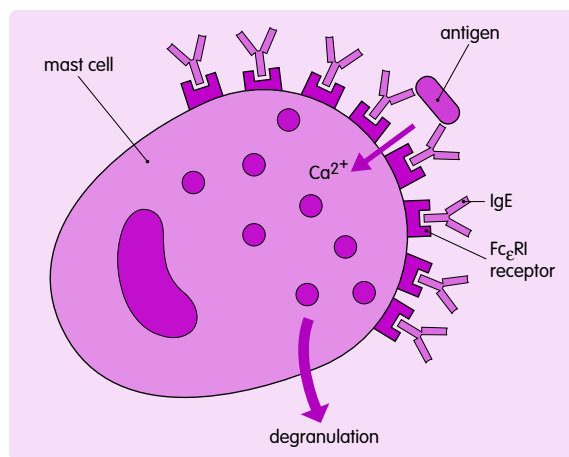


Fig. 1.6 Activation of mast cells by immunoglobulin E (IgE). IgE, produced by plasma cells, binds via its Fc domain to receptors on the mast cell surface. Cross-linking of these receptors by an antigen causes an influx of calcium ions (Ca^{2+}) into the cell. Calcium ions cause a rapid degranulation of inflammatory mediators from the mast cell.

Fig. 1.7 Mast cell mediators and their actions

Mediator		Action
Primary	Histamine	Increased capillary permeability, vasodilatation, smooth muscle contraction
	Serotonin	Increased capillary permeability, vasodilatation, smooth muscle contraction, platelet aggregation
	Heparin	Anticoagulation (see p. ●●), modulates tryptase
	Proteases	Tryptase: Activates complement (C3)
		Chymase: Increased mucus secretion
	Eosinophil chemotactic factor	Chemotactic (cells move towards site of production) for eosinophils
	Neutrophil chemotactic factor	Chemotactic for neutrophils
	Acid hydrolases	Degradation of extracellular matrix
Secondary	Platelet-activating factor	Platelet aggregation and activation, increased capillary permeability, vasodilatation, chemotactic for leucocytes, neutrophil activation
	Leukotrienes (C ₄ , D ₄ , B ₄)	Vasodilatation, smooth muscle contraction, mucus secretion, chemotactic for neutrophils
	Prostaglandins (D ₂)	Vasodilatation, smooth muscle contraction, chemotactic for neutrophils, potentiation of other mediators
	Bradykinin	Increased capillary permeability, vasodilatation, smooth muscle contraction, stimulation of pain nerve endings
	Cytokines	Various

Mast cells contain many preformed (primary) mediators that are stored in granules. They can also synthesize new (secondary) mediators when they are activated.

APPs is enhanced by cytokines secreted by macrophages and endothelial cells. The two main APPs are C-reactive protein (CRP) and serum amyloid A (SAA).

The extent of the rise in the plasma concentration of different APPs varies:

- Increased 50% above normal levels: caeruloplasmin
- Increased several fold above normal levels: α_1 -glycoprotein, α_1 -proteinase inhibitor, haptoglobin, fibrinogen
- 100–1000-fold increase: CRP, SAA.

The concentration of other plasma proteins, most notably albumin and transferrin, falls.

C-reactive protein

Levels of CRP rise within hours of tissue injury or infection. The actions of CRP are outlined in Fig. 1.8. CRP elevation can be slight (e.g. cerebrovascular accident), moderate (e.g. myocardial infarction) or marked (e.g. bacterial infections).

TNF- α , IL-1 and IL-6 released by macrophages stimulate the liver to produce the acute phase proteins.



Serum amyloid A

SAA levels rise within hours of tissue injury or infection. SAA may function as an opsonin. Persistent elevation of SAA can lead to its deposition in tissues in amyloidosis (see p. ●●).

Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR) is an index of the acute phase response. It is especially representative of the concentration of fibrinogen and α -globulins. Elevated fibrinogen levels cause red cells to form stacks (rouleaux), which sediment more rapidly than individual blood cells.

Fig. 1.8 The soluble proteins of innate immunity

	Protein	Notes
Secreted agents	Lysozyme	Bactericidal enzyme in mucus, saliva, tears, sweat and breast milk Cleaves peptidoglycan in the cell wall
Innate antimicrobial serum agents	Lactoferrin	Iron-binding protein that competes with microorganisms for iron, an essential metabolite
	Complement	Group of ~20 proenzymes Activation leads to an enzyme cascade, the products of which enhance phagocytosis and mediate cell lysis Alternative pathway can be activated by non-specific mechanism
	Mannan-binding lectin	Activates the complement system
	C-reactive protein	Acute phase protein, produced by the liver Serum concentration rises >100-fold in tissue-damaging infections Binds C-polysaccharide cell wall component of bacteria and fungi Activates complement via classical pathway Opsonizes for phagocytosis
Proteins produced by cells of the innate system	Interferon- α Interferon- β	Produced by virally infected cells Induces a state of viral resistance in neighbouring cells by: <ul style="list-style-type: none"> • Inducing genes that will destroy viral DNA • Inducing MHC class I expression
	Interferon- γ	Mainly produced by activated NK cells Activates NK cells and macrophages

MHC, major histocompatibility complex; NK, natural killer.

In chronic inflammation, high CRP and ESR persist. The resulting catabolism of muscle and fat may lead to severe weight loss.



The acute phase response

The acute phase response provides us with chemical markers of inflammation that can be measured. In a child presenting with abdominal pain a CRP can aid the clinician in their diagnosis. A normal CRP can allow more conservative management whereas a raised CRP would indicate an inflammatory response and necessitate urgent treatment, such as surgery in differentiating the abdominal pain in constipation from that of appendicitis.

ESR takes more time than CRP to become elevated, and is a useful marker measured in chronic inflammatory diseases.

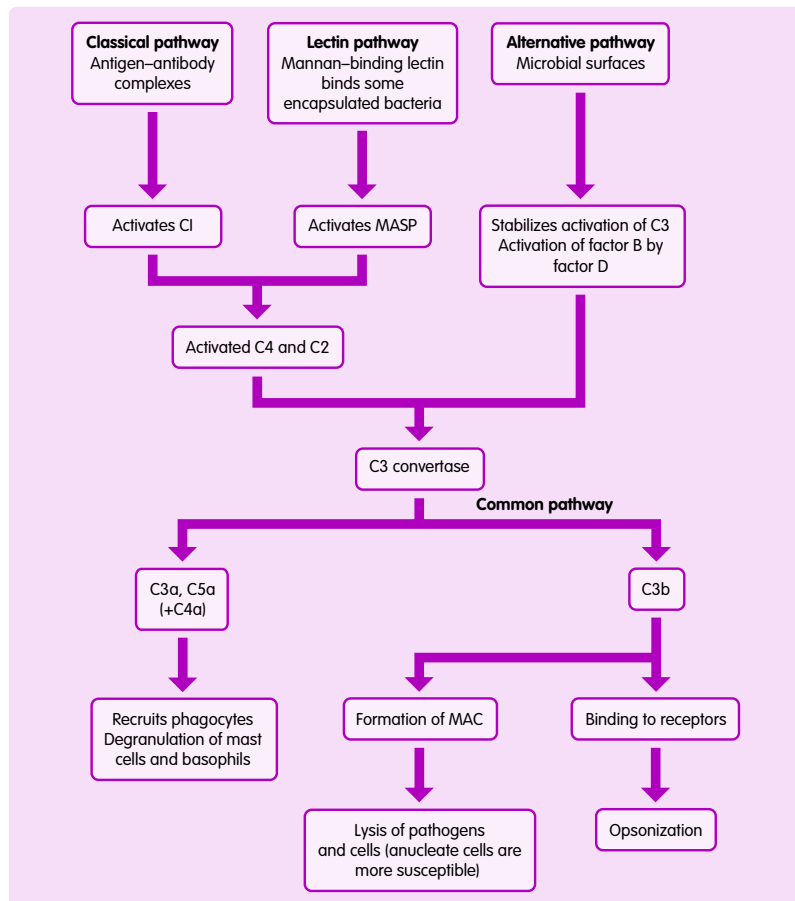
The complement system

The complement system—so-called because scientists compared its *complementary* actions to the function of antibody—is, in fact, much older in evolutionary terms than antibody and is equally important.

Complement is a collection of over 20 serum proteins that are always at high levels in the blood of the healthy individual. The complement system may seem complex with all the alphanumerical naming and active and inactive components. Thinking about it simply, it is a system that has three methods of activating a common pathway, which in turn has three results or effectors. The reason for the large number of proteins is to allow amplification; many of the components of complement are proenzymes that, when cleaved, activate more complement.

The three pathways that activate the complement system are the classical, the alternative and the lectin. All pathways result in the activation of the complement component C3 to C3 convertase. An overview of the complement system is given in Fig. 1.9.

Fig. 1.9 Overview of the complement system. Cell lysis by complement is due to formation of the membrane attack complex (MAC). This is formed when C5b, C6, C7, C8 and C9 bind together to form a 10-nm pore in the cell surface. MASP, mannan-binding lectin associated serine protease.



The classical pathway

The classical pathway was discovered first and involves the activation of complement by the Fc portion of antibody. IgM is particularly good at activating complement as it is a pentamer (has five Fc portions):

- Fc activates C1
- C1 activates C2 and C4
- C2 and C4 activate C3 (C3 convertase).

The alternative pathway

C3 is an unstable molecule and without inhibition spontaneously breaks down to the very reactive C3b; C3b reacts to two common chemical functional groups, the amino and hydroxyl groups. C3b is therefore neutralized quickly by water. However, with many pathogens made up of proteins and carbohydrates that contain these functional groups, C3b attaches to the pathogen and is not broken down. C3b then reacts with more complement components to form C3bBb; this is C3 convertase.

The lectin pathway

Mannan-binding lectin (MBL), which is normally found in serum, binds to MBL-associated serine proteases (MASP). This complex bears structural homology to the C1 complex. When MBL binds to carbohydrate on the surface of bacteria, MASP is activated. MASP then acts on C4 and C2 to generate the C3 convertase of the classical pathway.

C3 convertase

With the production of C3 convertase, all three pathways converge. C3 convertase has enzymatic effects against C3 and enables the production of large quantities of C3b, thus producing a major amplification step in the complement pathway.

Effectors of complement

C5 is cleaved into C5a and C5b, and C5b triggers the activation of C6–C9. These form the membrane attack complex (MAC). The MAC attacks pathogens by inserting a hole in their cell membrane; the

Fig. 1.10 Functions of complement

Function	Notes
Cell lysis	Insertion of MAC causes lysis of Gram-negative bacteria Nucleated cells are more resistant to lysis because they endocytose MAC
Inflammation	C3a, C4a, C5a cause degranulation of mast cells and basophils C3a and C5a are chemotactic for neutrophils
Opsonization	Phagocytes have C3b receptors, which means that they are able to phagocytose antigen coated in C3b
Solubilization and clearance of immune complexes	Complement prevents immune complex precipitation and solubilizes complexes that have already been precipitated Complexes coated in C3b bind to CR1 on red blood cells The complexes are then removed in the spleen

MAC, *membrane attack complex*.

pathogen then dies via osmotic lysis. The MAC appears to be the only way the immune system has of killing one family of bacteria, the *Neisseria* (a family that includes meningococcus and gonococcus).

The cleaved fragments C3b and C5b are anaphylotoxins which are chemoattractant for other immune cells which follow the concentration gradient to the infection. Complement also opsonizes bacteria as macrophages have receptors for C3b.

These functions are summarized in Fig. 1.10.

Inhibitors of complement

As we have seen, complement can activate spontaneously through the alternative pathway. Complement is regulated by inhibitory molecules which are necessary to prevent complement-mediated damage of healthy cells. There are nine complement inhibitors which act at various levels throughout the pathway:

- Membrane cofactor protein, complement receptor type 1, C4b-binding protein and factor H: these prevent assembly of C3 convertase
- Decay accelerating factor: this accelerates decay of C3 convertase
- C1 inhibitor: inhibits C1
- Factor I and membrane cofactor protein: cleave C3b and C4b
- CD59 (protectin): prevents the formation of the membrane attack complex (MAC).

Hereditary angioedema

Deficiency in even one of these inhibitory components can result in significant disease. For example, deficiency in C1 inhibitor results in hereditary angioedema (HAE; see photograph), a condition where there is activation of the classical pathway with minimal stimulation. This is of particular significance if the stimulation is in the larynx as it can lead to uncontrolled swelling. This laryngeal oedema can obstruct the airway and without infusion of C1 inhibitor can prove fatal.



RECOGNITION MOLECULES

We have already come across the recognition molecules MBL and C1q in the complement system. These are found in solution in the serum and are classified as collectins, being composed of collagen-like and lectin portions. Lectins are any protein that binds sugar molecules, usually on the surface of bacteria, e.g. MBL binds to the sugar mannose.

Another group of non-specific receptors for pathogens are the Toll-like receptors, transmembrane receptors found on antigen-presenting cells. Each Toll-like receptor recognizes a different type of pathogenic molecule, e.g. TLR4 binds lipopolysaccharide present in bacterial membranes and also fungi. Activation stimulates the production of cytokines and co-stimulatory molecules which facilitate an adaptive immune response.

The immunoglobulin domain

B and T cell surface receptors are members of the immunoglobulin gene superfamily. Genes in this family code for proteins composed of motifs called immunoglobulin domains. All molecules in the immunoglobulin superfamily extend from the surface of cells. They are flexible and include specialist domains; the antigen receptor site on B cell receptors is an example.

Members of this gene family include:

- Immunoglobulin (B cell receptor)
- T cell receptor
- MHC molecules
- T cell accessory molecules such as CD4
- Certain adhesion molecules, e.g. ICAM-1, ICAM-2 and VCAM-1.

Each domain is approximately 110 amino acids in length. The polypeptide chain in each domain is folded into seven or eight antiparallel beta strands. The strands are arranged to form two opposing sheets, linked by a disulphide bond and hydrophobic interactions. This compact structure is called the immunoglobulin fold.

Structure of B and T cell surface antigen receptors

Structure of immunoglobulin

The B cell surface receptor is a membrane-bound immunoglobulin (mIg) molecule. mIg recognizes the conformational structure (shape) of antigenic

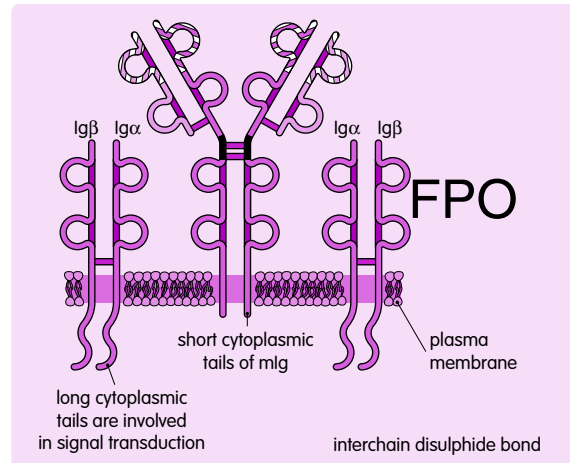


Fig. 1.11 Structure of the B cell surface receptor. Membrane-bound immunoglobulin is non-signalling. It associates with two Ig- α /Ig- β heterodimers (members of the immunoglobulin gene superfamily), which have long cytoplasmic domains capable of transducing a signal.

epitopes. Ig is composed of two light and two heavy chains. In the B cell receptor (Fig. 1.11), mIg associates with two Ig- α /Ig- β dimers (members of the immunoglobulin gene superfamily). Signal transduction through the mIg is thought to be mediated by the Ig- α /Ig- β heterodimers.

Ig is also secreted by plasma cells (see p. ●●). The extracellular portion of mIg is identical in structure to secretory Ig. mIg differs from secreted Ig (sIg) because it has transmembrane and cytoplasmic portions that anchor it to the membrane. Different Ig classes can be expressed on the same B cell and may indicate the stage of development of the B cell, e.g. a mature, but antigenically unchallenged, B cell expresses both mIgM and mIgD. The antigenic specificity of all of the mIg molecules expressed on any given B cell is the same.

Antigen recognition by T cells differs from antigen recognition by B cells:

- T cells recognize antigen only when it is associated with a molecule of the MHC
- T cells recognize peptide fragments of an antigen in association with MHC molecules; these fragments of antigen are processed by APCs before they are presented to the T cell.

The T cell surface antigen receptor consists of the T cell receptor (TCR) associated with CD3. The TCR is a heterodimer, comprising α - and β -chains, or γ - and δ -chains. Approximately 95% of T cells express

$\alpha\beta$ -receptors. The TCR is structurally similar to the immunoglobulin Fab region (see p. ••). Each chain comprises two immunoglobulin domains, one variable and one constant, linked by a disulphide bond. As in the variable domains of immunoglobulin, three variable regions on each chain combine to form the antigen-binding site.

CD3 is made up of three polypeptide dimers, consisting of four or five different peptide chains. The dimers are $\gamma\epsilon$, $\delta\epsilon$ and $\zeta\zeta$ (found in 90% of CD3 molecules) or $\zeta\eta$. The γ -, δ - and ϵ -chains are members of the Ig gene superfamily. The TCR recognizes and binds antigen, and CD3, functionally analogous to the Ig- α /Ig- β heterodimer in B cells, is involved in signal transduction (Fig. 1.12).

The major histocompatibility complex (MHC)

Major histocompatibility complex (MHC) is a generic term for a group of molecules produced by higher vertebrate species. The human leucocyte antigen (HLA) system is the human MHC.



The MHC genes

A complete set of MHC alleles inherited from one parent is referred to as a haplotype.



MHC genes exhibit a high degree of polymorphism, i.e. they exhibit considerable diversity (there are more than 100 identified alleles for human leucocyte antigen B (HLA-B)). This means that most individuals will be heterozygous at most MHC loci and that any two randomly selected individuals are very unlikely to have identical HLA alleles. Diversity of the MHC increases the chance that a person will be able to mount an adaptive response against a pathogen. The genetic loci are tightly linked, so that one set is inherited from each parent. The genes are divided into three regions, each region encoding one of the three classes of the MHC: class I, class II and class III (Fig. 1.13). The MHC alleles exhibit codominance, which means that both alleles are expressed.

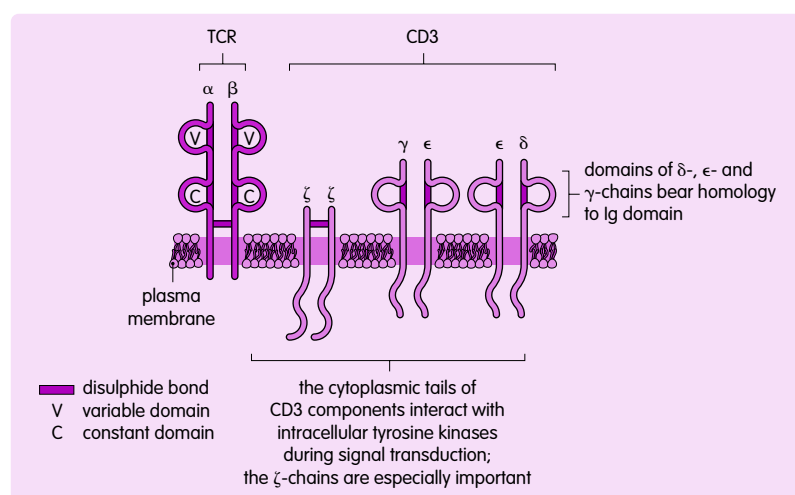


Fig. 1.12 Structure of the T cell surface antigen receptor. Negative charges on the transmembrane portion of CD3 components interact with positive charges on the T cell receptor (TCR). This maintains the complex. Antigen is detected by the TCR, but the signal is transduced by CD3.

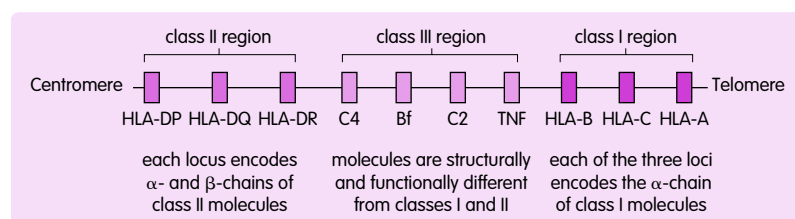


Fig. 1.13 Genetic organization of the human leucocyte antigen (HLA) complex. Only the classical genes are shown. The HLA complex is located in a 3–4 megabase sequence on the short arm of chromosome 6.

Structure and function of the MHC

Class I and class II MHC molecules are glycoproteins expressed on the cell surface and consist of cytoplasmic, transmembrane and extracellular portions (Fig. 1.14). Both class I and class II molecules exhibit broad specificity in their binding of peptide. The polymorphism of the MHC is largely concentrated in the peptide binding cleft. A summary of the dif-

ferences between class I and class II MHC molecules is shown in Fig. 1.15.

MHC restriction

T cells are only able to recognize antigen in the context of self-MHC molecules (self-MHC restriction). CD8⁺ T cells recognize antigen only in association with class I MHC molecules (class I MHC

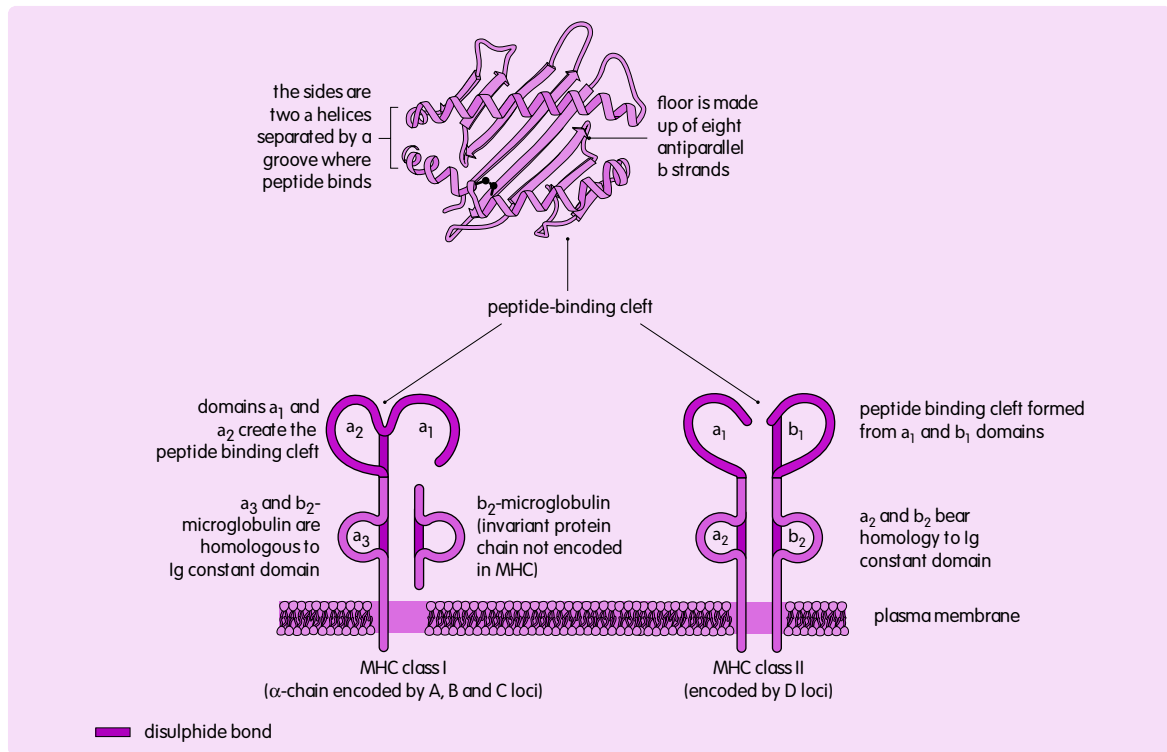


Fig. 1.14 Structure of class I and class II major histocompatibility molecules (MHC). The peptide binding cleft of a class I molecule is also shown as seen from above.

Fig. 1.15 Differences between class I and class II major histocompatibility molecules

	Class I	Class II
Size of bound peptide	8–9 amino acids	13–18 amino acids (binding cleft more open)
Peptide from	Cytosolic antigen	Intravesicular or extracellular antigen
Expressed by	All nucleated cells, especially T cells, B cells, macrophages, other antigen-presenting cells, neutrophils	B cells, macrophages, other antigen-presenting cells, epithelial cells of the thymus, activated T cells
Recognized by	CD8 ⁺ T cells	CD4 ⁺ T cells

restricted). CD4⁺ cells recognize antigen only in association with class II MHC molecules (class II MHC restricted).

Antigen processing and presentation

MHC molecules do not present whole antigen, the antigen being degraded into peptide fragments before binding can occur. There are different pathways of antigen processing for class I and class II MHC; these pathways are summarized in Fig. 1.16.

Professional APCs process and present antigen to CD4⁺ T cells in association with class II molecules. These cells express high levels of class II MHC molecules. Professional APCs include:

- Dendritic cells, including Langerhans' cells
- Macrophages
- B cells.

Structure and function of CD4 and CD8

CD4 and CD8 are 'accessory' molecules that play an important role in the T cell–antigen interaction. CD4 and CD8 have two important functions:

- They bind MHC class II and class I molecules, respectively, thereby strengthening the T cell–antigen interaction
- They function as signal transducers.

The role of CD4 and CD8 in antigen–receptor binding is shown in Fig. 1.17.

GENERATION OF ANTIGEN RECEPTOR DIVERSITY

Scientists know that there are approximately 20⁸ possible antigens, each requiring a corresponding

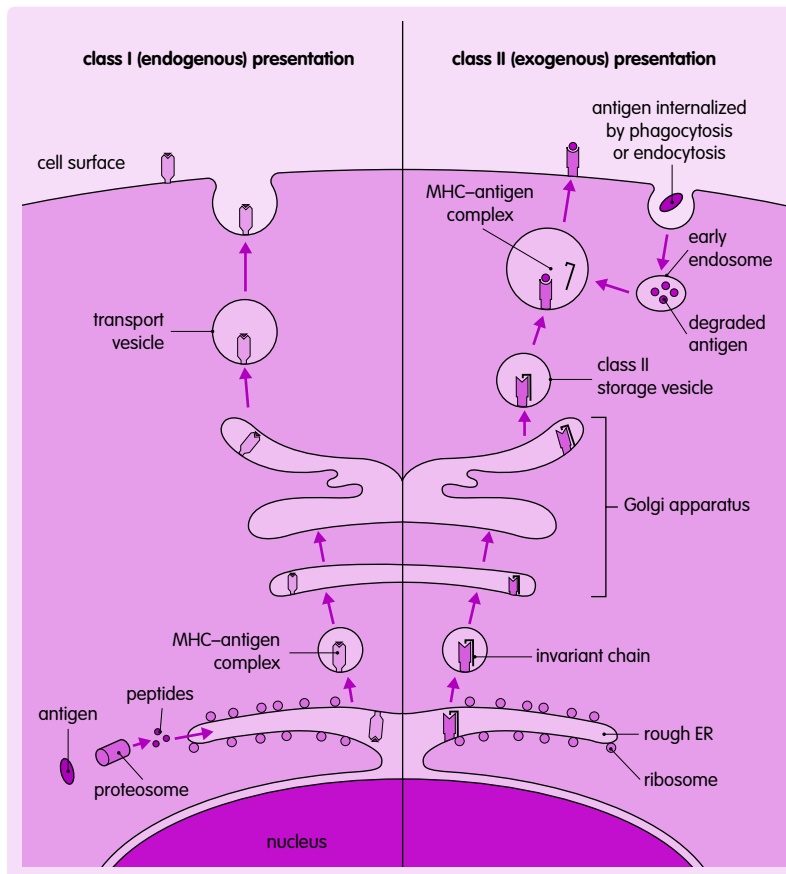
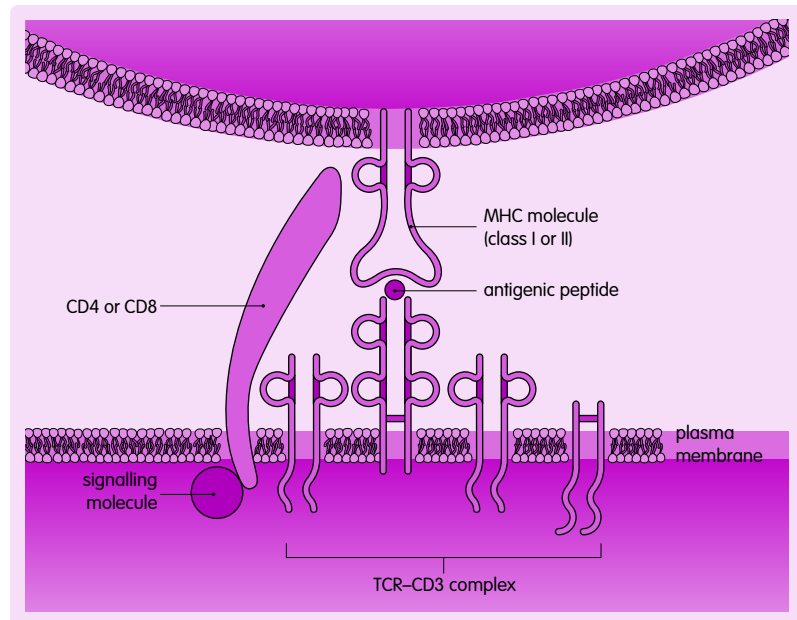


Fig. 1.16 Routes of antigen processing. Class I molecules present endogenous antigens. Cytosolic antigen is degraded by proteasomes and transported into the rough endoplasmic reticulum (ER), where peptides are loaded onto class I molecules. The MHC–peptide complex is transported via the Golgi apparatus to the cell surface. Class II molecules present exogenous antigens that have been phago- or endocytosed into intracellular vesicles. The MHC molecule is transported from the rough ER to the vesicle by the invariant chain (Ii). It is displaced from the MHC molecule by processed antigen, which is then presented at the cell surface. MHC, major histocompatibility complex.

Fig. 1.17 The role of CD4 and CD8 in T cell receptor (TCR)–major histocompatibility complex (MHC) antigen interaction. CD4 or CD8 is closely associated with the TCR complex. They bind MHC in a restricted fashion (CD8 to class I only, CD4 to class II only). Binding is antigen independent and strengthens the bond between TCR and a complementary peptide–MHC complex. Molecules associated with CD4 or CD8 are then able to transduce a signal.



receptor. The human genome contains only 30,000 genes and so each receptor cannot be coded by a single gene. Instead this diversity is achieved by genetic recombination, a process where segments of information are cut and pasted from the gene.

T cell receptor and immunoglobulin are the only genes to undergo genetic recombination.



Rearrangement of gene segments allows antibodies with an immense variety of specificity to be produced from a relatively small amount of DNA.

gene segments are clusters of exons, each of which encodes either a domain or a hinge region of the constant region.

Following rearrangement, the clonal progeny of each B cell will produce Ig of a single specificity. Rearrangement is completed and functional Ig chains are produced before the B cell encounters antigen (Fig. 1.18). The presence of multiple V, D (heavy chain only) and J gene segments, and the apparently random selection of these segments, generates considerable diversity, which can be calculated (Fig. 1.19).

A similar process occurs in T cells: α - and γ -chain variable domains have V and J segments; β - and δ -chains have V, D and J segments.

Junctional diversity

The formation of junctions between the various gene segments produces an opportunity for increased diversity, where nucleotides are added or subtracted at random to form the joining segments.

Junctional flexibility and N-nucleotide addition

When exons are spliced, there are slight variations in the position of segmental joining. In addition, up to 15 nucleotides can be added to the D–J and the V–DJ joints. This occurs only in heavy chains and is catalysed by terminal deoxynucleotidyl transferase (TdT).

Genetic rearrangements

Before genetic recombination occurs, we say the gene segments are in germline configuration. Rearrangement only occurs in the variable domain (those that code for the active site) as the other segments of the receptor remain constant. Each variable domain is encoded by a random combination of one of each of the V, D (heavy chain only) and J exons. Following genetic rearrangement, one exon remains which codes for a variable domain. The C exons encode the constant regions. Heavy-chain C

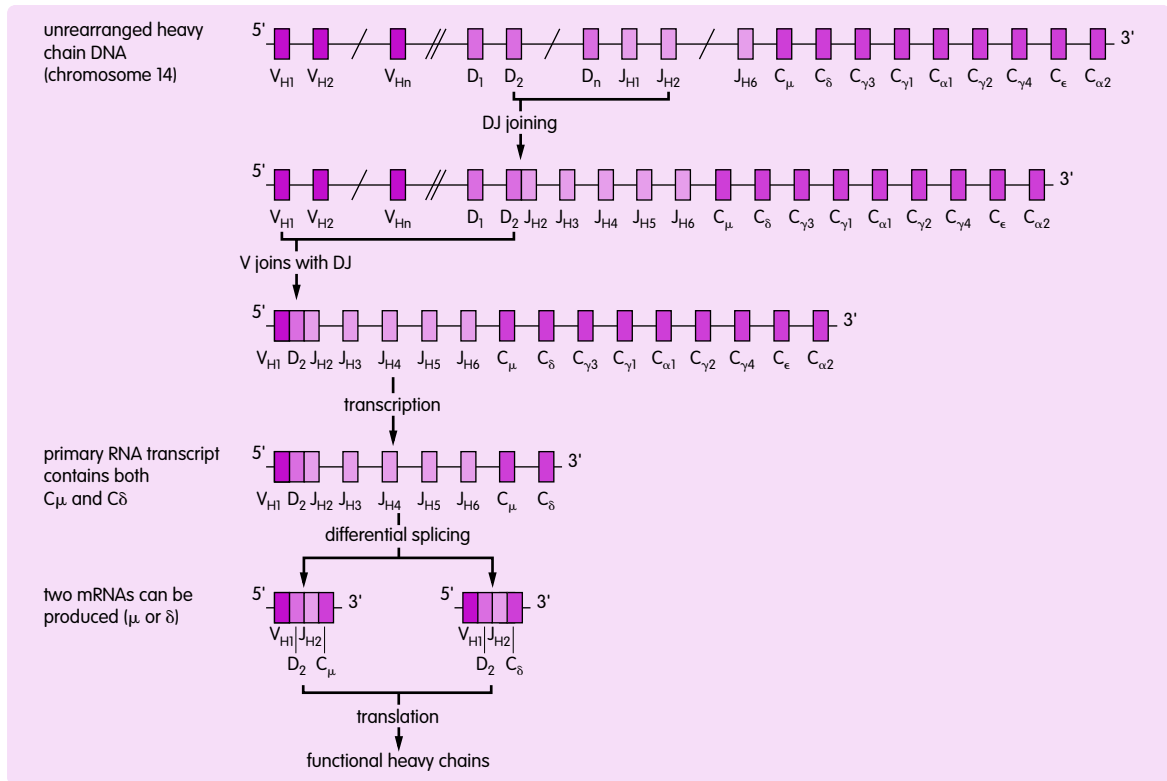


Fig. 1.18 Rearrangement of the heavy chain is similar to that of the light chain, although the join between D and J segments occurs first. In an unstimulated B cell, the heavy-chain mRNA that is transcribed contains both the C_μ and C_δ segments. The mRNA can be differentially spliced such that both IgM and IgD will be produced. They will both exhibit the same antigen binding specificity.

Fig. 1.19 Calculation of antibody diversity

Mechanism of diversity	Number of combinations		
	κ light chain	λ light chain	Heavy chain
Random joining of gene segments	$100 \times 5 = 500$	$100 \times 6 = 600$	$75 \times 30 \times 6 = 13,500$
Random chain associations	$(500 + 600) \times 13,500 = 1.5 \times 10^7$		

Given the fact that light chain can associate with any heavy chain, and from the number of gene segments present in germline DNA, it is possible to calculate the number of different molecules that can be produced. The extent of the contribution of junctional flexibility, N-nucleotide addition and somatic hypermutation is not known but will be significant.

Both junctional flexibility and N-nucleotide addition can disrupt the reading frame, leading to non-functional rearrangements. However, formation of productive rearrangements increases antibody diversity. The V-J, V-DJ and VD-J joints fall within the antigen-binding region of the variable domain. Therefore, diversity generated at these

joints will impact on the antigen specificity of the Ig molecule.

Somatic hypermutation

Somatic hypermutation is a process that increases the affinity of antibody for its antigen and is also called affinity maturation. B cells that are dividing by mitosis

to increase in number in order to combat infection are allowed to undergo mutation in their variable domain (the only cells in the body permitted to do so). Some mutations decrease the antibody's specificity for the antigen and apoptosis is stimulated in these cells; others result in antibody of increased specificity—these are positively selected for. Antibodies produced later in the primary immune response, and in the secondary immune response, will therefore have an increased affinity for antigen.

The TCR does not exhibit somatic hypermutation. This is probably because T cells do not recognize self-peptides, recognizing only self-MHC. Diversity is generated only in developing T cells, which can be deleted if they are either self-reactive or non-functional.

Class switching

This is the process whereby a single B cell can produce different classes of Ig that have the same specificity. The mechanism is not well understood but involves 'switch sites'—DNA sequences located upstream from each heavy chain C gene segment (except C_δ). Possible mechanisms include:

- Differential splicing of the primary transcript (see Fig. 1.18)
- A looping out and deletion of intervening heavy chain C gene segments (and introns)
- Exchange of C gene segments between chromosomes.

This process underlies the class switch from IgM in the primary response to IgG, IgA or IgE in the secondary response. Cytokines are important in controlling the switch.

nating extracellular pathogens. Antigen can be cleared from the host by a variety of effector mechanisms, which are dependent on antibody class or isotype (see p. ●●):

- Activation of complement, leading to lysis or opsonization of the microorganism
- Antibody-dependent cell-mediated cytotoxicity (ADCC)
- Neutralization of bacterial toxins and viruses
- Mucosal immunity (IgA-mediated).

Activated and differentiated B cells, known as plasma cells, produce antibodies. An overview of B cell activation is given in Fig. 1.20. B cells are activated within follicles found in secondary lymphoid structures, e.g. lymph nodes and spleen. B cells become activated only if they encounter specific antigen. During proliferation, variable regions of the immunoglobulin genes undergo somatic hypermutation (see p. ●●). This process occurs in the germinal centre of the follicle. Follicular dendritic cells present antigen, to which the B cells with the highest affinity will bind. This causes the expression of bcl-2, which prevents B cells undergoing apoptosis. Therefore, the highest-affinity clones are positively selected. In order for B cells to produce antibody, they require help from T cells. Activated T helper cells provide the help needed by producing cytokines (IL-2, IL-4, IL-5, IL-6). This acts as a further method of regulation within the immune system, as both B cells and T cells need exposure to the offending antigen in order for a response to be evoked. In addition, as self-reactive T cells are deleted in the thymus, the chance of autoimmunity is reduced. An overview of clonal selection of B cells is given in Fig. 1.21.

THE ADAPTIVE IMMUNE SYSTEM

Recognition molecules and their diversity are important for the generation of a specific, adaptive immune response. The adaptive immune response can be humoral or cell-mediated.

HUMORAL IMMUNITY

B cells and antibody production

The humoral immune response is brought about by antibodies, which are particularly efficient at elimi-

T-cell-dependent and T-cell-independent antigens

The process shown in Fig. 1.20 illustrates the need for T cells in the activation of a humoral response. The antigens that trigger this process are therefore known as T-cell-dependent antigens. Not all antigens require T cells to produce an antibody response. T-cell-independent antigens, including many microbial constituents, are able to stimulate B cells directly or with the help of non-thymus-derived accessory cells. This is particularly true for polysaccharides, which form the capsules of many bacteria, e.g. *Pneumococcus* and *Haemophilus*.

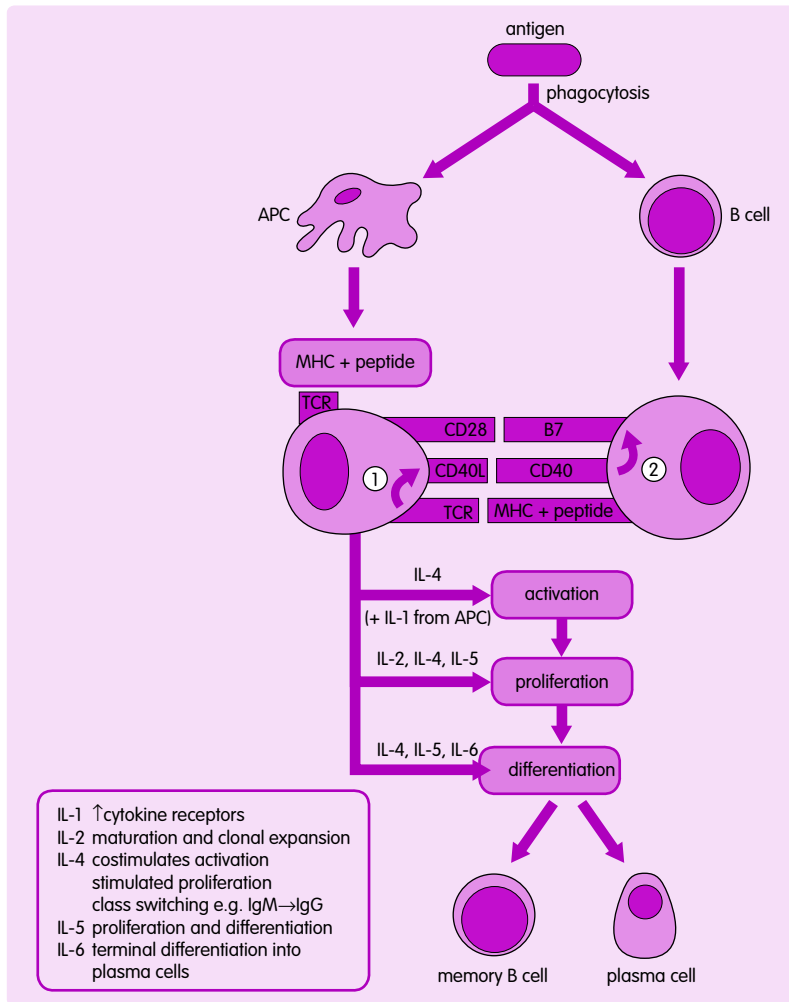


Fig. 1.20 Overview of the humoral immune response. Activated and differentiated B cells, known as plasma cells, produce antibody. B cells are activated by antigen in a T-cell-independent or dependent fashion (only T-cell-dependent antigens are shown). T helper cells are primed by antigen-presenting cells (APCs), which present antigen in conjunction with MHC class II molecules. B cells are stimulated by antigen interacting with B cell receptors. Primed T helper cells interact with B cells that also express antigen–MHC complexes. This interaction induces a sequence of surface receptor binding and cytokine production that results in B-cell activation, proliferation and differentiation. (1) Binding of the T cell receptor (TCR) to MHC induces the T cell to produce CD40L, which binds to CD40 on the B cell, producing a major stimulatory signal. (2) CD28 on the T cell then interacts with B7 on the B cell (co-stimulatory signal). Cytokines are also involved; their actions are shown in the diagram.

Structure and function of antibody

The structure of immunoglobulin is shown in Fig. 1.22. Immunoglobulin molecules (using IgG as an example) are composed of two identical heavy and two identical light chains, linked by disulphide bridges. The light chains consist of one variable and one constant domain, while the heavy chain contains one variable and three constant domains. Digestion of IgG with papain produces two types of fragment:

1. Two Fab fragments (bind antigen) consisting of the light chain and two domains of the heavy chain (denoted VH and CH1)
2. One Fc fragment (binds complement) consisting of the remainder of the heavy chain (CH2 and CH3).

The light chain

The light chain comprises two domains:

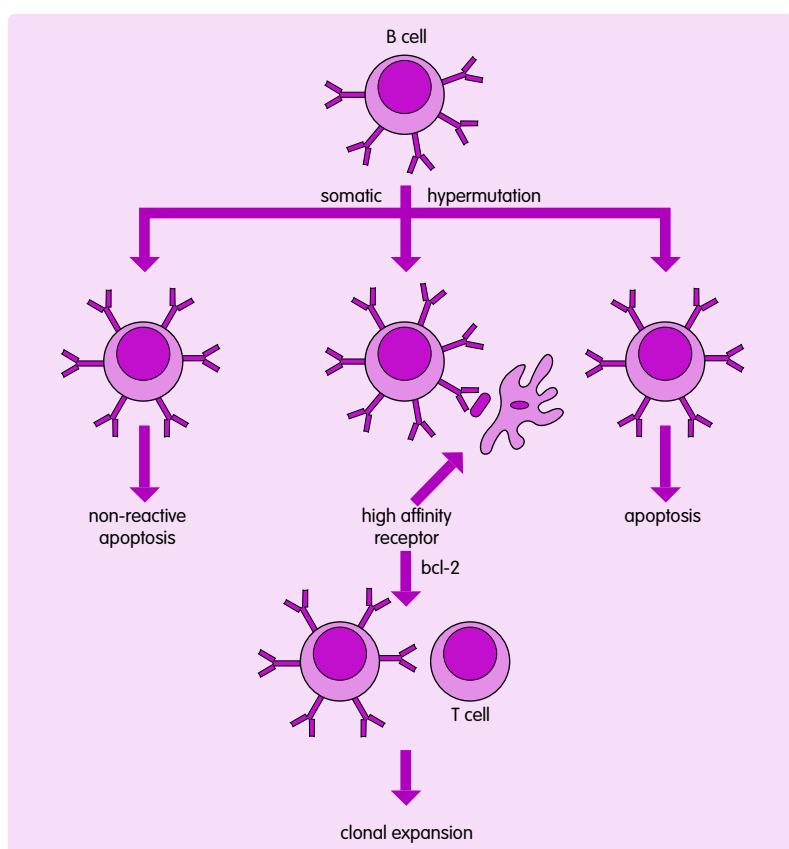
- The amino (N) terminal domain is variable and is the site of antigen binding
- The constant domain at the carboxy (C) terminal.

The constant region can be κ or λ , but both light chains within an Ig molecule will be the same; ~60% of human light chains are κ .

The heavy chain

The heavy chain has a variable domain attached to several constant domains. There are five classes of immunoglobulin (Ig) in humans: IgG, IgA, IgM, IgE and IgD. The heavy chain determines the immunoglobulin class. The heavy chain can be γ (IgG), α (IgA), μ (IgM), ϵ (IgE) or δ (IgD). IgG, IgA and IgD

Fig. 1.21 Clonal selection of B cells. During B cell activation, the antigen-binding region of the immunoglobulin gene undergoes hypermutation. Clonal selection ensures that cells that produce the best antibody are selected and that non-functional or self-reactive B cells are deleted. This process occurs within the germinal centres of lymphoid follicles.



have three constant domains with a hinge region; IgM and IgE have four constant domains but no hinge region.

The variable domain

Each variable domain exhibits three regions that are hypervariable. The hypervariable regions on both light and heavy chains are closely aligned in the immunoglobulin molecule. Together, they form the antigen-binding site and therefore determine the molecule's specificity.

The hinge region

The hinge is a peptide sequence located between the first and second constant domains in the heavy chain. It allows the Fab regions to move against the Fc region from 0 to 90°. This allows greater interaction with epitopes. The hinge region is also the site of the interchain disulphide bonds.

Classes of antibody

The different properties of the immunoglobulin classes are shown in Fig. 1.23. Different Ig classes

and subclasses are specific to each species. IgG, IgE and IgD are monomeric, secreted IgA (sIgA) is usually present as a dimer, and secreted IgM as a pentamer. The sIgA molecule is made up of two IgA monomers: a J chain and a secretory piece. The IgA dimer (J chain) is produced by submucosal plasma cells and enters the mucosal epithelial cell via receptor-mediated endocytosis, binding to the poly-Ig receptor. Having passed from the basal to the luminal surface of the epithelial cell, the IgA dimer is secreted across the mucosa, with part of the poly-Ig receptor (the secretory piece) still attached.

The functions of antibodies

The functions of Igs are shown in Fig. 1.24.

Lymphatic drainage and lymph nodes

Lymph nodes are secondary lymphoid organs. They provide a site for lymphocytes to interact with antigen and other cells of the immune system.

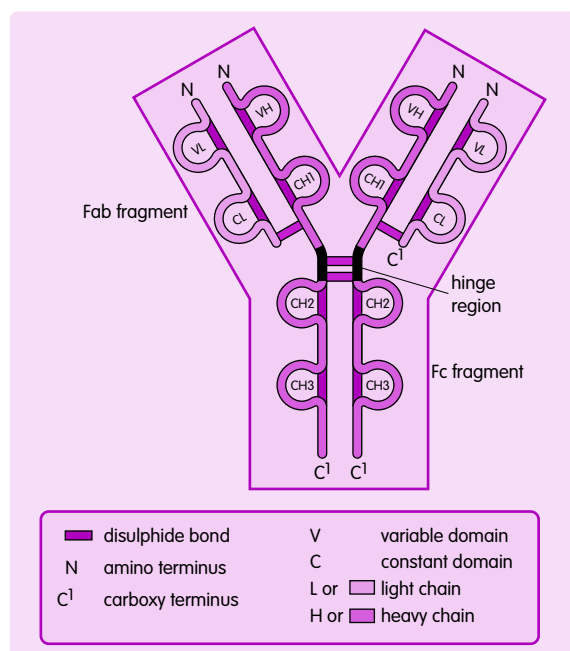


Fig. 1.22 Structure of IgG. Immunoglobulins are composed from two identical light and two identical heavy chains. The chains are divided into domains, each of which is an immunoglobulin fold. The variable domains form the antigen-binding site. Digestion of the immunoglobulin molecule with papain produces an Fc portion (which binds complement) and two Fab portions (which bind antigen).

At the arterial end of capillaries, water and low-molecular-weight solutes leak out into tissue spaces to create interstitial fluid. Most interstitial fluid returns to the venous circulation at the venous end of capillaries (due to pressure gradients). The remainder leaves the interstitial space via the lymphatic system. Once interstitial fluid has entered a lymphatic vessel it is known as lymph. Lymphatic vessels are present in almost all tissues and organs of the body.

Lymphatic circulation

The lymphatic system acts as a passive drainage system to return interstitial fluid to the systemic circulation; lymph is not pumped around the body. Lymph vessels therefore contain numerous valves to prevent backflow of lymph. Afferent lymph vessels carry lymph into lymph nodes. They empty into the subcapsular sinus and lymph percolates through the node. Each node is drained by only one efferent vessel.

Lymph returns to the circulation at lympho-venous junctions. These are located at the junction of the right subclavian vein and right internal jugular vein (which empties the right lymphatic duct) and at the junction of the left subclavian vein and left internal jugular vein (which empties the thoracic duct).

Fig. 1.23 Properties of the five immunoglobulin (Ig) classes

	IgG	IgA	IgM	IgE	IgD
Physical properties					
Molecular weight (kDa)	150	300	900	190	150
Serum concentration (mg/mL)	13.5	3.5	1.5	0.0003	0.03
Number of subunits	1	2	5	1	1
Heavy chain	γ	α	μ	ϵ	δ
Subclasses	4	2	—	—	—
Biological activities					
Present in secretions	X	✓	✓	X	X
Crosses placenta	✓	X	X	X	X
Complement fixation	✓	✓	✓✓✓	X	X
Binds phagocytic receptors	✓	X	✓	X	X
Binds mast cell receptors	✓	X	X	✓	X
Other features					
Main role	Main circulatory Ig for secondary immune response	Major Ig in secretions	Main Ig in primary immune response	Allergy and antiparasitic response	Expressed on naïve B cell; function not known

Fig. 1.24 Summary of the functions of immunoglobulins

Function	Notes
Opsonization	Phagocytic cells have antibody (Fc) receptors, thus antibody can facilitate phagocytosis of antigen
Agglutination	Antigen and antibody (IgG or IgM) clump together because immunoglobulin can bind more than one epitope simultaneously. IgM is more efficient because it has a high valency (10 antigen-binding sites)
Neutralization	Binding to pathogens or their toxins prevents their attachment to cells
Antibody-dependent cell-mediated cytotoxicity (ADCC)	The antibody-antigen complex can bind to cytotoxic cells (e.g. cytotoxic T cells, NK cells) via the Fc component of the antibody, thus targeting the antigen for destruction
Complement activation	IgG and IgM can activate the classical pathway; IgA can activate the alternative pathway
Mast cell degranulation	Cross-linkage of IgE bound to mast cells and basophils results in degranulation
Protection of the neonate	Transplacental passage of IgG and the secretion of sIgA in breast milk protect the newborn

sIgA, secretory immunoglobulin A; NK, natural killer.

Lymph nodes

Lymph nodes act as filters, 'sampling' lymphatic fluid for bacteria, viruses and foreign particles. APCs, loaded with antigen, also migrate through lymph nodes. They are present throughout the lymphatic system, often occurring at junctions of the lymphatic vessels. Lymph nodes frequently form chains, and may drain a specific organ or area of the body.

Lymph nodes act as sites for initiation of the adaptive immune response. Antigen is sampled, processed and presented by several professional APCs (macrophages and dendritic cells).

Lymphocyte recirculation

Lymphocytes move continuously between blood and lymph. Efferent lymph contains more lymphocytes than afferent lymph because:

- Antigenic challenge results in stimulation and proliferation of lymphocytes
- Lymphocytes enter the lymph node directly from blood.

Lymphocyte recirculation is essential for a normal immune response (Fig. 1.25). Approximately 1–2% of the lymphocytic pool recirculates each hour. This increases the chances of an antigenically committed lymphocyte encountering complementary antigen.

Lymphocytes tend to recirculate to similar tissues. For example, an activated lymphocyte that

has migrated from the skin to a local lymph node is most likely to migrate back to the skin following transport in the blood. Similarly, lymphocytes activated in mucosal-associated lymphoid tissue (MALT) will return to MALT. This recirculation is governed by the expression of molecules on both the lymphocyte and surface endothelium. These molecules, called integrins, confer specificity to lymphocyte recirculation. This fine tuning of lymphocyte recirculation is known as lymphocyte homing. Areas of endothelium through which lymphocytes migrate are known as high endothelial venules (HEVs). Lymphocytes activated in MALT express $\alpha_4\beta_7$ integrins that interact with MadCAM-1, an adhesion molecule only expressed on HEVs in MALT.

Lymphadenopathy

Lymph nodes can become enlarged (lymphadenopathy) for several reasons, including infection. Causes of lymphadenopathy are outlined on page 22.



Lymphadenopathy can be a sign of infection. Understanding the drainage of lymph can lead you to the source of infection.

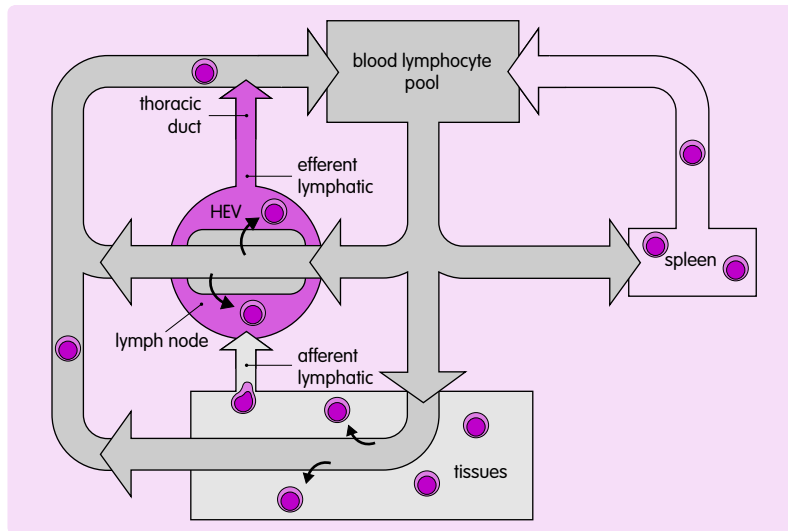


Fig. 1.25 Lymphocyte recirculation. Lymphocytes can enter lymph nodes via specialized high endothelial venules or in lymph. They leave the node in lymph that is returned to the systemic circulation via the right lymphatic duct or thoracic duct. HEV, high endothelial venule.

Mucosal-associated lymphoid tissue (MALT)

MALT consists of unencapsulated subepithelial lymphoid tissue found in the gastrointestinal, respiratory and urogenital tracts (Fig. 1.26). It can be subdivided into:

- Organized lymphoid tissue, e.g. tonsils, appendix, Peyer's patches
- Diffuse lymphoid tissue located in the lamina propria of intestinal villi and lungs.

Organized lymphoid tissue

Respiratory tract

MALT in the nose and bronchi includes the:

- Lingual, palatine and nasopharyngeal tonsils
- Adenoids
- Bronchial nodules.

The respiratory system is exposed to a large number of organisms every day, most of which are cleared by the mucociliary escalator. Microorganisms that are not removed are presented by dendritic cells in the bronchi and stimulate germinating centres.

Gastrointestinal tract

Peyer's patches are organized submucosal lymphoid follicles present throughout the large and small intestine, being particularly prominent in the lower ileum. The structure of a Peyer's patch is shown in Fig. 1.27.

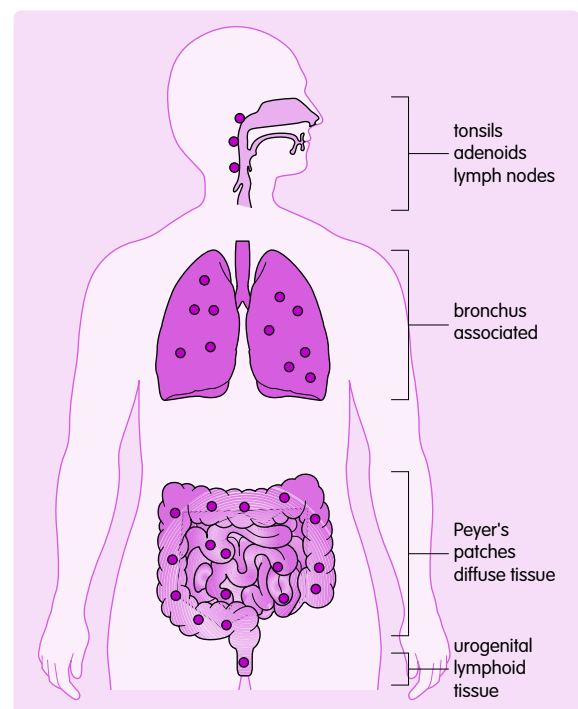
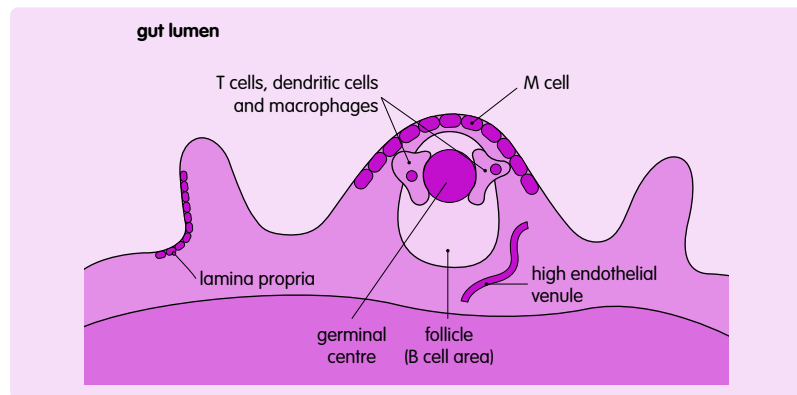


Fig. 1.26 Anatomical location of mucosal-associated lymphoid tissue (MALT). MALT is found in the nasal cavity, throat, respiratory tract, gastrointestinal tract and urogenital tract. Immune cells activated in MALT will home only to other mucosal sites.

Fig. 1.27 Structure of a Peyer's patch. Peyer's patches are found in the gastrointestinal tract. Microbes are transported across specialized epithelial M cells in pinocytotic vesicles into a dome-shaped area. Antigen-presenting cells then process and present antigen to T cells. T helper cells can then activate B cells within the follicle.



Lymphocyte trafficking in MALT

Mucosal lymphocytes generally recirculate within the mucosal lymphoid system. This occurs through recognition between specific adhesion molecules on the surfaces of lymphocytes from Peyer's patches and corresponding ligands on the venular endothelium.

CELL-MEDIATED IMMUNITY

Cell-mediated immunity is mediated by T lymphocytes, macrophages and NK cells. The cell-mediated immune system is involved in the elimination of:

- Intracellular pathogens and infected cells (mainly viruses, mycobacteria and fungi)
- Tumour cells
- Foreign grafts.

The thymus plays an important role in cell-mediated immunity because it is the site of T cell maturation.

The thymus gland

The thymus is important for the production of T lymphocytes. T lymphocyte differentiation begins in the bone marrow (see p. ●●) before early precursor cells migrate to the thymus. In the thymus, immature T lymphocytes undergo random recombination of their T cell receptor genes. Some of the resulting T cell receptors will be specific for pathogens and others for normal self-antigens. The role of the thymus is to select for cells that recognize self-MHC, and negatively select those T cells that recognize self-antigen with self-MHC.

The thymus is a gland with two lobes, located in the anterior part of the superior mediastinum, posterior to the sternum and anterior to the great vessels and upper part of the heart. It can extend superiorly into the roof of the neck and inferiorly into the anterior mediastinum. It receives its blood supply from the inferior thyroid and internal thoracic arteries. Each lobe is surrounded by a capsule and divided into multiple lobules by fibrous septa known as trabeculae. Each lobule is divided into two regions (Fig. 1.28):

- An outer cortex
- An inner medulla.

Immature thymocytes (T cell progenitors) enter the thymus gland via the cortex, where they rapidly proliferate and rearrange their T cell receptor genes. The thymus expresses many of the body's proteins (e.g. insulin) so that T cells which recognize this self-antigen can be forced to undergo apoptosis—so-called negative selection. T cells that are able to bind MHC to some extent will proliferate—positive selection. A much smaller and more mature group of thymocytes survives to enter the medulla. Thymocytes continue to mature in the medulla and eventually leave the thymus, via postcapillary venules, as mature, antigen-specific, immunocompetent T cells. In total, only 1–5% of thymocytes in the thymus reach maturity, the remainder undergoing programmed cell death (apoptosis).

Stromal cells of the thymus

The remainder of the thymic lobule is composed of a network of epithelial cells, known collectively as stromal cells. They interact with developing thymo-

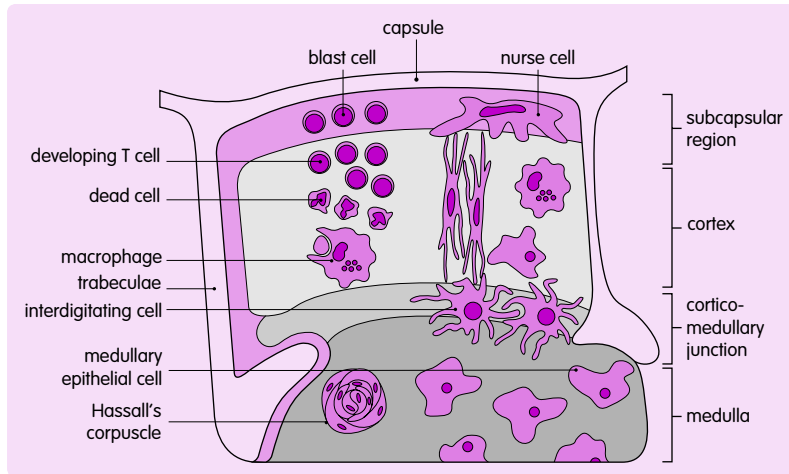


Fig. 1.28 Structure of a thymic lobule. The thymus is a bilobed gland, surrounded by a collagenous capsule, which is subdivided into lobules. Developing T cells (thymocytes) move from the subcapsular region to the medulla during maturation. Several different types of stromal cell support them. Many thymocytes undergo apoptosis (particularly in the cortex) and are phagocytosed by macrophages.

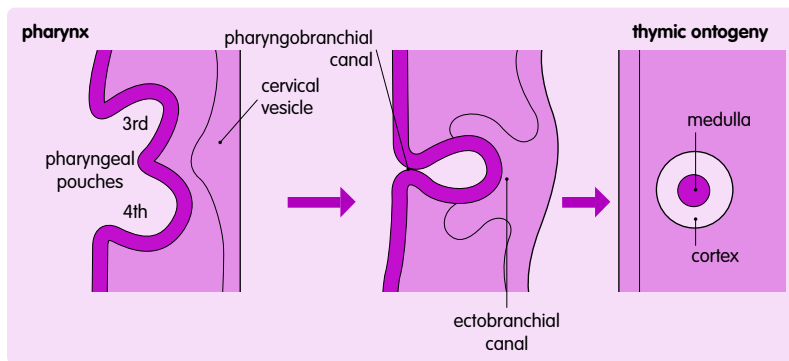


Fig. 1.29 Embryological development of the thymus. The thymus develops from the third (and possibly fourth) pharyngeal pouch. This forms the medulla, which is surrounded by the ectobranchial canal formed from the cervical vesicle. The thymus is developed by 8 weeks of gestation.

cytes and produce several hormones that are essential for their differentiation and maturation.

Embryological origin of the thymus

The human embryonic thymus develops from the third pharyngeal pouch during week 4 or 5 of gestation (Fig. 1.29). The thymus gland is formed by week 8, and is fully differentiated and producing viable lymphocytes by week 17. The third pharyngeal pouch also gives rise to the parathyroid glands. Lymphoid stem cells are produced by the fetal liver and spleen, and by bone marrow from 6 months' gestation.

Thymic hypoplasia

Although it continues to grow until puberty, the relative size of the thymus gland decreases over this period. After puberty there is a real reduction in size and, by adulthood, it is composed largely of adipose tissue and continues to produce far fewer T lympho-

cytes. This means it is harder for adults to recover from immunological damage caused by, for example, HIV or immunosuppressive drugs.

In DiGeorge syndrome, the thymus fails to develop. Consequently, there is an absence of circulating T cells and a reduction in cell-mediated immunity. The mother provides no passive T cell immunity after birth and infants present early with infections.

T lymphocytes

Functions of different T cell phenotypes

The different types of T cell can be differentiated by cell-surface molecules and function. There are two different types of T cell receptor (TCR), which have different functions. T cells expressing $\alpha\beta$ -TCRs account for at least 95% of circulatory T cells. They

become cytotoxic, helper or suppressor cells and, unless specified otherwise, account for all the T cells mentioned in this book. T cells expressing a $\gamma\delta$ -TCR are present at mucosal surfaces and their specificity is biased towards certain bacterial and viral antigens. Some $\gamma\delta$ -T cells can recognize antigen independently of an APC. These T cells are usually cytotoxic in their actions. They differ from NK cells because they detect antigen rather than the presence or absence of MHC class I molecules. They are part of the adaptive system, because their action is specific and shows evidence of immunological memory.

T helper cells

T helper (Th) cells play a key role in the development of the immune response:

- They determine the epitopes that are targeted by the immune system via their interactions with antigen in conjunction with class II MHC molecules on APCs
- They determine the nature of the immune response directed against target antigens, e.g. cytotoxic T cell response or antibody response
- They are required for normal B cell function (see p. ●●).

Most Th cells are CD4⁺ and can be divided into four subsets on the basis of the cytokines they secrete:

1. Th0
2. Th1
3. Th2
4. Treg.

Th0 cells arise as a result of initial short-term stimulation of naïve T cells; they are capable of secreting a broad spectrum of cytokines. Prolonged stimulation results in the emergence of Th1 and Th2 subsets. The cytokines released by the Th1 and Th2

subsets modulate one another's secretion. The different cytokine profiles of the Th1 and Th2 subsets reflect their different immunological functions (Fig. 1.30). The fourth type of helper T cell has a regulatory role. If autoreactive T cells manage to escape negative selection in the thymus, they need to be inhibited in the peripheral tissues. Regulatory T cells are capable of preventing this immune response. Their action is unknown but is thought to be via cytokines, including transforming growth factor- β (TNF- β) IL-5, IL-6 and IL-10.

Cytotoxic T cells

Most cytotoxic T (Tc) lymphocytes are CD8⁺ and recognize antigen in conjunction with class I MHC molecules (endogenous antigen). They lyse target cells via the same mechanisms as NK cells (see p. ●●).

Development of T cells

T cell precursors are produced in the bone marrow and are transported to the thymus for development and maturation. The aim of T cell development and maturation is to select T cells with receptors that can recognize foreign antigens in conjunction with self-MHC. Cells with non-functioning receptors or that are strongly self-reactive are destroyed (Fig. 1.31).

Positive selection

Positive selection occurs in the thymic cortex. T cells that are capable of binding self-MHC are allowed to live, i.e. they are positively selected for, and T cells that do not recognize self-MHC die. Furthermore, T cells that interact with MHC class I lose their CD4 (they are now CD8 T cells) and T cells that interact with MHC class II lose their CD8 (becoming CD4 T cells); this is MHC restriction. T cells that do not interact with the MHC molecules undergo

Fig. 1.30 Differences between the T helper 1 (Th1) and T helper 2 (Th2) cell subsets

	Th1 cells	Th2 cells
Cytokines secreted	IL-2, IL-3, IFN- γ , TNF- β	IL-3, IL-4, IL-5, IL-10, IL-13
Functions	<ul style="list-style-type: none"> • Responsible for classical cell-mediated immunity reactions such as delayed-type hypersensitivity and cytotoxic T cell activation • Involved in responses to intracellular pathogens • Activate macrophages 	<ul style="list-style-type: none"> • Promote B cell activation • Involved in allergic diseases and responses to helminthic infections • Involved in responses to intracellular pathogens

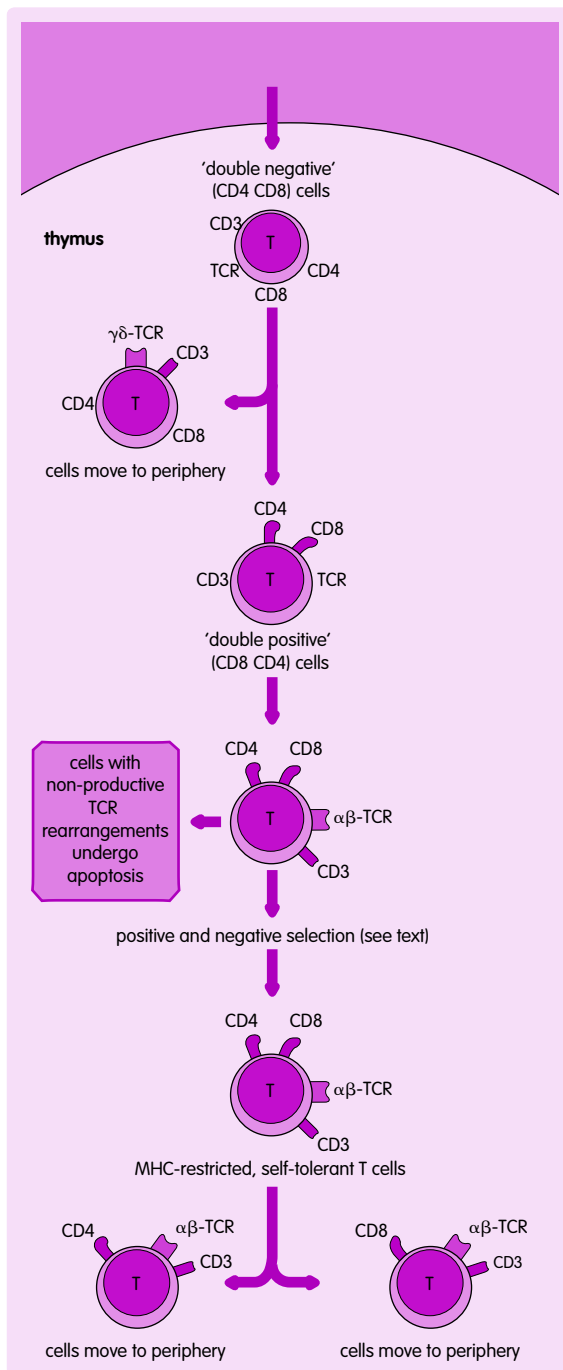


Fig. 1.31 Development of T cells in the thymus. Cells entering the thymus to become T cells are negative for CD4, CD8, CD3 and the T cell receptor (TCR). Rearrangement of the genes encoding the TCR will produce three cell lines: (1) CD4⁺ αβ-TCR; (2) CD8⁺ αβ-TCR; and (3) CD4⁻ CD8⁻ γδ-TCR. The β- or γ-chain genes rearrange first. If a functional β-chain is formed, both CD4 and CD8 are upregulated and the α-chain gene rearranges. The resultant T cells are positively selected if their TCR is functional, but negatively selected if they react too strongly. The majority of thymocytes will undergo apoptosis due to positive or negative selection. MHC, major histocompatibility complex.

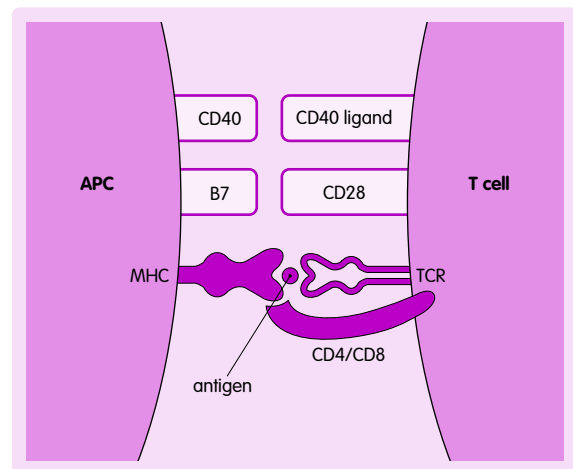


Fig. 1.32 Activation of T cells. Several interactions with antigen-presenting cells (APCs) are required to activate T cells. The T cell receptor (TCR) and CD4 or CD8 bind to MHC and antigen. CD28 on the T cell binds to B7 on the APC, providing a co-stimulatory signal. MHC, major histocompatibility complex.

apoptosis, as they do not receive a protective signal as a result of the TCR–MHC interaction.

Negative selection

T cells that are positively selected, but have high affinity for MHC molecules and self-antigen, undergo negative selection.

T cell activation

T cells are activated by interactions between the TCR and peptide bound to MHC. Activation also requires a 'second message' from the antigen-presenting cell. This process is shown in Fig. 1.32. Once T cells are activated they produce a wide range of molecules with several functions. These are primarily cytokines, which may be pro- or anti-inflammatory (see Fig. 1.30) or involved in activation of other immune cells.

Superantigens

T cells can be activated in a non-specific fashion by superantigens. Superantigens cross-link between the V- β domain of the TCR and a class II MHC molecule on an antigen-presenting cell. Cross-linking is independent of the peptide binding cleft but depends on the framework region of the V- β domain. This means that one superantigen is able to activate about 5% of T cells, far more than normal antigen. An example of a T cell superantigen is staphylococcal enterotoxin.

Superantigens result in polyclonal activation effectively 'crowding-out' the specific, protective immune response. A consequence of polyclonal

activation can be autoimmune disease. Superantigen can also result in the deletion of a large number of T cells by inducing negative selection in the thymus.

Toxic shock syndrome

Toxins produced by staphylococci and streptococci can act as superantigens, producing the clinical picture of 'toxic shock syndrome', where a seemingly innocuous stimulus such as a graze can lead to fever, a diffuse macular rash, hypotension and shock.