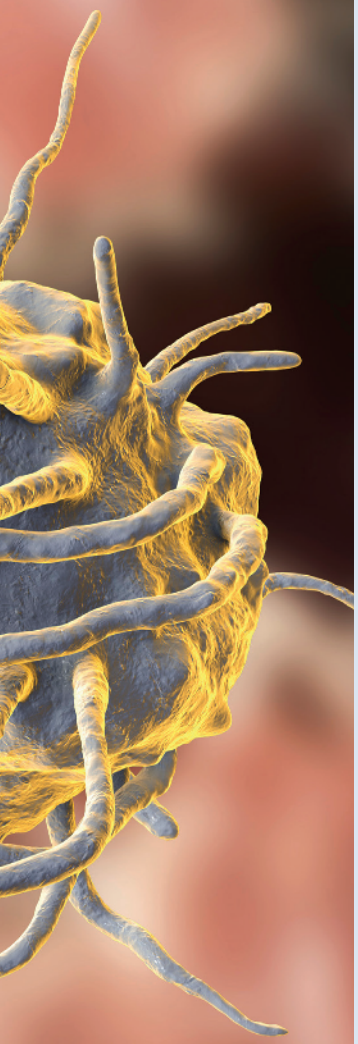
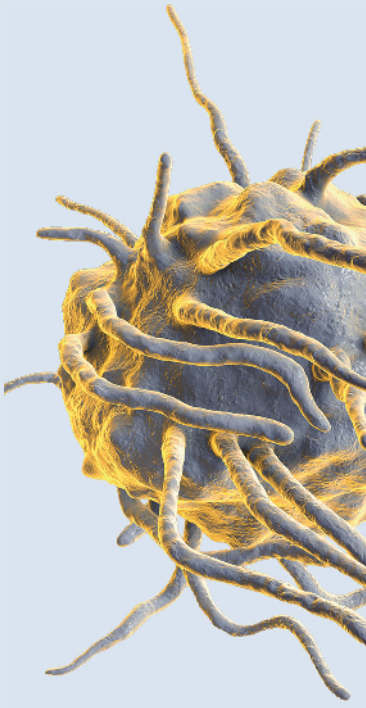


CHAPTER 1

Basic Components: Structure and Function

Key topics



Visit the companion website at **www.wiley.com/go/misbah/immunology** to download cases on these topics.

Chapel and Haeney's Essentials of Clinical Immunology, Seventh Edition. Edited by Siraj A. Misbah, Gavin P. Spickett, and Virgil A.S.H. Dalm.

1.1 Introduction

The immune system evolved as a defence against infectious diseases. Individuals with markedly deficient immune responses, if untreated, succumb to infections in early life. There is, therefore, a selective evolutionary pressure for a really efficient immune system. Although innate systems are fast in response to pathogens, the evolution to adaptive responses provided greater efficiency. However, a parallel evolution in pathogens means that all species, plants, insects, fish, birds and mammals have continued to improve their defence mechanisms over millions of years, giving rise to some redundancies as well as resulting in apparent complexity. The aim of this chapter is to provide an initial description of the molecules involved, moving on to the role of each in the immune processes rather than the more traditional sequence of anatomical structure, cellular composition and then molecular components. It is hoped that this gives a sense of their relationship in terms of immediacy and dependency as well as the parallel evolution of the two immune systems. An immune response consists of **five parts**:

- Recognition of material as foreign and dangerous.
- An early innate (non-specific) response to this recognition.
- A slower specific response to a particular antigen, known as an adaptive response.
- Non-specific augmentation of this response.
- A memory of specific immune responses, providing a quicker and larger response when that particular antigen is encountered the second time.

Innate immunity, though phylogenetically older and important in terms of speed of response, is less efficient. Humoral components (soluble molecules in the plasma) and cells in blood and tissues are involved. Such responses are normally accompanied by inflammation and occur within a few hours of stimulation (Table 1.1).

Adaptive immune responses are also divided into humoral and cellular responses. Adaptive humoral responses result in the generation of antibodies reactive with a particular antigen. Antibodies are proteins with similar structures, known collectively as immunoglobulins (Ig). They can be transferred passively to another individual by injection of serum. In contrast, only cells can transfer cellular immunity. Good examples of cellular immune responses are the rejection of a graft by lymphoid cells as well as graft-versus-host disease, where viable transferred cells attack an immunologically compromised recipient that is unable to fight back.

Antibody-producing lymphocytes, which are dependent on the bone marrow, are known as B cells. In response to antigen stimulation, B cells will mature to antibody-secreting plasma cells. Cellular immune responses are dependent on an intact thymus, so the lymphocytes responsible are known as thymus-dependent (T) cells. The developmental pathways of both cell types are fairly well established (Fig. 1.1).

The recognition phase is common to both adaptive and innate immunity. It involves professional cells, known as classical dendritic cells (DCs), that recognize general pathogen features or specific antigenic molecules, process the antigens and present antigen fragments to the other cells of the immune system, as well as initiating non-specific inflammation to the pathogen. In the **effector phase**, neutrophils and macrophages (innate immunity) and antibodies and effector T lymphocytes (adaptive immunity) eliminate the antigen.

In terms of disease, like other organs, the immune system may fail (immunodeficiency), may become malignant (lymphoid malignancies) or may produce aberrant responses (such as in autoimmunity or allergy). This chapter describes the normal immune system in order to lay the basis for discussing these ways in which it can go wrong and so cause disease.

1.2 Key molecules

Many types of molecules play vital roles in both phases of immune responses; some are *shared by both the innate and the adaptive systems*. Antigens are substances that are recognized by immune components. Detection molecules on innate cells recognize general patterns of 'foreignness' on non-mammalian cells, whereas those on adaptive cells are specific for a wide range of very particular molecules or fragments of molecules. Antibodies are not only the surface receptors of B cells (BCRs) that recognize specific antigens, but, once the appropriate B cells are activated and differentiate into plasma cells, antibodies are also secreted into blood and body fluids in large quantities to prevent that antigen from causing damage. T cells have structurally similar receptors for recognizing antigens, known as T-cell receptors (TCRs). Major histocompatibility complex (MHC) molecules provide a means of self-recognition and also play a fundamental role in T lymphocyte effector functions.

Effector mechanisms are often dependent on messages from initiating or regulating cells; soluble mediators, which carry messages between cells, are known as interleukins, cytokines and chemokines.

Table 1.1 Components of innate and adaptive immunity

Features	Innate	Adaptive
Foreign molecules recognized	Structures shared by microbes, recognized as patterns (e.g. repeated glycoproteins), PAMPs	Wide range of very particular molecules or fragments of molecules on all types of extrinsic and modified self-structures
Nature of recognition receptors	Germline encoded – limited PRRs	Somatic mutation results in a wide range of specificities and affinities
Speed of response	Immediate	Time for cell movement and interaction between cell types
Memory	None	Efficient
Humoral components	Complement components	Antibodies
Cellular components	Dendritic cells, neutrophils, macrophages, NK cells, NKT cells, B1 cells, epithelial cells, mast cells	Lymphocytes – T (Th1, Th2, Th17, Tregs), B
iNKT cells, γδ T cells		

NK, natural killer; PAMPs, pathogen-associated molecular patterns; pattern-recognition receptors (PRRs); Tregs, regulatory T cells.

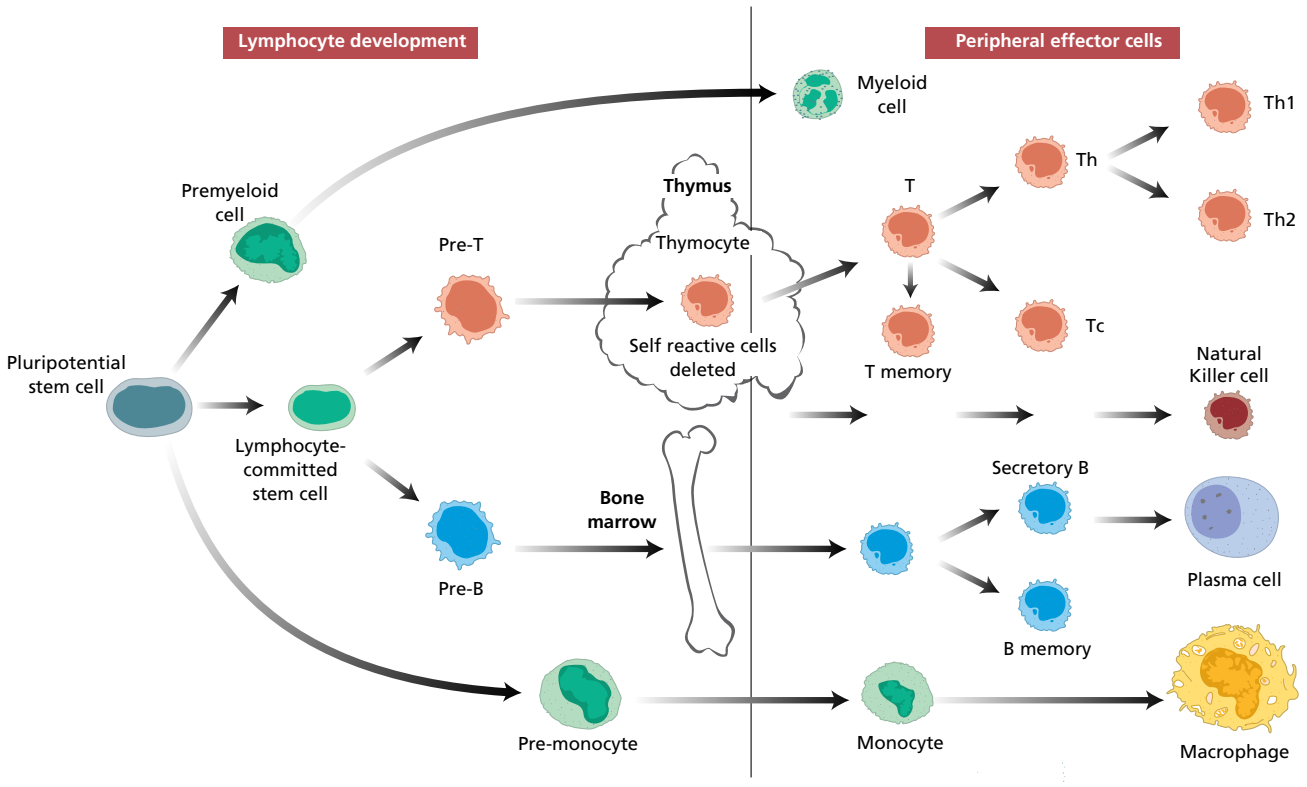


Fig. 1.1 Development of different types of blood cells from a pluripotential stem cell in the bone marrow. The developmental pathway for natural killer (NK) cells is shown separately because it is thought that NK cells may develop in both the thymus and the bone marrow.

1.2.1 Molecules recognized by immune systems

Foreign substances are recognized by both the innate and adaptive systems, but in different ways, using different receptors (see section 1.2.2). The innate system is activated by 'danger signals', due to pattern-recognition receptors on DCs recognizing conserved microbial structures directly, often repeated polysaccharide molecules, known as **pathogen-associated molecular patterns**. Toll-like receptors (receptors that serve a

similar function to toll receptors in drosophila) make up a large family of **non-antigen-specific** receptors for a variety of individual bacterial, viral and fungal components such as DNA, lipoproteins and lipopolysaccharides. Activation of DCs by binding to either of these detection receptors leads to inflammation and *subsequently activation of the adaptive system*.

Phagocytic cells also recognize particular patterns associated with potentially damaging materials, such as lipoproteins and other charged molecules or peptides.

Traditionally, **antigens** have been defined as molecules that interact with components of the adaptive system, i.e. T- and B-cell recognition receptors and antibody. *An antigenic molecule may have several antigenic determinants (epitopes)*; each **epitope** can bind with an individual antibody, and a single antigenic molecule can therefore provoke many antibody molecules with different binding sites. Some low-molecular-weight molecules, called **haptens**, are unable to provoke an immune response themselves, although they can react with existing antibodies. Such substances need to be coupled to a carrier molecule in order to have sufficient epitopes to be antigenic. For some chemicals, such as drugs, the carrier may be a host (auto) protein. The tertiary structure, as well as the amino acid sequence, is important in determining antigenicity. Pure lipids and nucleic acids are poor antigens, although they do activate the innate system and can be inflammatory.

Antigens are conventionally divided into thymus-dependent and thymus-independent antigens. **Thymus-dependent antigens** require T-cell participation to provoke the production of antibodies; most proteins are examples. **Thymus-independent antigens** require no T-cell cooperation for antibody production; they directly stimulate specific B lymphocytes by virtue of their ability to cross-link antigen receptors on the B-cell surface, produce predominantly IgM and IgG_2 antibodies and provoke poor immunological memory. Such antigens include bacterial polysaccharides, found in bacterial cell walls. Endotoxin, another thymus-independent antigen, not only causes specific B-cell activation and antibody production, but also acts as a stimulant for all B cells regardless of specificity.

Factors other than the intrinsic properties of the antigen can also influence the quality of the immune response (Table 1.2). Substances that improve an immune response to a separate, often rather weak, antigen are known as **adjuvants**. The use of adjuvants in humans, important in vaccines against infective agents and tumours, is discussed in Chapter 7, section 7.3.2.

Superantigen is the name given to those foreign proteins that are not specifically recognized by the adaptive system but do activate large numbers of T cells regardless of specificity, via direct action with an invariant part of the TCR (see Chapter 2, section 2.4.2).

Self-antigens are not recognized by DCs, so inflammation and co-stimulation of T cells (see section 1.4.1) is not induced. There are mechanisms to control any aberrant adaptive responses to self-antigens, by prevention of the production of specific receptors and regulation of the response if the immune system is fooled into responding (see Chapter 5).

Table 1.2 Factors influencing the immune response to an antigen, i.e. its immunogenicity

1	Nature of molecule: Protein content Size Solubility
2	Dose: Low doses provoke small amounts of antibody with high affinity and restricted specificity Moderate doses provoke large amounts of antibody but mixed affinity and broad specificity High doses provoke tolerance
3	Route of entry: ID, IM, SC→regional lymph nodes IV→spleen Oral→Peyer's patches Inhalation→bronchial lymphoid tissue
4	Addition of substances with synergistic effects, e.g. adjuvants
5	Genetic factors of recipient animal: Species differences Individual differences

ID, intradermal injection; IM, intramuscular injection; IV, intravenous injection; SC, subcutaneous injection.

1.2.2 Recognition molecules

There are several sets of detection molecules on DCs (Table 1.3): pattern-recognition receptors (PRRs), such as Toll-like receptors, as well as chemotactic receptors and phagocytic receptors. **PRRs** may be soluble or attached to cell membranes. Mannan-binding lectin is a protein that binds sugars on microbial surfaces; if attached to a macrophage, it acts as a trigger for phagocytosis and, if soluble, it activates the complement cascade, resulting in opsonization. Others belonging to this family are less well defined.

Toll-like receptors (TLRs) are part of this family too and are expressed either on the cell surface or intracellularly on endosomal membranes (Table 1.4). These are evolutionarily conserved proteins found on macrophages, DCs and neutrophils. At least ten different TLRs are found in humans, each TLR recognizing a range of particular motifs on pathogens, such as double-stranded RNA of viruses (TLR3), lipopolysaccharides of Gram-negative bacterial cell walls (TLR4), flagellin (TLR5) and bacterial DNA (TLR9), all highly conserved motifs unique to microorganisms. Upon binding to their ligands, TLRs induce signal transduction, via a complex cascade of intracellular adaptor molecules and kinases, culminating in the induction of nuclear factor kappa B transcription factor (NFκB)-dependent gene expression and the induction of proinflammatory cytokines (Fig. 1.2). The clinical consequences of a defective TLR pathway are discussed in Chapter 3, section 3.4.1 (see Box 1.1 in this chapter also).

Inflammasomes are a complex of intracellular proteins that are assembled in response to sensing pathogen-associated

Table 1.3 Markers on dendritic cells

	Immature dendritic cells	Mature myeloid dendritic cells
Function	Antigen capture	Antigen presentation to immature T cells for specific differentiation
Co-stimulatory molecule expression, e.g. CD80, CD86	Absent or low	++
Adhesion molecules, e.g. ICAM-1	Absent or low	++
Cytokine receptors, e.g. IL-12R	Absent or low	++
Pattern-recognition receptors, e.g. mannose receptor	++	–
MHC class II:		
Turnover	Very rapid	Persist >100 h
Density	Reduced (approx. 1×10^6)	Very high (approx. 7×10^6)

ICAM-1, Intercellular adhesion molecule-1; IL, interleukin; MHC, major histocompatibility complex.

Table 1.4 Location and ligands for toll-like receptors (TLRs)

TLR	Location	Ligand
TLR1, TLR2	Cell surface	Bacterial lipopeptides, peptidoglycan
TLR3	Endosomal membrane	ds viral RNA
TLR4	Cell surface	Lipopolysaccharide
TLR6	Cell surface	Bacterial lipopeptides
TLR7	Endosomal membrane	ssRNA
TLR8	Endosomal membrane	ssRNA
TLR9	Endosome	CpG DNA

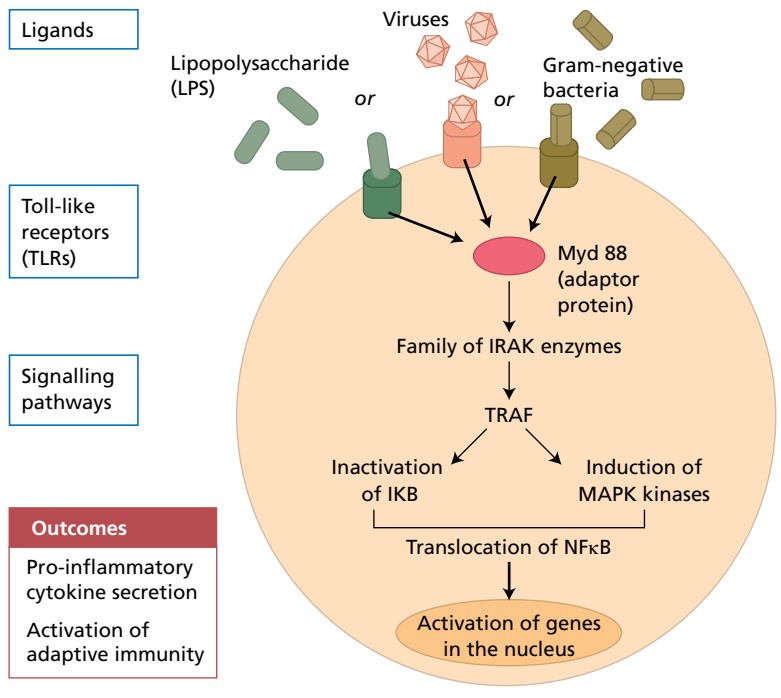


Fig. 1.2 Sequential cellular events induced by engagement of Toll-like receptors on dendritic cells, neutrophils and macrophages by microbial ligands. IKB, inhibitor kappa B; IRAK, interleukin-1 receptor-associated kinase; MAPK, mitogen-activated protein kinase; TRAF, TNF receptor-associated factor.

Box 1.1 Clinical consequences of a defective Toll-like receptor pathway

In humans, deficiency of IRAK-4 (interleukin-1 receptor-associated kinase) or MyDD88, key intracellular molecules responsible for TLR signal transduction (Fig. 1.2), is associated with recurrent pyogenic bacterial infections accompanied by failure to mount an appropriate acute-phase response (see Chapter 3, Case 3.6).

Mice lacking TLR4 are exceptionally susceptible to infection with Gram-negative bacteria.

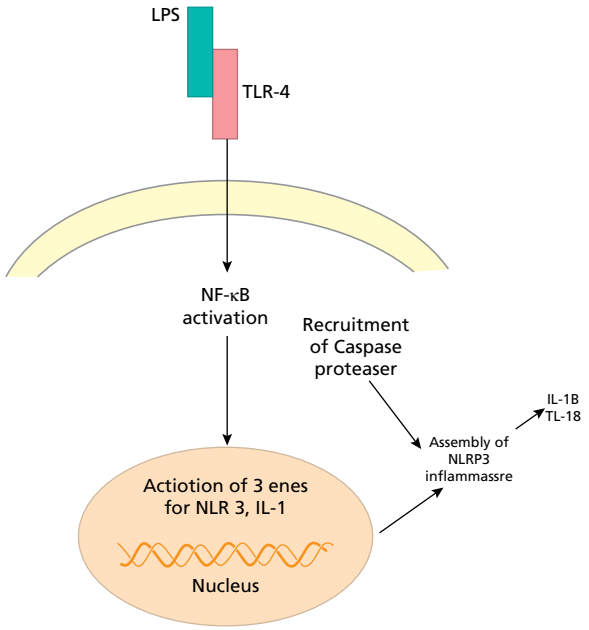


Fig. 1.3 Schematic representation of NLRP3 inflammasome activation.

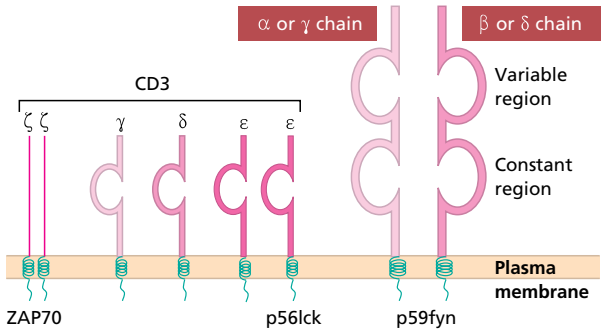


Fig. 1.4 Diagram of the structure of the T-cell receptor (TCR). The variable regions of the alpha (α) and beta (β) chains make up the T idiotype, i.e. antigen/peptide-binding region. The TCR is closely associated on the cell surface with the CD3 protein that is essential for activation.

molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), derived from external pathogens or damaged host cells (Fig. 1.3). A typical inflammasome is built around a scaffold containing NOD-like receptors (NLRs). An example of a clinically relevant, well-characterized inflammasome is NLRP3, also known as cryopyrin. NLRP3 plays a key role in innate immunity by orchestrating the release of proinflammatory cytokines, in particular interleukin (IL)-1 and IL-18. Mutations in the gene encoding cryopyrin are associated with a number of hereditary periodic fever syndromes (see Chapter 10), which are effectively treated by inhibitors of the IL-1 pathway (e.g. Anakinra).

CD1 molecules are invariant proteins (MHC-like and associated with β_2-microglobulin – see later) that are present on DCs and epithelial cells. CD1 combine with lipids, which are poor antigens and not usually well presented to the adaptive immune system, and so act as recognition molecules for the intestine and other microbial-rich surfaces. CD1 present lipids to the immune cells of the gut in particular, namely non-MHC-restricted natural killer (NK) T cells and γδ T cells in the epithelium.

Antigenic epitopes, having been processed by DCs, are recognized by cells of the adaptive system by means of specific receptors. *Each T cell, like B cells, is pre-committed to a given epitope*. It recognizes this by one of two types of **TCRs**, depending on the cell's lineage and thus its effector function. T cells have either αβTCR – a heterodimer of alpha (α) and beta β) chains – or γδTCR – a heterodimer of gamma γ and delta (δ) chains. αPTCR cells predominate in adults, although 10% of T cells in epithelial structures are of the γδTCR type. In either case, TCRs are associated with several transmembrane proteins that make up the cluster differentiation 3 (CD3) molecule (Fig. 1.4), to make the CD3–TCR complex responsible for taking the antigen recognition signal inside the cell (signal transduction). Signal transduction requires a group of intracellular tyrosine kinases (designated p56 lck, p59 fyn, ZAP 70) to join with the cytosolic tails of the CD3–TCR complex and become phosphorylated. Nearby accessory molecules, CD2, LFA-1, CD4 and CD8, are responsible for increased adhesion (see section 1.2.6), but are not actually involved in recognizing presented antigenic fragments.

The genes for TCR chains are on different chromosomes: β and γ on chromosome α7 and α and δ on chromosome 14. Each of the four chains is made up of a variable and a constant domain. The variable regions are numerous (although less so than immunoglobulin-variable genes); they are joined by D and J region genes to the invariant (constant) gene by recombinases, RAG1 and RAG2, *the same enzymes used for making antigen receptors on B cells and antibodies* (section 1.4.1). The **diversity of T-cell antige0.n receptors** is achieved in a similar way for immunoglobulin, although *TCRs are less diverse since somatic mutation is not involved*; perhaps the risk of 'self-recognition' would be too great. The diversity of antigen binding is dependent on the large number of V genes and the way

in which these may be combined with different D and J genes to provide different V domain genes. The similarities between TCRs and BCRs led to the suggestion that the genes evolved from the same parent gene and both are *members of a 'supergene' family*. Unlike immunoglobulin, TCRs are not secreted and are not independent effector molecules.

A particular TCR complex recognizes a processed antigenic peptide in the context of MHC class I or II antigens (section 1.4.1), depending on the type of T cell; helper T cells recognize class II with antigen, and this process is enhanced by the surface accessory protein CD4 (see later) and intracellular signals. Cytotoxic T cells (CTL/Tc) recognize antigens with class I (see section 1.3.1) and use CD8 accessory molecules for increased binding and signalling. Since the number of variable genes available to TCRs is more limited, reactions with antigen might not be sufficient if it were not for the increased binding resulting from these **accessory mechanisms**. Recognition of processed antigen alone is not enough to activate T cells. Additional signals, through soluble cytokines (interleukins), are needed; some of these are generated during 'antigen processing' (see section 1.4.1).

MHC molecules were originally known as 'histocompatibility antigens' because of the vigorous reactions they provoked during mismatched organ transplantation. However, these molecules are known to play a fundamental role in immunity by presenting antigenic peptides to T cells. Histocompatibility antigens in humans (known as human leucocyte antigens, HLAs) are synonymous with the MHC molecules. MHC molecules are cell-surface glycoproteins of two basic types: class I and class II (Fig. 1.5). They exhibit extensive genetic polymorphism with multiple alleles at each locus. As a result, genetic variability between individuals is very great and most unrelated individuals possess different MHC (HLA) molecules. This means that it is very difficult to obtain perfect HLA matches between unrelated persons for transplantation (see Chapter 8). The **extensive polymorphism in MHC molecules** is best explained by the need of the immune system to cope with an ever-increasing range of pathogens adept at evading immune responses (see Chapter 2).

The TCR of an individual T cell will only recognize antigen as a complex of antigenic peptide and self-MHC (Fig. 1.6). This process of dual recognition of peptide and MHC molecule is known as MHC restriction, since the MHC molecule restricts the ability of the T cell to recognize antigen (Fig. 1.6). The importance of MHC restriction in the immune response was recognized by the award of the Nobel Prize in Medicine to Peter Doherty and Rolf Zinkernagel, who found that virus-specific CTLs would only kill cells of the same particular allelic form of MHC molecule.

MHC class I antigens are subdivided into three groups: A, B and C. Each group is controlled by a different gene locus within the MHC region on chromosome 6 (Fig. 1.7) in humans (different in mice). The products of the genes at all three loci are chemically similar. All MHC class I antigens (see Fig. 1.5) are made up of an a heavy chain, controlled by a gene in the relevant MHC locus, associated with a smaller chain called β_2-microglobulin, controlled by a gene on chromosome 12. The differences between individual MHC class I antigens are due to variations in the a chains; the β_2-microglobulin component is constant. The detailed structure of class I antigens was determined by X-ray crystallography. This shows that small antigenic peptides (approx. nine amino acids long) can be tightly bound to a groove produced by the pairing of the two extracellular domains (a_1 and a_2) of the a chain. The *affinity (tightness of binding) of individual peptide binding depends on the nature and shape of the groove*, and accounts for the MHC restriction mentioned earlier.

MHC class II antigens have two heavy chains, α and β, both coded for by genes in the MHC region of chromosome 6. The detailed structure of MHC class II antigens was also

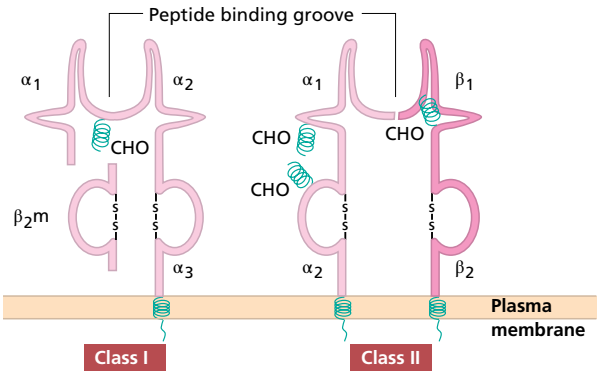


Fig. 1.5 Diagrammatic representation of major histocompatibility complex (MHC) class I and class II antigens. CHO, carbohydrate side chain; β_2m, β_2-microglobulin.

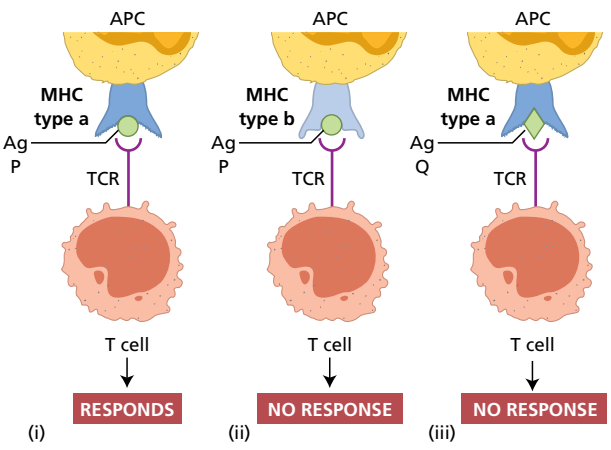


Fig. 1.6 Major histocompatibility complex (MHC) restriction of antigen recognition by T cells. T cells specific for a particular peptide and a particular MHC allele (i) will not respond if the same peptide were to be presented by a different MHC molecule as in (ii) or as in (iii) if the T cell were to encounter a different peptide. APC, antigen-presenting cell; TCR, T-cell receptor.

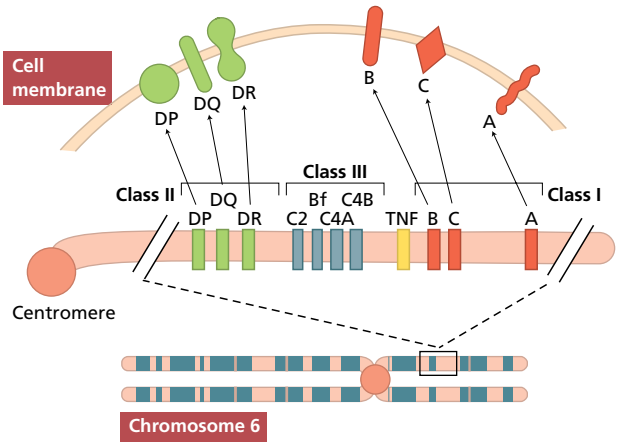


Fig. 1.7 Major histocompatibility complex on chromosome 6; class III antigens are complement components. TNF, tumour necrosis factor.

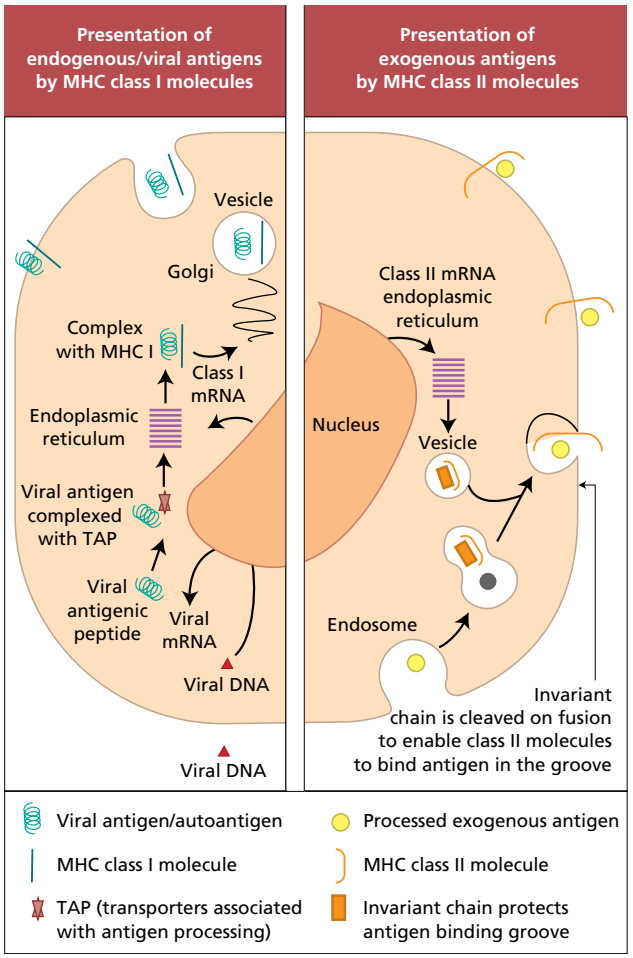


Fig. 1.8 Different routes of antigen presentation, depending on the nature of the antigen. MHC, major histocompatibility complex.

determined by X-ray crystallography. It has a folded structure similar to class I antigens, with the peptide-binding groove found between the α and β chains (see Fig. 1.5). Whereas most nucleated cells express class I molecules, *expression of class II molecules is restricted to a few cell types*: DCs, B lymphocytes, *activated* T cells, macrophages, *inflamed* vascular endothelium and some epithelial cells. However, other cells (e.g. thyroid, pancreas, gut epithelium) can be induced to express class II molecules under the influence of interferon (IFN)-γ released during inflammation. In humans, there are three groups of variable class II antigens: the loci are known as HLA-DP HLA-DQ and HLA-DR.

In practical terms, there are different mechanisms by which antigens in different intracellular compartments can be captured and presented to $CD4^+$ or $CD8^+$ T cells (Fig. 1.8). **Endogenous antigens** (including viral antigens that have infected host cells) are processed by the endoplasmic reticulum and presented by MHC class I-bearing cells exclusively to $CD8^+$ T cells. Prior to presentation on the cell surface, endogenous antigens are broken down into short peptides, which are then actively transported from the cytoplasm to endoplasmic reticulum by proteins. These proteins act as a shuttle and are so named 'transporters associated with antigen processing' (TAP-1 and TAP-2). TAP proteins (also coded in the MHC class II region) deliver peptides to MHC class I molecules in the endoplasmic reticulum, from whence the complex of MHC and peptide is delivered to the cell surface. Mutations that affect function in either TAP gene prevent surface expression of MHC class I molecules.

In contrast, **exogenous antigens** are processed by the lysosomal route and presented by MHC class II antigens to $CD4^+$ T cells (Fig. 1.8). As with MHC class I molecules, newly synthesized MHC class II molecules are held in the endoplasmic reticulum until they are ready to be transported to the cell surface. While in the endoplasmic reticulum, class II molecules are prevented from binding to peptides in the lumen by a protein known as MHC class II-associated invariant chain. The invariant chain also directs delivery of class II molecules to the endosomal compartment, where exogenous antigens are processed and made available for binding to class II molecules.

The **MHC class III region** (see Fig. 1.7) contains genes encoding proteins that are involved in the complement system (see section 1.4.1): the early components C4 and C2 of the classical pathway and factor B of the alternative pathway. Some inflammatory proteins, e.g. tumour necrosis factor (TNF), are also encoded in adjacent areas. Invariant MHC-like proteins, such as CD1 lipid-recognition receptors (see earlier), are not coded for on chromosome 6, despite being associated with β_2-microglobulin.

In contrast to TCRs, the antigen BCRs are **surface-bound immunoglobulin** molecules that can be secreted as soluble molecules. As with TCRs, they have predetermined specificity for epitopes and are therefore extremely diverse. *The immune system has to be capable of recognizing all pathogens, past and future*. Such diversity is provided by the way in which all three types of molecules, TCR, BCR and antibody, are produced.

The **basic structure of the immunoglobulin** molecule is shown in Fig. 1.9. It has a four-chain structure: two identical

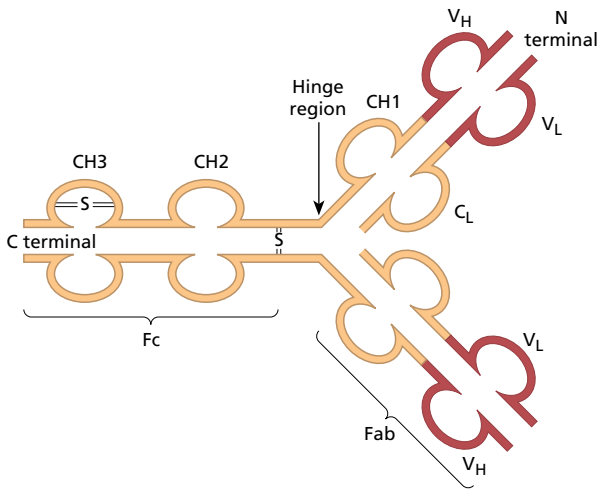


Fig. 1.9 Basic structure of an immunoglobulin molecule. Domains are held in shape by disulfide bonds, though only one is shown. $C_{1\text{-}3}$, constant domain of a heavy chain; C_L, constant domain of a light chain; =S=, disulfide bond; V_H, variable domain of a heavy chain; V_L, variable domain of a light chain.

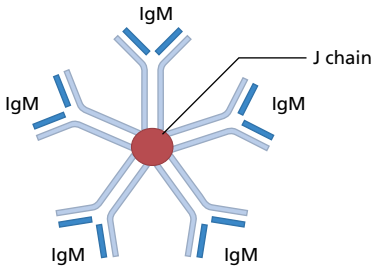


Fig. 1.10 Schematic representation of immunoglobulin (Ig)M pentamer (MW 800 kDA).

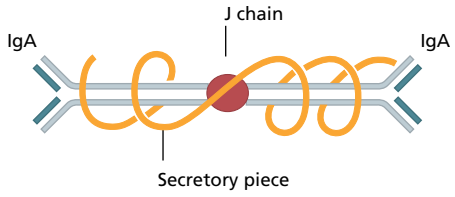


Fig. 1.11 Schematic representation of dimeric secretory immunoglobulin (Ig)A (MW 385 kDA).

heavy (H) chains (mol. wt. 50 kDa) and two identical light (L) chains (mol. wt. 25 kDa). Each chain is made up of domains of about 110 amino acids held together in a loop by a disulphide bond between two cysteine residues in the chain. The domains have the same basic structure and many areas of similarity in their amino acid sequences. The heavy chains determine the isotype of the immunoglobulin, resulting in pentameric IgM (Fig. 1.10), dimeric IgA (Fig. 1.11) or monomeric IgG.

The amino (N) terminal domains of the heavy and light chains include the **antigen-binding site**. The amino acid sequences of these N-terminal domains vary between different antibody molecules and are known as variable (V) regions. Most of these differences reside in three hypervariable areas of the molecule, each only 6–10 amino acid residues long. In the folded molecule, these hypervariable regions in each heavy and light chain come together to form, with their counterparts on the other pair of heavy and light chains, the antigen-binding site (Fig. 1.9). The structure of this part of the antibody molecule is unique to that molecule and is known as the **idiotypic determinant**. In any individual, approximately 10^6–10^7 different antibody molecules could be made up by 10^3 different heavy chain variable regions associating with 10^3 different light chain variable regions, though there are even more epitopes due to further variation during the later processing (see section 1.4.1).

The part of the immunoglobulin chain next to the V region in either heavy or light chains is the constant (C) region; this is made up of one domain in a **light chain** (C_L) and three or four in a **heavy chain** (C_H) (Fig. 1.9). There are two alternative types of C_L chain, known as kappa (κ) and lambda (λ); an antibody molecule has either two κ or two λ light chains, *never one of each*. Of all the antibodies in a human individual, roughly 60% contain κ and 40% contain λ light chains. There are no known differences in the functional properties between κ and λ light chains. In contrast, there are several possible different types of C_H domain, each with important functional differences (Table 1.5). The heavy chains determine the **isotype** of the antibody and the ultimate physiological function of the particular antibody molecule. Once the antigen-binding site has reacted with its antigen, the molecule undergoes a change in the conformation of its heavy chains in order to take part in effector reactions, depending on the isotype of the molecule.

The processes by which the components of this supergene family are produced are identical for TCR and BCR and are known as **recombination.** Immunoglobulin production, whether for BCR or antibody production, is the same initially. As for the TCR, the genes for the different chains in a BCR are carried on different chromosomes (Fig. 1.12). Like those coding for other macromolecules, the genes are broken up into coding segments (exons) with intervening silent segments (introns). The heavy chain gene set on chromosome 14 is made up of small groups of exons representing the constant regions of the heavy chains – e.g. mu (μ) chain – and a very large number of V region genes, perhaps as many as 10^3. Between the V and C genes are two small sets of exons, D and J (Fig. 1.12). In a single B cell, one V region gene is selected, joined to one D and J on the same chromosome; the VDJ product is then joined at the level of RNA processing to Cμ when the B cell is making IgM. The cell can make IgG by omitting the Cμ and joining VDJ to a Cγ. Thus, the cell can make IgM, IgD and IgG/A/E in sequence, while still using the same variable region. *The same enzymes are used for the TCRs, and coded for by two recombination-activating genes controlling VDJ gene recombination: RAG1 and RAG2.* Disruption of the RAG1 or RAG2

Table 1.5 Immunoglobulin classes and their functions

Isotype	Heavy chain	Serum concentration*	Main function	Complement fixation†	Placental passage	Reaction with Fc receptors‡
IgM	μ	0.5–2.0	Neutralization and opsonization	+++	–	L
IgG_1	γ1	5.0–12.0	Opsonization	+++	++	M, N, P, L, E
IgG_2	γ2	2.0–6.0		+	±	P, L
IgG_3	γ3	0.5–1.0	Opsonization	+++	++	M, N, P, L, E
IgG_4	γ4	0.1–1.0		–	+	N, L, P
IgA_1	α1	0.5–3.0	Neutralization at mucosal surfaces	–	–	M, N
IgA_2	α2	0.0–0.2		–	–	–
IgD	δ	Trace	Lymphocyte membrane receptor	–	–	–
IgE	εΣ	Trace	Mast cell attachment	–	–	B, E, L

*Normal adult range in g/L.
†Classical pathway.
‡Fc receptors on: basophils/mast cells, B; eosinophils, E; lymphocytes, L; macrophages, M; neutrophils, N; platelets, P.

function in infants who have mutations in these genes causes profound immune deficiency, characterized by absent mature B and T cells, as neither TCRs or BCRs can be produced. On a different chromosome (either chromosome 22 for λ chains or chromosome 2 for κ chains) in the same cell, a V gene is joined to a J gene (there is no D on the light chain) and then the VJ product is joined at the RNA level to the Cκ or Cλ (Fig. 1.12).

The **wide diversity of antigen binding** is dependent on the large number of V genes and the way in which these may be combined with different D and J genes to provide different rearranged VDJ gene segments. Once V, D and J rearrangement has taken place to produce a functional immunoglobulin molecule, *further V region variations are introduced only at a much later stage,* when antibodies rather than BCRs are produced by the process of somatic mutation in germinal centres.

Natural killer cells also have recognition molecules. These cells are important in killing virally infected cells and tumour cells. They have to be able to recognize these targets and distinguish them from normal cells. They recognize and kill cells that have reduced or absent MHC class I, using two kinds of receptors – inhibitory (KIR) and activating (KAR) – to estimate the extent of MHC expression. They also have one type of Fc IgG (Fcγ) receptor, that for low-affinity binding of IgG antibodies, and so NK cells are able to kill some cells with large amounts of antibody on their surfaces. Further subsets of NK-like cells that contribute to innate immunity

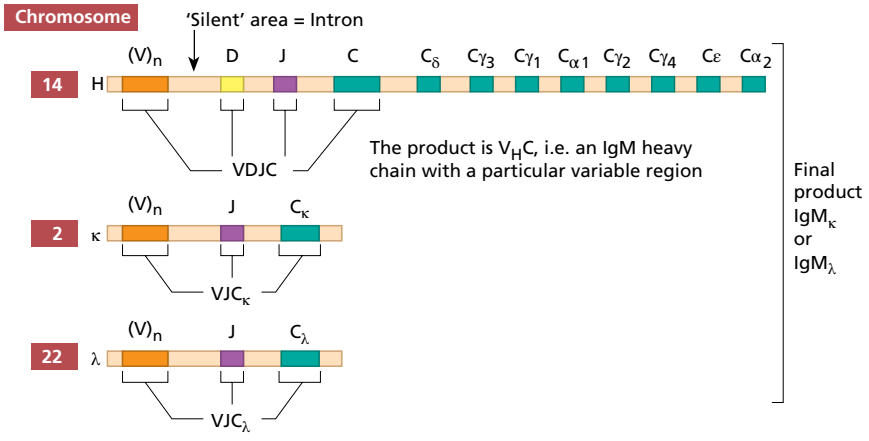


Fig. 1.12 Immunoglobulin genes (see text for explanation).

include NKT cells and invariant NKT cells (section 1.3.6); these are thought to be particularly important in tumour immunology (section 1.5.1).

The major purpose of the complement pathways is to provide a means of removing or destroying antigens, regardless of whether or not these are coated with antibody. This requires that *complement components recognize* damaging material such as immune complexes (antigen combined with antibodies) or foreign antigens. The complement pathways are discussed in more detail in section 1.3.5.

1.2.3 Accessory molecules

The binding of a processed antigen–MHC class II complex on an antigen-presenting cell (APC) to the corresponding TCR provides an insufficient signal for T-cell activation; the binding of accessory molecules on the two cell surfaces provides additional stimuli. Accessory molecules are lymphocyte surface proteins, distinct from the antigen-binding complexes, which are necessary for **efficient binding, signalling and homing**. *Accessory molecules are invariant, non-polymorphic proteins*. Each accessory molecule has a particular ligand – a corresponding protein to which it binds. These ligands are present on all cells that require close adhesion for functioning; for example, there are those on T cells for each of the many cell types that can activate or respond to T cells (APCs, endothelial cells, etc.); similar ligands are present on B cells for efficiency of T-cell help as well as stimulation by follicular DCs.

There are several families of accessory molecules, but the most important appear to be the **immunoglobulin supergene family of adhesion molecules**, which derives its name from the fact that its members contain a common immunoglobulin-like structure. Members of the family strengthen the interaction between APCs and T cells (Fig. 1.13); those on T cells include CD4, CD8, CD28, CTLA-4, CD45R, CD2 and lymphocyte function antigen 1 (LFA-1). For interaction with B cells, CD40 ligand and ICOS are important for class switching (see section 1.4.3). Adhesion molecules, for binding leucocytes (both lymphocytes and polymorphonuclear leucocytes) to endothelial cells and tissue matrix cells, are considered in section 1.2.6. On B cells, such molecules include CD40 (ligand for CD40L, now named CD154; see Chapter 3, Case 3.2), B-7-1 and B7-2 (ligands for CD28).

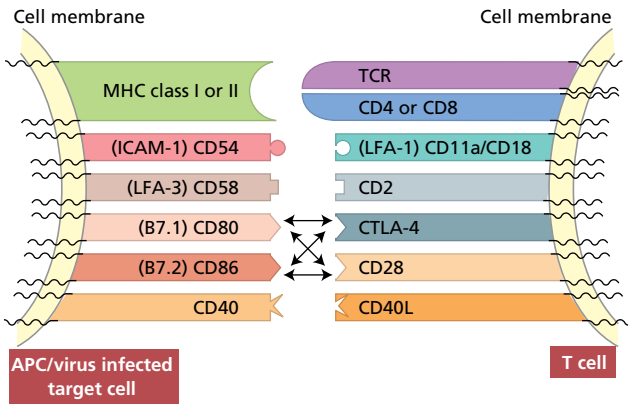


Fig. 1.13 Diagrammatic representation of adhesion molecules on T cells and their ligands on antigen-presenting cells (APCs)/virus-infected target cells. MHC, major histocompatibility complex; TCR, T-cell receptor.

1.2.4 Effector molecules for immunity

There are humoral and cellular effector molecules in both the innate and the adaptive immune systems (Table 1.6). Several of the same mechanisms are used in both types of immune responses, especially in killing of target cells, suggesting that the evolution of immune responses has been conservative in terms of genes, though with much redundancy to ensure the life-preserving nature of the immune systems in the face of rapid evolution of pathogenic microbes.

Antibodies

Antibodies are the best-described effector mechanisms in adaptive immunity. They are the **effector arm of B cells** and are secreted as soluble molecules by plasma cells in large

Table 1.6 Effector molecules in immunity

	Innate	Adaptive
Humoral	Complement components for opsonization or lysis	Specific antibodies for opsonization and phagocytosis or lysis with complement
Cellular	Perforin in NK cells creates pores in target cell membranes	Perforin in cytolytic (CD8) T cells creates pores in specific target cell membranes, allowing entry of granzymes to cause apoptosis
		NKT cells induce apoptosis by perforin production
	Granzymes in NK cells induce apoptosis in target cells	
	Lysosomes in phagocytic vacuoles result in death of ingested microbes	
	Preformed histamine and related vasoactive substances as well as leukotrienes in mast cells	

NK, natural killer

quantities, to be carried in the blood and lymph to distant sites. As shown in Table 1.5, there are five major isotypes of antibodies, each with different functions (see also Box 1.2).

IgM is a large molecule whose major physiological role is intravascular neutralization of organisms (especially viruses). **IgM** has five complement-binding sites, resulting in excellent complement activation and subsequent removal of the antigen–antibody–complement complexes by complement receptors on phagocytic cells or complement-mediated lysis of the organism (see section 1.4).

IgG is a smaller immunoglobulin that penetrates tissues easily. Placental transfer is an active process involving specific placental receptors for the Fc portion of the IgG molecule, termed FcRn (Fc receptor of the neonate). The FcRn receptor is also present on epithelial and endothelial cells and is an important regulator of IgG metabolism (see Chapter 7, section 7.4 and Fig. 7.8). Of the four subclasses, IgG_1 and IgG_3 activate complement most efficiently and are responsible for clearing most protein antigens, including the removal of microorganisms by phagocytic cells (see section 1.5). IgG_2 and IgG_4 react predominantly with carbohydrate antigens (in adults) and are relatively poor opsonins (promoters of phagocytosis).

IgA is the major mucosal immunoglobulin. Attachment of the 'secretory piece' prevents digestion of this immunoglobulin in the intestinal and bronchial secretions. IgA_2 is the predominant subclass in secretions and neutralizes antigens that enter via these mucosal routes. IgA, the monomeric IgA in serum, is capable of neutralizing antigens that enter the circulation, but IgA_1 is sensitive to bacterial proteases and therefore less useful for host defence at mucosal surfaces. IgA has additional functions via its receptor (FcaR or CD89), present on mononuclear cells and neutrophils, for activation of phagocytosis, inflammatory mediator release and antibody-dependent cell-mediated cytotoxicity (ADCC) (see section 1.5).

There is little free **IgD or IgE** in serum or normal body fluids, since these act as surface receptors on mature B cells or mast cells, respectively.

As mentioned previously, mechanisms of recombination in immunoglobulin production, whether for BCR or antibody production, are the same initially (Fig. 1.12). Once V, D and J region rearrangement has taken place, **further variation is introduced when antibodies are made**, by the introduction of point mutations in the V region genes. This process, known as **somatic hypermutation,** occurs in the lymphoid germinal centres and is critically dependent on activation-induced cytidine deaminase (AID), an enzyme responsible for deamination of DNA. Somatic hypermutation helps to increase the possible number of combinations and accounts for the enormous diversity of antibody specificities (10^{14}), which by far exceeds the number of different B cells in the body (10^{10}).

Box 1.2 Immunoglobulin isotypes and their significance

IgM is phylogenetically the oldest class of immunoglobulin. It is a large molecule (Fig. 1.10) and penetrates poorly into tissues. IgM has five complement-binding sites, which results in excellent activation of the classical complement pathway.

IgG is smaller and penetrates tissues easily. It is the only immunoglobulin to provide immune protection to the neonate (Table 1.5) as IgG is actively transported across the placenta. There are four subclasses of IgG, with slightly different functions.

IgA is the major mucosal immunoglobulin – sometimes referred to as 'mucosal antiseptic paint'. IgA in mucosal secretions consists of two basic units joined by a J chain (Fig. 1.11); the addition of a 'secretory piece' prevents digestion of this immunoglobulin in the intestinal and bronchial secretions.

IgD is synthesized by antigen-sensitive B lymphocytes and is not secreted, acting as a cell-surface receptor for activation of these cells by the specific antigen relating to the B-cell receptor; it is essential for activation of antigen-responsive B cells.

IgE is produced by plasma cells, but is taken up by specific IgE receptors on mast cells and basophils. IgE then provides an antigen-sensitive way of expelling intestinal parasites by increasing vascular permeability and inducing chemotactic factors via mast cell degranulation (see section 1.7).

Cytokines and chemokines

Cytokines are soluble mediators secreted by macrophages or monocytes (monokines) or lymphocytes (lymphokines). These mediators act as **stimulatory or inhibitory signals** between cells; those between cells of the immune system were known as interleukins (a phrase that has fallen out of general usage since the range of soluble molecules has widened so tremendously, though the individual names persist to avoid confusion). As a group, cytokines share several common features (see Box 1.3). Among the array of cytokines produced by macrophages and T cells, IL-1 and IL-2 are of particular interest due to their pivotal role in amplifying immune responses. IL-1 acts on a wide range of targets (Table 1.7), including T and B cells. In contrast, the effects of IL-2 are largely restricted to lymphocytes. Although IL-2 was originally identified on account of its ability to promote the growth of T cells, it has similar trophic effects on IL-2 receptor-bearing B and NK cells. The considerable overlap between actions of individual cytokines and interleukins is summarized in Table 1.8.

Cytokines that induce chemotaxis of leucocytes are referred to as **chemokines**, a name derived from chemo + kine, i.e. something chemical to help movement. Some cytokines and interleukins have been redefined as chemokines as their function becomes clearer, e.g. IL-8 = CXCL8. Chemokines are structurally similar proteins of small molecule size (8–10 kDa), which are able to diffuse from the site of production to form a local concentration gradient along which granulocytes and

Box 1.3 Common features of cytokines

- Their half-lives are short, so any potential harm due to persistent action is controlled.
- They are rapidly degraded as another method of regulation and thus difficult to measure in the circulation.
- Most act locally within the cell's microenvironment, which confines their action to a particular site.
- Some act on the cell of production itself, promoting self-activation and differentiation through high-affinity cell-surface receptors.
- Many cytokines are pleiotropic in their biological effects, i.e. affecting multiple organs in the body.
- Most exhibit biologically overlapping functions, illustrating the redundancy of the group. For this reason, therapeutic targeting of individual cytokines in disease has had limited success so far (effects of deletion of individual cytokine genes are listed in Table 1.8).

Table 1.7 Actions of interleukin-1

Target cell	Effect
T lymphocytes	Proliferation
	Differentiation
	Lymphokine production
	Induction of IL-2 receptors
B lymphocytes	Proliferation
	Differentiation
Neutrophils	Release from bone marrow
	Chemoattraction
Macrophages	
Fibroblasts	Proliferation/activation
Osteoblasts	
Epithelial cells	
Osteoclasts	Reabsorption of bone
Hepatocytes	Acute-phase protein synthesis
Hypothalamus	Prostaglandin-induced fever
Muscle	Prostaglandin-induced proteolysis

IL, interleukin

lymphocytes can migrate towards the stimulus. There are two types of movement: migration of leucocytes to sites of inflammation and that of differentiating cells moving to a specific activation site (see section 1.2.5); chemokines are involved in both. There are therefore two main types: the i**nflammatory chemokines** (CXC), coded for by genes on chromosome 17 and attractants for granulocytes, and the **homeostatic chemokines**, acting as attractants for lymphocytes (CC) and coded by genes on chromosome 4. The corresponding receptors on inflammatory cells are designated CXCR on neutrophils and CCR on lymphocytes; there are exceptions!

Molecules for lysis and killing

The other major sets of effector molecules are the cytolytic molecules, though less is known about their diversity or mechanisms of action. They include **perforin**, a C9-like molecule present in secretory lysosomes in CD8 T cells and in NK cells that polymerizes to form pores to enable large proteins to enter the cell. These cell types also secrete **granzymes**, enzymes that induce apoptosis in target cells (Table 1.6). Macrophages and polymorphonuclear leucocytes also contain many substances for the destruction of ingested microbes, some of which have multiple actions, such as TNF. The duplication of the functions of this essential phylogenetically ancient protein during evolution underlines the continued development of mammalian immunity to keep up with microbial invaders.

1.2.5 Receptors for effector functions

Without **specific cytokine receptors** on the surface of the target cells, cytokines are ineffective; this has been demonstrated in those primary immune deficiencies in which gene mutations result in absence or non-functional receptors, such as the commonest X-linked form of severe combined immune deficiency (see Chapter 3, Case 3.5), IL-12 receptor or IFN-γ receptor deficiencies (see Chapter 3). Some cytokines may have unique receptors, but many others share a common structural chain, such as the γ-chain in the receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-23, suggesting that *these arose from a common gene originally*. There are other structurally similar cytokine receptors, leading to the classification of these receptors into five families of similar types of receptors, many of which have similar or identical functions, providing a safety net (redundancy) for their functions, which are crucial for both the innate and adaptive immune systems.

Chemokine receptors from a family of G protein-coupled receptors – meaning that they are transmembrane and able to activate internal signalling pathways. These receptors also function as differentiation 'markers', as they become expressed as an immune reaction progresses and cells move in inflammatory responses.

Receptors for the Fc portions of immunoglobulin molecules (FcR) are important for effector functions of phagocytic cells and NK cells. There are at least **three types of Fcγ receptors**: FcRγl are high-affinity receptors on macrophages and neutrophils that bind monomeric IgG for phagocytosis; FcRγII are low-affinity receptors for phagocytosis on macrophages and neutrophils and for feedback inhibition on B cells; and FcRγIII on NK cells as mentioned earlier. There are also FcRn involved in the transfer of IgG across the placenta and these receptors

Table 1.8 Clinically important cytokines grouped by effect on immune or inflammatory responses, to show source and site of action

Cytokines	Action
(a) Promotion of non-specific immunity and inflammation	
Interleukin-17 (IL-17)	Increases chemokine production for inflammatory cells
Interleukin-1 (IL-1)	(See Table 1.7)
Interleukin-6 (IL-6)	Growth and differentiation of T, B and haematopoietic cells
	Production of acute-phase proteins by liver cells
Interleukin-8 (now CXCL8)	Chemotaxis and activation of neutrophils and other leucocytes
Interferon-α (IFN-α)	Antiviral action by activation of NK cells, upregulation of MHC class I antigens on virally infected cells, inhibition of viral replication
Interleukin-5 (IL-5)	Activation of B cells, especially for IgE production
	Activation of eosinophils
Tumour necrosis factor (TNF)	Promotion of inflammation by: activation of neutrophils, endothelial cells, lymphocytes, liver cells (to produce acute-phase proteins)
	Interferes with catabolism in muscle and fat (resulting in cachexia)
Interferon-γ (IFN-γ)	Activation of macrophages, endothelial cells and NK cells. Increased expression of MHC class I and class II molecules in many tissues; inhibits allergic reactions (↓IgE production)
(b) Lymphocyte activation, growth and differentiation, i.e. specific immunity	
Interleukin-2 (IL-2)	Proliferation and maturation of T cells, induction of IL-2 receptors and activation of NK cells
Interleukin-4 (IL-4) and interleukin-5 (IL-5)	Induction of MHC class II, Fc receptors and IL-2 receptors on B and T cells
	Induction of isotype switch in B cells Facilitation of IgE production (mainly IL-4) Activation of macrophages Proliferation of bone marrow precursors
Interleukin-12 (IL-12)†	Synergism with IL-2; regulates IFN-y production Activation of NK cells
Interleukin-13 (IL-13)	Actions overlap with IL-4, including induction of IgE production
	IL-13 receptor acts as a functional receptor for IL-4
Interleukin-15 (IL-15)	Similar to IL-12
Interleukin-16 (IL-16)	Chemotaxis and activation of CD4 T cells
(c) Colony stimulation of bone marrow precursors	
GM-CSF	Stimulates growth of polymorph and mononuclear progenitors
G-CSF	Stimulates growth of neutrophil progenitors
M-CSF	Stimulates growth of mononuclear progenitors
(d) Regulatory cytokines	
Interleukin-10 (IL-10); also called cytokine synthesis inhibitory factor‡	Inhibition of cytokine production Growth of mast cells
Transforming growth factor-ß (TGF-ß)	Antiinflammatory Inhibits cell growth

Table 1.8 (*Continued*)

Cytokines	Action
(e) Chemokines	
Interleukin-8 (IL-8)	See under section (a)
RANTES (regulated on activation, normal T cell expressed and secreted)	Chemoattractant for eosinophils, monocytes
Monocyte chemotactic protein (MCP 1, 2, 3)	Chemoattractant for monocytes
Exotaxin	Chemoattractant for eosinophils; synergistic with IL-5

Evidence from murine models. See appendix for web address for update on knockout mice.
†IL-12 family of cytokines includes IL-23 and IL-27.
‡IL-10 family includes IL-19, IL-20 and IL-22.
G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; Ig, immunoglobulin; M-CSF, macrophage colony-stimulating factor; MHC, major histocompatibility complex; NK, natural killer.

are involved in IgG recirculation and catabolism. IgE receptors are found on mast cells, basophils and eosinophils for triggering degranulation of these cells. IgA receptors ensure the transport of polymeric IgA across the mucosal cells and other, possibly important functions are slowly being defined.

Complement receptors for fragments of C3 produced during complement activation also provide a mechanism for phagocytosis and are found on macrophages and neutrophils. However, there are several types of **complement receptors**: those on red blood cells for transport of immune complexes for clearance (CR1), those on B cells and follicular DCs in lymph nodes to trap antigen to stimulate a secondary immune response (CR2) (see section 1.4.3), and those on macrophages, neutrophils and NK cells to provide adhesion of these blood cells to endothelium, prior to movement into tissues (CR3).

1.2.6 Adhesion molecules

Adhesion molecules comprise another set of cell surface glycoproteins with a pivotal role, not only in immune responses by **mediating cell-to-cell adhesion**, but also for **adhesion between cells and extracellular matrix proteins**. Adhesion molecules are grouped into two major families: (i) integrins and (ii) selectins (Table 1.9). The migration of leucocytes to sites of inflammation is dependent on three key sequential steps mediated by adhesion molecules (Fig. 1.14): rolling of leucocytes along activated endothelium is selectin dependent; tight adhesion of leucocytes to endothelium is integrin dependent; and transendothelial migration occurs under the influence of chemokines. Cytokines also influence the selectin- and integrin-dependent phases.

Integrins are heterodimers composed of non-covalently associated α and β subunits. Depending on the structure of the β subunit, integrins are subdivided into five families (β_1 to β_5 integrins). β_1 and β_2 integrins play a key role in leucocyte–endothelial interaction. β_1 integrins mediate lymphocyte and monocyte binding to the endothelial adhesion receptor called vascular cell adhesion molecule (VCAM-1). β_2 integrins share a common β chain (CD18) that pairs with a different α chain (CD11a, b, c) to form three separate molecules (CD11a CD18, CD11b CD18, CD11c CD18); they also mediate strong binding of leucocytes to the endothelium. Examples in other systems include β_3 to β_5 integrins mediating cell adhesion to extracellular matrix proteins such as fibronectin and vitronectin in the skin and laminin receptor in muscle.

The **selectin** family is composed of three glycoproteins designated by the prefixes E (endothelial), L (leucocyte) and P (platelet) to denote the cells on which they were first described. Selectins bind avidly to carbohydrate molecules on leucocytes and endothelial cells and regulate the homing of the cells to sites of inflammation (see section 1.6.1, Chapter 11, section 11.1 and Table 1.10).

1.3 Functional basis of innate responses

The aim of an immune response is to destroy foreign antigens, whether these are inert molecules or invading organisms. To reach the site of invasion and destroy the pathogens, the components of the immune systems have to know where to go and to how to breach the normal barriers, such as the endothelial cells of the vascular system. Humoral factors (such as antibodies and complement) are carried in the blood and enter tissues following an increase in permeability associated with **inflammation**. Immune cells (innate and antigen specific) are actively attracted to a site of inflammation and enter the tissues via specific sites using active processes of adhesion.

Table 1.9 Examples of clinically important adhesion molecules

Adhesion molecule	Ligand	Clinical relevance of interaction	Consequences of defective expression
β_1 integrin family			
VLA-4 (CD49d-CD29) expressed on lymphocytes, monocytes	VCAM-1 on activated endothelium	Mediates tight adhesion between lymphocytes, monocytes and endothelium	Impaired migration of lymphocytes and monocytes into tissue. Defective expression of either ß1integrins or VCAM-1 has not yet been described in humans. However, blockade of ß1integrins by natalizumab, a therapeutic monoclonal antibody, is associated with a high risk of progressive multifocal leucoencephalopathy, a severe viral infection of the brain
β_2 integrin family			
CD18/CD11 expressed on leucocytes	ICAM-1 on endothelium	Mediates tight adhesion between all leucocytes and endothelium	Defective expression of CD18/CD11 is associated with severe immunodeficiency, characterized by marked neutrophil leucocytosis, recurrent bacterial and fungal infection, and poor neutrophil migration into sites of infection
β_3 integrin family			
Expressed on platelets	Fibrinogen	Interacts during clotting	Clotting disorder Glanzmann's disease
Selectin family			
E-selectin (CD62E) expressed on activated endothelial cells	Sialyl Lewis X (CD15) on neutrophils, eosinophils	Mediates transient adhesion and rolling of leucocytes on monocytes	Defective expression of CD15 is associated with severe immunodeficiency – clinical features similar to CD18 deficiency. Mice deficient in both E- and P-selectin exhibit a similar clinical phenotype
L-selectin (CD62L) expressed on all leucocytes	CD34, Gly CAM on high endothelial venules	L-selectin mediates transient adhesion and rolling of leucocytes in lymph nodes, and also acts as a homing molecule directing lymphocytes into lymph nodes	L-selectin-deficient mice exhibit reduced leucocyte rolling and impaired lymphocyte homing

ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; VLA, very late activation antigen.

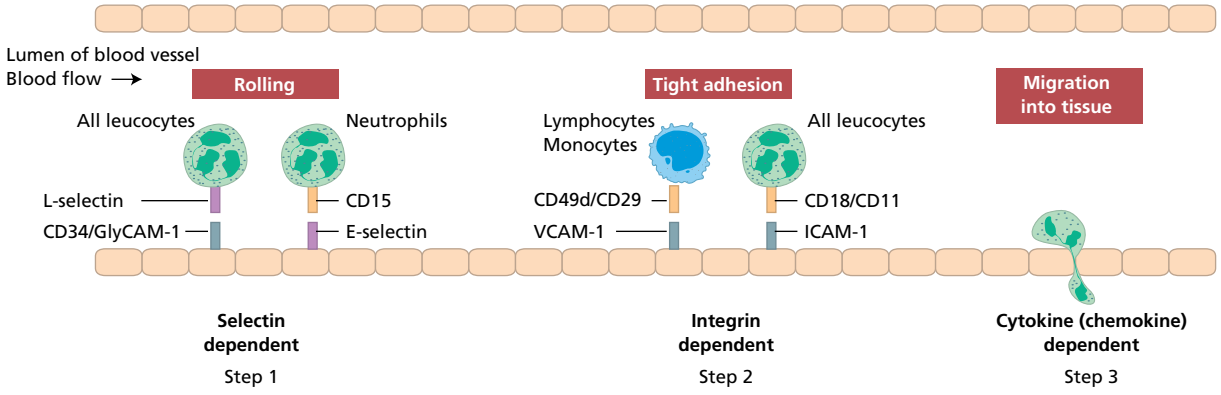


Fig. 1.14 Adhesion molecules and leucocyte–endothelial interactions.

Table 1.10 Proteins controlling classical and alternative complement pathways*

Protein	Function	Clinical consequences of deficiency
Circulating inhibitors		
C1 esterase inhibitor	Binds to activated C1r, C1s, uncoupling it from C1q	Uncontrolled activation of classical pathway leading to hereditary angioneurotic oedema
Factor H	Binds C3b displacing Bb; co-factor for factor I	Total deficiency causes recurrent bacterial infection, glomerulonephritis and renal failure; partial deficiency with familial (atypical) haemolytic uraemic syndrome; a particular allele with adult macular degeneration
Factor I	Serine protease that cleaves C3b; acts synergistically with factor H	As for factor H
Membrane inhibitors		
Complement receptor 1 (CR1; CD35)	Receptor for C3b	Protect mammalian cells. Low CR1 numbers on red cells in SLE is a consequence of fast turnover
Decay accelerating factor (DAF; CD55)	Accelerates decay of C3b Bb by displacing Bb	DAF deficiency alone does not cause disease
Protectin (CD59)	Inhibits formation of lytic pathway complex on homologous cells; widely expressed on cell membranes	In combination with DAF deficiency leads to paroxysmal nocturnal haemoglobinuria (see Chapter 16, section 16.2.4)

*This is not an exhaustive list.
SLE, systemic lupus erythematosus.

Non-specific immunity is older, in evolutionary terms, than antibody production and antigen-specific T cells. The major cells involved in the innate system are phagocytic cells (macrophages and polymorphonuclear leucocytes), which remove antigens including bacteria, and DCs, which are the first cells to react to invaders. The major humoral components of the four complement pathways can either directly destroy an organism or initiate/facilitate its phagocytosis. DCs recognize pathogens in order to provide a rapid initial cytokine response (such as interferon-a in a viral infection by plasmacytoid DCs) and to process antigen for presentation to specific TCRs alongside MHC for activation (classical DCs) (section 1.4.1).

1.3.1 Endothelial cells

The endothelium forms a highly active cell layer lining the inside of blood vessels and thus is present in all tissues. In addition to its critical role in maintaining vasomotor tone, the **endothelium** is closely involved in inflammation, wound healing and the formation of new blood vessels (angiogenesis). Immunologically, endothelial cells are intimately involved in interactions with leucocytes prior to leaving the circulation to enter sites of tissue damage (Fig. 1.14). The endothelium also plays an important role in regulating the turnover of IgG, through the presence of FcRn, a receptor that prevents IgG from undergoing lysosomal degradation (see section 1.2.4 and Chapter 7, section 7.4). The immunological importance of the endothelium is summarized in Box 1.4.

Box 1.4 Immunological importance of the endothelium

- Expresses a wide range of molecules on the cell surface (E-selectin, intercellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule [VCAM-1], complement receptors) and thus plays a critical role in leucocyte–endothelial interactions (Fig. 1.14).
- Major site of immunoglobulin (Ig)G turnover due to FcRn (Fc receptor of the neonate).
- Forms an important part of the innate immune response by expressing Toll-like receptors to recognize foreign pathogens.
- Capable of antigen presentation.

1.3.2 Neutrophil polymorphonuclear leucocytes

Neutrophils are short-lived cells that play a major role in the body's defence against acute infection. They synthesize and express adhesion receptors so that they can stick to, and migrate out of, blood vessels into the tissues. Neutrophils move in response to **chemotactic agents** produced at the site of inflammation; substances include CXCL8, complement-derived factors (such as C3a and C5a), kallikrein, cytokines released by THh1 cells and chemotactic factors produced by mast cells.

Neutrophils are **phagocytic** cells. They are at their most efficient when activated after entering the tissues. Morphologically,

the process of phagocytosis is similar in both neutrophils and macrophages. Neutrophils are able to kill and degrade the substances that they ingest. This requires a considerable amount of energy and is associated with a 'respiratory burst' of oxygen consumption, increased hexose monophosphate shunt activity and superoxide production. Genetically defective respiratory burst activity is associated with chronic granulomatous disease (see Chapter 3).

1.3.3 Macrophages

Macrophages and monocytes represent the mononuclear phagocytic system, which, along with DCs, forms the cells of the innate system. Lymphoid and myeloid cells are derived from closely related stem cells in the bone marrow (Fig. 1.1); each cell lineage has a different colony-stimulating factor and, once differentiated, they have entirely different functions. Polymorphonuclear leucocytes develop in the bone marrow and emerge only when mature. *Monocytes circulate for only a few hours before entering the tissues, where they may live for weeks or months as mature macrophages or DCs*. **Macrophages differentiate** in the tissues, principally in subepithelial interstitial and lymphatic sinuses in liver, spleen and lymph nodes, sites where antigens gain entry. Tissue macrophages are heterogeneous in appearance, in metabolism and also in function; they include freely mobile alveolar and peritoneal macrophages, fixed Kupffer cells in the liver and those lining the sinusoids of the spleen. When found in other tissues, they are called histiocytes.

A major function of the mononuclear phagocyte system is to phagocytose-invading organisms and other antigens. Macrophages have prominent lysosomal granules containing acid hydrolases and other degradative enzymes with which to destroy phagocytosed material. That material may be an engulfed viable organism, a dead cell, debris, an antigen or an immune complex. In order to carry out their functions effectively, macrophages must be 'activated'; in this state, they show increased **phagocytic and killing** activity. Stimuli include cytokines (see section 1.2.5), substances that bind to other surface receptors (such as IgG: Fc receptors, TLRs for endotoxin and other microbial components, receptors for bacterial polysaccharides and for soluble inflammatory mediators such as C5a; see Fig. 1.15). Activation may result in release of cytokines from monocytes or DCs such as TNF or IL-1, which may cause further damage in already inflamed tissues.

1.3.4 Dendritic cells

Classical or **myeloid dendritic cells** are mononuclear cells derived from bone marrow precursors and closely related to monocytes. There are many subsets, but there are differences between these subsets in mice compared with humans and other primates, particularly in their surface markers. So only those relating to humans are described here, though clearly their corresponding functions have been described in all mammalian species studied so far.

Immature dendritic cells are ubiquitous, particularly in epithelia that serve as a portal of entry for microbes, where they capture antigens as well as reacting to pathogen components quickly, within a few hours of invasion. Subsequently, the activated DCs migrate to draining lymph nodes and mature to present antigen to cells of the adaptive system (Fig. 1.16).

DCs have a range of **functions**: as well as processing antigens (Fig. 1.8), they are able to recognize and respond to pathogens by secreting IFN-α, produce IL-12 and chemokines as well as causing the differentiation of immature T cells to a

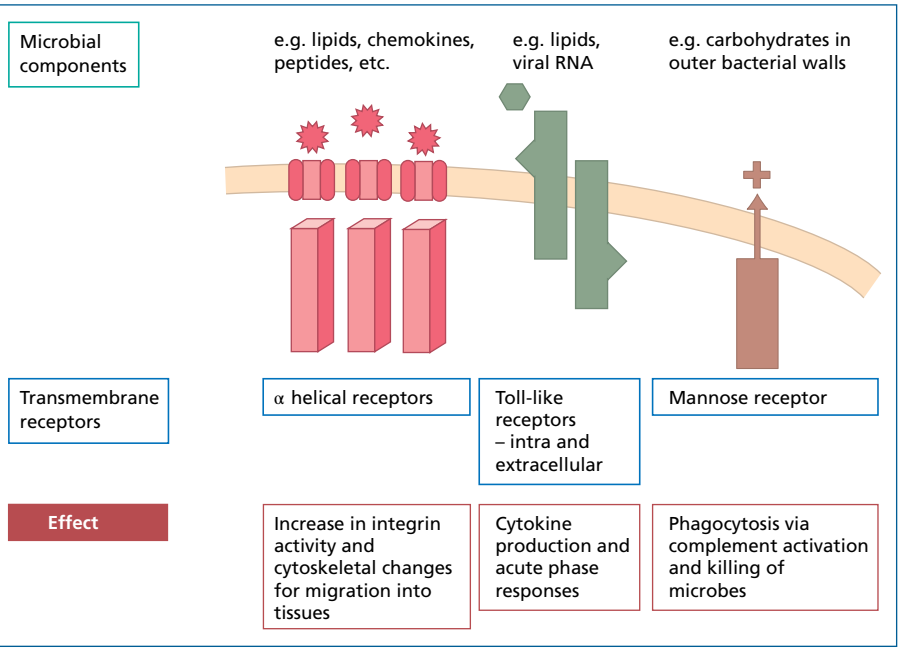


Fig. 1.15 Receptors and functions of mononuclear phagocytic cells.

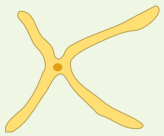
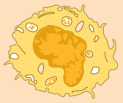
Cell	Appearance	Site	Mobility	Present to:
Interdigitating dendritic cells		Paracortex of lymph node	Mobile	T cells
Langerhans' cells		Skin	Mobile	T cells
Veiled cells		Lymph	Mobile	T cells
Follicular dendritic cells		Lymph node follicles	Static	B cells
Macrophages		Lymph node medulla Liver (Kupffer cells) Brain (astrocytes)	Mobile Static Static	T and B cells
B cell (especially if activated)		Lymphoid tissue	Mobile	T cells

Fig. 1.16 Antigen-presenting cells and their associated sites.

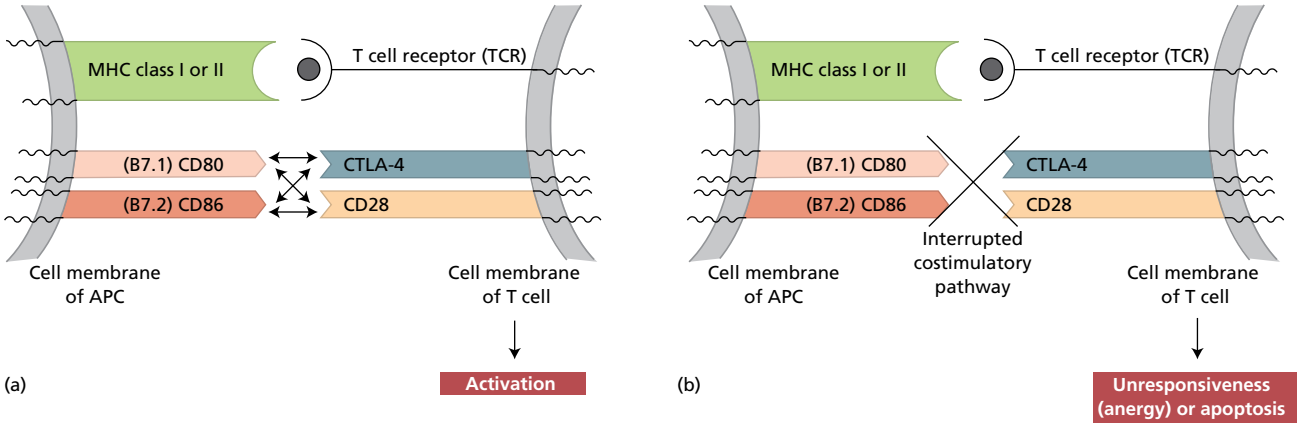


Fig. 1.17 (a, b) Role of co-stimulatory pathway in T-cell activation. APC, antigen-presenting cell; MHC, major histocompatibility complex.

variety of effector T cells. Depending on the environment of the cell, which is not entirely understood, mature DCs can activate $CD4^+$ cells to become Th1, Th2, Th17, CTLs and regulatory T cells (Tregs) or to induce apoptosis and so induce tolerance (see section 1.4). Immature and mature DCs have different sets of surface proteins (which act as distinct markers), in keeping with their different functions (see Table 1.3), depending on whether they are immature (for antigen capture/sensing pathogens via PRRs) or mature (for presentation of antigen to T cells).

The interaction between DCs and T cells is strongly influenced by a group of cell surface molecules that function as **co-stimulators**: CD80 (also known as B7-1) and CD86 (B7-2) on the activated DC, each of which engages with counter receptors on the T-cell surface referred to as CD28 and CTLA-4. *A functional co-stimulatory pathway is essential for T-cell activation*. In the absence of a co-stimulatory signal, interaction between DCs and T cells leads to T-cell unresponsiveness (Fig. 1.17). The importance of the co-stimulatory pathway is underlined by the ability of antagonists to co-stimulatory molecules to interrupt immune responses both *in vitro* and *in vivo*. This observation has been exploited therapeutically in mice with advanced lupus, in which treatment with a CTLA-4 conjugated protein to block CD28 leads to significant improvement in disease activity. Translation to human therapeutic monoclonal antibodies has succeeded despite a rocky start (see Chapter 7, Case 7.3). Abatacept, a CTLA-4 fusion protein, is licensed for the treatment of rheumatoid arthritis. T-cell activation by DCs also depends on cytokines secreted by activated DCs such as IL-12.

Processed antigen is presented to T cells complexed with the MHC class II antigens on the APC surface, since T cells do not recognize processed antigen alone. The most efficient APCs are the **interdigitating dendritic cells** found in the T-cell regions

of a lymph node (Figs 1.15 and 1.17). These dendritic cells have high concentrations of MHC class I and II molecules, co-stimulatory molecules (CD80, CD86) as well as adhesion molecules on their surfaces (Table 1.3) and limited enzymatic powers, which enable antigen processing but not complete digestion. Being mobile, they are able to capture antigen in the periphery and migrate to secondary lymphoid organs, where they differentiate into mature dendritic cells and interact with naive T cells. These cells are known as Langerhans cells when present in the skin.

These cells differ from the **follicular dendritic cells** in the follicular germinal centre (B-cell area) of a lymph node (see Figs 1.15 and 1.17). Follicular dendritic cells have receptors for complement and immunoglobulin components and their function is to trap immune complexes and to feed them to B cells in the germinal centre. This is part of the secondary immune response, since preexisting antibodies are used, accounting for B-cell memory.

Plasmacytoid DCs are found in blood and mucosal-associated lymphoid tissues and can secrete large quantities of type I IFNs in response to viral infections. However, their precise role and repertoire and therefore clinical significance remain unclear.

The few DCs in the blood are typically identified and enumerated in flow cytometry. Three types of DCs have been defined in human blood and these are the $CD11_c^+$ myeloid DCs, the $CD141^+$ myeloid DCs and the CD303+ plasmacytoid DCs (as per the IUIS Nomenclature committee). Dendritic cells in blood are less mature and have no projections from their surface (dendrites). Still, they can perform complex functions, including chemokine production in $CD11_c^+$ myeloid DCs, cross-presentation of antigen in $CD\ 141^+$ myeloid DCs and IFN-a production in $CD303^+$ plasmacytoid DCs.

Monocyte-derived DCs, also known as **myeloid** DCs, are activated (mature) DCs that are found in inflammatory sites, from whence they travel to draining lymph nodes. As with other mature DCs, they express co-stimulatory molecules and so can activate T cells. Under certain circumstances they can even secrete TNF-α and nitric oxide. The ability to culture these cells from human blood monocytes has led to the concept of DC vaccines for cancers.

Activated B cells themselves are also able to present antigen (Fig. 1.16).

1.3.5 Complement

The complement system consists of a series of heat-labile serum proteins that are activated in turn. The components normally exist as soluble inactive precursors; once activated, a complement component may then act as an enzyme (Fig. 1.19), which cleaves several molecules of the next component in the sequence (rather like the clotting cascade). Each precursor is cleaved into two or more fragments. The **major fragment** has two biologically active sites: one for

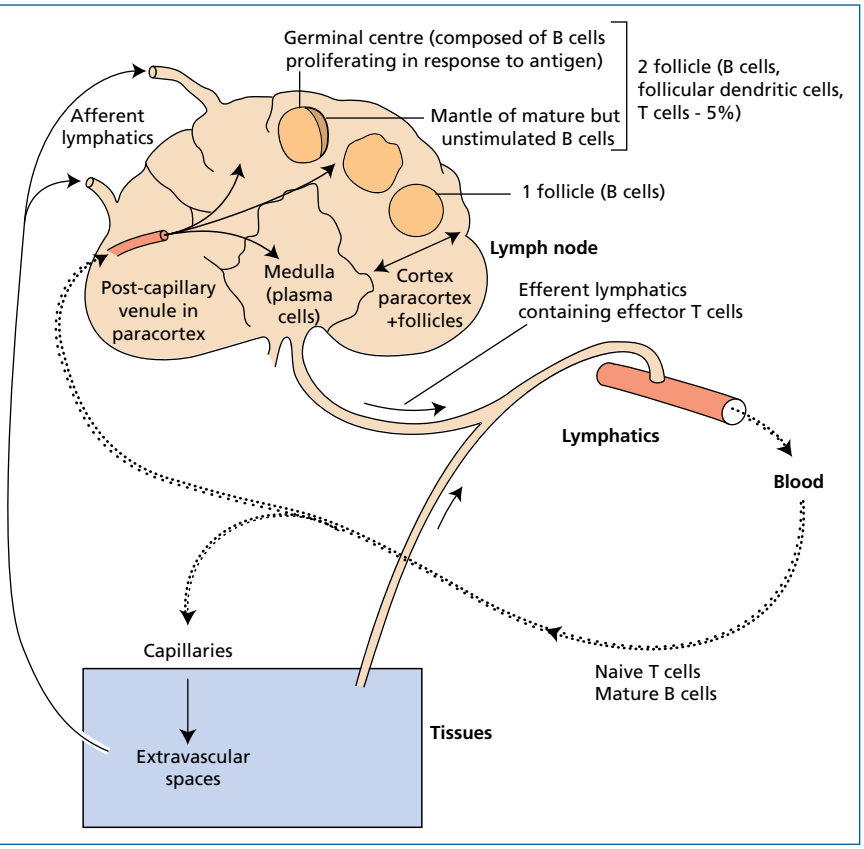


Fig. 1.18 Organization of spleen.

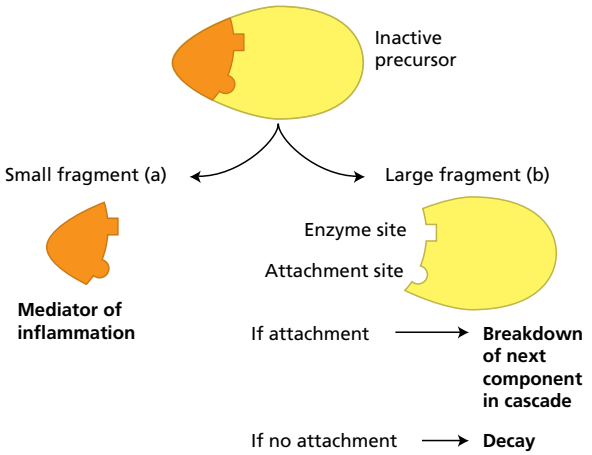


Fig. 1.19 Basic principle underlying the cleavage of complement components.

binding to cell membranes or the triggering complex and the other for enzymatic cleavage of the next complement component (Fig. 1.20). Control of the sequence involves spontaneous decay of any exposed attachment sites and specific inactivation by complement inhibitors. **Minor fragments** (usually prefixed 'a') generated by cleavage of components have important biological properties in the fluid phase, such as the chemotactic activity of C5a.

The history of the discovery of the complement pathways has made the terminology confusing. Several of the components have numbers, but they are not necessarily activated in numerical order; the numbering coincides with the order of their discovery and not with their position in the sequence. Activated components are shown with a bar over the number of the component (e.g. C1 is activated to $C\overline{1}$) and fragments of activated components by letters after the number (e.g. C3 is split initially into two fragments, C3a and C3b).

The major purpose of the complement pathways is to provide a means of removing or destroying antigen, regardless of whether or not it has become coated with antibody (Fig. 1.20). The **lysis** of whole invading microorganisms is a dramatic example of the activity of the complete sequence of complement activation, but that is not necessarily its most important role. The key function of complement is probably the **opsonization** of microorganisms and immune complexes; microorganisms coated (i.e. opsonized) with immunoglobulin and/or complement are more easily recognized by macrophages and more readily bound and phagocytosed through IgG: Fc and C3b receptors.

Similarly, immune complexes are opsonized by their activation of the classical complement pathway (see later); individuals who lack one of the classical pathway components suffer from immune complex diseases (see section 1.6). Soluble complexes are **transported** in the circulation from the inflammatory site by erythrocytes bearing CR1, which bind to the activated C3 (C3b) in the immune complex. Once in the spleen or liver, these complexes are removed from the red cells, which are then recycled (Fig. 1.21).

Minor complement fragments are generated at almost every step in the cascade and contribute to the **inflammatory response**. Some increase vascular permeability (C3a), while others attract neutrophils and macrophages for subsequent

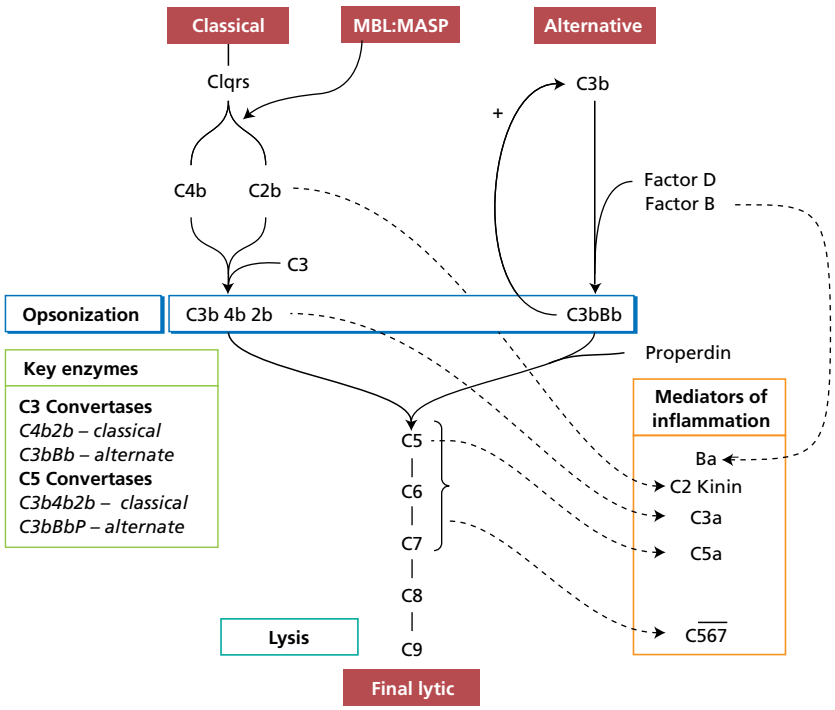


Fig. 1.20 Functions of complement pathways. MASP, MBL-associated serine protease; MBL, mannan-binding lectin.

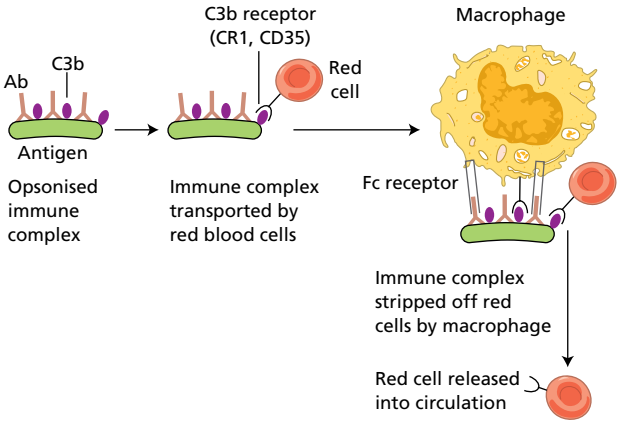


Fig. 1.21 Transport of immune complexes by erythrocytes to macrophages in liver and spleen.

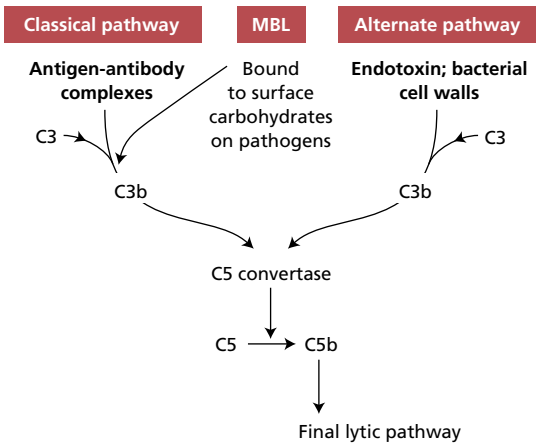


Fig. 1.22 Complement pathways and their initiating factors. MBL, mannan-binding lectin.

opsonization and phagocytosis (C5a) (Fig. 1.20). C5a not only promotes leucocytosis in the bone marrow, but mobilizes and attracts neutrophils to the inflammatory site where it increases their adhesiveness; it also upregulates complement receptors CR1 and CR3 on neutrophils and macrophages to maximize phagocytosis.

Complement **activation** occurs in two phases: activation of the C3 component, followed by activation of the 'attack' or lytic sequence. The critical step is a cleavage of C3 by complement-derived enzymes termed 'C3 convertases'. The cleavage of C3 is achieved by three routes, the classical, alternative and lectin pathways, all of which can generate C3 convertases but in response to different stimuli (Fig. 1.22). The pivotal role of C3 in complement activation is underlined by patients with a deficiency of C3, who cannot opsonize pathogens or immune complexes, predisposing them to bacterial infection as well as immune complex diseases.

The **classical pathway** was the first to be described. It is activated by a number of substances, the most widely recognized being antigen–antibody complexes where the antibody is either IgM or IgG (Fig. 1.22). The reaction of IgM or IgG with its antigen causes a conformational change in the Fc region of the antibody to reveal a binding site for the first component in the classical pathway, C1q. C1q is a remarkable, collagen-like protein composed of six subunits, resembling a 'bunch of tulips' when seen under the electron microscope. C1q reacts with Fc via its globular heads; attachment by two critically spaced binding sites is needed for activation. The Fc regions of pentameric IgM are spaced so that one IgM molecule can activate C1q; in contrast, IgG is relatively inefficient because the chance of two randomly sited IgG molecules being the critical distance apart to activate C1q is relatively low. IgA, IgD and IgE do not activate the classical pathway.

Once C1q is activated, C1r and C1s are **sequentially bound** to generate enzyme activity (C1 esterase) for C4 and C2 (see Fig. 1.20), splitting both molecules into 'a' and 'b' fragments. The complex C4b2b is the classical pathway C3 convertase. Other fragments released are C4a, C2a and a vasoactive peptide released from C2. C4b2b cleaves C3 into two fragments, C3a possessing anaphylatoxic and chemotactic activity and C3b that binds to the initiating complex and promotes many of the biological properties of complement. The C 4b2b3b complex so generated is an enzyme, C5 convertase, which initiates the final lytic pathway (the 'attack' sequence).

The **alternative pathway** is phylogenetically older than the classical pathway. It is relatively inefficient in the tissues, and high concentrations of the various components are required. The central reaction in this pathway, as in the classical one, is the activation of C3, but the alternative pathway generates a C3 convertase without the need for antibody, C1, C4 or C2. Instead, the most important activators are bacterial cell walls and endotoxin (Fig. 1.22).

The initial **cleavage** of C3 in the alternative pathway happens continuously and **spontaneously** (see Fig. 1.22), generating a low level of C3b. C3b is an unstable substance and, if a suitable acceptor surface is not found, the attachment site in C3b decays rapidly and the molecule becomes inactive. If, however, an acceptor surface (bacterial cell walls and endotoxin) is nearby, the C3b molecules can bind and remain active. C3b is then able to use factors D and B of the alternative pathway to produce the active enzyme 'C3bBb'. This latter substance has two properties. It can break down more C3, providing still more C3b; this is known as the 'positive feedback loop' of the alternative pathway (Fig. 1.20). Alternatively, C3bBb becomes stabilized in the presence of properdin to form the C5 convertase of the alternative pathway.

There are thus two ways of producing **C5 convertase**. In the classical pathway C5 convertase is made up of C3b, C4b and C2b, while in the alternative pathway it is produced by C3b, Bb and properdin (Fig. 1.20).

The third pathway of complement activation is initiated by **mannan-binding lectin** (MBL, also known as mannan-binding protein), a surface receptor (see Fig. 1.20) shed into the circulation, binding avidly to carbohydrates on the surface of microorganisms. MBL is a member of the collectin family of

C-type lectins, which also includes pulmonary surfactant proteins A and D. MBL is structurally related to C1q and activates complement through a serine protease known as MASP (MBL-associated serine protease), similar to C1r and C1s of the classical pathway. Inherited deficiency of MASP-2 has been shown to predispose to recurrent pneumococcal infections and immune complex disease.

All pathways converge on a common **final lytic** pathway ('attack' sequence) of complement involving the sequential attachment of the components C5, C6, C7, C8 and C9 and resulting in lysis of the target cell such as an invading organism or a virally infected cell. The lytic pathway complex binds to the cell membrane and a transmembrane channel is formed. This can be seen by electron microscopy as a hollow, thin-walled cylinder through which salts and water flow, leading to the uptake of water by a cell, swelling and destruction. During the final lytic pathway, complement fragments are broken off. C5a and the activated complex C567 are both potent mediators of inflammation. C5a, along with C3a, are anaphylotoxins, i.e. they cause histamine release from mast cells with a resulting increase in vascular permeability. C5a also has the property of being able to attract neutrophils to the site of complement activation (i.e. it is chemotactic; see Fig. 1.20).

The **control of any cascade sequence** is extremely important, particularly when it results in the production of potentially self-damaging mediators of inflammation. The complement pathway is controlled by three mechanisms (see Box 1.5).

These mechanisms ensure that the potentially harmful effects of complement activation remain confined to the initiating antigen without damaging autologous (host) cells. Table 1.10 lists some of the clinically important complement regulatory proteins. When considering their role in pathology, there are important caveats (see Box 1.5).

Box 1.5 Physiological control of complement

- A number of the activated components are inherently unstable; if the next protein in the pathway is not immediately available, the active substance decays.
- There are a number of specific inhibitors, e.g. C1 esterase inhibitor, factor I, factor H.
- There are proteins on cell membranes that block the action of complement:
  - By increasing the rate of breakdown of activated complement components, e.g. DAF (decay accelerating factor, CD55), MCP (monocyte chemotactic protein, CD46).
  - By binding C5b678 and preventing C9 from binding and polymerizing, e.g. CD59.

1.3.6 Antibody-dependent cell-mediated cytotoxicity

ADCC is a mechanism by which antibody-coated target cells are destroyed by cells bearing **low-affinity FcγRIII receptors** – NK cells ($CD16^+$), monocytes, neutrophils (see section 1.2.4 and Fig. 1.23) – without involvement of the MHC. Clustering of several IgG molecules is required to trigger these low-affinity receptors to bind, resulting in secretion of IFN-γ and discharge of granules containing perforin and granzymes, as found in CTLs. The overall importance of ADCC in host defence is unclear, but it represents an additional mechanism by which bacteria and viruses can be eliminated.

1.3.7 Natural killer cells

NK cells look like large granular lymphocytes and are found in blood, liver and secondary lymphoid organs particularly the spleen and mucosal-associated lymphoid tissue (MALT). They can kill target cells, even in the absence of antibody or antigenic stimulation. The name '**natural killer**' reflects the fact that, unlike the adaptive system, they do not need prior activation but have the relevant recognition molecules on their surfaces already. Non-specific agents, such as mitogens, IFN-γ and IL-12, can activate them further. NK cells form an integral part of the early host response to viral infection (Fig. 1.24). The exact mechanism by which NK cells distinguish between infected and non-infected cells is not clear, but is likely to involve **cell-surface receptors** (Fig. 1.25). NK cells express two types of surface receptor (see section 1.2.2). Expression of MHC class I proteins by most normal cells prevents NK cells from killing healthy cells. Interference with this inhibition, by virally induced down-regulation or alteration of MHC class I molecules, results in NK-mediated killing, either directly (secretion of granzymes or perforin), by FcRIII and ADCC or by secretion of IFN-γ and TNF-α.

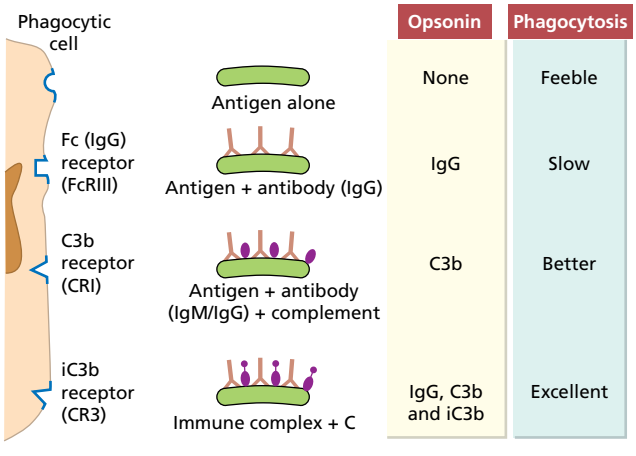


Fig. 1.23 Opsonins and the relationship to phagocytosis.

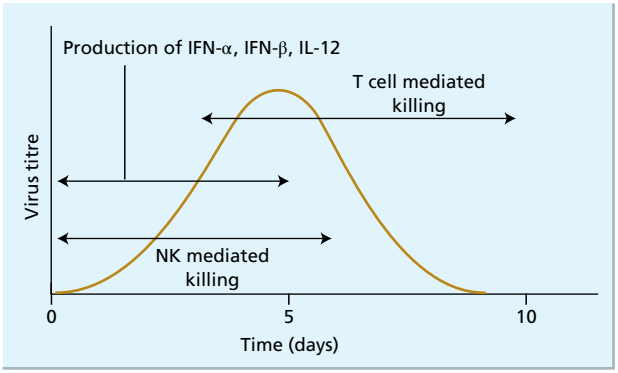


Fig. 1.24 Role of cells in early immune response to virus infection. Early – innate immune cells produce type I interferons (IFN) and interleukin (IL)-12. Late – T-cell-mediated killing by antigen-specific cells: cytotoxic T cells (CTLs). NK, natural killer.

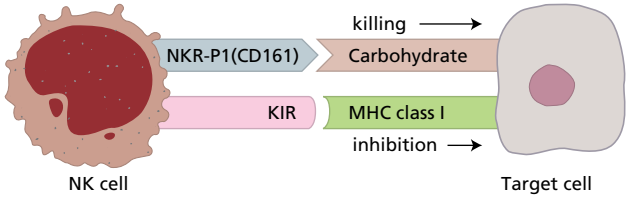


Fig. 1.25 Natural killer (NK) cell recognition of target cells. NK cell killing is mediated by engagement of the receptor NKR-P1 with its carbohydrate ligand on the target cell. This is inhibited by the interaction between the inhibitory receptor (KIR) and major histocompatibility complex (MHC) class I on the target cell.

NK cells are not immune cells in the strictest sense because, like macrophages, they are not clonally restricted; in addition, they show **little specificity** and they have no memory. The range of their potential targets is broad. Animals and rare patients with deficient NK cell function have an increased incidence of certain tumours and viral infections. A subset of NK cells, NKT cells, is therefore important in 'immune' surveillance against tumours (section 1.5.1). The human immunodeficiency X-linked lymphoproliferative syndrome is an example in which Epstein–Barr virus (EBV)-driven tumours are associated with absent NKT cells (see Chapter 2, section 2.3.1).

1.3.8 Innate lymphoid cells

Innate lymphoid cells (ILCs) are tissue-resident lymphocytes that do not express antigen receptors. They are best regarded as innate T cells. Based on their cytokine-secreting profile and functions, three groups of ILCs are recognized (Table 1.11). The cytokine profile of ILC1, ILC2 and ILC3 groups of lymphocytes mirrors that of $CD4^+$ Th1, Th2 and Th17 cells, respectively, with a similar overlap of functions. This classification is not mutually exclusive, with ILCs exhibiting sufficient plasticity to change phenotypes and functional capacities in response to changes in cytokines and transcription factors.

In contrast to adaptive T-cell responses, which take several days, ILCs act promptly in response to cytokine signals during an immune response. Beyond the immune system, ILCs play a key role in metabolic homeostasis and tissue repair. ILCs resident in the intestine and lungs also express receptors for neurotransmitters and neuropeptides, thus playing an important role in cross-talk between mucosal surfaces and the nervous system.

1.4 Functional basis of the adaptive immune responses

Antigen-specific effector lymphocytes are of two types: B cells and T cells. B cells are ultimately responsible for antibody production and act as APCs in secondary immune responses. There are several types of T cells that act as effector cells with several different functional activities (Table 1.12). Some T cells have a regulatory rather than effector role, in terms of assisting maturation of other cell types or regulating immune responses. T-cell functions of help, killing or regulation may depend on different stimuli, resulting in different cytokines being produced with predominantly activating or inhibitory effects.

The factors regulating a normal immune response (see later Box 1.7) are complex and include antigen availability, specific suppression by T cells and the balance of cytokines produced (section 1.4.2).

1.4.1 Antigen processing

The first stage of an antigen-specific immune response involves capture and modification of that antigen by specialized

Table 1.11 Classification of innate lymphoid cells (ILCs)

	ILC1	ILC2	ILC3
Key transcription factor required for development	T-bet	GATA-3	RORϒt
Secreted cytokine profile	Interferon-ϒ	Interleukin (IL)-4, IL-5, IL-13	IL-17, IL-22
Function	First line of defence against intracellular pathogens, e.g. viruses, toxoplasma	Defence against parasites	Defence against extracellular bacteria, fungi; regulation of adaptive Th17 cell responses
Key cell surface molecules	CD45, CD127, CD161, IL1R	CD45, CD161, CRTH2 (chemoattractant receptor)	CD45, CD127, CD117, CD161

Table 1.12 Lymphocytes involved in adaptive immune responses

Cell type	Function of cell	Product of cell	Function of product
B	Produce antibody	Antibody	Neutralization
	Antigen presentation		Opsonization
			Cell lysis
Th2	B-cell antibody production Activate T_C	Cytokines IL-3, -4, -5, -10, -13	Help B and T_C cells
Th1	Inflammation: initiation and augmentation	IL-2, IFN-γ, TNF	Inflammatory mediators
TRegs	B-cell antibody production suppress T_C	Suppressor factor(s), e.g. TGF-β	Suppress Th and therefore indirectly B and T_C
Tc	Lysis of antigenic target cells	IFN-γ	Enhances MHC expression
			Activates NK cells
		Perforins	Disrupts target cell membranes
NKT	Target cell killing	IL-4, IFNγ	
Th17	Inflammation	IL-17A, IL-17F and IL-22	Host defence against bacteria and fungi via IL-17s attracting neutrophils

IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; T_C, cytotoxic T cell (CTL); TGF, transforming growth factor; Th1 and Th2, helper T-cell types; Th17, effector T cells secreting inflammatory cytokines; TNF, tumour necrosis factor; TRegs, regulatory T cells.

cells, DCs prior to presentation to the immune cells. *This is not an antigen-specific process, unlike the subsequent restricted binding of antigen to lymphocytes predetermined to react with that antigen only*. Antigen is processed, then carried and 'presented' to lymphocytes. T cells cannot recognize antigen without such processing into small peptides and presentation in relation to self-MHC. Since activation of T cells is essential for most immune responses, antigen processing is crucial. The specialized cells involved are dendritic cells (and some monocytes) for a primary immune response and B cells for a secondary immune response when the antigen has been recognized and responded to on a previous occasion.

1.4.2 T-cell-mediated responses

As mentioned earlier, $CD4^+$ T cells have many functions and there are T-cell subsets that reflect this. Furthermore, the function of a particular T cell can change, depending on the environment in which it finds itself. Conventional $CD4^+$ cells that have alpha-beta chains in their TCRs (αβ T cells) are the predominant type in the blood and lymphoid circulations. They can become helper cells, of which there are presently three types: Th0, Th1 and Th2. Th0 are thought to be the precursor naive T cells, as they are able to secrete a wide variety of cytokines. Th1 are proinflammatory T cells and Th2 assist Tc activation and antibody production. $CD8^+$ T cells, which depend on 'help' from $CD4^+$ T cells for antigen specificity, are one of several types of killing (cytotoxic) cells and are particularly important in the control or elimination of viruses. Th17 cells are proinflammatory and are thought to have evolved to aid in host defence against bacteria and fungi, via their production of inflammatory cytokines IL-17A, IL-17F and IL-22. Tregs control immune responses, particularly aberrant responses such as autoimmunity. In addition, there is a group of more primitive T cells (sometimes called 'unconventional' T cells) that include gamma-delta T cells (γδ T cells), found mainly in relation to the mucosa particularly the gut, and NKT cells, which are important for regulation and recognizing lipid (often tumour) antigens.

T-cell help

T-cell help is always antigen specific. Only helper T cells that have responded to antigen previously presented in the context of MHC class II can subsequently help those $CD8^+$ T cells or $CD19^+$ B cells that are already committed to the same antigen (Burnet's clonal selection theory). **Helper T cells** recognize both antigen and MHC class II antigens as a complex on the presenting cells, via their specific TCR. They then recognize the same combination of antigen and the particular class II antigen on the responding cell. **Co-stimulation** is essential for T-cell activation and accessory molecules are vital (Fig. 1.17).

MHC class II molecules play an important role in the activation of helper T cells. T cells from one individual will not cooperate with the APCs, T cells or B cells from a different person (i.e. of a different HLA type). Certain MHC class II molecules on the presenting cells fail to interact with particular antigens (as a prelude to triggering helper T cells) and so fail to trigger an adaptive immune response to that stimulus. This provides a

mechanism for the **genetic regulation of immune responses** (originally attributed to distinct immune response genes). The MHC class II thus helps to determine the responsiveness of an individual to a particular foreign antigen, since they interact with the antigen before T-cell help can be triggered.

When helper T cells meet an antigen for the first time, there is a limited number that can react with that antigen to provide help; these stimulated T cells therefore undergo blast transformation and **proliferation**, providing an increased number of specific helper T cells when the animal is re-exposed, i.e. an expanded clone. In addition, specific memory T cells differentiate.

Memory T cells (which bear the surface marker CD45RO) have increased numbers of adhesion molecules (LFA-1, CD2, LFA-3, ICAM-1; see section 1.2.6) and a higher proportion of high-affinity receptors for the relevant antigen. Memory cells are therefore easily activated and produce high concentrations of IL-2 to recruit more helper T cells of both types, Th1 and Th2 (see later in the chapter). Thus T-cell memory is a combination of an increase of T cells (quantitative) as well as a qualitative change in the efficiency of those T cells, providing a more rapid immune response on second and subsequent exposure as well as a more vigorous response.

Antigen-specific **cell-mediated effector responses** are carried out by T lymphocytes. T cells can lyse cells expressing specific antigens (cytotoxicity), release cytokines that trigger inflammation (delayed-type hypersensitivity), take part in antibody production or regulate immune responses (regulation). **Distinct T-cell populations** mediate these types of T-cell responses: $CD8^+$ Tc cytotoxic cells, $CD4^+$ Th1 cells, $CD4^+$ Th2 and $CD4^+$ Treg cells, respectively.

T-effector cells

$CD4^+$ effector T cells are grouped into four distinct subgroups depending on their cytokine profile. Th1 cells secrete TNF and IFN-γ and consequently mediate inflammation. In contrast, Th2 cells predominantly secrete IL-4, IL-5, IL-10 and IL-13 (Fig. 1.26), stimulate vigorous antibody production and activate Tc. T cells expressing cytokine profiles common to **both Th1 and Th2** cells are designated Th0. It is unclear how a naive T cell selects which cytokine profile to secrete, but there is evidence to suggest that exposure to certain cytokines is an important influence. Exposure to IL-4 and IL-6 stimulates development of Th2 cells, while IL-12 and IFN-y result in a developing T cell acquiring Th1 properties. Recent evidence suggests that $CD8^+$ T cells are also capable of secreting cytokine profiles typical of these cell types.

In humans, a Th1 cytokine profile is essential for protection against intracellular pathogens, while a Th2 cytokine profile is associated with diseases characterized by overproduction of antibodies including IgE. The clinical consequences of inducing a **particular Th response** are strikingly illustrated in patients with leprosy, an infectious disease caused by Mycobacterium leprae, an intracellular bacterium. Patients who mount a protective Th1 response develop only limited disease (tuberculoid leprosy), since their macrophages are able to control M. leprae efficiently. In contrast, patients who produce a predominant Th2 response develop disabling lepromatous leprosy, since without the limitation provided by Th1 inflammation, antibody alone is ineffective in tackling an intracellular pathogen.

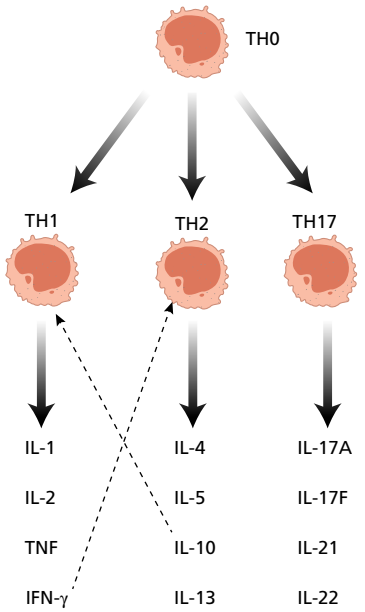


Fig. 1.26 Th1 and Th2 cells and their cytokine profiles; broken arrows indicate inhibition.

T cells for inflammation – Th1 cells and Th17 cells

Both Th1 and Th17 cells are involved in delayed-type hypersensitivity (DTH) reactions to antigens. The tuberculin test (Mantoux test) is a good example of a DTH response. Individuals who have previously been infected with Mycobacterium tuberculosis mount a T-cell response that evolves over 24–72 hours following intradermal injection of tuberculin. This is clinically manifest as local swelling and induration; biopsy of the site reveals both types of T cell as well as macrophage infiltration. The histology of tissue granulomas in tuberculosis, leprosy and sarcoidosis are all examples of DTH. Like the induction of T-cell help, the effector functions in **delayed hypersensitivity** vary with MHC polymorphisms.

Th17 cells are a proinflammatory subset of THCs defined by production of inflammatory cytokines IL-17A, IL-17F and IL-22. Th17 cells are thought to have evolved to aid in host defence against extracellular and intracellular bacteria as well as fungi. IL-17 is important for the recruitment of neutrophils (and possibly eosinophils), but the precise role in inflammation in systemic inflammatory diseases such as rheumatoid arthritis is not yet clear. Th17 cells require the proinflammatory cytokines transforming growth factor (TGF)-β1, IL23 and IL-6, secreted by DCs, for their development, rather than the IL-12 needed for Th1 development.

T-cell lysis

$CD8^+$ CTLs lyse cells infected with virus and possibly those tumour cells with recognizable tumour antigens too. Such cytotoxicity is antigen specific and only cells expressing the relevant viral proteins on their surfaces are killed (see Fig. 1.6), so obeying the rules of the clonal selection theory. Since infected cells express surface viral proteins prior to the assembly of new virus particles and viral budding, **cytotoxic T cells** are important in the recovery phase of an infection, destroying the infected cells before new virus particles are generated. CTLs lyse target cells by means of secretory lysosomes (granules) containing perforin and granzymes (and granulysin capable of antimicrobial activity). The lysosomes fuse with the outer membrane of the target cell and discharge the contents via a synaptic cleft, resulting in death of the target cell. Other methods are used by CTL to cause programmed cell death (apoptosis) that do not involve the secretory lysosomes.

In contrast to $CD4^+$ helper T cells, $CD8^+$ CTLs recognize viral antigens together with MHC class I molecules (rather than MHC class II) on both dendritic cells for activation and target cells for effector function. They show exquisite specificity for self-MHC molecules, in that they can lyse only cells expressing the same MHC class I molecules. MHC class I molecules may affect the **strength of the effector CTL response** to a particular virus, providing further strong evidence for the evolution of a polymorphic MHC system, so that immune responses to pathogens vary to protect the species. All endogenous antigens (including viral antigens) are presented in the context of MHC class I antigens (see Fig. 1.8). This combination on the dendritic cells directly activates $CD8^+$ T cells and provides the appropriate target cells for virally induced T-cell cytotoxicity, as well as mechanisms for graft rejection and tumour surveillance. The relevance of $CD8^+$ T cells to transplantation is discussed in Chapter 8. $CD8^+$ T cells are also involved in autoimmune diseases; the T-cell epitopes of endogenous self-antigens are presented by DCs in the same way and a process known as cross-presentation allows 'B-cell epitopes' of self-antigens to be presented by DCs to T cells to provide T -cell help to B cells.

Regulatory T cells

After initial scepticism in the 1980s regarding the existence of suppressor T cells (renamed regulatory T cells), there is now good evidence to support the presence of several subsets of Tregs with distinct phenotypes that play key roles in immunoregulation by dampening down a wide range of immune responses, including responses to self-antigens, alloantigens and tumour antigens, as well as to pathogens and commensals. A number of immunoregulatory cells have been described, but it is likely that the $CD4^+$ Tregs, identified by high levels of the IL-2 receptor alpha chain ($CD25^+$) and the FOXP3 transcription factor, are the most important for maintaining peripheral tolerance. These **natural regulatory T cells** (natural Tregs) develop in the thymus in response to self-antigens; they maintain peripheral self-tolerance and so prevent autoimmunity. However similar cells, **induced (i) Tregs** (producing IL-10), can be generated from precursors outside the thymus in response to environmental antigens; these cells maintain tolerance to non-self components such as gut flora. Both types seem to be interchangeable with Th17, depending on the cytokines and other mediators such as in inflammation caused by pathogens or $CD8^+$ autoimmune cells.

It is thought that Tregs act by producing immunosuppressive cytokines such as TGFβ and IL-10, as well as direct cell-to-cell contact resulting in apoptosis of the target cell.

The development of Treg cells is under the control of the gene called FOXP3, which encodes a transcription repressor protein specifically in $CD4^+$, $CD25^+$ T cells in the thymus as well as in the periphery. Mutations in the FOXP3 gene result in severe autoimmune disease and allergy (Box 1.6).

Natural killer T cells

A few T cells also express some of the markers of NK cells and are therefore known as NKT cells. These cells form a separate lineage, though they are $CD3^+$. They have α TCR chains, with limited diversity, but are also able to **recognize lipids in conjunction with CD1**, MHC class I-like molecules of equally restricted diversity. They rapidly produce many cytokines after stimulation and thus influence diverse immune responses, such as augmenting the proliferation of

Box 1.6 Evidence that $CD4^+CD25^+$ T cells are important in immunoregulation

- Depletion of CD4+CD25+ T cells in humans, due to mutations in the FOXP3 gene, is associated with the rare IPEX syndrome – immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome – characterized by autoimmune diabetes, inflammatory bowel disease and severe allergy.
- Regulatory T cells (Tregs) determine the disease prognosis in hepatitis B virus (HBV) infection – high levels lead to viral progression and impaired immune response.
- Clinical improvement after allergen immunotherapy for allergic rhinitis and asthma has been associated with the induction of interleukin (IL)-10 and transforming growth factor (TGF)-β producing Foxp3 expressing $CD4^+CD25^+$ T cells, resulting in suppression of Th2 cytokines.
- Corticosteroid therapy in asthma acts on Tregs, in part to increase IL-10 production, while vitamin D_3 and long-acting beta-agonists enhance IL-10 Treg function.
- The antiinflammatory therapeutic effects of low-dose IL-2 are mediated through an expansion of Tregs.

Tregs in an IL-4-dependent manner. They can also promote cell-mediated immunity to tumours and infectious organisms, while paradoxically they can suppress the T-cell responses associated with autoimmune disease, graft-versus-host disease or allograft rejection. The exact mechanisms by which these cells carry out such contrasting functions are not known. Absence of NKT cells in a particular form of primary immunodeficiency known as X-linked lymphoproliferative disease (XLP) is associated with the development of EBV-driven lymphoma, suggesting an important role in responses to this particular virus and to tumours.

1.4.3 Antibody production

Antibody production involves at least three types of cell: APCs, B cells and helper T cells (Table 1.12).

B cells

Antibodies are synthesized by B cells and their mature progeny, plasma cells. B cells are readily recognized because they express **immunoglobulin on their surface**, which acts as the BCR (see section 1.2.2). During development, B cells first show intracellular μ chains and then surface IgM μ with one light chain – κ or λ. These cells are able to switch from production of IgM to one of the other classes as they mature, so that they later express IgM and IgD and, finally, IgG, IgA or IgE, a process known as isotype switching. The final type of surface immunoglobulin determines the class of antibody secreted; surface and secreted immunoglobulin are identical. This immunoglobulin maturation sequence fits with the kinetics of an antibody response: the primary response is mainly IgM and the secondary response predominantly IgG (Fig. 1.27). **Isotype switching** is mediated by the interaction of several important proteins: for example, CD40 on the B-cell surface engages with its ligand (CD40L) on activated T cells (Fig. 1.28), under the influence of IL-4. Deficiency of either molecule (CD40 or CD40L) in mice and humans leads to a severe immunodeficiency characterized by inability to switch from IgM to IgG production, with consequently low serum concentrations of IgG and IgA for protection against infections but normal or even high serum IgM (hence called a hyper-IgM syndrome), accompanied by poor germinal centre formation and inability to produce memory B cells (see Chapter 3, Case 3.2).

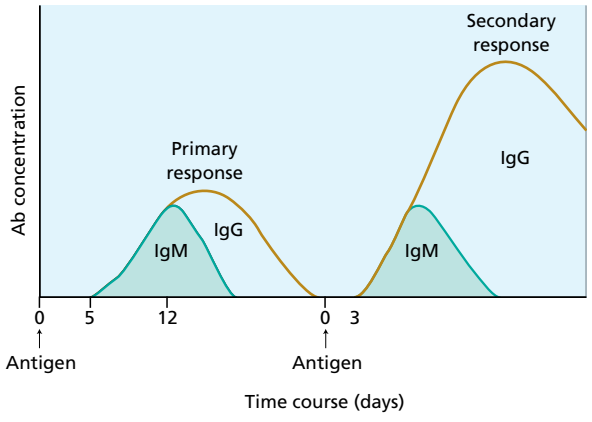


Fig. 1.27 Primary and secondary antibody (Ab) responses following antigenic stimulation.

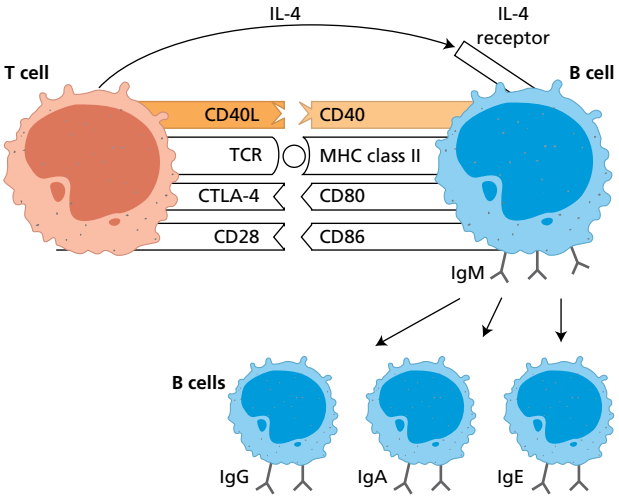


Fig. 1.28 Interaction between CD40L on T cells and CD40 on B cells, under the influence of interleukin (IL)-4, leading to isotype switching. MHC, major histocompatibility complex; TCR, T-cell receptor.

Each B cell is committed to the production of an antibody that has a unique VH–VL combination (see section 1.2.4). This uniqueness is the basis of Burnet's clonal selection theory, which states that each B cell expresses a surface immunoglobulin that acts as its antigen-binding site. Contact with antigen and factors released by $CD4^+$ T helper cells (IL-4, -5, -13) stimulates the B cells to divide and differentiate, generating more antibody-producing cells, all of which make the same antibody with the same V_H–V_L pair. Simultaneously, a population of **B memory cells** is produced that expresses the same surface immunoglobulin receptor. The result of these cell divisions is that a greater number of antigen-specific B cells become available when the animal is exposed to the same antigen at a later date; this is known as **clonal expansion** and helps to account for the increased secondary response.

As well as being quicker and more vigorous (Fig. 1.27), secondary responses are more efficient. This is due to the production of antibodies that bind more effectively to the antigen, i.e. have a higher affinity. There are two reasons for this. First, as antigen is removed by the primary response, the remaining antigen (in low concentration) reacts only with those cells that have high-affinity receptors. Second, the **rapid somatic mutation, which accompanies B-cell division in the germinal centre**, provides B cells of higher affinity, a process known as 'affinity maturation'. C3 fragments play a key role in the secondary antibody response by interacting with the co-stimulation receptors on B cells.

A minority subset of B cells respond directly to antigens called **T-independent antigens** (see section 1.2.1). These antigens have repeating, identical antigenic determinants and provoke predominantly IgM antibody responses. These responses are relatively short-lived and restricted in specificity and affinity, due to the lack of T-cell involvement. A few T-independent antigens provoke non-specific proliferation of memory B cells and are therefore known as polyclonal B-cell mitogens.

A given B cell produces particular V_H and V_L domains and all the daughter cells of that B cell produce the same V_H and V_L. Initially, the B cell produces intracellular antigen-specific IgM, which then becomes bound to the surface of the cell (surface immunoglobulin) and acts as the antigen receptor for that cell; the B cell is then 'antigen responsive'. On exposure to that antigen, a committed B cell fixes the isotype (or class) of immunoglobulin that it will produce and divides; all the progeny produce identical immunoglobulin molecules (known as monoclonal immunoglobulins). Many of these cells then mature into plasma cells, while others act as APCs (section 1.4.1) or memory B cells.

1.5 Physiological outcomes of immune responses

Once the immune response is initiated, the end result depends on the nature and localization of the antigen, on whether the predominant response has been humoral or cell mediated, on the types of effector T cells and/or antibodies provoked and on whether the augmentation processes have been involved.

1.5.1 Killing of target cells (virally infected/tumour cells)

Target cells killed as a result of an immune response include organisms and cells bearing virally altered or tumour-specific antigens on their surfaces. They may be killed directly by antigen-specific mechanisms such as antibody and complement, ADCC following binding of specific antibody or antigen-specific CTL.

Cytokine production results in activation of NK cells, neutrophils and macrophages and subsequently non-specific killing by mechanisms similar to those in adaptive immunity (see section 1.2.3).

1.5.2 Direct functions of antibody

Although some forms of antibody are good at neutralizing particulate antigens, many other factors, such as the concentration of antigen, the site of antigen entry, the availability of antibody and the speed of the immune response, may influence antigen removal (Box 1.7).

Neutralization is one direct effect of antibody and IgM and IgA are particularly good at this. A number of antigens, including diphtheria toxin, tetanus toxin and many viruses, can be neutralized by antibody. Once neutralized, these substances are no longer able to bind to receptors in the tissues; the resulting antigen–antibody complexes are usually removed from the circulation and destroyed by macrophages.

Box 1.7 Some factors affecting immune responses

Antigen

- Biochemical nature: polysaccharide antigens tend to elicit a predominant immunoglobulin (Ig)M + IgG_2 response in contrast to protein antigens, which elicit both cellular and humoral responses.
- Dose: in experimental animals large doses of antigen induce tolerance.
- Route of administration: polio vaccine administered orally elicits an IgA antibody response than intramuscular injection. Some antigens/allergens given orally can induce tolerance.

Antibody

- Passive administration of antibody can be used to modulate immune responses, e.g. maternal administration of antibodies to the red cell Rh antigen is used to prevent haemolytic disease of the newborn by removing fetal red cells from the maternal circulation.

Cytokines

- Cytokines released by Th1/Th2 lymphocytes influences type of immune response. Th1 cytokines favour development of cellular immunity, while Th2 cytokines favour antibody production.

Genes

- Major histocompatibility complex (MHC) genes help to control immune responses to specific antigens, e.g. studies in mice have identified strains that are high responders to certain antigens but poor responders to others. This is mirrored in humans by the strong link between certain MHC genes and the development of certain autoimmune diseases.
- Non-MHC genes also influence immune responses, e.g. mutations in the recombinase gene responsible for immunoglobulin and T-cell receptor gene rearrangement result in severe combined immunodeficiency in babies.

Although the physiological function of IgE antibody is unknown, it may have a role in the expulsion of parasites from the gastrointestinal tract. IgE antibody is normally bound to tissue mast cells. Attachment of antigen to IgE antibodies results in mast cell triggering, and release of a number of mediators of tissue damage (see Fig. 1.29 and Chapter 4).

1.5.3 Indirect functions of antibody

Opsonization is the process by which an antigen becomes coated with substances (such as antibodies or complement) that make it **more easily engulfed** by phagocytic cells. The coating of soluble or particulate antigens with IgG antibodies renders them

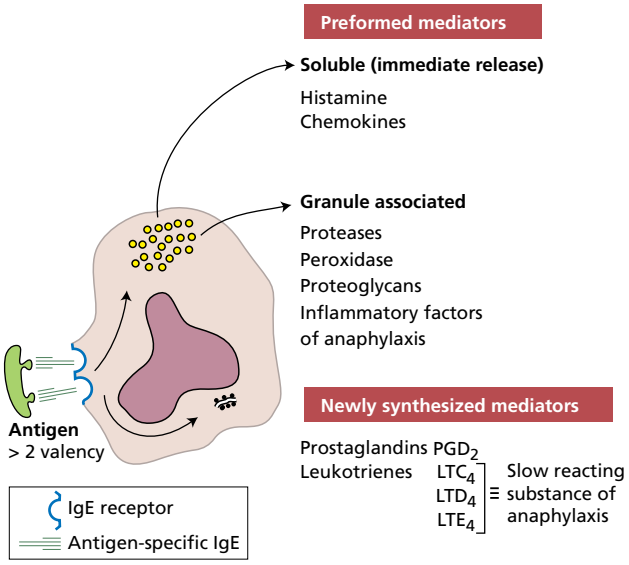


Fig. 1.29 Mechanisms in immunoglobulin (Ig)E-mediated hypersensitivity.

susceptible to cells that have surface receptors for the Fc portions of IgG (FcRIII) (Fig. 1.23). Neutrophils and macrophages both have these Fc receptors and can phagocytose IgG-coated antigens; however, this process is relatively inefficient if only Fc receptors are involved. The activation of complement by antibody (via the classical pathway) or by bacterial cell walls (via the alternative pathway) generates C3b on the surface of microorganisms and makes them susceptible to binding by several types of C3 receptors on macrophages and neutrophils (see Fig. 1.23). C3 receptors are very efficient in triggering phagocytosis.

1.5.4 Regulation

As discussed previously, termination of an ongoing immune response and regulation of the size of the response are crucial if collateral damage is to be prevented. While much is known of the regulation of the complement pathways, the science of cell-mediated regulation is in its infancy (after a false start in the 1980s). Several cell types are thought to have a regulatory function, involving at least three possible mechanisms (cell lysis, induced apoptosis or downregulation via cytokines). Natural and induced T regs (section 1.4.2) seem to be the most important cells at present; absence of these cells, or excess of their counterparts, Th17 cells, results in autoimmune diseases, severe inflammation and allergies. Other cell types involved also include NKT cells.

1.6 Tissue damage caused by the immune system

1.6.1 Inflammation: a brief overview

Inflammation is defined as increased vascular permeability accompanied by an infiltration of 'inflammatory' cells, initially polymorphonuclear leucocytes (usually neutrophils) and later macrophages, lymphocytes and plasma cells. **Vascular permeability** may be increased (resulting in oedema) by a number of agents, which include complement fragments such as C3a, C5a, factor Ba and C2 kinnin. Some fragments (C3a, C5a and C567) also attract neutrophils and mobilize them from the bone marrow; cytokines generated by activated DCs, T cells and macrophages, such as IL-1, IL-6, TNF and IL-12, have similar properties, as well as activating vasodilation to increase blood flow (resulting in erythema). Inflammatory chemokines also attract a variety of cells to **migrate into tissues**.

The triggering of mast cells via IgE is also a method of causing inflammation, due to release of histamine and leukotrienes (which are quite distinct from cytokines) that increase vascular permeability and attract eosinophilic polymorphonuclear leucocytes too. This is discussed further in Chapter 4.

The inflammatory cytokines (IL-1, IL-6 and TNF) also provoke increased synthesis of particular serum proteins in the liver. The proteins are known as **acute-phase proteins** and include proteins that act as mediators (as in opsonization – C3 and C4 complement components, C-reactive protein [CRP]), enzyme inhibitors (α_1-antitrypsin) or scavengers (haptoglobin); the increased serum concentrations of such proteins are helpful in resolving inflammation. In practical terms, serial measurements of CRP give a useful indication of the extent and persistence of inflammation; since the half-life of CRP is only a few hours, changes in serum levels reflect rapid changes in inflammation (such as after antibiotic therapy) sufficiently quickly to be clinically useful. This is in contrast to fibrinogen (another acute-phase protein and the major factor in the erythrocyte sedimentation rate, ESR), where changes are much slower and therefore not useful clinically.

Unfortunately, the recognition of antigen by antibodies, B cells or T-effector cells can cause incidental tissue damage as well as the intended destruction of the antigen. Reactions resulting in tissue damage are often called **hypersensitivity** reactions; Gell and Coombs defined four types (Table 1.13) and this classification (though arbitrary) is still useful to distinguish types of immunological mechanisms. *Most hypersensitivity reactions are not confined to a single type: they usually involve a mixture of mechanisms.*

Immediate hypersensitivity (type I) reactions are those in which antigen interacts with preformed antigen-specific IgE bound to tissue mast cells or basophils. IgE responses are usually directed against antigens that enter at epithelial surfaces, i.e. inhaled or ingested antigens. Specific IgE production requires helper T cells and is regulated by T-cell-derived cytokines; IL-4 and IL-13 stimulate IgE production, while IFN-γ is inhibitory. The balance between help and suppression depends on many variables, including the route of administration of the antigen, its chemical composition, its physical nature, whether or not adjuvants were employed and the genetic background of the animal. Following the interaction of cell-surface IgE and allergen, **activation of the mast cell** causes the release of pharmacologically active substances (see Chapter 4). Type I reactions are rapid; for example, if the antigen is injected into the skin, 'immediate hypersensitivity' can be seen within 5–10 minutes as a 'wheal-and-flare reaction',

Table 1.13 Types of hypersensitivity – mechanism, examples of disease and relevant therapy

Types	Mechanism	Therapy	Disease example
Immediate (type I)	IgE production	Antigen avoidance Neutralization of IgE – e.g. Omalizumab – monoclonal antibody binding free IgE and to B cells with surface IgE	Anaphylaxis Atopic diseases
	Mast cell degranulation	Mast cell stabilizers (disodium cromoglycate)	
	Mediators: histamine	Antihistamines	
	Leukotrienes	Leukotriene receptor antagonists, e.g. Montelukast	
	Granule-associated mediators	Corticosteroids	
Cell-bound antigen (type II)	IgG/IgM autoantibodies		
	Complement lysis	Immune suppression and/or plasma exchange to remove antibodies	Cold autoimmune haemolytic anaemia Myasthenia gravis
	Opsonization leading to neutrophil activation	Plasmapheresis Splenectomy	Goodpasture's syndrome Warm autoimmune haemolytic anaemia Immune thrombocytopenic purpura
	Metabolic stimulation	Correct metabolism	Graves' disease
	Blocking antibodies	Replace factors missing due to atrophy	Pernicious anaemia Myxoedema Infertility (some cases)
Immune complex (type III)	High concentrations of immune complexes, due to persistent antigen and antibody production, leading to complement activation and inflammation	Removal/avoidance of antigen if possible	Serum sickness Extrinsic allergic alveolitis Lepromatous leprosy
		Antiinflammatory drugs: non-steroidals, corticosteroids	Systemic lupus erythematosus
		Immune suppression: cyclophosphamide	Cutaneous vasculitis
Delayed-type hypersensitivity (type IV)	TH1 cytokine production and macrophage activation	Block cytokine production: Ciclosporin Azathioprine	Graft rejection Graft-versus-host disease
		Antiinflammatory: many inflammatory conditions Corticosteroids Reduce macrophage activity: Corticosteroids	Tuberculosis, tuberculoid leprosy Contact dermatitis
		Remove antigen	

Ig, immunoglobulin

where the resulting oedema from increased vascular permeability is seen as a wheal and the increased blood flow as a flare. In humans, there is a familial tendency towards IgE-mediated hypersensitivity, although the genes related to this 'atopic tendency' do not determine the target organ or the disease. Clinical examples of type I reactions include anaphylactic reactions due to insect venoms, peanuts and drugs, as well as the atopic diseases of hay fever and asthma (see Chapter 4).

Type II reactions are initiated by antibody reacting with antigenic determinants that form **part of the cell membrane**. The consequences of this reaction depend on whether or not complement or accessory cells become involved, and whether the metabolism of the cell is affected (Fig. 1.30). IgM and IgG can be involved in type II reactions. The best clinical examples are some organ-specific autoimmune diseases (see Chapter 5) and immune haemolytic anaemias (see Chapter 16 and Table 1.13).

Although type II reactions are mediated by autoantibodies, T cells are also involved. For example, in Graves' disease, which is known to be due to autoantibodies stimulating thyroid-stimulating hormone (TSH) receptors, specific reactive T cells are present also. Although these T cells are instrumental in promoting antibody production (primary effect), they are unlikely to cause tissue damage since the lymphocytic infiltration is mild and consists of B cells too. **Secondary autoantibodies** to antigens are released following tissue damage such as the antibodies to thyroid peroxidase. In contrast, the autoreactive T cells cloned from patients with rheumatoid arthritis and multiple sclerosis have a **primary role** in tissue damage.

Type III reactions result from the presence of immune complexes in the circulation or in the tissues. Localization of **immune complexes** depends on their size, their charge, the nature of the antigen and the local concentration of complement. If they accumulate in the tissues in large quantities, they may activate complement and accessory cells and produce extensive tissue damage. A classic example is the Arthus reaction, where an antigen is injected into the skin of an animal that has been previously sensitized. The reaction of preformed antibody with this antigen results in high concentrations of local immune complexes; these cause complement activation and neutrophil attraction and result in local inflammation 6–24 hours after the injection. Serum sickness is another example: in this condition, urticaria, arthralgia and glomerulonephritis occur about 10 days after initial exposure to the antigen. This is the time when maximum amounts of IgG antibody, produced in response to antigen stimulation, react with remaining antigen to form circulating, soluble immune complexes (Fig. 1.31). As these damaging complexes are formed, the antigen concentration is rapidly lowered; the process only continues as long as the antigen persists and thus is usually self-limiting. Further clinical examples include systemic lupus erythematosus (SLE; see Chapter 5), glomerulonephritis (see Chapter 9) and extrinsic allergic alveolitis (see Chapter 13).

Type IV reactions are initiated by T cells that react with antigen and release **Th1 cytokines**. Cytokines attract other cells, particularly macrophages, which in turn liberate lysosomal enzymes and Th17 cells. The resultant acute lesions consist of **infiltrating lymphocytes**, **macrophages** and occasionally eosinophil polymorphonuclear leucocytes. Chronic lesions show necrosis, fibrosis and, sometimes, granulomatous reactions. An understanding of mechanisms that lead to tissue damage helps to find relevant therapy (Table 1.13).

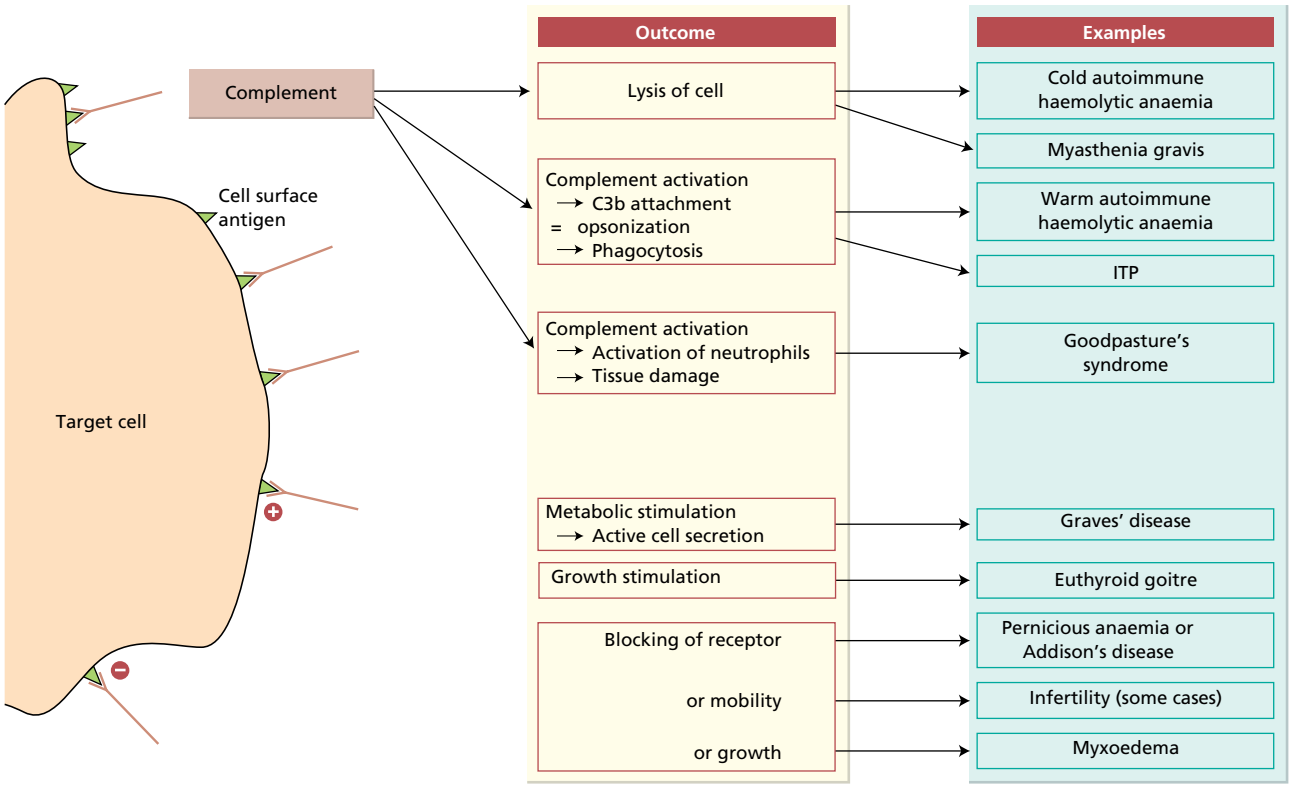


Fig. 1.30 Clinical consequences of cell-bound hypersensitivity. ITP, immune thrombocytopenic purpura.

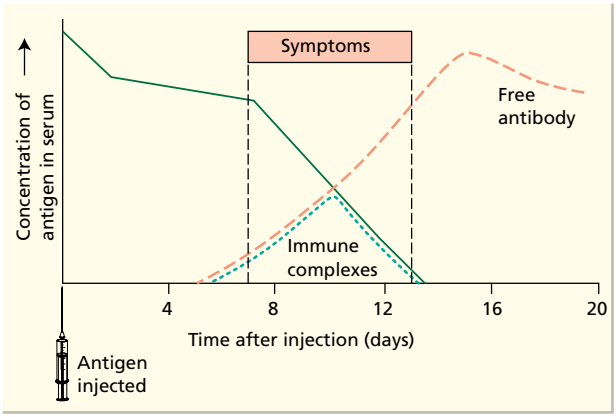


Fig. 1.31 Immune complex formation in acute serum sickness.

1.7 Organization of the immune system: an overview

All lymphoid cells originate in the bone marrow. The exact nature of the uncommitted lymphoid stem cell is unclear (though it is $CD34^+$). An understanding of the developmental pathways is important, not only to clarify the physiology of the normal immune response, but because some immunodeficiency states represent maturation arrest of cells in their early stages of development (see Chapter 3) and some forms of therapy, such as bone marrow transplantation and gene therapy, depend on the identification and use of stem cells.

Lymphoid progenitors destined to become T cells migrate from the bone marrow into the cortex of the **thymus**. Under the influence of stromal cells and Hassalls' corpuscles in the thymic cortex, further differentiation into mature T cells occurs. The passage of T cells from the thymic cortex to medulla is associated with the acquisition of characteristic surface glycoprotein molecules so that medullary thymocytes eventually resemble mature, peripheral T cells. T-cell development in the thymus is characterized by a process of **positive selection** in the thymic cortex; T cells that recognize self-MHC proceed to full maturation. In contrast, T cells that do not recognize self-MHC do not develop any further. **Negative selection** happens in the thymic medulla. Those maturing T cells that recognize and bind to peptides of self-antigens with high affinity are selected out (negative selection) and kill themselves by apoptosis (programmed cell death). Deletion of self-reactive, developing T cells in the thymus is an important mechanism by which autoimmune disease is prevented (Chapter 5). Von Boehmer has succinctly summarized the role of the thymus in T-cell selection: 'the thymus selects the useful, neglects the useless and destroys the harmful' (a reference to autoreactive T cells). The nature of the T cells that survive is variable in terms of final tissue distribution. Those with αβ TCRs have wide-ranging antigen-binding capacity and are distributed to all tissues (including mucosae) as well as circulating according to the nature of their V regions: some to skin, others to the gut or reproductive tract or respiratory mucosa.

In contrast, B-cell development occurs in the **bone marrow** and is closely dependent upon interactions between surface glycoproteins on non-lymphoid stromal cells (such as stem cell factor, SCF) and specific receptors on B-cell precursors (in the case of SCF, Kit tyrosine kinase). Activation of Kit by SCF triggers the early stages of B-cell development; later stages of B-cell development occur under the influence of cytokines secreted by stromal cells, principally IL-7.

The thymus and the bone marrow are **primary lymphoid organs**. They contain cells undergoing a process of maturation from stem cells to antigen sensitivity and restriction. *This process of maturation is independent of antigenic stimulation within the animal*. In contrast, secondary lymphoid organs are those that contain antigen-reactive cells in the process of recirculating through the body. They include lymph nodes, spleen, bone marrow (in part) and mucosal-associated lymphoid tissues. Antigenic stimulation changes the relative proportions of the mature cell types in secondary tissues.

Peripheral T and B cells circulate in a definite pattern through the **secondary lymphoid organs** (Fig. 1.32). Most of the recirculating cells are T cells and the complete cycle takes about 24 hours; some B cells, including long-lived memory B cells, also recirculate. Lymphocyte circulation is strongly influenced by chemokine receptors on the lymphocyte surface that act as homing agents. There are also adhesion molecules directing cells to their respective ligands on high endothelial venules of lymph nodes and mucosal tissue. For instance, L-selectin is a surface glycoprotein on lymphocytes responsible for homing into lymph nodes (see section 1.2.6 and Tables 1.9 and 1.14). Recently, expression of the sphingosine-1-phosphate receptor (S1PR) on lymphocytes and its interaction with its ligand, sphingosine-1-phosphate, has been shown to be a major determinant of lymphocyte trafficking. Inhibition of lymphocyte egress by S1PR agonists has been therapeutically exploited in the treatment of multiple sclerosis.

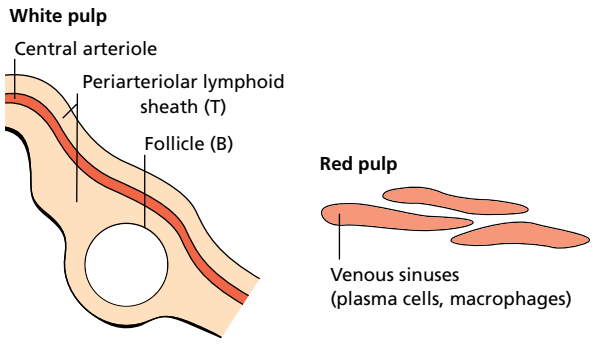


Fig. 1.32 Recirculation pathways of lymphocytes. The majority of naive T cells entering the lymph node cortex from blood will leave the node immediately via efferent lymphatics. Naive T cells that recognize specific antigens differentiate into effector T cells and reenter the circulation. B-cell recirculation follows a similar route: B cells that encounter specific antigens proliferate to form germinal centres; memory B cells form a surrounding marginal zone.

Table 1.14 Key molecular determinants of lymphocyte recirculation

Lymphocyte receptor	Ligand	Function
CD62L (L-selectin)	CD34, glycam	Mediates adhesion and lymphocyte rolling on endothelium
CCR7	CCL21 on lymph node HEV	Lymphocyte homing to lymph nodes
CCR9 (gut tropic)	CCL25 on intestinal HEV	Lymphocyte homing to intestine
Sphingosine-1-phosphate receptor (S1PR)	Sphingosine-1-phosphate	Lymphocyte egress from lymph nodes; S1PR agonists are licensed for the treatment of multiple sclerosis

Lymph node architecture is well adapted to its function (Fig. 1.18). The **lymphatic network**, which drains the extravascular spaces in the tissues, is connected to the lymph nodes by lymphatic vessels; these penetrate the lymph node capsule and drain into the peripheral sinus, from which further sinuses branch to enter the lymph node, passing through the cortex to the medulla and hence to the efferent lymphatic vessel. This sinus network provides an excellent filtration system for antigens entering the lymph node from peripheral tissues (Fig. 1.18).

The **cortex** contains primary follicles of B lymphocytes, surrounded by T cells in the 'paracortex'. There is a meshwork of interdigitating cells throughout the lymph node. Antigen is filtered and then presented to lymphoid cells by these interdigitating cells. On antigen challenge, the 'primary' follicles of the lymph node develop into 'secondary' follicles. In contrast to primary follicles, secondary follicles contain germinal centres. These comprise mainly B cells with a few helper T cells and a mantle zone of the original primary follicle B cells. B cells in a secondary follicle are antigen activated and more mature; most have IgG on their surfaces, whereas those B cells in the primary follicle and mantle zone are less mature, bearing both IgD and IgM. Activated B cells migrate from the follicle to the medulla, where they develop into plasma cells in the **medullary cords** before releasing antibody into the efferent lymph.

The architecture of the spleen is similar. The white pulp around arterioles is arranged into T- and B-cell areas with primary and secondary follicles (Fig. 1.18). Antigen challenge results in expansion of the white pulp with B-cell activation and the development of secondary follicles. Plasma cells migrate to the red pulp.

1.8 Conclusions

The aim of this chapter is to give an overview of the normal workings of the immune system, so that the pathological processes involved in diseases are easily understood. The subsequent chapters are clinically based, devoted either to the immunological conditions of an organ or a particular type of immunological disease (allergy, autoimmune diseases or immunodeficiency). An understanding of the molecular basis of immunology as well as the cells involved in the four types of immunological mechanisms will assist the reader with the immunopathogenesis of each condition leading to the relevant treatment options.