Section 1

1 Notes on the Immune System

Tracey J. Lamb

Department of Pediatrics, Emory University School of Medicine, Atlanta, USA

This chapter provides some background to the immune system, outlining the cells involved in carrying out immune responses, the receptors mediating recognition of foreign antigens (such as those carried by parasitic organisms) and the effector mechanisms activated to destroy parasites and contain infection. This outline is not a comprehensive account of the workings of the immune system; instead, these notes focus on the aspects of the immune system that are most relevant to the chapters which follow on specific parasite infections.

Readers are encouraged to refer to the suggestions for further reading cited at the end of this chapter, or to one of the many comprehensive textbooks published, such as *Janeway's Immunobiology*, for a more detailed account of specific aspects of the immune system.

1.1 The immune system

The body has external physical barriers to prevent infection, such as the skin, the production of sweat containing salt, lysozyme and sebum, and the mucous membranes, which are covered in a layer of mucous that pathogens find hard to penetrate. If these barriers are breached, the body will then mount an immune response and mobilise immune cells to destroy the intruder.

Immune responses are carried out by a variety of different immune cells, all of which initially arise from progenitor stem cells in the bone marrow (Figure 1.1). While most cells mature in the bone marrow, T cells undergo additional development in the thymus. The number of immune cells in the body (homeostasis) is regulated through tight controls on haematopoiesis in the bone marrow, an environment rich in growth factors (such as colony-stimulating factors) and cytokines that support the growth and differentiation of immune cells. The bone marrow and thymus are known as the primary lymphoid organs, because they are the primary sites of immune cell development and maturation.

Immunity to Parasitic Infection, First Edition. Edited by Tracey J. Lamb.

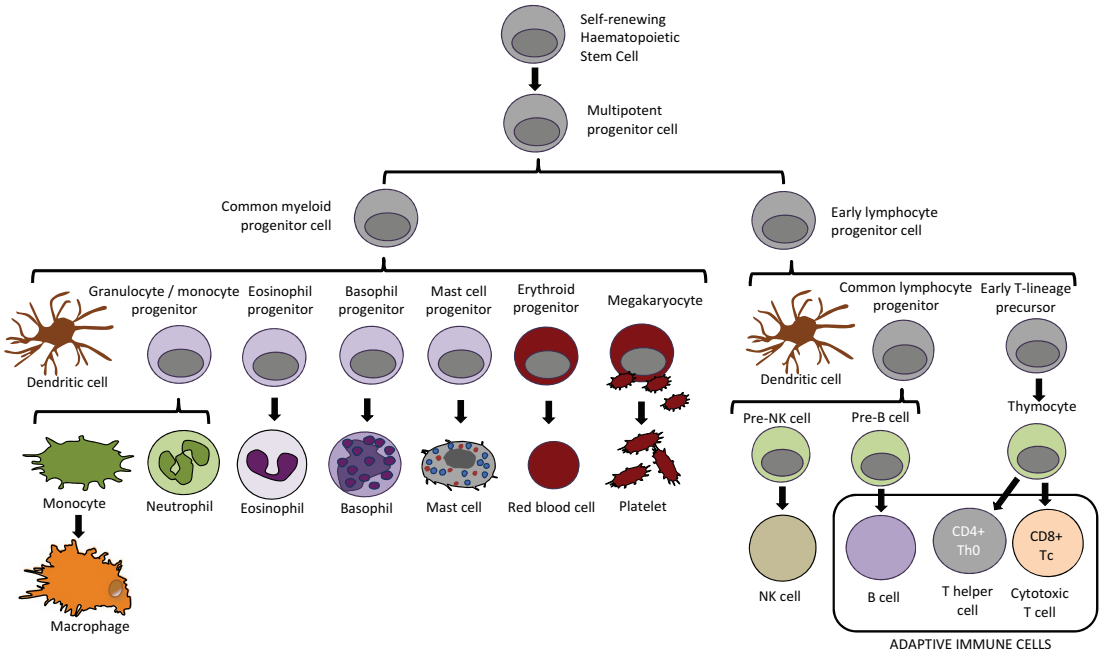


Figure 1.1 Innate and adaptive immune cells of the human body. All cells are derived from self-renewing haematopoietic stem cells in the bone marrow, and they arise from myeloid or lymphoid progenitors. Dendritic cells can develop from both lineages and also differentiate from monocytes (pathway not shown).

Once mature, immune cells exit the bone marrow (or the thymus, in the case of T cells) and take up residence in highly organised structures composed of both immune and non-immune cells, known as the secondary lymphoid organs (Figure 1.2). Although immune responses are initiated at the point where the body's external barrier has been breached, the establishment of an immune

Some of the main lymphoid organs in the human body			
	Organ	Location	Function
SECONDARY	Tonsils	At the back of the throat	Detection of ingested or inhaled pathogens
PRIMARY	Thymus	Above and in front of the heart	Maturation and selection of T cells
SECONDARY	Spleen	Upper left quadrant of the abdomen	Filtration of the blood for invading pathogens
PRIMARY	Bone Marrow	Bone cavities	Main location for haematopoiesis
SECONDARY	Lymph nodes and lymphatics	Widely distributed throughout the body	Detection of invading pathogens at sites distal to other lymphoid organs

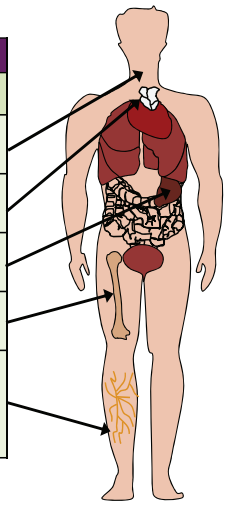


Figure 1.2 Lymphoid organs in the human body. Immune cell development occurs in the primary lymphoid organs, whereas secondary lymphoid organs are the sites where immune responses are coordinated.

Table 1.1 Functions of the innate and adaptive arms of the immune system.

Innate Immune Cells	Adaptive Immune Cells
Immune recognition	
Immune effector mechanisms	
Immune regulation	
	Immunological memory

response – particularly the adaptive arm of the immune response – occurs in the secondary lymphoid organs draining the site of infection.

The immune system has evolved a number of effector mechanisms capable of destroying pathogenic organisms. Immune responses can be classified as innate or adaptive (see Table 1.1). The innate arm of the immune system recognises pathogens non-specifically and generates immediate generic mechanisms of pathogen clearance. The adaptive arm of the immune system is more specific for individual pathogens, and it takes a number of days to develop.

There is a high degree of 'cross-talk' between the innate and adaptive arms of the immune system. In general, an adequate adaptive immune response is only activated after initiation by the cells of the innate immune system; conversely, innate immune effector mechanisms become more efficient by interaction with an active adaptive immune response.

1.2 Innate immune processes

The innate immune system is able to mount an immediate immune response to a foreign pathogen, or to whatever is 'dangerous' to the human body as embodied by Matzinger's 'Danger hypothesis'. Innate immune responses are generic and mounted upon recognition of pathogen-associated molecular patterns (PAMPs) commonly found in molecules that are part of, or produced by, pathogenic organisms. PAMPs are recognised by pattern recognition receptors (PRRs – see Figure 1.3), primarily (but not exclusively) expressed on (and in) phagocytic antigen presenting cells (APCs) such as macrophages, dendritic cells (DCs) and some types of granulocytes. PRRs can also recognise host molecules containing damage-associated molecular patterns (DAMPs) – molecules that are often released from necrotic cells damaged by invading pathogens.

1.2.1 Inflammation

Once recognition of PAMPs or DAMPs occurs, a series of innate immune processes are activated by innate immune cells that contribute to pathogen destruction. 'Inflammation' is a generic term used to describe the dilation and increased permeability of the blood vessels in response to leukotrienes and prostaglandins secreted by phagocytes upon pathogen recognition. Inflammation results in increased blood flow and in the loss of fluid and serum components from capillaries into tissue, as well as the extravasation of white blood cells to the breached area. Superficially, inflammation is responsible for the visible symptoms swelling, pain and redness in infected tissue.

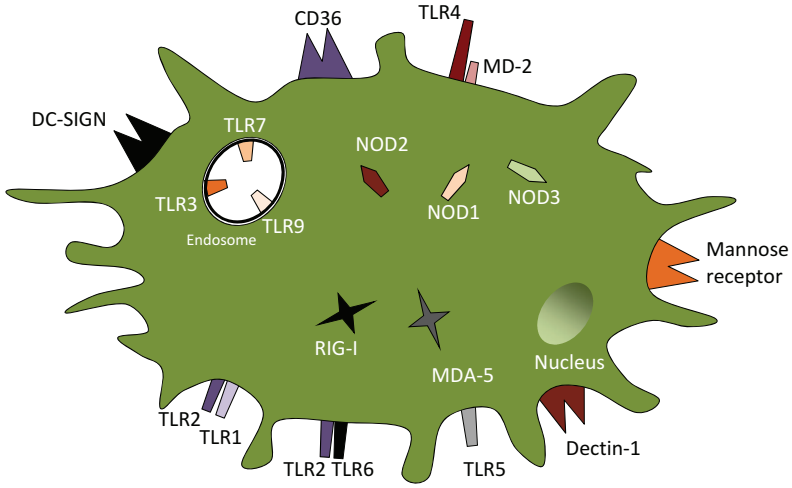


Figure 1.3 Some of the main pattern recognition receptors found on antigen presenting cells. Abbreviations: DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; MD2, myeloid differentiation factor-2; MDA-5, myeloid differentiation-associated gene-5; NOD, nucleotide-binding oligomerisation domain; RIG-I, retinoic acid inducible gene-I; TLR, Toll-like receptor.

1.2.2 The acute phase response

The acute phase response is initiated by activation of macrophages upon ligation of PRRs with pathogen-associated molecules. This term is used to describe the production of several different proteins which enhance the containment and clearance of invading pathogens.

The production of acute phase cytokines (interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)) are collectively called endogenous pyrogens, because they stimulate the induction of prostaglandin E2, which acts on the hypothalamus to induce fever. Fever is effective in inhibiting the growth of some pathogens and can also enhance the performance of phagocytes. When uncontrolled, however, fever can be damaging to the body.

IL-6 acts on the liver to induce the production of acute phase proteins which include C-reactive protein, serum amyloid protein and mannose binding lectin (MBL). Acute phase proteins opsonise invading pathogens, promoting their phagocytosis and activating the complement pathway to induce pathogen lysis – the latter a particular feature of mannose-binding lectin (MBL) which activates the lectin-pathway of complement (see below).

1.2.3 Anti-microbial peptides

Anti-microbial peptides vary in length from between 12 and 50 amino acids, and are ionically charged molecules (anionically or cationically). In mammals, there are two large families of anti-microbial peptides: defensins and cathelicidins. These peptides can opsonise pathogens, attaching and inserting into the membrane to modify the membrane fluidity and form a pore that lyses and

destroys the pathogen. It has also been suggested that some anti-microbial peptides exert their anti-microbial effects by translocating across the pathogen membrane and inhibiting essential enzymes necessary for nucleic acid and protein synthesis, effectively killing the pathogen by starvation. Anti-microbial peptides are effective against some protozoan pathogens as well as against bacteria.

1.3 The complement cascade

The complement cascade involves several different components activated in sequence leading to the generation of a cytopathic 'membrane attack complex' (MAC). The MAC is a structure that is able to form a pore in the membrane of the invading pathogen, leading to damage and lysis (Figure 1.4). Complement can be activated by three different pathways: the classical pathway, the alternative pathway and the lectin pathway:

- The **classical pathway** is linked to the adaptive arm of the immune system and is activated by antibody recognition of pathogens (specifically IgM or IgG) (Figure 1.4).
- The **alternative pathway** is an innate antibody-independent mechanism activated by a variety of 'danger signals' and spontaneous hydrolysis of C3.
- The **lectin pathway** depends on the binding of MBL to surface proteins of invading pathogens that contain mannose residues.

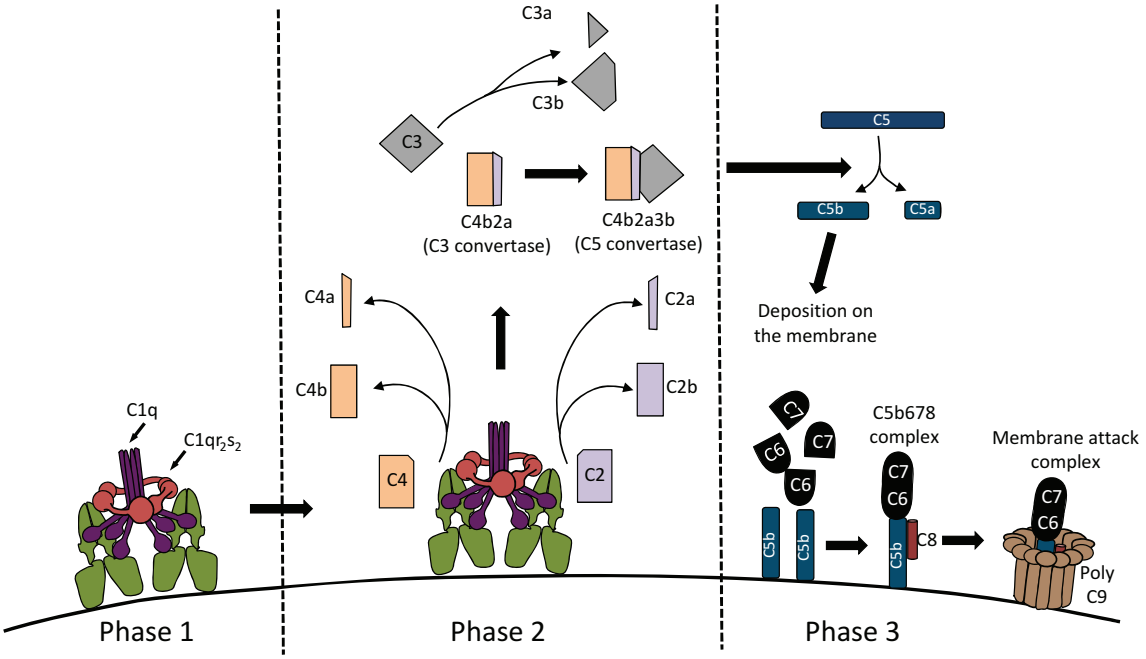


Figure 1.4 The complement cascade. The cascade involves nine components (C1-C9) and can be split into three phases: the first phase involves the attachment of C1 to antibodies opsonising the surface of a pathogen; the second phase leads to cleavage of the C2 and C4 components and the formation of C3 convertase, which in turn cleaves C3 to form C5 convertase; the third phase involves the cleavage of C5 by C5 convertase and the deposition of C5b on the surface of the pathogen. C5b activates the formation of the membrane attack complex (MAC) which creates a pore in the membrane, leading to lysis.

On a molecular level, MBL shares similarity to the complement component C1q, enabling this reaction to occur. Five per cent of the world's population have polymorphisms in the gene encoding MBL which leave people with low levels of MBL. Although the lectin pathway of complement activation has been little studied, it is known to play a role in defence against some protozoan parasites, such as *Cryptosporidium* (see Chapter 5). Although *Cryptosporidium* can activate both the classical and lectin pathways of complement, it is the lectin pathway that is most effective at destroying the parasite.

Upon activation of complement the deposition of C5b on the surface of the invading pathogen leads to lysis via the assembly of the MAC. Other components of complement (in particular C3b) are opsonising agents. Phagocytic cells express complement receptors that can detect pathogens opsonised in complement fragments, promoting phagocytosis and pathogen clearance. The by-products of the cleavage of complement components C3 and C5, C3a and C5a (Figure 1.4) are also called anaphylotoxins, and these are potent inflammatory molecules that induce degranulation of mast cells and basophils, leading to the vasodilatory effects and vascular leakage associated with granule release from these cells.

Unwanted complement activation can be damaging to the host tissues, so it is necessary to regulate the process of complement activation. This regulation is carried out by several soluble and membrane-bound complement regulatory proteins, which regulate different points of activation in the complement pathway. C1 inhibitor (C1-INH) controls activation of complement via the classical and lectin-binding pathways, by associating with the C1 complex and causing the separation of C1r and C1s from C1q (see Figure 1.4). Further down the complement pathway, Factor H is able to hinder the formation of C3 convertase, while carboxypeptidase N inactivates the C3a and C5a fragments from cleavage of C3 and C5 respectively. Membrane-bound complement regulatory proteins include decay-accelerating factor (DAF or CD55), which accelerates the decay of C3 convertases, rendering them ineffective at cleaving C3.

1.4 Innate recognition

Innate immune recognition of pathogens and pathogen-associated molecules occur via several families of pattern recognition receptors (PRRs) (Figure 1.3), as well as receptors that recognise molecules that opsonise pathogens, such as MBL or anaphylotoxins (C3a and C5a). For many pathogens (in particular parasitic organisms), there is no complete picture of PAMP-containing molecules and the PRRs that initiate an innate immune response upon infection. However, it is known that innate immune responses can cross strain-specificity within a species of pathogen and, indeed, also species-specificity, because the patterns recognised by PRRs are often commonly occurring repetitive sequences.

The main PRRs that have been studied with respect to parasitic infection are the Toll-like receptors (TLRs) and some of the C-type lectin receptors, but a role for more recently discovered PRRs cannot be ruled out at present. Although APCs are generally the first type of immune cell to recognise pathogens via PRRs,

many other types of immune and non-immune cells of the body have been found to express PRRs to some extent. The repertoire of PRRs expressed – and, correspondingly, the type of pathogen that can be recognised by individual cell types – varies.

1.5 Pattern recognition receptors

TLRs were first discovered in *Drosophila* fruit flies, and they are thought to be a microbe-detection system conserved widely throughout the animal kingdom. There are now ten described members of this family in humans and twelve in mice. The expression of individual TLRs varies with cell type, and not all TLRs are expressed on the cell surface: some are expressed intracellularly on the endoplasmic reticulum (ER) membrane.

TLRs do not always work individually, but some become activated as dimeric complexes. For example, TLR2 recognises ligands by forming heterodimers with TLR1 or TLR6, and it is also known to act as a co-receptor for the scavenger receptor CD36. Similarly, TLR4 recognises lipopolysaccharide (LPS) when complexed with myeloid differentiation factor 2 (MD2). PAMPs recognised by TLRs are found on a diverse range of molecules, some of which are listed in Table 1.2.

C-type lectins are an important family of PRRs that are likely to play an important, if understudied, role in parasitic diseases. They recognise carbohydrate motifs found on glycoproteins of both protozoan and helminth parasites. Examples of C-type lectins include the mannose receptor, which recognises MBL produced by the liver in response to IL-6 during the acute phase response and Dectin-1, which recognises zymosan.

Other PRRs that have been characterised in viral and bacterial infections and may have some role in parasitic infection, include the cytoplasmic nucleotide-oligomerisation domain (NOD)-like receptor family. The members of this family contain the protein-binding motifs caspase activation and recruitment domain (CARD), a pyrin domain and/or baculovirus inhibitor of apoptosis protein repeat (BIR). Current defined ligands for the NOD receptors include bacterial peptidoglycans and uric acid, an inflammatory by-product that forms upon degradation of hypoxanthine released during shizogeny of malaria-infected red blood cells (see Chapter 3).

1.5.1 Signalling events activated upon ligation of PRRs

Ligation of PRRs with pathogens or pathogen products activates signalling pathways that lead to the transcription of genes encoding products involved in the inflammatory immune response. Signalling pathways emanating from TLR ligation have been particularly well characterised. TLR signalling is mediated by sequential phosphorylation of kinases, brought together via adaptor proteins that bind to the TLR upon activation. The adaptor proteins involved in TLR signalling pathways contain Toll/Interleukin-1 receptor (TIR)-domains. TLR signalling pathways are classified into myeloid differentiation factor of

Table 1.2 Some innate receptors commonly used for recognition of pathogens.

Recognition receptor	Receptor name	Cellular location	Examples of molecules recognised
Toll-like receptors	TLR1	Surface	Triacyl lipoprotein
	TLR2	Surface	Zymosan (glucan from yeast cell wall) GPI anchors (*Plasmodium*, *Trypanosomes*)
	TLR3	Endosomal	Nucleic acids: double stranded RNA
	TLR4	Surface	LPS (gram negative bacteria) Lipoteichoic acid (gram positive bacteria)
	TLR5	Surface	Flagellin
	TLR6	Surface	Diacyl lipoprotein
	TLR7 (human TLR8)	Endosomal	Nucleic acids: single stranded RNA
	TLR9	Endosomal	Nucleic acids: CPG (bacteria) Haemozoin (*Plasmodium*)
	TLR10	Endosomal	Unknown
	TLR11	Surface	Profilin (*Toxoplasma)*
Nod-like receptors NALPS	NOD1	Cytoplasmic	iE-DAP (peptidoglycan component of gram negative bacteria)
	NOD2	Cytoplasmic	MDP (peptidoglycan component of both gram positive and gram negative bacteria)
	NOD3	Cytoplasmic	Uric acid
RIG-I-like receptors	RIG-I	Cytoplasmic	Nucleic acids: short double stranded RNA fragments (<1 kb)
	MDA5	Cytoplasmic	Nucleic acids: long double stranded RNA fragments (>2 kb)
C-type lectin receptors	DC-SIGN	Surface	Mannose-type carbohydrates
	Mannose receptor	Surface	MBL (opsonic protein that binds to carbohydrates of pathogens)
	Dectin-1	Surface	Zymosan (glucan from yeast cell wall)
Co-receptors	CD14	Surface	Co-receptor for LPS
Scavenger receptors	CD36	Surface	Lipids *Pf*EMP-1 (malaria)

Abbreviations: CPG, -C-phosphate-G- DNA; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; GPI, glycosylphosphatidylinositol; iE-DAP, g-D-glutamyl-mesodiaminopimelic acid; LPS, lipopolysaccharide; MBL, mannose-binding lectin; MDA5, melanoma differentiation-associated gene 5; MDP, muramyl dipeptide; NOD, nucleotide-domain oligomerisation domain-containing proteins; *Pf*EMP-1, *Plasmodium falciparum* erythrocyte membrane protein-1; RIG-I, retinoic acid inducible gene-I.

88kD (MyD88)-dependent (all TLRs except TLR3) or TIR-domain-containing adaptor inducing IFN-β (TRIF)-dependent (TLR3 and TLR4).

TLR4 can signal through both MyD88- and TRIF- pathways (Figure 1.5). When MyD88 is recruited to the TLR4 upon ligation, it interacts with IL-1R-associated kinases (IRAKs) – initially IRAK4, then IRAK1 and IRAK2. These then associate with TNFR-associated factor (TRAF)-6, which in turn activates TGF-β-activated kinase 1 (TAK-1) and the transcription of pro-inflammatory genes such as TNF-α (via NF-κB activation) and IL-12 (via MAP kinase activation). Additionally, TLR4 recruits a third adaptor, called TRIF-related adaptor molecule (TRAM), to activate TRIF, which in turn complexes with TRAF3, TANK-binding

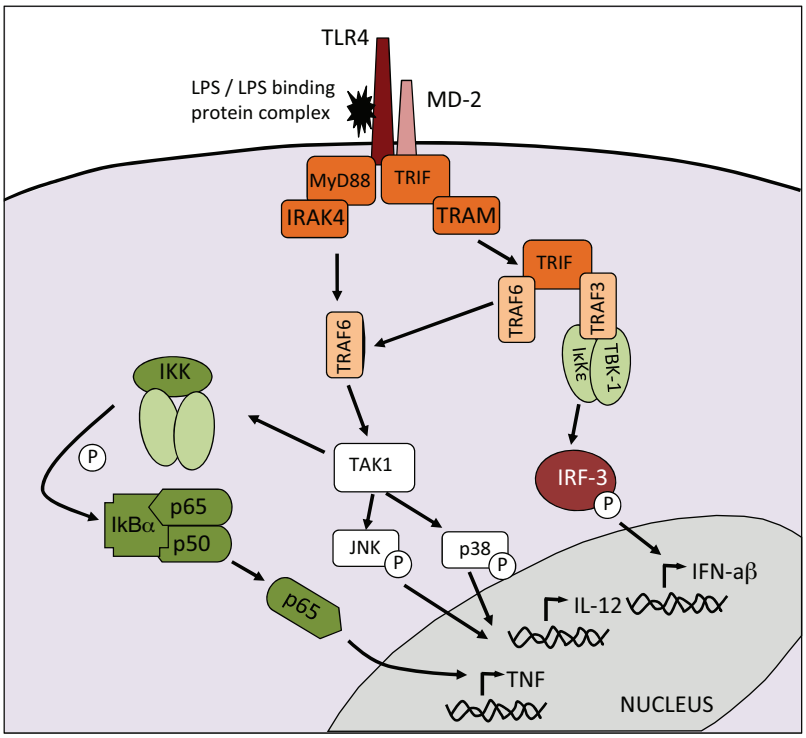


Figure 1.5 Signalling pathways activated by ligation of TLR4. The TLR-4 receptor can signal through both MyD88- and TRIF-dependent pathways to activate the transcription of pro-inflammatory genes in the nucleus.
Abbreviations: IFN, interferon; IKK, IκB kinase; IL, interleukin; IRAK, IL-1 receptor-associated kinase; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MyD88, myeloid differentiation primary response gene (88); TAK, TGF-β-activated kinase; TLR, Toll-like receptor; TNF, tumour necrosis factor; TRAM, TRIF-related adaptor molecule; TRAF, TNF receptor-associated factor; TRIF, TIR-domain-containing adapter-inducing interferon-β.

kinase (TBK-1) and IKK-ε to facilitate the eventual transcription of genes encoding type 1 interferons (IFN-α and IFN-β – not to be confused with type 2 IFN-γ). Thus ligation of TLR4 leads to the transcription of pro-inflammatory cytokines and the initiation of the innate immune response.

1.6 Innate immune cells

1.6.1 Macrophages

Macrophages are a heterogeneous population of cells that reside in most tissues of the body. They arise from monocyte precursors but are terminally differentiated when resident in a tissue. Tissue-resident cells with macrophage-like properties include alveolar macrophages ('dust cells') in the lungs, Kupffer cells in the liver, histiocytes in the connective tissues and microglial cells in the central nervous system, which may play a role in the pathogenesis of cerebral malaria. The combination of surface markers expressed and the location

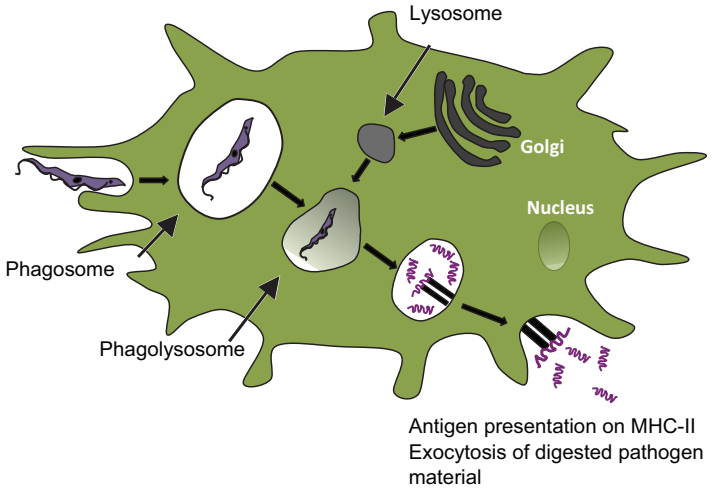


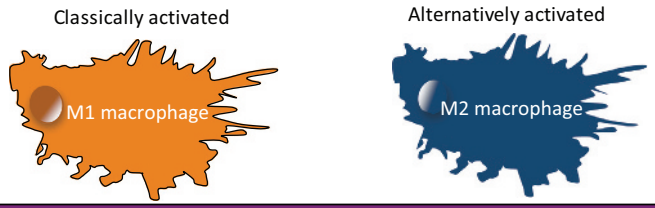
Figure 1.6 Phagocytosis leads to uptake and digestion of extracellular pathogens and their products. The pathogen is engulfed in a phagosome, which then fuses with acidic lysosomes which bud from the Golgi and contain enzymes such as hydrolases for digestion of the endocytosed material. The digested material is expelled from the cell in a process known as exocytosis, but some peptides will be loaded onto major histocompatibility complexes (MHC) for presentation to T cells.

of the macrophages isolated can define the type of macrophages being studied. In mice, some of the generic markers used to define macrophages include F4/80, CD11b and the glycoprotein CD68, the latter found intracellularly in the cytoplasm.

Macrophages are phagocytic cells, and continuously clear senescent erythrocytes and apoptotic cells from the body. The capacity of macrophages to phagocytose, digest and destroy invading pathogens once activated by PRR ligation (Figure 1.6) is aided by a number of different opsonins, notably antibodies, complement fragments and acute phase proteins. Macrophages also play an important immunoregulatory role, both by the secretion of cytokines and chemokines and also as effective APCs that can express peptide-loaded Major Histocompatibility Complex (pMHC) to activate T cells.

The different phenotypes adopted by macrophages is dependent on the molecular cues they receive from the local environment. In parasitic infection, macrophages are often divided into classically activated macrophages (abbreviated to M1 macrophages) and alternatively activated macrophages (abbreviated to M2 macrophages). This division arises after exposure to pro-inflammatory Th1 conditions commonly associated with protozoan infections (M1), or to type 2 inflammation commonly found in helminth infections (M2). M1 and M2 macrophages are quite different, both functionally and on a molecular level (Figure 1.7).

In the context of classical activation, M1 macrophages typically express and up-regulate the receptor for the pro-inflammatory cytokine IFN-γ. They are thus able to respond to the IFN-γ present in a type 1 response. The ability of



Comparison of classically and alternatively activated macrophages

Function	M1 macrophages	M2 macrophages
Cytokine production	TNF, IL-1, IL-6 (acute phase response) IL-8 (neutrophil chemoattractant) IL-12, IL-23 (pro-inflammatory)	IL-4 and IL-13 (down-regulation of type 1 infalmmation)
Distinguishing molecules expressed	iNOS NADPH oxidase IDO	Arignase-1 Chitinase-like enzymes (Ym-1 and Ym-2) RELM-α Upregulated mannose receptor (CD206) and Dectin-1
Phagocytic capacity	Highly phagocytic	Reduced phagocytocytic capacity
Immunoregulatory capacity	Elevated expression of co-stimulatory molecules CD80 and CD86 Able to present antigen to T cells	Up-regulation of MHC-II but poor antigen presentation capacity
Destruction of protozoan parasites	Good producers of toxic nitrogen and oxygen radicals Destruction of intracellular pathogens	Poor at producing toxic nitrogen and oxygen radicals Permissive to infection with *Leishmania* Associated with susceptibility to *Trypanosoma brucei*
Destruction of helminth parasites	Not generally stimulated in helminth infection	Contribute to the immune mechanisms leading to the clearance of parasitic nematodes

Abbreviations: IDO, indoleamine 2,3 dioxygenase; IL, interleukin; iNOS, inducible nitric oxide synthase; MHC, major histocompatibility complex; NADPH, reduced form of nicotineamide adenine dinucleotide phosphate^{+}; TNF, tumour necrosis factor; RELM, resistin-like molecules

Figure 1.7 A comparison of classically activated (M1) and alternatively activated (M2) macrophages.

M1 macrophages to phagocytose protozoan pathogens and digest them is enhanced in response to IFN-γ.

The signalling pathway emanating from the IFN-γ receptor leads to the transcription of a number of IFN-γ-inducible genes that are involved in the destruction of phagocytosed pathogens. These include the up-regulation of enzymes which can generate nitrogen and oxygen derivatives toxic to invading pathogens. This process is known as respiratory burst, and it is mediated by inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO), nicotineamide adenine dinucleotide phosphate (NADPH) oxidase (which generates superoxide) and superoxide dismutase (which generates hydrogen peroxide from superoxide). In M1 macrophages, IFN-γ also up-regulates the expression of indoleamine 2,3-deaminase (IDO), a rate-limiting enzyme in the kynurenine pathway for the degradation of tryptophan, an amino acid essential for the growth of many intracellular pathogens.

M1 macrophages are prolific producers of pro-inflammatory cytokines, including those associated with the acute phase response (TNF, IL-1 and IL-6) and

the neutrophil chemoattractant IL-8. Upon secretion of IL-8, phagocytic neutrophils migrate into the infected tissue and help to clear infection. The secretion of IL-12 by activated M1 macrophages also amplifies the Th1 response due to the polarising effect of this cytokine on CD4+ T cells (see below).

Alternatively activated M2 macrophages are generated in type 2 inflammatory environments and become polarised in response to IL-4. In helminth infections, IL-4 produced by basophils and mast cells in response to chitin (a polymeric component of the body of helminth parasites) may contribute to the development of M2 macrophages. Other type 2 cytokines, such as IL-13 and IL-21, can also polarise macrophages towards an M2 phenotype. Ym-1 (also called chitinase3-like3) and Ym-2 are chitinase-like enzymes secreted by M2 macrophages, but they do not possess chitinase activity. Other molecules associated with an M2 phenotype include increased surface expression of C-type lectins (in particular the mannose receptor and dectin-1 (Table 1.2)) and the expression of resistin-like molecule-α (RELM-α), the latter produced in response to IL-13.

M2 macrophages also produce arginase-1 (Arg1), an enzyme which catalyses the amino acid arginine to ornithine. Since ornithine is a precursor of collagen, a constituent of the extracellular matrix, it has been hypothesised that M2 macrophages may facilitate repair of tissue mechanically damaged by helminths. When uncontrolled, however, excessive deposition of extracellular matrix may lead to fibrosis, a condition that occurs in the liver in the context of strong Th2 responses to trapped Schistosome eggs (Chapter 16). Although associated with helminth infections, M2 macrophages can also be found in protozoan infections. In *Leishmania* (Chapter 7) and trypanosome (Chapter 8) infections, they are associated with susceptibility to infection.

1.6.2 Granulocytes

Granulocytes are composed of a granulated cytoplasm containing granules rich in immunomodulatory molecules. There are four types of granulocytes in the body: neutrophils, eosinophils, basophils and mast cells. With the exception of mast cells, which are largely confined to the tissues (particularly in the gut), granulocytes can be found in the peripheral blood circulation. They form an important part of the body's defence against helminth parasites although, when activated by innocuous antigens, they are responsible for hypersensitivity reactions such as allergic responses (see below).

1.6.2.1 Neutrophils

Neutrophils are the most abundant type of granulocyte in the bloodstream. They are also called polymorphonuclear cells (PMNs), due to their characteristic multi-lobed nucleus. Neutrophil granules stain with both acidic and basic dyes, and they contain a variety of lytic enzymes. Primary granules (azurophilic) contain peroxidase, elastase, lysozyme and hydrolytic enzymes, whereas secondary granules contain collagenase and lysozyme. The bone marrow can release an increased number of neutrophils in response to infection, leading to a transient neutrophil leukocytosis.

Neutrophils are generally one of the first cell types recruited to an area of acute inflammation. They are attracted by a number of chemotactic factors, including IL-8 (also called KC) and leukotrienes secreted by macrophages, and anaphylotoxins derived from the complement cascade. Like macrophages, neutrophils are proficient phagocytic cells. Upon activation, neutrophils degranulate; the release of substances within the neutrophil granules is generally toxic to invading pathogens. Neutrophils can also release neutrophil-extracellular traps (NETs), which are extracellular fibres composed of DNA that can bind to pathogens and more effectively target the delivery of granule contents.

1.6.2.2 Eosinophils

Like neutrophils, eosinophils are motile phagocytic cells that can migrate into the tissue in response to inflammatory stimuli. The granules of eosinophils stain with the acidic dye eosin red ('eosin-ophil'), and contain potent immune mediators such as eosinophil cationic protein, major basic protein and eosinophil peroxidase. Eosinophils express the high-affinity receptor for IgE, FcεRI, and cross-linking of FcεRI by IgE complexed with multivalent antigen leads to eosinophil activation. Eosinophils have long been recognised for their role as effector cells in the anti-helminthic immune response, and releasing the granule contents on the surface of macroparasites such as Schistosomes can damage their surface coat (see Chapter 16).

1.6.2.3 Mast cells

Mast cells are tissue-resident and often defined by their location, either as mucosal mast cells (gastrointestinal tract and lung) or connective tissue mast cells (all other tissues). Mast cells are important mediators of allergic immune responses by virtue of the presence of vasodilator histamine in the granules. Histamine works in concert with other granule substances such as serotonin, prostaglandins and leukotrienes, all of which increase vascular permeability, vasodilation and smooth muscle contraction in the area of release. In addition, mast cell granules are full of chemotactic factors for neutrophils and eosinophils, recruiting these cell types the site of activation.

Mast cells can degranulate in response to binding of anaphylotoxins (the complement fragments C3a and C5a). Like eosinophils, mast cells are also activated by cross-linking of the high affinity receptor for IgE (FcεRI) by IgE/antigen complexes. In addition, mast cells also express FcγRIII, a receptor which can bind to IgG/antigen complexes.

1.6.2.4 Basophils

Mast cells are not the only granulocytes involved in hypersensitivity reactions such as allergic immune responses. Although the least common type of granulocyte in the body, basophils share some similarities with mast cells and are also important contributors to hypersensitivity reactions. The granules of basophils stain with basic dyes ('bas-ophil') and also contain histamine. Although they have previously been considered to be non-phagocytic, this view has been challenged by recent evidence in models of helminth infection (Chapter 14) and in allergy; it is now thought that basophils are important APCs, particularly in the

polarisation of CD4+ T cells towards a Th2 phenotype. Basophils are thought to contain stores of pre-formed IL-4 and they may be an important early source of this cytokine during priming of T cells.

Like mast cells, basophils can express FcεRI (which binds to IgE with high affinity) and the IgG receptors FcγRIII (CD16) and FcγRII (CD32) that bind to IgG. However, although cross-linking of FcεRI by IgE/antigen complexes leads to degranulation, the events following IgG binding on basophils is still unclear, since this can lead to an inhibitory rather than a stimulatory effect. Basophils also harbour a receptor for IgD, and ligation by cross-linked IgD can lead to the release of IL-4. Similar to mast cells, basophils can also degranulate in an antibody-independent manner in response to the binding of anaphylotoxins.

1.6.3 Dendritic cells

Dendritic cells (DCs) are key cells that link the innate and adaptive arms of the immune system. They take their name from the numerous extensions, or 'dendrites', that they possess. Like macrophages, DCs can differentiate from monocytes, and they have the ability to recognise PAMPs on pathogens and pathogen-associated molecules via the expression of PRRs. DCs are 'professional' APCs, whose main function is to process and present antigens to naïve and memory T cells. They provide activating signals such as co-stimulation and secrete cytokines to help shape adaptive immune responses and induce the expansion of clonal polarised CD4+ T cells.

DCs are defined by their expression of the integrin CD11c, but they also express an array of other surface markers. The heterogeneity within DCs has led to the description of a number of different sub-populations of DCs. Myeloid DCs are derived from the myeloid lineage during haematopoiesis (Figure 1.1). In mice, myeloid DCs can be separated by the presence or absence of the expression of the CD8 α-chain molecule.

In humans, DCs under study are normally derived from the peripheral blood. Markers used to define subsets within the myeloid lineage include expression of the FcγRIII (CD16) and the blood DC antigens (BDCA) BDCA1 and BDCA3. No subpopulation of human DCs expressing CD8 has yet been identified. However, DC subsets expressing low and high levels of the integrin CD11b appear to correspond functionally to mouse CD8+ and CD8-DCs respectively.

Three other types of DC that may be of relevance to parasitic infection include CD103+DCs, Langerhans cells and plasmacytoid DCs. CD103+ expressing DCs are concentrated in the mucosal areas of the body, such as the respiratory tract and the intestine. CD103+DCs have been shown to produce the immunoregulatory cytokine transforming growth factor (TGF)-β, and they have the capability of expanding T regulatory (Treg) cells; therefore, they are sometimes referred to as 'regulatory DCs'.

Langerhans cells are specialised DCs that reside in the skin, and are defined by the expression of the C-type lectin Langerin (CD207). They are an important DC subset involved in processing and presenting antigen from the epidermis.

Plasmacytoid DCs are an important source of type I interferons (IFN-α and IFN-β) in viral infection. Morphologically, they look similar to plasma cells (antibody secreting B cells), but their capacity to present antigen led to their designation as a DC subset. Plasmacytoid DCs may contribute to the initiation of adaptive immune responses in some protozoan infections.

When DCs are tissue resident, they take up antigen from the extracellular environment via the processes of macropinocytosis and phagocytosis. Prior to activation, immature DCs express few MHC or co-stimulatory molecules on their surface, and correspondingly they have poor capacity to stimulate T cells. However, upon recognition of antigen via PRRs or receptors recognising opsonic molecules (e.g. antibody/Fc receptors or complement/complement receptors), DCs become activated (or 'mature'). Mature DCs are often found in lymphoid tissue; they have a low capacity for antigen uptake, but express high levels of peptide-loaded MHC and co-stimulatory molecules, and they have a high capacity for stimulating T cells.

The ability of different DC subsets to activate and polarise T cells is not identical. In mice, CD8+DCs and CD8–DCs have differing roles in T cell activation. CD8+DCs and CD103+DCs (but not CD8–DCs) can cross-present antigen (see below). Furthermore, some studies have observed differences in the type of CD4+ T cells expanded by different types of DC. While CD103+ 'regulatory' DCs have a tendency to expand Tregs, Th1 cells appear to be preferentially expanded by CD8+DCs; CD4+ T cells activated by CD8-DCs are more polarised towards a Th2 phenotype. In part, this could be due to the propensity of CD8+ (but not CD8–) DCs to secrete high levels of IL-12p70 upon activation, a critical cytokine involved in polarisation of CD4+ T cells to a Th1 phenotype.

1.6.4 Natural killer (NK) cells

Natural killer cells can be found in both lymphoid and non-lymphoid tissues throughout the body. As suggested by the name, NK cells are cytotoxic and are able to mediate lysis of infected cells by the targeted release of granules containing perforin and granzymes. In addition to a direct cytotoxic role, NK cells have a high immunomodulatory capacity, and they secrete cytokines to skew the immune response when activated. In some protozoan infections, they are considered to be an important innate source of IFN-γ facilitating up-regulation of the IL-12 receptor on naïve CD4+ T cells during priming, in turn permitting responsiveness to APC-secreted IL-12 and the expansion of Th1 cells.

In addition to the secretion of cytokines, activated NK cells also secrete chemokines such as macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4) and Regulated upon Activation, Normal T cell Expressed (RANTES/CCL5). This attracts other immune cells to the infected tissue and helps to focus immune defence mechanisms at the site of infection.

NK cells can be activated in response to ligation of receptors for the macrophage-derived cytokines IL-12 and IL-18. However, degranulation of NK cells is not indiscriminate, as this would undoubtedly result in extensive tissue damage. The identification and targeted lysis of infected cells by activated NK

cells is complicated by the fact that NK cells do not express a clonotypic receptor. Instead, infected target cells are recognised by NK cells via a cumulation of signals derived from multiple inhibitory and activatory receptors on the NK cell surface.

In general, NK cells become activated when activatory signals (ligation of receptors bearing immunoreceptor tyrosine-based activation motif (ITAM)) outweigh inhibitory signals (ligation of receptors bearing immunoreceptor tyrosine-based inhibitory motif (ITIM)). Antibody opsonisation of pathogens can facilitate the activation of NK cells via the ligation of FcγRIII, an activatory receptor on the NK cell surface (Figure 1.8 A). This leads to antibody-dependent cytotoxicity (ADCC) and lysis of the opsonised cell or parasite. In a similar way, ligation of other activatory receptors expressed on NK cells during infection may activate NK cells, although ligands recognised in parasitic infections have not been fully elucidated.

NK cells can also become activated by the lack of 'self' molecules, such as MHC-I, expressed on the surface of infected cells. In some infections with

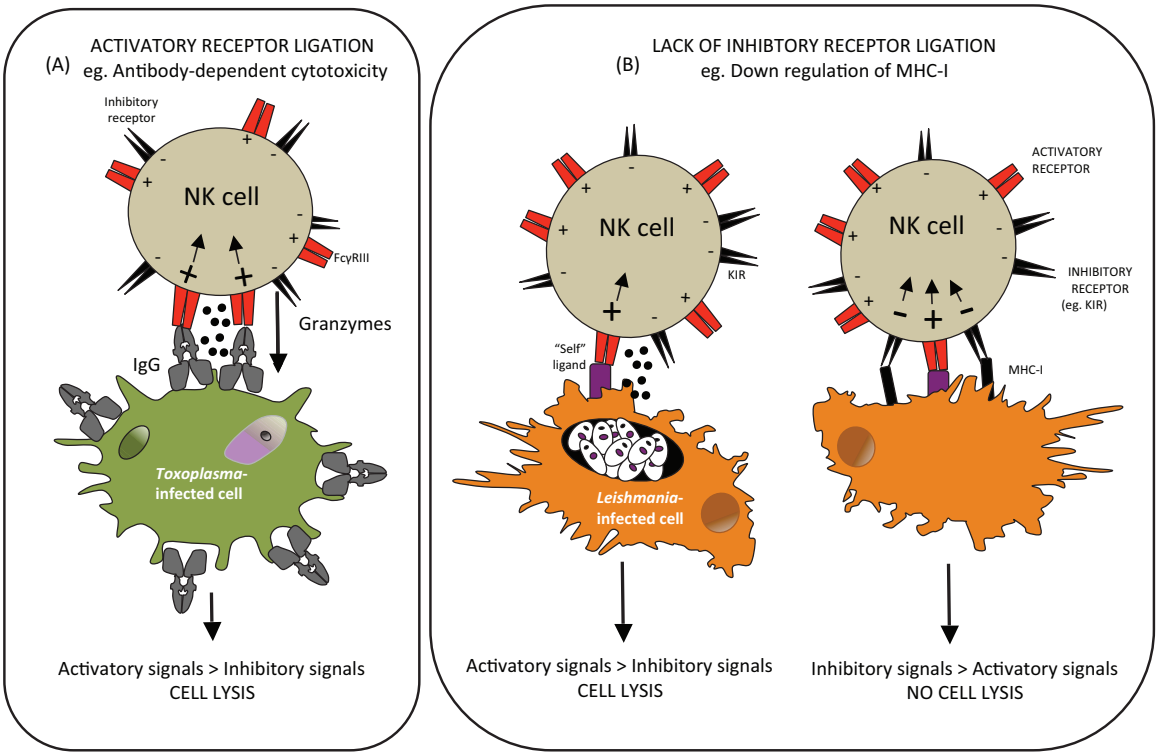


Figure 1.8 Activation of NK cells. Activation of NK cells occurs when activatory signals outweigh inhibitory signals. This can occur by the specific ligation of activatory receptors not normally activated, such as ligation of FcγRIII by IgG on an opsonised pathogen (A) or when inhibitory receptors, such as those normally ligated by MHC-I expressed on most nucleated cells, do not receive signals (B). This latter can occur in situations such as infection with intracellular pathogens that down-regulate MHC-I expression to avoid immune detection.
Abbreviations: Ig, immunoglobulin; KIR, natural killer cell immunoglobulin-like receptor; NK, natural killer; MHC, major histocompatibility complex.

intracellular pathogens, the expression of MHC-I is down-regulated on the surface of infected cells (in parasitic infection, one example is *Leishmania* infection in macrophages). Some of the inhibitory receptors expressed on NK cells (for example some of the natural killer cell immunoglobulin-like receptors (KIRs)) ligate with MHC-I molecules, delivering inhibitory signals that prevent activation of NK cells. The failure to ligate these inhibitory molecules delivers insufficient inhibitory signals to the NK cell, and the NK cells become activated as a result, degranulating to cause lysis of infected cells (Figure 1.8B).

The efficiency of NK cells in the immune response can be enhanced by interactions with phagocytic cells such as DCs and macrophages. These interactions are stabilised by the ligation of integrins such as CD11a/18 (leukocyte functioning antigen, LFA-1), CD11b/CD18 and CD11c/18 expressed on the surface of the NK cell, which can bind to cellular adhesion molecules (ICAMs) expressed on DCs and macrophages.

1.7 Communication in the immune system

Immune cells secrete and respond to a network of proteins known as cytokines. Some of the main cytokines involved in parasitic infection are shown in Table 1.3. The ability to respond to any particular cytokine is determined by the expression of cytokine receptors that can initiate signalling pathways to activate gene transcription in the nucleus. The Janus kinase (Jak)-signal transducer and activator of transcription (STAT) signalling system is a common pathway used to transmit signals from cytokine receptors to the nucleus. This system consists of several autophosphorylating Jak molecules that can phosphorylate the cytoplasmic tails of cytokine receptors. This, in turn, allows the binding of different combinations of STATs, which dimerise and translocate into the nucleus, where they switch on gene transcription.

Many cytokine receptors are dimeric, and the chains making up some of the cytokine receptors are promiscuous. For example, the common γ chain (CD132) is shared by a number of cytokine receptors (notably the receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21), and the IL-4R chain (IL-4Rα) pairs with IL-α13R to convey signals in response to IL-13.

1.8 Adaptive immunity

The adaptive immune response differs from the innate immune response because it has specificity and the ability to form immunological memory. Specificity in the immune system is mediated by antigen-specific antibodies and clonotypic receptors: the T cell receptor (TCR) on T cells and the B cell receptor (surface-bound antibody) on B cells. 'Adaptive immunity' is therefore a term used to describe immune responses carried out by T cells (both CD4+ T helper cells and CD8+ cytotoxic T cells) and B cells.

Antibodies/BCRs and TCRs have variable regions which dictate the differences in binding to specific antigen sequences, known as epitopes. Antibodies and BCRs can recognise epitopes on proteins from tertiary or linear structures of

Table 1.3 Some of the main cytokines involved in parasitic infections.

Cytokine	Main sources	Main functions
IL-1	Macrophages; Epithelial cells	Pro-inflammatory; macrophage activation; acute phase response; induction of fever.
IL-2	T cells	T cell proliferation; clonal expansion of T cells.
IL-3	T cells	Differentiation of basophils from progenitor cells.
IL-4	Basophils; Mast cells; Th2 cells; B cells	Polarisation of Th2 cells; proliferation factor for B cells; isotype switching to IgE; development of M2 macrophages.
IL-5	Th2 cells; Mast cells	Differentiation and expansion of eosinophils; isotype switching in B cells to IgA.
IL-6	Macrophages; Endothelial cells; Th2 cells	Acute phase response; induction of fever; growth and differentiation of adaptive immune cells.
IL-8	Macrophages	Chemoattractant for neutrophils.
IL-9	Th2 cells Th9 cells	Expansion and recruitment of mast cells.
IL-10	Macrophages Dendritic cells T cells B cells	Immunoregulatory cytokine that dampens most immune responses.
IL-12	Dendritic cells; Macrophages	Bioactive IL-12p70 composed of a p35 and a p40 subunit is pro-inflammatory; monomeric or dimeric p40 subunits inhibitory to IL-12R signalling; IL-12p70 is one of the main inducers of Th1 cells; activation of NK cells.
IL-13	Th2 cells	Mucous production; promotion of tissue fibrosis; development of M2 macrophages; antagonism of Th1 responses; promotion of eosinophil reactivity and airway hyper-responsiveness in allergic asthma; isotype switching to IgE in human B cells.
IL-15	Macrophages	Proliferation of NK cells; promotion of CD8+ memory T cell survival.
IL-17	Th17 cells $\gamma\delta$ T cells	Promotion of neutrophilic immune responses.
IL-18	Macrophages	Activation of NK cells; production of IFN-γ by NK cells; promotion of Th1 responses.
IL-21	Th2 cells Th17 cells NKT cells	Promotion CD8+ cytotoxic T cell responses; suppression of IgE production; down-regulation of IgE-mediated allergic responses; isotype switching of human B cells to IgG1 and IgG3.
IL-22	Activated NK cells; Human Th22 cells	Protects tissues from damage; enhances the innate immune responsiveness and regeneration of non-haematopoeitic cells such as epithelial cells and keratinocytes.
IL-23	Dendritic cells	Composed of the p40 subunit of IL-12 and a p19 subunit; polarisation of CD4+ T cells to Th17.

Table 1.3 (*Continued*)

Cytokine	Main sources	Main functions
IL-25	Th2 cells; Mast cells; Epithelial cells	Induces the production of Th2-associated cytokines; stimulates the expansion of eosinophils via Th2 cell induction.
IL-27	Dendritic cells; Macrophages	Heterodimer composed of two subunits: Epstein-Barr induced gene 3 (EBI3) and IL-27 p28; synergises with IL-12 to promote expansion of Th1 cells; suppresses the development of Th17 cells.
IL-33	Epithelial cells Endothelial cells Mast cells	Promotes Th2 cytokine release from granulocytes and Th2 cells.
IFN-γ	NK cells Th1 cells; CD8+ T cells	Increase expression of vascular adhesion molecules for cell trafficking; increase the efficiency of phagocytes; promotion of NK cell activity; isotype switching of mouse B cells to IgG2a.
TNF	Dendritic cells; Macrophages; NK cells Th1 cells	Formally TNF-α (TNF-β now lymphotoxin); induced as part of the acute phase response; pro-inflammatory cytokine that can induce cachexia and temperature dysregulation; increase expression of vascular adhesion molecules for cell trafficking.
TGF-β	Dendritic cells; Macrophages, Treg cells	Immunoregulatory cytokine that can dampen most immune responses; isotype switching of B cells to IgA.
TSLP	Fibroblasts; Epithelial cells Stromal cells	Activation of Langerhans cells; maturation of CD11c+ myeloid DCs.

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; NK, natural killer; TGF, transforming growth factor; TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.

proteins, and the repertoire of sequences that can be detected by antibodies/BCRs is so vast that most sequences can be recognised. TCRs only recognise linear epitopes in the context of cell surface-bound Major Histocompatibility complexes (MHC)(see below) on APCs. Therefore, linear T cell receptor epitopes are restricted by MHC haplotype. As such, in any individual, the variability of the MHC molecules in the genome determines the epitope sequences that can be detected by that individual's T cells.

Once activated, antigen-specific adaptive immune cells undergo clonal expansion, resulting in a population of cells with identical antigen receptors and antigen specificities. Once the immune response has cleared the pathogen from the body, the antigen-specific cell population contracts in number via programmed cell death (apoptosis). However, some antigen-specific T and B cells remain in the body as long-lived memory cells which are capable of responding to a second infection of the same pathogen more efficiently than their naïve counterparts. Maximising the numbers and efficiency of these memory cells ('immunological memory') is the target of vaccination against parasitic infection (Chapter 25)

1.9 The role of the MHC in the immune response

The MHC is a polygenic family of glycoproteins that present peptides from digested pathogen proteins to TCRs on T cells. The MHC genes were originally defined in the context of their role in the compatibility and rejection of transplanted tissues (hence the name 'histo-compatibility'). In addition to the presence of several MHC genes in the human genome, each MHC gene is polymorphic, with multiple variants in the human species, and expression is co-dominant between the alleles inherited from each parent. The particular combination of MHC variant genes in a human is known as the MHC haplotype.

The MHC can be classified into MHC-I and MHC-II. MHC-I is expressed on most nucleated cells, whereas MHC-II is generally restricted to haematopoietic cells – in particular, APCs such as DCs, macrophages and granulocytes. APCs play a crucial role in the initiation of T cell responses because of their ability to present antigen on MHC-II molecules on the cell surface. MHC-II is also expressed on B cells to enable the acquisition of help from CD4+ T helper cells (see below).

The two classes of MHC differ in composition: MHC-I is composed of a polymorphic α chain with three domains, the structure of which is stabilised by dimerisation with a second non-polymorphic molecule called β2-microglobulin. MHC-II molecules are composed of two chains – the α- and the β- chain – each with a constant and variable domain.

The variable domains of each class of MHC molecule form the peptide-binding groove, a structure which varies in amino acid sequence. The peptide-binding groove binds to a peptide epitope using a combination of hydrogen bonding and ionic interactions which anchor the peptide into the groove. The sequence of the peptide-binding groove influences the size and sequence of the peptide that can be loaded and presented. The size of the peptide that can be presented by MHC molecules differs by class: MHC-I-associated peptides are generally 8–10 amino acids long, whereas MHC-II-associated peptides are slightly longer at 15–24 amino acids.

The variability among MHC molecules affects the peptide sequence or 'sequence motif' that can be presented to T cells. It is the combination of peptide sequence and the sequence of the MHC residues surrounding the peptide-binding groove that is recognised by each clonotypic TCR. Thus, the recognition of a linear T cell epitope presented in the context of the MHC is partially determined by the residues in the MHC molecule and is 'MHC-restricted'; individual T cell receptors react with peptides complexed with some variants of MHC molecules but not others.

MHC-I molecules present peptides that are intracellularly derived (for example, viral particles in virally-infected cells, tumour antigens or peptides derived from intracellular bacteria or protozoan parasites), whereas MHC-II presents antigens derived from the extracellular environment. This makes sense when viewed in the context of the types of T cells that become activated by the different classes of MHC molecule: the CD8 molecule on cytotoxic T cells

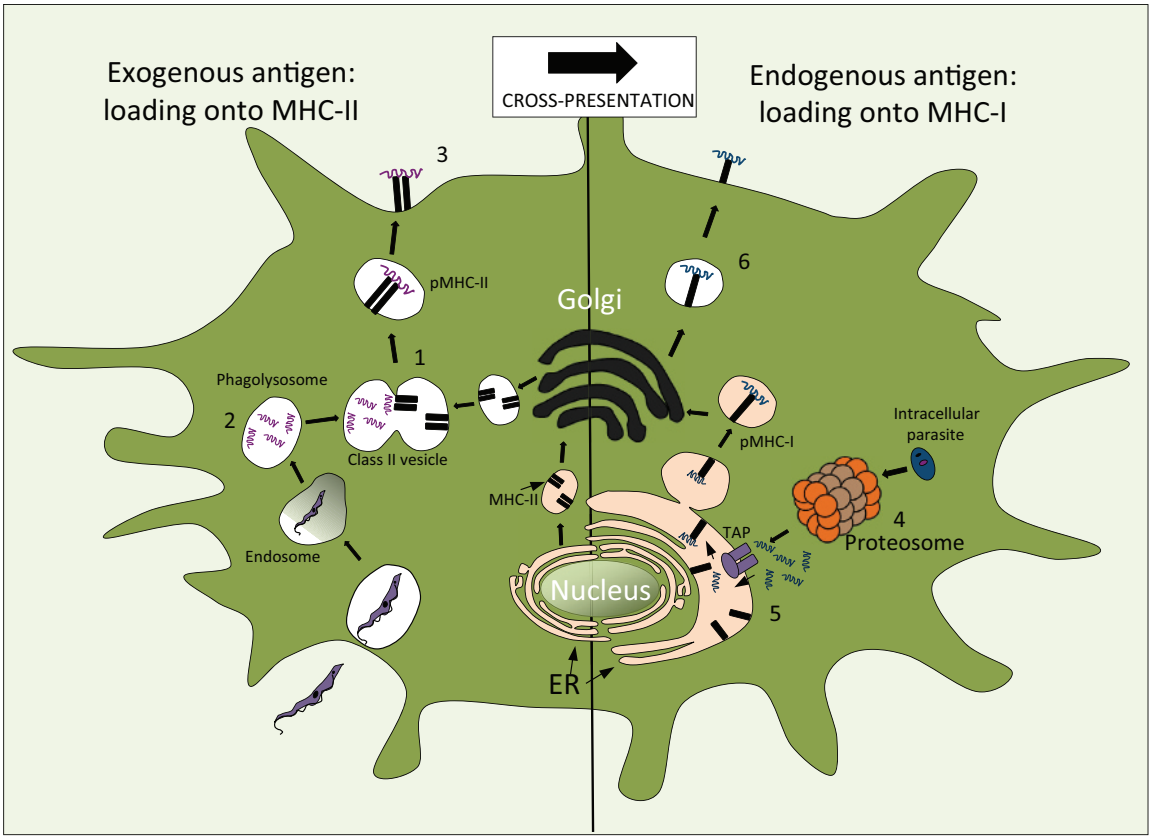


Figure 1.9 MHC processing pathways. MHC-II is loaded in specialised vesicles (Class II vesicles) (1) that fuse with phagolysosomes containing endocytosed digested pathogen particles (2) before peptide-loaded MHC-II traffics to the cell surface for display to T cells (3). Peptides from intracellular pathogens are generated by a multi-subunit structure known as the proteosome (4), and are pumped into the ER of the cell by TAP molecules (5), where MHC-I is loaded before trafficking to the cell surface via the Golgi (6). Cross-presentation, whereby endocytosed material 'crosses over' to the MHC-I loading pathway, occurs by mechanisms that are not fully understood.
Abbreviations: ER, endoplasmic reticulum; MHC, major histocompatibility complex; TAP, transporters associated with antigen processing.

can bind to MHC-I molecules, facilitating activation by pMHC-I, whereas the CD4 molecule on T helper cells can bind to the MHC-II molecules, facilitating activation by pMHC-II. Correspondingly, the lytic properties of CD8+ T cells can be implemented for lysis of infected cells expressing intracellularly-derived peptides, whereas the orchestration of multiple types of immune cells reacting against extracellular pathogens can be achieved by activation of CD4+ T cells.

Epitopes are loaded onto MHC molecules inside APCs, and different MHC-loading pathways have been determined according to the source of the antigen (Figure 1.9). However, this schema is simplified; the process of cross-presentation (see below), allowing CD8+ T cells to become 'primed' by DCs, demonstrates that the extracellularly-derived pathogen proteins can be endocytosed and 'cross over' to the MHC-I loading pathway in the endoplasmic reticulum.

1.10 T cell activation and cellular-mediated immunity

The main subsets of T cells are defined by their expression of CD4 (T helper cells) or CD8 (cytotoxic T cells). T helper cells provide 'help' to other cells of the immune system, such as the amplification of macrophage functions, the isotype switching of B cells or the amplification of CD8+ cytotoxic T cell functions. Cytotoxic T cells lyse cells infected with intracellular pathogens.

1.10.1 Three signals are required for CD4+ T cell activation

The activation of CD4+ T cells initially requires binding between T cells and APCs. This interaction is stabilised by the adhesion molecule leukocyte function-associated antigen (LFA)-1 on the T cell surface, and cellular adhesion molecules such as intercellular adhesion molecule (ICAM)-1 on the APC. Naïve CD4+ T cells become fully activated to become effector CD4+ T cells in response to three main signals received from the APC (Figure 1.10). The first is the ligation of the TCR with pMHC-II. The TCR is made up of two chains – α and β – neither of which contain intracytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs). Therefore, signals are transmitted from the TCR via a complex of ITAM-containing CD3 molecules associated with the α and β chains making up the TCR.

The second signal received from the APC is the ligation of CD28 by co-stimulatory molecules of the B7 family (B7.1/CD80 and B7.2/CD86). Although

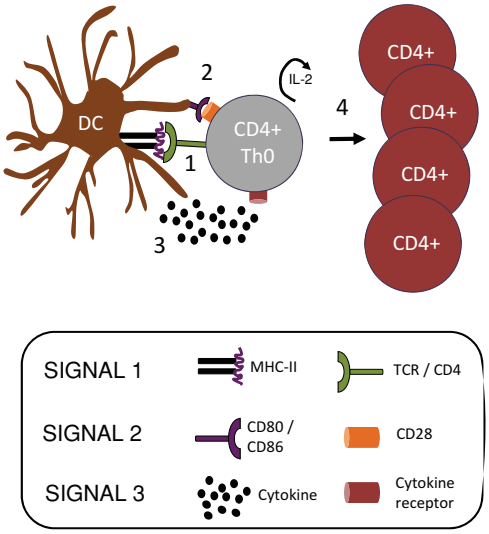


Figure 1.10 Molecular interactions leading to CD4+ T cell activation. CD4+ T cells require three main signals from APCs for activation. The first is received from ligation of the TCR by pMHC-II (1). The second signal is received by ligation of the co-stimulation molecule CD28 with CD80 or CD86 expressed on the APC (2), and this leads to the production of IL-2, allowing the T cell to proliferate. The third signal is received by cytokine receptors and determines the polarisation of the CD4+ T cell into a specific subtype (3). Upon receipt of all three of these signals, the CD4+ T cell expands and differentiates (4).
Abbreviations: APC, antigen presenting cell; pMHC-II, peptide loaded major histocompatibility-II molecules; TCR, T cell receptor.

Table 1.4 Cytokines required for polarisation of CD4+ T cell subsets.

T cell subset	Polarising cytokine
Th1	IL-12p70
Th2	IL-4
Th9	TGF-β and IL-4
Th17	TGF-β, IL-6, IL-21, IL-23
Th22	IL-6, TNF-α
iTreg	TGF-β

Abbreviations: IL, interleukin; iTreg, inducible T regulatory cell; TGF, transforming growth factor; Th, T helper; TNF, tumour necrosis factor.

this is regarded as one of the main co-stimulatory signals, many other molecules can contribute to the co-stimulatory signals required for full T cell activation upon ligation. Co-stimulation is critical to induce the expression of IL-2 and to up-regulate expression of the α-chain of the IL-2 receptor. Since T cell expansion is critically dependent on IL-2, this endows the T cell with the ability to proliferate. Activation of CD4+ T cells in the absence of co-stimulation leads to T cell anergy, whereby the T cells cannot proliferate.

Once CD4+ T cells have become activated, the proliferation of T cells gives rise to a critical mass of antigen-specific T cells to effectuate an immune response. Since all T cells arising from the original activated T cell are clonal, expansion is referred to as 'clonal expansion'.

T cell polarisation is facilitated by a third signal received from the APC – the ligation of cytokine receptors by APC-derived cytokines (Table 1.4). Signals received through cytokine receptors result in Jak-STAT signalling and the activation of transcription factors that promote the secretion of specific cytokines associated with the various polarised CD4+ T cell subsets (described in more detail below).

1.10.2 Cross-presentation and cross-priming of CD8+ T cells

Primed CD8+ T cells can traffic to areas of the body to lyse cells infected with intracellular pathogens. These are normally viruses, but also can be intracellular bacteria or parasites such as *Toxoplasma gondii* (Chapter 4) or *Trypanosoma cruzi* (Chapter 9). At the site of infection, they recognise infected cells via the expression of pMHC-I presenting peptides derived from the intracellular pathogen. However, naïve CD8+ T cells must be primed before they can function efficiently, in part because they also require co-stimulation to become fully activated. CD8+ T cells become primed by DCs in the secondary lymphoid organs.

DCs are associated with the endocytosis of foreign antigen from extracellular pathogens via phagocytosis. They also primarily present extracellularly-derived antigen complexed with MHC-II molecules for presentation to CD4+ T cells. This begs the question: how can DCs possibly prime CD8+ T cells that require presentation of peptide epitopes from intracellularly-derived antigen complexed with MHC-I? The process by which DCs present peptide derived

from intracellular pathogens with which they are not directly infected is called 'cross-presentation'. The priming of CD8+ T cells by DCs is essential, not just for defence against intracellular pathogens, but also to prime and generate memory CD8+ T cells induced by vaccination.

Cross-presentation involves the crossing of endocytosed extracellularly-derived peptides from the endocytic pathway for loading onto MHC-II molecules to the proteosome-derived pathway for peptide loading onto MHC-I molecules in the endoplasmic reticulum (Figure 1.9). The molecular events mediating cross-presentation are described in the References for Further Reading. However, the method of antigen uptake by the DC can influence this process; receptor-mediated phagocytosis by Fc receptors (see below) and some of the C-type lectins (Figure 1.3) can feed endocytosed peptides into the MHC-I loading pathway. Although several types of phagocytes can cross-present antigen, only certain subsets of DC can cross-present antigen to prime of CD8+ T cells. In mice, cross-presentation and priming is thought to be restricted to CD8+DCs and CD103+DCs.

Priming of CD8+ T cells also requires 'help' from CD4+ T helper cells in addition to ligation of the TCR with pMHC-I on DCs. The exact nature of CD4+ T cell help is still unclear, but it is thought to involve recruitment of naïve CD8+ T cells to the pMHC-I DC via secretion of chemotactic factors, up-regulation of appropriate co-stimulatory molecules to deliver signal 2 to the CD8+ T cells, and IL-2 production to assist in clonal expansion of the primed CD8+ T cells (Figure 1.11). The ligation of CD40L on the CD4+ T cell by CD40 on the DC is known to be critical in the provision of CD4+ T cell help during cross-priming of CD8+ T cells. Thus, DCs can simultaneously prime CD4+ T helper and CD8+ cytotoxic T cells and act as a bridge, allowing CD4+ T cells to provide help in the priming of CD8+ T cells.

1.10.3 CD4+ T cell phenotypes

During priming of CD4+ T cells, the cytokines secreted by APCs can polarise the CD4+ T cells into one of several different phenotypes. Originally, the field of CD4+ T cell polarisation centred around a Th1/Th2 paradigm, whereby CD4+ T cells were thought to differentiate into either Th1 (pro-inflammatory IFN-γ secreting CD4+ T cells) or Th2 (anti-inflammatory CD4+ T cells secreting several cytokines, of which IL-4 was the main protagonist). The cross-regulatory nature of IFN-γ and IL-4 was considered a factor in the observed dominance of either response, Th1 responses during infection with microorganisms such as viruses, bacteria and parasites, or Th2 responses during allergic reactions and infection with macroparasites such as helminths. Since the original description of the Th1/Th2 paradigm in the 1980s, the polarisation of CD4+ T cells is now known to be more complex than the Th1/Th2 paradigm, with the identification of several other distinct types of CD4+ T cells, including Tregs and, more recently, Th17, Th9 and Th22 cells.

Each subset of CD4+ T cells secretes a distinct profile of cytokines (Figure 1.12) and provides a different form of 'help' to amplify specific effector mechanisms of the immune system. Thus, the effector mechanisms mediated by IFN-γ secreted by Th1 cells are effective at clearing protozoan parasites, whereas IL-4

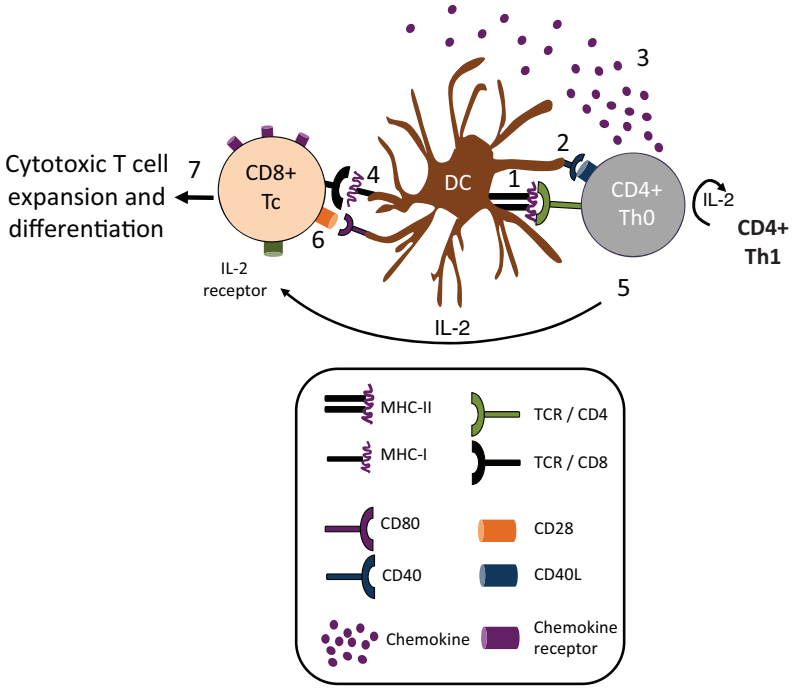


Figure 1.11 CD4+ T cell help is required for cross-presentation and priming of CD8+ cytotoxic T cells.
CD8+ or CD103+DCs can display endocytosed antigen on pMHC-II molecules(1) for recognition by CD4+ T cells. The ligation of CD4+ T cell-expressed CD40L with CD40 expressed on the DC (2) permits CD4+ T cell help in cross-priming of CD8+ T cells. CD4+ T cells secrete chemokines to attract CD8+ T cells to the DC (3), where the TCR of the CD8+ T cell recognises endocytosed antigen that has 'crossed over' to the MHC-I loading pathway, presented as pMHC-I on the DC surface (4). CD4+ T cells induce the up-regulation of co-stimulatory molecules, which allows CD4+ T cell production of IL-2 to support CD8+ T cell proliferation (5), as well as co-stimulation of the CD8+ T cell (6). These events lead to the expansion of CD8+ T cells to exogenously-derived antigen (7).
Abbreviations: DC, dendritic cell; IL, interleukin; MHC, major histocompatibility complex; TCR, T cell receptor.

secreted by Th2 cells activates effector mechanisms that can provide protection against helminths. Furthermore, all immune responses must be controlled because, when excessive, they can cause immunopathology. Regulation of immune responses is carried out by the immunoregulatory cytokines IL-10 and TGF-β, both of which can be produced by the Treg subset of CD4+ T cells.

1.10.3.1 Th1

Th1 cells are a feature of protozoan infections, and the signature cytokine of Th1 cells is INF-γ. T-box, expressed in T cells (T-bet), is a key transcription factor for the IFN-γ gene, and CD4+ T cells expressing T-bet are generally considered to be of a Th1 phenotype. APC-derived IL-12 is a key driver of Th1 polarisation. However, naïve CD4+ T cells (Th0) do not express receptors for IL-12 but up-regulate expression of the IL-12 receptor in response to stimulation from IFN-γ. Since IFN-γ is not produced in significant quantities by APCs, often the source of IFN-γ comes from other cells of the innate immune system. NK cells

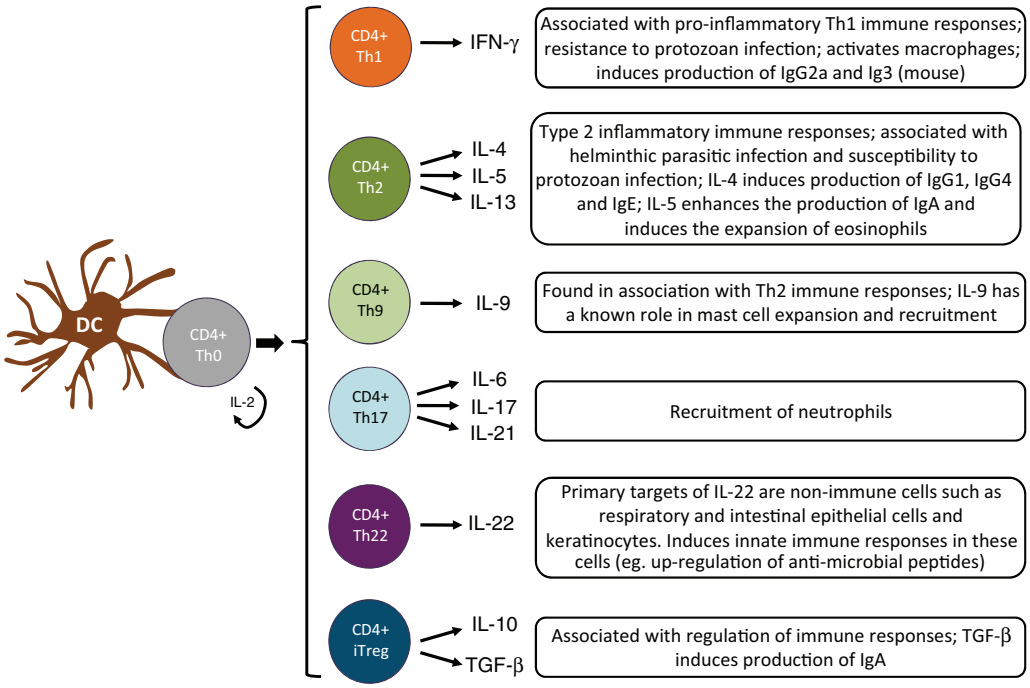


Figure 1.12 Functions of different CD4+ T cell phenotypes.

and γδ T cells (see below) are important sources of innate IFN-γ, and their activation can therefore play a key role in the development of pro-inflammatory immune responses via the expansion of Th1 cells.

Signalling through the IFN-γ receptor results in the transcription of IFN-inducible genes, the products of which enhance the microbicidal activity of a number of immune cells. In macrophages, reaponding to IFN-γ promotes phagocytosis and induces the production of the enzymes, mediating respiratory burst. The expression of MHC molecules is up-regulated in APCs, leading to enhanced antigen presentation. Furthermore, in mice IFN-γ induces B cells to isotype-switch to the IgG2a, a cytophilic antibody isotype effective at opsonising protozoan parasites.

1.10.3.2 Th2

Th2 cells are induced in helminth infections and in allergic responses. Although it is undoubtedly true that Th2 cells and Th1 cells are counter-regulatory, Th2 cells field of parasitology (in particular helminthology) has played a major role in determining that Th2 cells do not only arise when Th1 responses are absent; they can be actively induced, and one of the strongest inducers of Th2 cells can be found in the eggs of Schistosomes (Chapter 16). Counter-regulation of Th1 responses by Th2 associated cytokines is also not always absolute, with instances where some IL-4 can be necessary for the induction of a Th1 response (for example in *Cryptosporidium* infection – see Chapter 5).

The signature cytokine of Th2 cells is IL-4, although Th2 cells also produce significant quantities of IL-5 and IL-13 (Figure 1.12). Previously, Th2 cells were thought to be the major producers of IL-9 and T cell-derived IL-10, but this is no longer the case; IL-9 producing T helper cells have recently been designated as a separate subset, and IL-10 has now been observed to be produced in large quantities from most CD4+ T cell subsets, including Th1 cells under certain conditions.

Naïve CD4+ T cells polarise towards a Th2 phenotype in response to IL-4. DCs do not produce significant quantities of IL-4, and the early source of IL-4 from the innate immune system has remained an area of investigation. Recent studies suggest that basophils may play an important role in this regard (see Chapter 12).

The IL-4 receptor is composed of two chains (IL-4Rα and the common γ chain). IL-4R is also a component of the IL-13R. The IL-13R is responsive to IL-13 when paired with the IL-13R1 chain and both the IL-4R and the IL-13R signal through STAT6 activation. Pairing of the IL-13R1 chain with IL-13R2 rather than the IL-4Rα results in the formation of a soluble, high affinity binding protein that inhibits IL-13 signalling. Indeed understanding how positive signaling via IL-4R/IL-13R1 and inhibition by the IL-13R2 is regulated is not currently known but will be of great importance for many Th2-associated diseases including asthma, ulcerative colitis and schistosomiasis.

Th2-effector mechanisms centre around the effects of IL-4 on B cells. IL-4 is an important growth factor for B cells, and it induces isotype switching to IgE. Since IgE is a central molecule mediating degranulation of eosinophils, mast cells and basophils, Th2-induced isotype switching of B cells to IgE production is critical in supporting some of the main granulocyte-based effector mechanisms against helminth parasites. Th2 cytokines also polarise macrophages towards an M2 phenotype, possibly contributing to the repair of tissue damaged by helminth infection, but also leading to susceptibility of macrophages to infection with some protozoan parasites.

1.10.3.3 Th17

Th17 cells were first described as producers of IL-17, but more recently they have also been shown to also produce IL-21. The polarisation of Th17 cells was originally thought to depend on the production of IL-23, a pro-inflammatory cytokine related to IL-12 (see Table 3), by the activating APC. However, Th17 cells are now known to differentiate in response to IL-6 and TGF-β. The development of Th17 cells can be suppressed by both IFN-γ and IL-4. The main function of Th17 cells in infectious diseases is the recruitment of neutrophils to control infection, although they may also have other, as yet undiscovered, roles.

1.10.3.4 Th9 and Th22

IL-9 was historically considered to be a product of Th2 cells, but recently a subset of T helper cells that produces IL-9 alone has been identified and designated as a separate Th9 subset. Information regarding the contribution of Th9 cells and, indeed, the role of IL-9 in immune responses during parasitic

infection, is currently sparse. IL-9 is known to be involved in the expansion and recruitment of mast cells, in turn influencing the recruitment and activation of eosinophils via mast cell-derived release of eosinophil chemotactic factor. IL-9 is known to have a protective effect against *Trichuris* infection (Chapter 14) and promote mucous production and mast cell activation in schistosome infection (Chapter 16), but it is currently unclear whether the source of IL-9 in these infections is from Th2 or Th9 cells.

IL-22 is secreted by activated CD4+ T cells (now termed Th22 cells) that are found in immune responses in humans, but not in mice. The function of IL-22 (and Th22 cells) is still being investigated, but on a molecular level IL-22 is related to the immunoregulatory cytokine IL-10. It is known that the functional receptor for IL-22 is predominantly expressed on epithelial cells in the mucosal tract and keratinocytes in the skin. However, no expression of the IL-22 receptor has been detected on immune cells. Cells responsive to IL-22 can produce innate immune molecules, such as anti-microbial peptides and type 1 interferons, in response to ligation of the IL-22 receptor. Therefore, IL-22 may play a role in infection immunity via the induction of innate immune responses. Although the evidence suggests that IL-22 may contribute to the body's defence against bacterial infection, a role for IL-22, and for the Th22 cells that produce this cytokine, awaits elucidation in parasitic infection.

1.10.3.5 Tregs

Natural regulatory CD4+ T cells (nTreg) maintain immunological homeostasis, preventing activation of auto-reactive cells, while inducible Treg (iTreg) cells appear to control the magnitude of immune responses to exogenous antigenic challenge, including that posed by invading pathogens. Both nTregs and iTregs provide essential immunological control to prevent immune-mediated pathology. The discovery of Forkhead box protein 3 (Foxp3) as a definitive transcription factor for Treg cells has allowed investigators to identify, isolate and study the role of these cells in many immunological systems.

Markers such as the α-chain of the IL-2 receptor (CD25), inhibitory co-receptor cytotoxic T lymphocyte antigen-4 (CTLA-4), and the glucocorticoid-inducible tumour necrosis factor receptor (GITR) provide clues to the function and mechanisms used by Tregs, but these markers are shared with activated effector T cells. CD103, an αEβ7 T cell integrin also expressed on regulatory DCs, is required for cell-cell contact and is highly expressed on activated Treg cells. Neuropilin-1 (also called BCDA-4) is a receptor involved in axon guidance, angiogenesis and the activation of T cells, and it has also been identified on nTreg cells during *S. mansoni* infection. Originally identified through global gene expression studies, high expression of surface neuropilin-1 correlates with Foxp3 expression and is rapidly down-regulated following TCR-ligation.

iTregs are a more heterogeneous population of CD4+ T regulatory cells than nTregs. They are often distinguished by the panel of cytokines they secrete upon activation. Subsets of iTregs include 'Th3' cells that secrete IL-4 in addition to TGF-β and IL-10, and 'Tr1' cells that secrete TGF-β and IL-10 but not IL-4. Regulatory CD4+CD25+Foxp3− cells that can secrete IL-10 extend the repertoire of immunoregulatory CD4+ T cells.

Collectively, iTreg and nTreg cells represent a population of professional suppressor cells, with the unique and primary function to suppress immune responses and prevent immune-driven pathology. Tregs are able to suppress both Th1 and Th2 responses. In addition to the Treg-mediated suppression of the cell function and proliferation of other CD4+ T cell subsets, Tregs can also influence the function of macrophages, CD8+ cells, B cells and granulocytes.

1.10.4 Other T cells of the innate immune system

Other TCR-expressing T cells that may play a role in parasitic infections in addition to CD4+ T helper cells and CD8+ cytotoxic T cells are γδ T cells and NKT cells.

1.10.4.1 γδ T cells

γδ T cells are defined by the expression of TCRs that are composed of γ and δ chains rather than α and β chains. γδ T cells recognise monophosphate and diphosphate esters, and do not require presentation of these antigens by APCs to become activated. As such, they are capable of producing cytokines before T cells expressing TCRs composed of α and β chains (αβ T cells). In some protozoan infections, γδ T cells may be an important early source of IFN-γ for the up-regulation of IL-12 receptors on pre-Th1 cells. The repertoire of cytokines secreted by γδ T cells is not confined to IFN-γ, and these cells have been shown to also secrete IL-4, IL-10 and IL-17, depending on their environment.

1.10.4.2 NKT cells

NKT cells express a range of markers associated with NK cells, such as the NK cell receptor NK1.1, as well as αβ-TCRs, and they are therefore defined as both NK cells and T cells. In mice, NKT cells have a TCR that disproportionately uses the variable regions Vα14Vβ8. NKT cells can recognise lipids and glycolipids – in particular α-galactosylceramide (α-GalCer) – in the context of MHC-I-like molecule CD1d. Upon activation, NKT cells are flexible with regards to cytokine production and can produce both IL-4 and IFN-γ, depending on their environmental stimuli.

1.11 B cells and the humoral response

The predominant function of B cells is to produce antibodies, the main component of the humoral immune response. Upon activation, naïve B cells differentiate to become antibody-secreting cells called plasma cells. Some B cells differentiate to become long-lasting memory B cells that participate in memory responses upon challenge infections with the same pathogen.

B cells are a heterogeneous population of cells that can respond to antigen in a T-dependent or T-independent manner, the former requiring 'help' from CD4+ T cells. B cells can be divided into B1 and B2 cells B1- and B2-B cells. B1-B cells largely produce antibody to T-independent antigens, and they secrete IgM that can interact with multiple epitopes with a low affinity. B1-B cells are activated

by B cell mitogens that can directly initiate the division of B cells. They respond to polysaccharides found on pathogens such as the TLR-4 agonist LPS, a cell wall constituent of gram-negative bacteria. B1 cells also spontaneously secrete antibody and are the main B cell subset contributing to natural serum antibodies present in the bloodstream.

B2-B cells make up the majority of B cells in the body and require T cell help from CD4+ T cells to produce high-affinity, isotype-switched antibody. The acquisition of help from CD4+ T cells dictates that B2-B cells have a capacity for antigen presentation and, indeed, they can act as APCs. It is the B2 subset of B cells that form long-lasting memory B cells, a capability not shared with B1-B cells. The antibody secreted by B2-B cells recognises particular epitopes in pathogen-derived molecules with high specificity.

1.11.1 B cell activation against T-dependent antigens

The activation of B cells to produce antibody against T-dependent antigens occurs via the BCR, essentially surface-bound antibody. Naïve B cells express predominantly IgM and IgD on their surface, and the antigen-binding fragment of the BCRs on any one B cell are identical. Upon binding to multivalent antigen, cross-linking of the BCRs on the B cell surface induces internalisation of the antigen which becomes degraded and loaded onto MHC-II molecules for presentation to CD4+ T cells at the cell surface (Figure 1.13). This process is

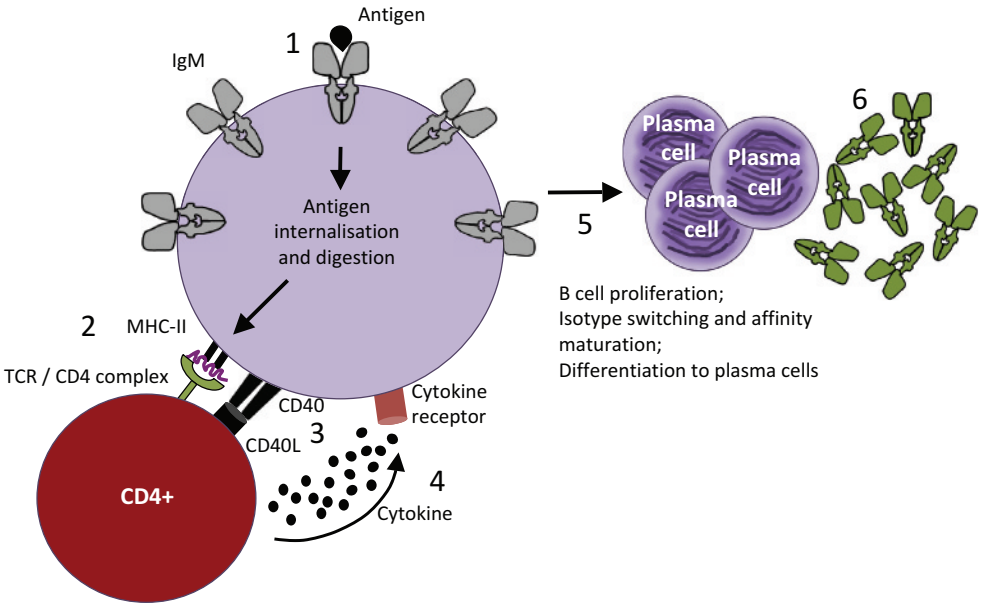


Figure 1.13 Activation of B cells towards T-dependent antigen. Ligation of the BCR by antigen (1) leads to internalisation and digestion of the antigen. Digested peptides are loaded onto MHC-II molecules, and peptide loaded MHC-II is displayed on the B cell surface for recognition by cognate CD4+ T cells (2). The ligation of CD40 by CD4+ T cell expressed CD40L (3) and the production of cytokines by the CD4+ T helper cell (4) leads to B cell activation and the eventual formation of plasma cells (5) that secrete isotype-switched, high-affinity antibodies (6).
Abbreviations: BCR, B cell receptor; MHC, major histocompatibility.

amplified by the presence of the B cell co-receptor (not shown), a complex of molecules consisting of CD19, CD21 and CD81. Indeed, expression of CD19 is often used to distinguish B cells from other immune cells, particularly in studies involving mice.

The recruitment of a cognate CD4+ T cell (i.e. one that can recognise the same antigen as the B cell) is essential for the B cell to receive signals derived from the T cell to initiate B cell proliferation, isotype switching and somatic hypermutation (a process known as "affinity maturation" which increases the affinity of the antibody for the antigen). The ligation of CD40 on the B cell surface with CD40 ligand (CD154) on the CD4+ T cell is essential to induce B cell proliferation and this interaction induces the up-regulation of co-stimulatory molecules on the B cell surface (Figure 1.13). This, in turn, increases the ability of the B cell to co-stimulate the CD4+ T cell, stimulating the production of T cell-derived cytokines such as IL-2, IL-4 and IL-5 that drive proliferation and isotype switching.

Isotype switching exchanges the constant regions of the heavy chain of the antibody molecules (Figure 1.14), leading to the production of specific types of antibody, each with different functions in the immune system (see below). The constant region of each isotype can react with specific Fc receptors, differentially expressed on the cells of the immune system. Affinity maturation alters the sequence of the variable region of the antibody, increasing the affinity of the antibody for the antigen.

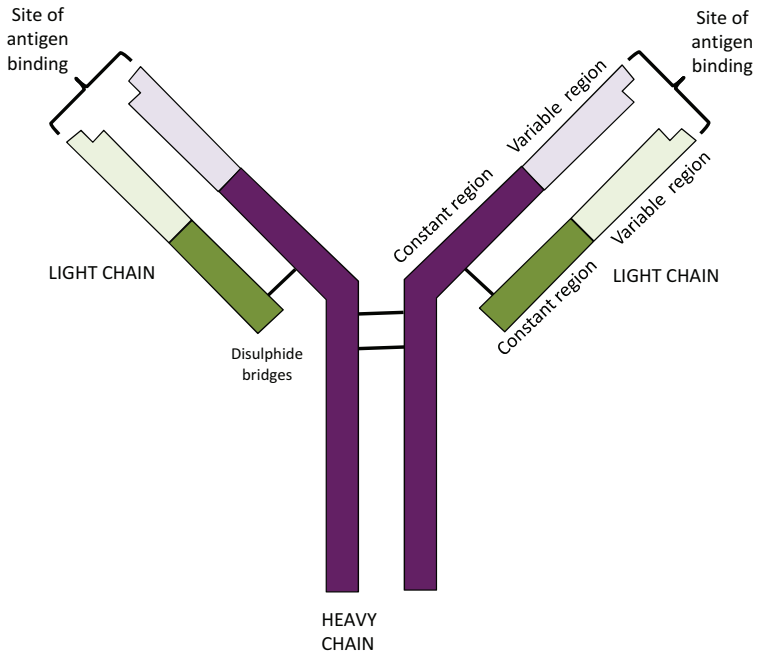


Figure 1.14 Generic structure of an antibody. Antibodies are composed of two heavy chains and two light chains, connected by disulphide bridges. Each heavy and light chain is composed of a constant region and a variable region, and the sites of antigen binding are composed of the variable regions of the heavy and light chains. The constant region of the heavy chains determines the isotype of the antibody and is the portion of the antibody that binds to Fc receptors.

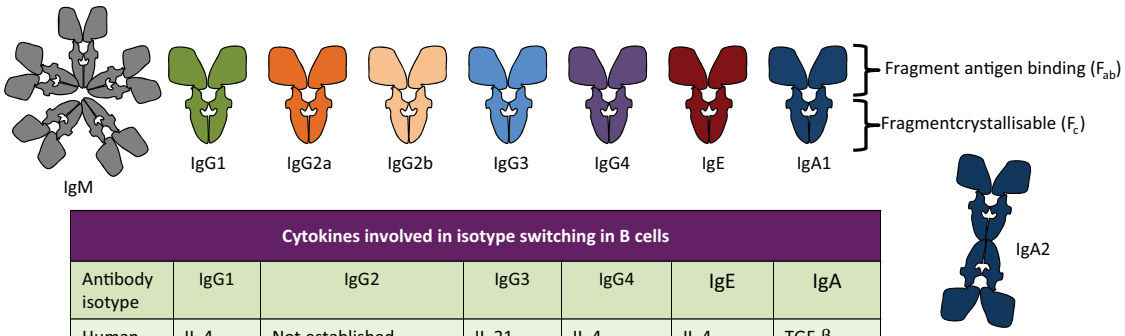
Antibody-secreting B cells (plasma cells) can secrete up to 2,000 antibodies per cell per second for 1–2 weeks, and this intensive output of protein is the reason that plasma cells have extensive rough endoplasmic reticulum and Golgi apparatus. Antibodies have a half-life and do not live forever in the circulation. However, plasma cells continuously secrete antibody once activated, and they maintain antibody in the circulation. Long-lived plasma cells (a type of B memory cell) can secrete antibodies for many years while resident in the bone marrow.

The production of antibody is not the only function of B2-B cells. They also play an important role as APCs in the activation of CD4+ T cells, and are also producers of cytokines. In particular, B cells have also become appreciated as important immunoregulatory cells, capable of producing the immunoregulatory cytokines IL-10 and TGF-β in certain situations.

1.11.2 Antibody isotypes

Antibodies are of distinct isotypes (or classes), designated according to the constant regions of the heavy chains from which they are composed (Figure 1.14). There are five classes of antibody isotypes: IgM, IgD, IgG, IgE and IgA. The IgG class is further divided into four subclasses. Isotype switching is determined by the CD4+ T cell-derived cytokines received during T cell help (Figure 1.15), and this is influenced by the subclass of CD4+ T cell that provides help for the B cell. Different isotypes of antibody can be concentrated in different locations of the body (eg. IgA is the major isotype at the mucosal surfaces).

Antibodies perform numerous effector functions in the immune system. Some of these functions are carried out by particular isotypes of antibody. The main



Cytokines involved in isotype switching in B cells							
Antibody isotype	IgG1	IgG2		IgG3	IgG4	IgE	IgA
Human	IL-4 IL-21	Not established		IL-21	IL-4 IL-10	IL-4 IL-13	TGF-β IL-5 IL-21
Mouse	IL-4 IL-5 IL-13	IgG2a: IFN-γ	IgG2b: TGF-β	IL-5	_____	IL-4	TGF-β IL-5

Abbreviations: IFN, interferon; Ig, immunoglobulin; IL, interleukin; TGF, transforming growth factor.

Figure 1.15 Cytokines involved in isotype switching. Monomeric antibodies can be split into two fragments of antigen binding (Fab) and a crystallisable fragment (Fc) when digested by the protease papain. All antibodies shown are monomeric, with the exception of IgM (pentamer) and IgA2(dimeric).

Table 1.5 Functions of antibody isotypes.

Function	Isotypes	Effects
Neutralisation	IgA IgG1, IgG2, IgG3, IgG4	Neutralisation of pathogen toxins
Opsonisation	IgG1 and IgG3 (IgG2a in mice)	Prevention of pathogen attachment and invasion Targeting of pathogens for phagocytosis Targeting of pathogens for antibody-dependent cell-mediated cytotoxicity (NK cells)
Activation	IgE IgD IgM IgG1 and IgG3	Mast cell degranulation IL-4 induction from basophils Complement activation

roles of antibodies can be categorised into neutralisation of pathogen toxins, opsonisation of pathogens and activation of immune effector mechanisms (Table 1.5). Some isotypes of antibody – in particular some subclasses of IgG – can perform more than one function effectively.

1.11.2.1 IgM

IgM is one of the first antibody isotypes secreted in a primary immune response. It is found on the surface of naïve B cells and is the major isotype secreted by B1-B cells in response to T-independent antigens. IgM normally does not undergo affinity maturation, so it is generally capable of binding to several different antigens with weak affinity. Soluble IgM forms pentameric structures, and it is the majority isotype in natural antibody found in the serum. This isotype is effective at activating the complement cascade.

1.11.2.2 IgD

IgD is found on the surface of naïve B cells prior to class switching. This isotype can also can be found in extremely low concentrations in the serum. The function of IgD has been debated for some time, but it was recently shown to bind to an unknown receptor on basophils. Complexes of IgD can lead to the production of IL-4 from basophils, and therefore this antibody isotype may be an important player in the production of innate-derived IL-4.

1.11.2.3 IgG

IgG is one of the main antibody isotypes in the body. Humans have four subclasses of IgG antibody (IgG1, IgG2, IgG3 and IgG4), of which IgG1 and IgG3 are cytophilic ('affinity for cells') and are particularly good opsonins. Mice also have four subclasses of IgG, but have two forms of IgG2 (IgG2a and IgG2b) and do not produce IgG4. C57BL/6, C57BL/10 and non-obese diabetic (NOD) mice express IgG2c instead of IgG2a, although commercial reagents to measure IgG2a often cross-react with the IgG2c isotype.

IgG is a multifunctional isotype, and the cytophilic nature of the IgG1 and IgG3 subclasses make the generation of these antibodies desirable for efficient pathogen clearance. The subclass of IgG produced is heavily influenced by the cytokine environment at the time of B cell priming (Figure 1.15). Of particular relevance for parasitic infections in mice, IgG1 is often used as an indicator of a Th2/IL-4 environment, whereas IgG2a is used as an indicator of a Th1/IFN-γ environment.

1.11.2.4 IgE

IgE is induced when B cells receive help from IL-4 producing Th2 cells (in both humans and mice). IgE is the main antibody isotype that induces degranulation of mast cells, eosinophils and basophils. Therefore, the production of IgE is the main isotype responsible for allergic hypersensitivity reactions. It is also a key isotype that facilitates anti-helminthic effector mechanisms mediated by the degranulation of granulocytes.

1.11.2.5 IgA

IgA is an important component of breast milk and is the major type of antibody found in the mucous secretions of the body, particularly in the gastrointestinal tract and in the respiratory tract. IgA plasma cells normally reside in the lamina propria of the gut. There they secrete dimeric IgA which is able to trancytose across the epithelium of the gut and into the lumen by complexing with the polymeric immunoglobulin receptor (pIgR). IgA1 is less abundant than IgA2 and can be found circulating in the serum. IgA performs an important role in defence against gastrointestinal parasites such as *Giardia* (Chapter 6).

1.11.3 Fc receptor recognition via Fc receptors

One of the main functions of antibody is to opsonise pathogens. Opsonised pathogens can be recognised by immune cells expressing Fc receptors that bind onto the Fc portion of antibodies. Cross-linking of Fc receptors by antibodies complexed by a multivalent antigen (or on the surface of a pathogen) activates intracellular signalling cascades. In the case of Fcγ receptors this occurs via an ITAM present on the cytoplasmic portion of the Fc receptor chain (FcγRII-A), or by association of the Fc receptor chain with an ITAM-containing Fcγ adaptor protein (Figure 1.16). Cross-linking of Fc receptors activates effector pathways for destruction of the opsonised pathogen. Immune cells expressing Fc receptors include APCs such as macrophages (endocytosis and digestion of the pathogen), NK cells (lysis by ADCC) or granulocytes (damage by exposure to the granule contents after induced granule release). The FcγRII-B receptors (both B1 and B2) are the exception, because the cytoplasmic tail of these receptors contain an ITIM that inhibits the signaling cascade.

Fc receptors are designated by Greek symbols that match the relevant antibody isotype recognised: IgG antibodies ligate Fcγ receptors, IgE antibodies ligate Fcε receptors, and IgA antibodies ligate Fcα receptors (Figure 1.16). However,

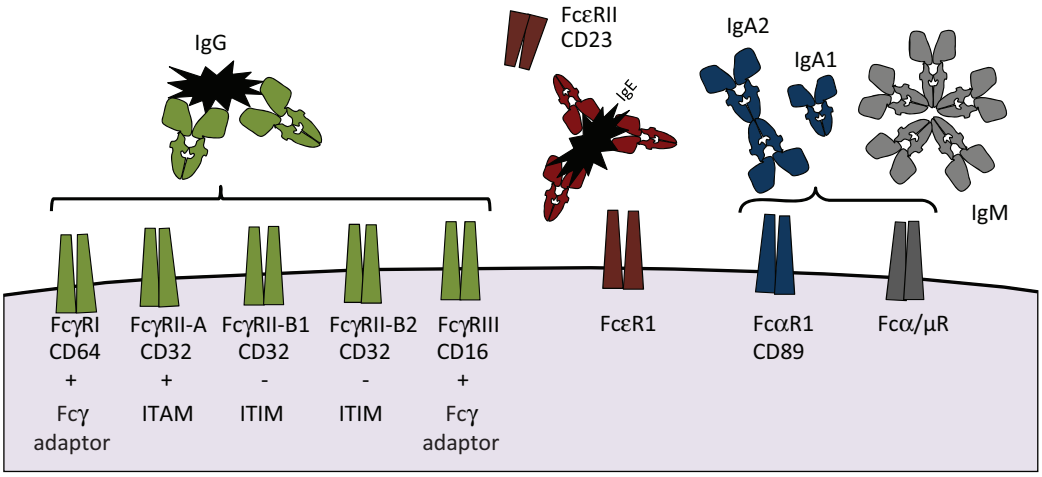


Figure 1.16 Soluble antibodies bind to Fc receptors expressed on the surface of effector cells. IgG is recognised by Fcγ, IgE is recognised by Fcε receptors, one of which is cleaved to form a soluble Fc receptor, and IgA is recognised by Fcα receptors. IgM is recognised by the Fcα/μR.
Abbreviations: Ig, immunoglobulin; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibitory motif.

Fcα/μR can bind both IgA and IgM. While most Fc receptors are cell-surface bound, one exception is FcεRII, an Fc receptor that binds IgE with low affinity. FcεRII is a C-type lectin and it can be cleaved into soluble FcεRII (also called CD23). The binding of IgE/antigen complexes with FcεRII is thought to promote further IgE production from B cells by promoting uptake of the antigen by APCs and presentation of the antigen to CD4+ T cells to provide B cell help.

1.12 Cell trafficking around the body

Parasitic infection can occur in different areas of the human body. As such, tissue-resident sentinel APCs, such as macrophages and DCs, must detect antigen in the breached area and traffic to the local lymph nodes to present antigen to adaptive immune cells for initiation of an adaptive immune response. For effective containment of the infection, effector-immune cells must then traffic towards the area of infection. The ability of immune cells to travel around the body in a controlled and specific manner is mediated by a group of molecules distinct from cytokines called chemokines.

Chemokines are produced by activated immune cells and tissue resident APCs. Secreted chemokines attract additional immune cells to the infected tissue. Immune cells move towards gradients of chemokines which are detected by G-protein-coupled chemokine receptors expressed on the cell surface. In this way immune responses can be targeted to particular areas of the body, as required.

Chemokines are classified into four different groups, according to the sequence of cysteine motifs found in the amino terminal of the molecule. Chemokines

containing CC and CXC motifs are the most common chemokines. The CC class of chemokines includes RANTES (CCL5) and eotaxin (CCL11) which are both important in the chemotaxis of T cells and eosinophils. CXC chemokines include IL-8 (CXCL8), secreted by macrophages to recruit neutrophils to the site of infection, and IP-10 (CXCL-10), secreted by endothelial cells and monocytes to attract T cells and NK cells. Lymphotactin (CXL1) and Fractalkine (CX_3CL1) belong the C and CX_3C classes of chemokines respectively, attracting DCs, NK cells (CXL1) and T cells (CX_3CL1).

Once immune cells are recruited to the site of infection, they must attach to the endothelium and leave the bloodstream to mediate effector functions in the infected tissue. The up-regulation of integrins on immune cells activated in the secondary lymphoid organs, and adhesion molecules on the endothelium, is normally induced by inflammatory cytokines and facilitates the accumulation of immune cells at sites of inflammation.

Integrins such as leukocyte functioning antigen (LFA)-1, CD11b:CD18 and CD11c:CD18, and adhesion molecules such as members of the selectin family (e.g. E-selectin or P-selectin) or cellular adhesion molecules (e.g. ICAM-1), allow attachment and traversal of the endothelium. Interactions between integrins and selectins initiate a rolling adhesion before cellular adhesion molecules support a tighter binding of the immune cell to the endothelium. Leukocyte extravasation, that is traversal between endothelial cells and into the tissue in a process known as diapedesis, completes the journey of the activated immune cells to the site of infection.

1.13 Cellular immune effector mechanisms

1.13.1 Phagocytosis and pathogen digestion

Phagocytosis ('cell-eating') is carried out by macrophages, granulocytes (in particular neutrophils) and DCs. Macrophages and neutrophils clear pathogens from the body by phagocytosis, while DCs phagocytose pathogens to enable digested pathogen fragments to be displayed on the cell surface, complexed with MHC molecules to activate T cells. Unlike phagocytosis of pathogens and their products, which activate macrophages to produce inflammatory molecules to recruit immune cells to the area of infection, the clearance of senescent or lysed (see below) cells undergoing programmed cell death does not lead to the generation of an inflammatory immune response.

Phagocytes generally engulf pathogens or pathogen products using extensions known as pseudopodia. The initial compartment formed is known as a phagosome (Figure 1.6). Acidification of the phagosomes to form phagolysosomes, occurs after fusion of lysosomes, Golgi-derived compartments full of acidic hydrolases. Conditions in phagolysosomes are optimal for the digestion and destruction of the engulfed pathogen. Peptides derived from digested pathogen proteins are loaded onto MHC-II molecules in Class II vesicles (Figure 1.9). These peptide loaded MHC II molecules are trafficked to the surface of the cell for presentation to CD4+ T cells.

1.13.2 Cellular-mediated lysis of pathogens

Lytic responses can be mediated by activation of complement (Figure 1.5). However cellular-mediated lysis of pathogenic organisms or infected cells is carried out by CD8+ cytotoxic T cells and NK cells. Although the molecules identifying target cells for lysis are different for each of these cell types (pMHC-I for CD8+ T cells or the lack of MHC-I or opsonic IgG antibodies/reduced levels of MHC-I on the infected cell surface for NK cells), the mechanisms used for lysis are similar in that both cell types release cytotoxic granules (which are modified lysosomes) stored in the cytoplasm.

CD8+ T cells are cross-primed by DCs in the secondary lymph nodes, and then traffic to the site of infected tissue by up-regulating the expression of chemokine receptors to detect chemokines produced in the area of infection. The TCR of primed CD8+ T cells is able to recognise infected cells within the tissue by their expression of pMHC-I molecules displaying peptide from the intracellular pathogen.

Upon recognition, the release of granzymes and perforins (serine proteases) from the cytotoxic granules of the CD8+ T cell creates pores in the target cell, resulting in apoptotic lysis. CD8+ T cells do not randomly release granule contents, but do so in a controlled manner. The enzymes within the granules induce programmed cell death (apoptosis) via activation of caspases rather than necrotic cell death, because the latter would induce inflammation via recognition of DAMPs by PRRs in the surrounding tissue. Apoptotic infected cells are then ingested by phagocytic cells recruited to the area of infection by chemokines.

NK cells similarly contain cytotoxic granules of pre-formed perforin and granzymes. NK cells act in a quicker time frame than CD8+ T cells because they are activated by invariant receptors; they do not require priming. The lytic activity of NK cells is amplified by exposure to pro-inflammatory cytokines such as IL-12.

1.13.3 Granuloma formation as a method of containment

Immune cells traffic to the site of infection attracted by chemokines that are produced as a result of the inflammatory immune response generated by cells already in the infected tissue. When microbes are not effectively destroyed by this action, immune cells can develop a structure known as a tissue granuloma. Granulomas can form in the environment of both type 1 and type 2 inflammation, although the type of immune response under way influences the composition of the immune cell types that form the granuloma. Macrophages (M1 or M2) are integral to granuloma formation and in type 1 granulomas they can fuse to form multinucleated giant cells. Both T and B cells can be found in a granuloma, and type 2 granulomas have a greater proportion of granulocytes.

Granulomas essentially act as cellular cages, containing the pathogen and, in some cases, causing death or inactivation of the pathogen by starvation due to restricted access to the components necessary for growth. Granulomas in

Table 1.6 Summary of immune effector mechanisms against parasitic infection.

Soluble			Cellular		
Component	Function	Innate or adaptive	Cell	Process	Innate or adaptive
Complement	Opsonin lysis	Innate and adaptive	Macrophages, dendritic cells Neutrophils Eosinophils	Phagocytosis	Innate
Acute phase proteins	Opsonin complement activation	Innate	CD8+ T cells	Cell lysis	Adaptive
Anti-microbial peptides	Opsonin lysis	Innate	NK cells	Cell lysis	Innate
Antibody	Opsonin complement activation	Adaptive	Granulocytes	Granuloma formation	Innate

parasitic infection are most commonly associated with schistosome infection, in particular the formation of type 2 granulomas around schistosome eggs trapped in the liver (see Chapter 16). In this case, the prolonged induction of Th2 inflammation, and the involvement of M2 macrophages, can lead to fibrosis in the liver. Granulomas can also form around some parasitic nematodes, although it has not been established whether this is a cause or a consequence of, nematode death. Type 1 granulomas are most commonly associated with tuberculosis as a method of containment of *Mycobacterium tuberculosis* bacteria in the lung. They also occur in Crohn's disease as an over-reaction to the commensal gut flora.

Some of the different immune effector mechanisms against parasitic infections are summarised in Table 1.6.

1.14 Hypersensitivity reactions

Hypersensitivity reactions are of relevance to this book, due to the powerful effects that parasitic infections, in particular helminth infections, can have on the development of allergies and allergic asthma. This is discussed in section 5 under the 'hygiene hypothesis' (Chapter 23), which attempts to explain why the incidence of allergies and asthma have increased in developed countries when compared with their developing counterparts. Hypersensitivity reactions are classified into four different types, with types 1, 2 and 3 occurring immediately and type 4 taking some time to develop fully (delayed hypersensitivity reactions).

1.14.1 Type 1 hypersensitivity reactions (immediate)

Type 1 reactions are mediated by cross-linking of FcεRI on the surface of mast cells by complexes of innocuous multivalent antigens ('allergens') with IgE (Figure 1.17 A). This leads to the release of vasoactive mediators, such as histamine, and symptoms of allergic reactions such as hay fever.

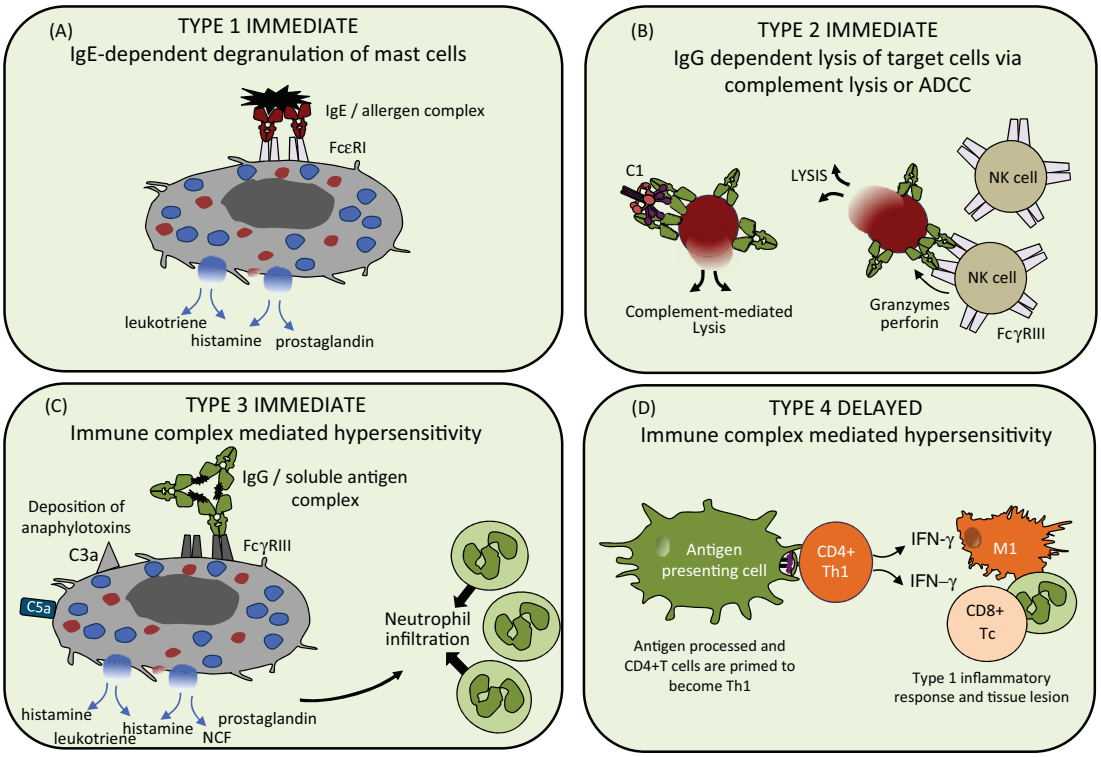


Figure 1.17 Hypersensitivity reactions. Hypersensitivity reactions can be classified into four types. Type 1 hypersensitivity reactions (A) arise from the degranulation of mast cells by IgE complexed with multivalent antigen. Type 2 hypersensitivity reactions lead to ADCC-mediated lysis of red blood cells and platelets opsonised by IgG or IgM (IgM not shown) (B). The deposition of immune complexes and neutrophil mediated inflammation cause type 3 hypersensitivity reactions (C). Cell-mediated delayed type hypersensitivity reactions are the result of activation of Th1-mediated inflammation in the tissue (D) Abbreviations: ADCC, antibody-dependent cytotoxicity; Ig, immunoglobulin; NCF, neutrophil chemotactic factor; NK, natural killer.

1.14.2 Type 2 hypersensitivity reaction (immediate)

Type 2 reactions result from the opsonisation of red blood cells and platelets by antibodies of the IgG or IgM isotype. These antibodies are made in response to drugs such as the antibiotic penicillin. The drugs bind onto the surface of red blood cells and platelets, serving as a target for opsonising antibodies resulting in ADCC from ligation of FcγRIII on NK cells by IgG or complement lysis from IgM opsonisation. Clearance of the opsonised cells leads to haemolytic anaemia (opsonised red blood cells) or thrombocytopaenia (opsonised platelets).

1.14.3 Type 3 hypersensitivity reactions (immediate)

Type 3 reactions are mediated by immune (IgG/ antigen) complexes that form in response to soluble antigen. The deposition of these complexes in various tissues causes an inflammatory response, initially mediated by mast cell degranulation in response to anaphylotoxins from the complement pathway

and cross-linking of FcγRIII, which leads to the degranulation of mast cells. This response is subsequently amplified by the infiltration of neutrophils into the tissue. Serum sickness from the administration of therapeutic antibodies, or anti-venom against snake bites and glomerulonephritis (deposition of immune complexes in the kidneys), are some examples of type 3 hypersensitivity reactions.

1.14.4 Type 4 hypersensitivity reaction (delayed)

Type 4 reactions are delayed type hypersensitivity reactions (DTH) that occur when inflammatory Th1 cytokines activate macrophages and cytotoxic CD8+ T cells, causing direct cellular damage. DTH is delayed due to the time required to activate the cellular component of the reaction. Examples of DTH reactions include rejection of transplanted tissue, and the delayed reaction to the tuberculin test normally used to determine whether an individual has been previously infected with *M. tuberculosis*.

REFERENCES FOR FURTHER READING

Introduction and general immunology

Mebius, RE and G Kraal (2005). **Structure and function of the spleen**. *Nature Reviews. Immunology* 5(8), 606–616.

Metcalf, D (2007). **On hematopoietic stem cell fate**. *Immunity* 26(6), 669–673.

Murphy, K *et al.* 2011). *Janeway's Immunobiology*. 8th edition. Published by Garland Science, New York

Willard-Mack, CL (2006). **Normal structure, function, and histology of lymph nodes**. *Toxicologic Pathology* 34(5), 409–424.

Innate immunity and recognition

Matzinger, P (1994). **Tolerance, danger, and the extended family**. *Annual Review of Immunology* 12, 991–1045.

Takeuchi, O & Akira, S (2010). **Pattern recognition receptors and inflammation**. *Cell* 140(6), 805–820.

Ehrnthaller, C *et al.* (2011). **New insights of an old defense system: structure, function, and clinical relevance of the complement system**. *Molecular Medicine* 17(3–4), 317–329.

Macrophages

Gordon, S. & Martinez, FO (2010). **Alternative activation of macrophages: mechanism and functions**. *Immunity* 32:593–604.

Hesse, M *et al.* (2001). **Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines *in vivo*: granulomatous pathology is shaped by the pattern of L-arginine metabolism**. *Journal of Immunology* 167(11), 6533–6544.

Mosser, DM & Edwards, JP (2008). **Exploring the full spectrum of macrophage activation**. *Nature Reviews. Immunology* 8, 958–969.

Pesce, J *et al.* (2006). **The IL-21 receptor augments Th2 effector function and alternative macrophage activation**. *The Journal of Clinical Investigation* 116(7), 2044–2055.

Rutschman, R *et al.* (2001). **Cutting edge: Stat6-dependent substrate depletion regulates nitric oxide production**. *Journal of Immunology* 166(4), 2173–2177.

Welch, JS *et al.* (2002). **TH2 cytokines and allergic challenge induce Ym1 expression in macrophages by a STAT6-dependent mechanism**. *The Journal of Biological Chemistry* 277(45), 42821–42829.

Granulocytes

Neutrophils

Mantovani, A *et al.* (2011). **Neutrophils in the activation and regulation of innate and adaptive immunity**. *Nature reviews. Immunology* 11(8), 519–531.

Eosinophils

Akuthota, P *et al.* (2008). **Immunoregulatory roles of eosinophils: a new look at a familiar cell**. *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 38(8), 1254–1263.

Mast Cells

Abraham SN & St John, AL (2010). **Mast cell-orchestrated immunity to pathogens**. *Nature reviews. Immunology* 10(6), 440–452.

Basophils

Cady, CT *et al.* (2010). **IgG antibodies produced during subcutaneous allergen immunotherapy mediate inhibition of**

basophil activation via a mechanism involving both FcgammaRIIA and FcγRIIB. *Immunology Letters* 130(1–2), 57–65.

Meknache, N *et al.* (2009). **Human basophils express the glycosylphosphatidylinositol-anchored low-affinity IgG receptor FcγRIIIB (CD16B)**. *Journal of Immunology* 182(4), 2542–2550.

Min, B & Paul, WE (2008). **Basophils and type 2 immunity**. *Current Opinion in Hematology* 15(1), 59–63.

Min, B *et al.* (2004). **Basophils produce IL-4 and accumulate in tissues after infection with a Th2-inducing parasite**. *The Journal of Experimental Medicine* 200(4), 507–517.

Perrigoue, JG *et al.* (2009). **MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity**. *Nature Immunology* 10(7), 697–705.

Sokol, CL *et al.* (2009). **Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response**. *Nature Immunology* 10(7), 713–720.

Yoshimoto, T *et al.* (2009). **Basophils contribute to T(H)2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells**. *Nature Immunology* 10(7), 706–712.

Dendritic cells

Banchereau, J & Steinman, RM (1998). **Dendritic cells and the control of immunity**. *Nature* 392(6673), 245–252.

MacDonald, KP *et al.* (2002). **Characterization of human blood dendritic cell subsets**. *Blood* 100(13), 4512–4520.

Scott, CL *et al.* (2011). **Intestinal CD103+ dendritic cells: master regulators of tolerance**? *Trends in Immunology* 32(9), 412–419.

Shortman, K & Heath, WR (2010). **The CD8+ dendritic cell subset**. *Immunological Reviews* 234(1), 18–31.

Ueno, H *et al.* (2011). **Targeting human dendritic cell subsets for improved vaccines**. *Seminars in Immunology* 23(1), 21–27.

Villadangos, JA & Shortman, K (2010). **Found in translation: the human equivalent of mouse CD8+ dendritic cells**. *The Journal of Experimental Medicine* 207(6), 1131–1134.

NK cells

Bryceson, YT *et al.* (2011). **Molecular mechanisms of natural killer cell activation**. *Journal of Innate Immunity* 3(3), 216–226.

Nakanishi, K *et al.* (2001). **Interleukin-18 regulates both Th1 and Th2 responses**. *Annual Review of Immunology* 19, 423–474.

Adaptive immunity

Kalia, V *et al.* (2006). **Differentiation of memory B and T cells**. *Current Opinion in Immunology* 18(3), 255–264.

Pulendran, B & Ahmed, R (2011). **Immunological mechanisms of vaccination**. *Nature Immunology* 12(6), 509–517.

MHC

Apostolopoulos, V *et al.* (2008). **MHC and MHC-like molecules: structural perspectives on the design of molecular vaccines**. *Human Vaccines* 4(6), 400–409.

Donaldson, JG & Williams, DB (2009). **Intracellular assembly and trafficking of MHC class I molecules**. *Traffic* 10(12), 1745–1752.

Kazansky, DB (2008). **MHC restriction and allogeneic immune responses**. *Journal of Immunotoxicology* 5(4), 369–384.

Landsverk, OJ *et al.* (2009). **MHC II and the endocytic pathway: regulation by invariant chain**. *Scandinavian Journal of Immunology* 70(3), 184–193.

T cell activation

Akdis, M *et al.* (2011). **Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases**. *The Journal of Allergy and Clinical Immunology* 127(3), 701–721 e701–770.

Amigorena, S & Savina, A (2010). **Intracellular mechanisms of antigen cross presentation in dendritic cells**. *Current Opinion in Immunology* 22(1), 109–117.

Kurts, C *et al.* (2010). **Cross-priming in health and disease**. *Nature reviews. Immunology* 10(6), 403–414.

CD4 T cell phenotypes

Mosmann, TR *et al.* (1986). **Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins.** *Journal of Immunology* 136(7), 2348–2357.

Murphy, KM & Reiner, SL (2002). **The lineage decisions of helper T cells**. *Nature Reviews. Immunology* 2(12), 933–944.

Th1

Boehm, U *et al.* (1997). **Cellular responses to interferon-γ**. *Annual Review of Immunology* 15, 749–795.

Lazarevic, V & Glimcher, LH (2011). **T-bet in disease**. *Nature Immunology* 12(7), 597–606.

Th2

Grunig, G *et al.* (1998). **Requirement for IL-13 independently of IL-4 in experimental asthma**. *Science* 282(5397), 2261–2263.

Heller, F *et al.* (2005). **Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution**. *Gastroenterology* 129(2), 550–564.

McDonald, SA *et al.* (2004). **Protection against the early acute phase of *Cryptosporidium parvum* infection conferred by interleukin-4-induced expression of T helper 1 cytokines**. *Journal of Infectious Diseases* 190(5), 1019–1025.

Wills-Karp, M *et al.* (1998). **Interleukin-13: central mediator of allergic asthma**. *Science* 282(5397), 2258–2261.

Zhang, JG *et al.* (1997). **Identification, purification, and characterization of a soluble interleukin (IL)-13-binding protein. Evidence that it is distinct from the cloned Il-13 receptor and Il-4 receptor alpha-chains**. *The Journal of Biological Chemistry* 272(14), 9474–9480.

Th17

Park, H *et al.* (2005). **A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17)**. *Nature Immunology* 6(11), 1133–1141.

Harrington, LE *et al.* (2005). **Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages**. *Nature Immunology* 6(11), 1123–1132.

Cruz, A *et al.* (2006). **Cutting edge: IFN-γ regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection**. *Journal of Immunology* 177(3), 1416–1420.

Lubberts, E *et al.* (2000). **IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion**. *The Journal of Clinical Investigation* 105(12), 1697–1710.

Th9 and Th22

Noelle, RJ & Nowak, EC (2010). **Cellular sources and immune functions of interleukin-9)**. *Nature reviews. Immunology* 10(10), 683–687.

Veldhoen, M *et al.* (2008). **Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset**. *Nature Immunology* 9(12), 1341–1346.

Wolk, K *et al.* (2010). **Biology of interleukin-22)**. *Seminars in Immunopathology* 32(1), 17–31.

T regulatory cells

Annacker, O *et al.* (2005). **Essential role for CD103 in the T cell-mediated regulation of experimental colitis**. *The Journal of Experimental Medicine* 202(8), 1051–1061.

Belkaid, Y & Rouse, BT (2005). **Natural regulatory T cells in infectious disease**. *Nature Immunology* 6(4), 353–360.

Bruder, D *et al.* (2004). **Neuropilin-1: a surface marker of regulatory T cells**. *European Journal of Immunology* 34(3), 623–630.

Fontenot, JD *et al.* (2003). **Foxp3 programs the development and function of CD4+CD25+ regulatory T cells**. *Nature Immunology* 4(4), 330–336.

Leithauser, F *et al.* (2006). **Foxp3-expressing CD103+ regulatory T cells accumulate in dendritic cell aggregates of the colonic mucosa in murine transfer colitis**. *The American Journal of Pathology* 168(6), 1898–1909.

Lim, HW *et al.* (2005). **Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells**. *Journal of Immunology* 175(7), 4180–4183.

Robertson, SJ *et al.* (2006). *In vitro* **suppression of CD8+ T cell function by Friend virus-induced regulatory T cells**. *Journal of Immunology* 176(6), 3342–3349.

Sakaguchi, S *et al.* (1995). **Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases**. *Journal of Immunology* 155(3), 1151–1164.

Sakaguchi, S & Sakaguchi, N (2005). **Regulatory T cells in immunologic self-tolerance and autoimmune disease**. *International Reviews of Immunology* 24(3–4), 211–226.

Sakaguchi, S *et al.* (2006). **Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease**. *Immunological Reviews* 212, 8–27.

Sakaguchi, S *et al.* (2006). **Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in self-tolerance and autoimmune disease.** *Current Topics in Microbiology and Immunology* 305, 51–66.

Sakaguchi, S (2005). **Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self**. *Nature Immunology* 6(4), 345–352.

Shevach, EM (2002). **CD4+ CD25+ suppressor T cells: more questions than answers**. *Nature Reviews. Immunology* 2(6), 389–400.

Taams, LS *et al.* (2005). **Modulation of monocyte/macrophage function by human CD4+CD25+ regulatory T cells**. *Human Immunology* 66(3), 222–230.

Wan, YY & Flavell, RA (2005). **Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter**. *Proceedings of the National Academy of Sciences of the United States of America* 102(14), 5126–5131.

Wilson, MS *et al.* (2005). **Suppression of allergic airway inflammation by helminth-induced regulatory T cells**. *The Journal of Experimental Medicine* 202(9), 1199–1212.

B cell activation and antibody production

Chen, K *et al.* (2009). **Immunoglobulin D enhances immune surveillance by activating antimicrobial, proinflammatory and B cell-stimulating programs in basophils**. *Nature Immunology* 10(8), 889–898.

Harris, DP *et al.* (2000). **Reciprocal regulation of polarized cytokine production by effector B and T cells**. *Nature Immunology* 1(6), 475–482.

Maizels, RM & Yazdanbakhsh, M (2003). **Immune regulation by helminth parasites: cellular and molecular mechanisms**. *Nature Reviews. Immunology* 3(9), 733–744.

Nimmerjahn, F & Ravetch, JV (2010). **Antibody-mediated modulation of immune responses**. *Immunological Reviews* 236, 265–275.

Nimmerjahn, F & Ravetch, JV (2007). **Fc-receptors as regulators of immunity**. *Advances in Immunology* 96, 179–204.

Snapper, CM *et al.* (1988). **Differential regulation of IgG1 and IgE synthesis by interleukin 4**. *The Journal of Experimental Medicine* 167(1), 183–196.

Snapper, CM *et al.* (1988). **IFN-γ stimulates IgG2 a secretion by murine B cells stimulated with bacterial lipopolysaccharide**. *Journal of Immunology* 140(7), 2121–2127.

Vos, Q *et al.* (2000). **B-cell activation by T-cell-independent type 2 antigens as an integral part of the humoral immune response to pathogenic microorganisms**. *Immunological Reviews* 176, 154–170.

Cell trafficking

Raman, D *et al.* (2011). **Chemokines in health and disease**. *Experimental Cell Research* 317(5), 575–589.

Immune effector mechanisms

Sandor, M *et al.* (2003). **Granulomas in schistosome and mycobacterial infections: a model of local immune responses**. *Trends in Immunology* 24(1), 44–52.

Schroder, K *et al.* (2004). **Interferon-γ: an overview of signals, mechanisms and functions**. *Journal of Leukocyte Biology* 75(2), 163–189.

Vivier, E *et al.* (2011). **Innate or adaptive immunity? The example of natural killer cells**. *Science* 331(6013), 44–49.

Zhang, N & Bevan, MJ (2011). **CD8(+) T cells: foot soldiers of the immune system**. *Immunity* 35(2), 161–168.

Hypersensitivity reactions

Akdis, CA (2006). **Allergy and hypersensitivity: mechanisms of allergic disease**. *Current Opinion in Immunology* 18(6), 718–726.

Kobayashi, K *et al.* (2001). **Immunopathogenesis of delayed-type hypersensitivity**. *Microscopy Research and Technique* 53(4), 241–245.