

# Immunology

- Introduction
- Overview of the immune system
- Cells and molecules of the immune system
- Initial response of the host to injury (the innate immune response)
- Development of adaptive immunity and immunological memory
- Effector mechanisms
- Organization of the immune system
- Antigen recognition
- The major histocompatibility system
- T-cell activation
- B-cell activation
- Immunological tolerance and autoimmunity
- Allergy and immediate hypersensitivity
- Organ and tissue transplantation
- Tumours induce immune responses
- The eye and the immune system
- Conclusion

## Introduction

Immunology is the study of host defence mechanisms. Even the simplest organisms have the ability to mount a variety of specific and non-specific responses to invasion or attack by foreign organisms. Where the host and pathogen meet and interact is where the immune response is initiated and the nature of both players determines the outcome. Thus humans can respond to pathogens in many different ways, depending on their genetic make-up, the type of foreign organism (e.g. virus, bacterium, fungus, etc.) and prevailing conditions or the setting in which the host is under threat.

The immune response is even determined by the nature of the tissue invaded. For instance, the eye (and the brain) respond when under foreign attack, but under certain circumstances the predicted response does not occur but is rather modulated by the tissue; this is called ‘immune privilege’ and is related to the special microenvironment and immunoregulatory mechanisms operating in these tissues.

Most of this chapter outlines the basic principles of immune responses, with reference to the eye as appropriate. At a very simple level, however, it must be remembered that the organism has very effective means to prevent host and pathogen meeting each other in the first place: namely, barriers such as the skin and mucous membranes with their surface coverings (including that most important layer of mucus present on mucosal surface tissues).

## Overview of the immune system

Immunity is defined as the ability of the host to protect itself against a foreign organism or pathogen. To do this it requires an *immune system* comprising the cells and molecules used in the host’s defence. For unicellular hosts this may simply mean certain molecules on the cell surface that enable it to recognize foreign organisms. However, for higher-order hosts the immune system is a highly organized network of tissues, cells and molecules.

Hosts defend themselves by mounting an *immune response* involving the activation and recruitment of the cells and molecules of the immune system. Currently, the immune system is considered in terms of its specificity: the *innate (natural or native) immune system* and the *acquired (adaptive) immune system*. In its first line of defence against attack, the host uses the innate immune system because this is rapidly mobilized and is not dependent on previous exposure

to the foreign invader, i.e. does not involve ‘memory’. This form of response is considered relatively *non-specific* in that the same sort of response occurs to most foreign organisms and even to injury itself. It involves activation of cells (such as macrophages, see p. 377) and molecules (such as complement, see p. 405) which are present in the tissues and fluids of all higher organisms.

Innate immunity, however, has some degree of specificity in that innate immune cells such as macrophages ‘sense’ pathogens through receptors that recognize generic molecular ‘patterns’ which characterize different types of pathogens (pathogen-associated molecular patterns or PAMPs). Thus there are receptors for broad groups of molecules present in different organisms such as viral DNA, fungal carbohydrates and bacterial endotoxins (see Ch. 8, p. 464). However, as stated above immune responses can be initiated not only by pathogens, but simply by injury, or by molecules such as plant pollens (causing allergies), and especially by ‘alloantigens’ (those molecules that differentiate individuals within a species and cause rejection of donor-unrelated transplants). Accordingly, Matzinger (2012) developed Janeway’s PAMP hypothesis to suggest that innate immunity has evolved to allow the host to sense ‘danger’ or damage to tissues and includes anything that threatens host or tissue viability, namely pathogens of all varieties, sterile trauma such as chemical or thermal injuries, non-self proteins such as those present in allografts, and even ‘self’ proteins if these have become ‘abnormal’ (as in the case of cancer cell proteins, prion proteins, and even self proteins (auto-antigens).

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Accordingly the molecular patterns associated with danger signals and tissue damage are known as danger- or damage-associated molecular patterns (DAMPs) and include PAMPs. Thus bacterial and viral products such single- or double-stranded RNA, endotoxin, complement fragments, reactive oxygen species, heat shock proteins, high mobility group box (HMGB) proteins, and many other molecules (sometimes called ‘alarmins’) can act as DAMPs.

The host-defence immune system thus includes:

- physicochemical barriers such as the skin, eyelids, tears (see Ch. 4)

- molecules normally present in body fluids such as blood, tears and aqueous humour (e.g. complement, lysozyme, antiproteases). Antibacterial defensins are also on this list of ocular surface proteins
- phagocytic and cytotoxic cells such as polymorphonuclear leucocytes, macrophages, eosinophilic granulocytes, natural killer (NK) cells
- molecules released by cells responding to attack and acting on other cells (cytokines), such as interleukins and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) (see below) and complement.

As indicated above, the innate immune system does not have ‘memory’ and so if, after successfully repelling pathogen invasion and clearing the pathogen from the system, a second attack by the same pathogen occurred, the organism would have to go through the same process again. However, innate myeloid immune cells and specifically one subset of specialized myeloid cells, termed dendritic cells (DCs), after devouring and digesting a pathogen, has the potential to present titbits of the devoured material to specialized immune cells, termed T cells, which once activated in the throes of battle with the pathogens (inflammation), become specialized not only to assist in clearing the pathogen (effector T cells) but also to become long-lived memory cells which can respond rapidly and with minimal fuss to a repeat challenge by that same pathogen. This is acquired immunity, which is exquisitely specific and not only involves T cells which can kill pathogens and infected cells but also can activate other immune cells, namely B cells, to produce very high levels of pathogen-specific killer molecules (antibodies) which are the basis of vaccination protocols now used against many common pathogens.

The acquired immune system includes:

- specific immune systems associated with barrier surfaces (the skin immune system and the mucosa-associated immune system, MALT) where memory T cells reside and have ‘first call’ on invading pathogens
- a constant source of naive T and B lymphocytes with potential to modulate their receptors to specifically recognize foreign organisms and molecules (antigens)

There has been a recent explosion of knowledge concerning the innate immune system (see below). After the initial discovery of the phagocytic leucocyte (macrophage) by the Russian zoologist Metchnikoff at the beginning of the 20th century, much of immunology was devoted to immunochemistry of antigens and antibodies and then, in the latter half of the 20th century, to developments in cellular immunology, involving cells of the adaptive immune system such as T cells and B cells. Specificity for pathogen recognition by innate immune cells was considered non-existent until Janeway and others identified receptors in the fruit fly *Drosophila* which were important not only for wing patterning in development but also for recognition of pathogens, such as fungi. His earlier proposal that there were classes of receptors recognizing broad groups of pathogens such as viruses, fungi, bacteria, including mycobacteria, and parasites was shown to be true by the discovery of Toll receptors. Other classes of pathogen-associated molecular patterns were later discovered. However, as Matzinger pointed out, pathogens were not the only agents which activated the innate immune system. Allergens also initiate immune responses, as do allografts, where infectious pathogens were considered not to play a part. Her concept of molecules being either 'dangerous' or 'harmless' has changed the thinking of immunologists in many ways. In addition, she has developed her concepts to consider that different tissues respond to the same insult immunologically in different ways: for instance, the skin and the liver might produce a modulated immune response to the same antigen. In one sense this is a rewriting of the notion of immune privilege, which has been long accepted as a modified immune response taking place in the eye (see p. 457).

- molecules that specifically counteract foreign antigens (antibodies); these proteins are known as immunoglobulins and there are five types (see p. 396)
- non-specific molecules (e.g. cytokines) released by antigen-specific cells (e.g. lymphocytes) which will recruit further myeloid cells to sustain the response as needed.

Specific immunity is described as *humoral* when antibodies (derived from B lymphocytes; see below) and complement are involved in removing the antigen or as *cell-mediated* (cellular) when T lymphocytes and macrophages are involved.

Immunity after infection is normally termed *active immunity* in that the host has responded actively to the stimulus. However, immunity may be transferred *passively* by antibodies or cells. Vaccination procedures that involve the administration of antibodies are termed *passive immunization*, while those that involve inducing a response to the antigen or even the attenuated live organism are termed *active immunization* (see Ch. 8).

The development of acquired immunity involves a number of discrete phases, including:

- an afferent phase in which the foreign antigen is transported from the site of entry and presented by specialized antigen-presenting cells (APCs) to the lymphocytes in the lymphoid tissue (see below)
- a phase of T-cell activation in which T cells are transformed from a resting to an active state
- an effector stage in which T cells induce other cells, such as B cells and macrophages, to remove the antigen. If the antigen remains intracellular, as with virus-infected cells, the T cells themselves attack the infected cell (cytotoxic T cells).

This is known as the *primary immune response* and is accompanied by the appearance of antigen-specific T cells and antibody-secreting B cells. As indicated above, the acquired immune system evolved to provide a memory-based rapid reaction force to heighten immune defence. Thus, on second exposure to the same pathogen, antigen-specific memory T and B cells are recruited much sooner and more efficiently, such that antibody levels are considerably higher than on the first exposure. This is known as the *secondary*

*immune response*. In addition, the type (isotype; see below) of antibody in secondary immune responses is different.

Throughout the development of the acquired immune response there are several checkpoints that prevent a runaway overwhelming inflammatory reaction. Most of these regulatory mechanisms are in place to prevent adaptive immune responses to self-antigens but they also participate in downregulatory responses to foreign antigens and help to restore homeostasis.

Innate immunity with its early warning, rapid-response system provides a reliable means of protecting the host against most extracellular organisms (pathogens) and is a property of every living being. It might be asked, therefore, why the acquired immune system has evolved? In part this is due to the remarkable ability of organisms to evade the immune system. Many pathogens reside and hide within the host cell, as in the case of protozoa such as *Toxoplasma*, obligate parasites such as *Chlamydia* or, more frequently, viruses. Viruses use the host cell machinery for survival and replication by incorporating their genome, or at least part of it, into the DNA of the host cell. This may lead to viral latency or persistence, but may also allow the immune system to recognize the infected cells by the expression of the foreign antigen on the surface of the cell in addition to the self molecules (also termed antigens because they can induce an immune response). It is the recognition of the foreign antigen in the context of self-antigen (i.e. together with self-antigens, the peptide-major histocompatibility complex (MHC); see p. 424) that led to the evolution of the acquired immune system. However, many pathogens continue to evade the immune system by subverting the function of the innate immune cells in which they reside or by becoming 'latent' as in the case of herpes simplex-infected neurones.

The innate immune system is the initial primary responder to pathogens or other challenges. The acquired immune system has developed a considerable degree of sophistication, based on memory of previous pathogen encounters, with an increasingly recognized range of different cell types: T and B lymphocytes; T and B cell subsets (e.g. T helper (Th), T cytotoxic (Tc), T and B regulatory (Treg and Breg) cells; and even subsets of these (e.g. Th0, Th1, Th2,

Th17) (see below), each designed for specific cellular functions. In addition, T and B cells have evolved in fundamentally different directions – T cells to deal with surface-bound antigen (usually cell-associated) and B cells to deal with soluble (extracellular) antigen. The sophistication and specificity of the acquired immune system involving T cells has thus been harnessed to assist the innate immune system in dealing more efficiently with extracellular organisms, for instance via B cells.

Certain basic concepts about immune mechanisms can therefore be derived from the above considerations:

- extracellular foreign antigen is normally cleared by the innate immune system, with some assistance from B-cell activity
- intracellular foreign antigen may evade the immune system and remain latent during the lifetime of the host unless (1) it kills the cell, is released and generates a second innate immune response to remove the pathogen; (2) it is expressed on the surface of the cell in the context of self-antigen and is recognized by the acquired immune system, which kills the infected cells and clears the pathogen (applies to many viruses, e.g. influenza); (3) the host immune system is compromised, in which case the intracellular pathogen proliferates freely, killing many cells and ultimately the host
- all cellular defence mechanisms involve interactions of cell surface molecules (receptors) with complementary molecules (ligands).

So far we have focused on *immunity*, defined as the response of the host to foreign organism. This suggests that all foreign organisms are pathogens but this is not so. Indeed the great majority of microbes and other living organisms are not pathogenic, as evidenced by the very large numbers of *commensal* organisms which colonize several regions of the body, particularly the GI tract (the microbiome) (Box 7-1), and indeed are required for normal health (homeostasis).

This lack of immune responsiveness to foreign antigens can be described as ‘tolerance’ of foreign organisms and indeed the immunological obverse of ‘immunity’ is ‘tolerance’. Tolerance as a defining characteristic can be applied to any antigen, but is especially applied to self-antigens, since clearly we do not

normally react to our own tissues. It has been realized that tolerance to self-antigens, and indeed to any antigen, is an active process in which certain T cells (T regulatory cells [Tregs]) are critical for its normal function. Many effector immune responses to infectious foreign antigens are controlled by the induction of a specific Tregs to that organism. Tregs are part of our normal circulating T-cell population and when Treg function goes awry, autoimmune diseases can ensue; diseases such as various forms of dry eye disease (Sjögren syndrome, see Ch. 9), rheumatoid arthritis and some forms of uveitis are considered autoimmune diseases. Indeed there is a growing recognition that both the innate and the acquired immune response co-evolved with microbes, each exerting counter-regulation on the other.

To summarize, acquired immune responses have certain features that are inherent to them:

- specificity – based on certain features/determinants (known as epitopes) of the antigenic structure
- non-responsiveness to many antigens (prototypically, self-antigens), also known as tolerance, the loss of which underpins autoimmune disease
- diversity – around  $10^9$  individual epitopes can be distinguished – this is known as the lymphocyte ‘repertoire’ and represents the range of potential foreign antigens to which immune responses can be generated
- memory – the secondary immune response, specific to that antigen and present in both B and T cells
- specialization – the immune response is customized to different microbes
- ability to downregulate – the immune response is strictly regulated/limited in magnitude and time through specific and non-specific mechanisms.

## Cells and molecules of the immune system

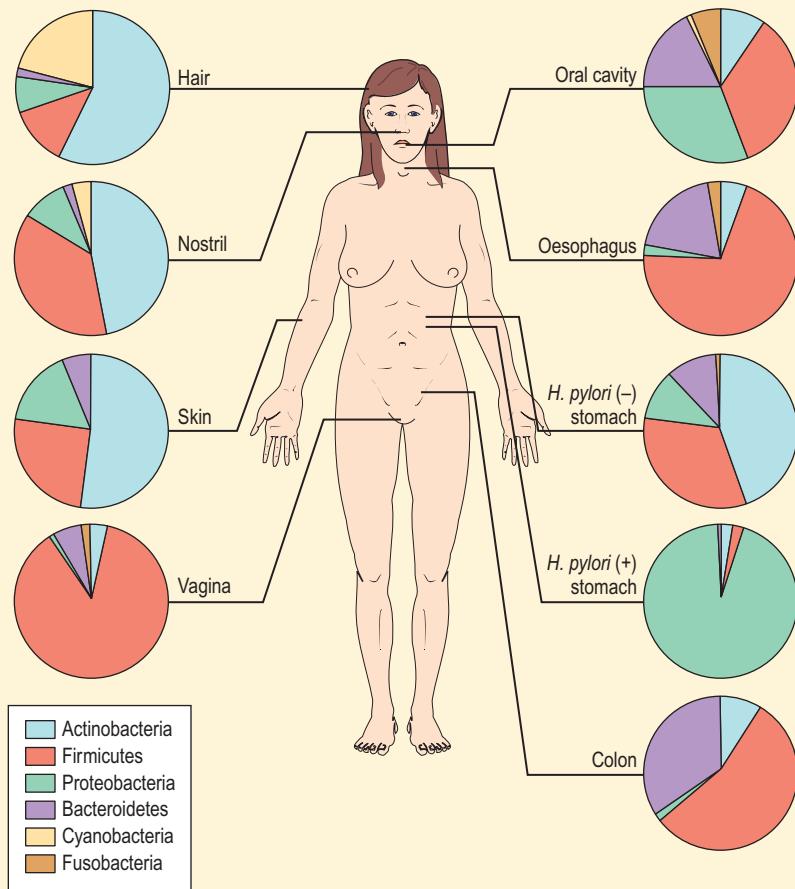
The cells of the immune system are mostly specialized to participate in processes belonging either to the innate or the acquired immune systems (Box 7-2). Some cells are central to both and the distinction between innate and adaptive immune cells is becoming blurred with the discovery of cells such as innate lymphoid cells (see p. 382 and Video 7-1).

**BOX 7-1 THE MICROBIOME**

The total number of separate genomes from microorganisms colonizing a specific site (known as an environmental 'niche') constitutes a 'microbiome' for that site. There are 10 times more microbial cells than human cells in the body and although it does not weigh much more than 500 grams, its importance to normal physiological processes is such that some regard it as a separate and essential 'organ'. Most of the organisms that constitute the microbiome cannot be cultured and have been identified by genetic mapping techniques. The most important role for the microbiome may be in immunoregulation and prevention of disease including autoimmune disease, diabetes, atherosclerosis, cancer and other diseases associated with chronic inflammation and

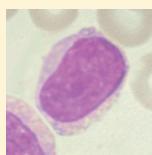
obesity. It may also have a role in psychiatric disease through its effects on neurotransmitter production.

High-throughput sequencing has revealed substantial intra-individual microbiome variation at different anatomical sites, and inter-individual variation at the same anatomical sites (see figure). However, the microbiome at specific anatomical sites can remain remarkably stable over time in each individual. The figure indicates the relative proportion of sequences determined at the taxonomic phylum level at eight anatomical sites. Certain features, such as the presence (+) or absence (-) of *Helicobacter pylori*, can lead to permanent and marked perturbations in community composition. (adapted from Costello et al., 2012).



**BOX 7-2 CELLS AND TISSUES OF THE INNATE AND ACQUIRED IMMUNE SYSTEMS**

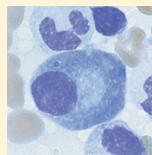

Mature T or B lymphocyte



6–9  $\mu\text{m}$ ; round or slightly indented nucleus; sparse cytoplasm; few granules; few mitochondria



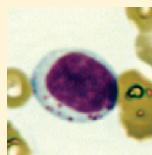
Plasma cell



5–30  $\mu\text{m}$ ; round or oval nucleus; abundant cytoplasm; no granules; abundant endoplasmic reticulum



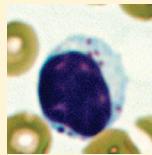
NK cell



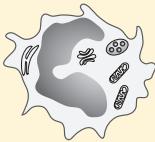
10–12  $\mu\text{m}$ ; round nucleus; abundant cytoplasm; many granules; scattered mitochondria



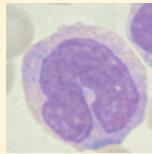
NKT cell



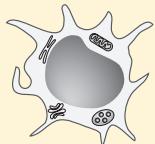
Phenotypically similar to NK cell



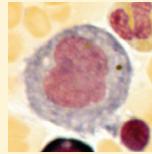
Monocyte



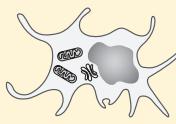
12–20  $\mu\text{m}$ ; round, oval, notched, or horseshoe-shaped nucleus; abundant cytoplasm; abundant granules; well-developed Golgi apparatus; abundant mitochondria



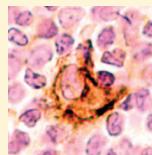
Macrophage



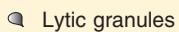
15–80  $\mu\text{m}$ ; elongated, indented, or oval nucleus; abundant cytoplasm; many granules and vacuoles; few mitochondria; abundant lysosomes



Dendritic cell



Irregularly shaped cell and nucleus; many cellular processes; few intracellular organelles; prominent mitochondria



Lytic granules



Mitochondria



Phagosomes



Golgi apparatus



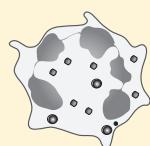
Smooth endoplasmic reticulum



Rough endoplasmic reticulum



Granules

**BOX 7-2 CELLS AND TISSUES OF THE INNATE AND ACQUIRED IMMUNE SYSTEMS—cont'd**

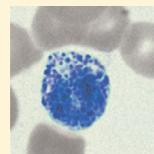
Neutrophil



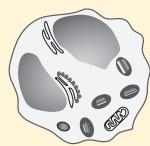
10–15  $\mu\text{m}$ ; 2–5 distinct nuclear lobes; abundant cytoplasm; numerous granules; few mitochondria; abundance of glycogen



Basophil



14–16  $\mu\text{m}$ ; 2–3 nuclear lobes; abundant cytoplasm; large, coarse granules; many ribosomes and mitochondria; abundance of glycogen



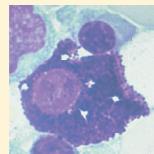
Eosinophil



12–17  $\mu\text{m}$ ; 2–3 nuclear lobes; abundant cytoplasm; large oval granules containing elongated crystallloid and smaller granules; extensive smooth endoplasmic reticulum; few mitochondria



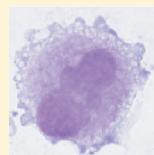
Mast cell



14–16  $\mu\text{m}$ ; non-segmented nucleus; abundant cytoplasm; many large granules; few mitochondria



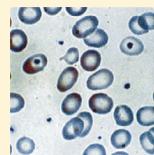
Megakaryocyte



35–160  $\mu\text{m}$ ; irregularly shaped nucleus; abundant cytoplasm; fine granules



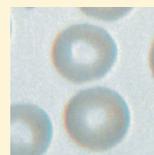
Platelet



1.5–3.5  $\mu\text{m}$ ; non-nucleated; granular cytoplasm



Erythrocyte



7.2  $\mu\text{m}$ ; non-nucleated; no organelles

(From Mak and Saunders, 2006, with permission from Elsevier.)

## THE MYELOID SYSTEM AND INNATE IMMUNITY

The cells directly involved in bacterial killing and removal of damaged host tissue at the site of entry of the foreign organism are the cells of the myeloid system. Some of these cells are recruited to the site of injury during acute inflammation. Their defining feature is the presence of cytoplasmic organelles (lysosomes) containing a battery of hydrolytic enzymes for both intracellular and extracellular killing.

### Neutrophilic granulocytes

Neutrophilic granulocytes (polymorphonuclear leucocytes) are the most common white cell in the circulation and are attracted to sites of inflammation by chemotaxis (see p. 387). These are fully differentiated cells with no capacity for proliferation. They are primary scavengers, causing much of their effect via release of free radicals and proteases from their numerous cytoplasmic granules and lysosomes. These include defensins, lysozyme, lactoferrin and oxidative enzymes (e.g. NADPH-dependent oxidases, myeloperoxidases and catalase), which are also present in ocular fluids such as tears. The half-life of neutrophils is 1–2 days.

This limited ‘clearing-the-bugs’ role has been revised recently with many new functions being ascribed to neutrophils. PMNs have been shown to use a remarkable mechanism for ‘trapping’ bacteria and other ‘dangerous’ material by extruding NETS (neutrophil extracellular traps), composed predominantly of nuclear DNA material, during the process of cell death. NETS are very ‘sticky’ and powerfully prevent bacterial dissemination. However, they can also have deleterious effects by trapping metastatic cells and protecting them from immune surveillance (see eFig. 7-2).

Neutrophils may also participate in the adaptive immune response through release of cytokines and extracellular vesicles which mediate intercellular communication.

### Myeloid mononuclear cells

Monocytes, macrophages and dendritic cells are cells of the mononuclear phagocyte system. Like neutrophils, these cells are derived from haematopoietic bone marrow stem cells and differentiate into a variety of tissue macrophages (histiocytes) with specific functions (Box 7-3).

Macrophages have many functions as part of the innate immune system: they phagocytose dead and damaged cells and organisms in inflammatory exudates; they release cytokines of various sorts, which may activate other cells such as lymphocytes and eosinophils; and they are involved in the acquired immune system as antigen-presenting cells (APCs), as effector cells involved in the process of cell lysis, and by removing antibody-coated (opsonized) cells and particulate material.

Macrophages are, however, not simple cells. There are several varieties of macrophages, some with ‘house-keeping’ roles as resident cells whose job is to remove dead and dying cells during the normal tissue cell turnover. Other macrophages are recruited to sites of inflammation, differentiating from circulating blood monocytes, and are highly active in tissue destruction. These cells are sometimes referred to as classically activated ‘M1’ macrophages with a strongly pro-inflammatory function, while resident and other macrophages are considered to be alternatively activated ‘M2’ macrophages and function more in healing processes including fibrosis and new vessel formation (eFig. 7-1).

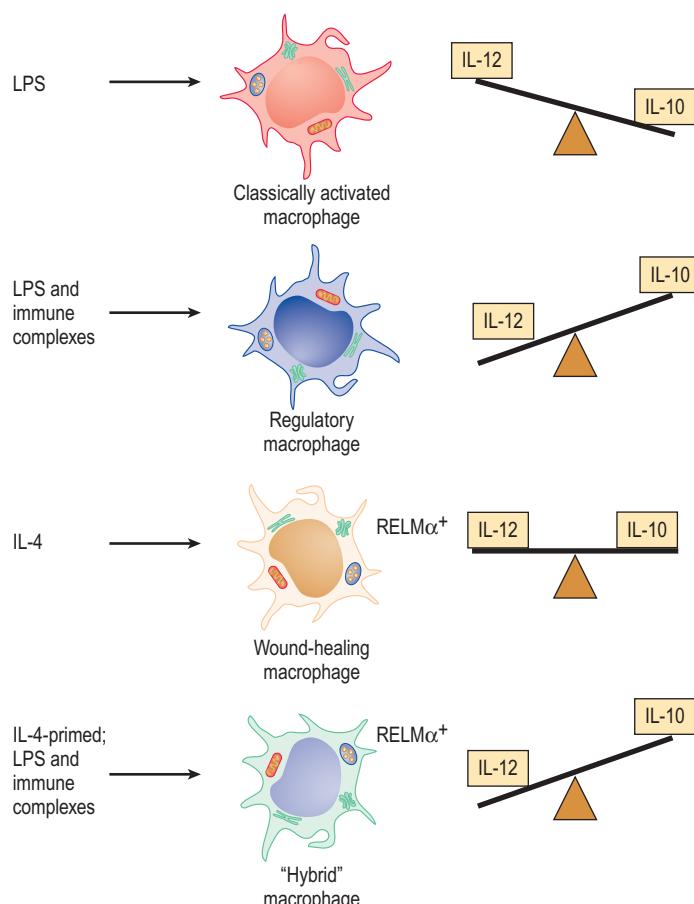
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Macrophages, like B cells, usually capture antigen (e.g. ‘opsonized’ bacteria) for processing and presentation to T cells via surface receptors such as the complement receptor and the Fc receptor. The Fc receptor on macrophages binds the Fc portion of antibody–antigen complexes while B cells express antibody (immunoglobulin) on their surface and use the Fab portion of the antibody to capture antigen. In both cases, preformed antibody to that antigen is needed and so the immune system must have already been exposed to antigen. It is clear therefore that macrophages and B cells are only effective as APCs in the later stages of an ongoing immune response.

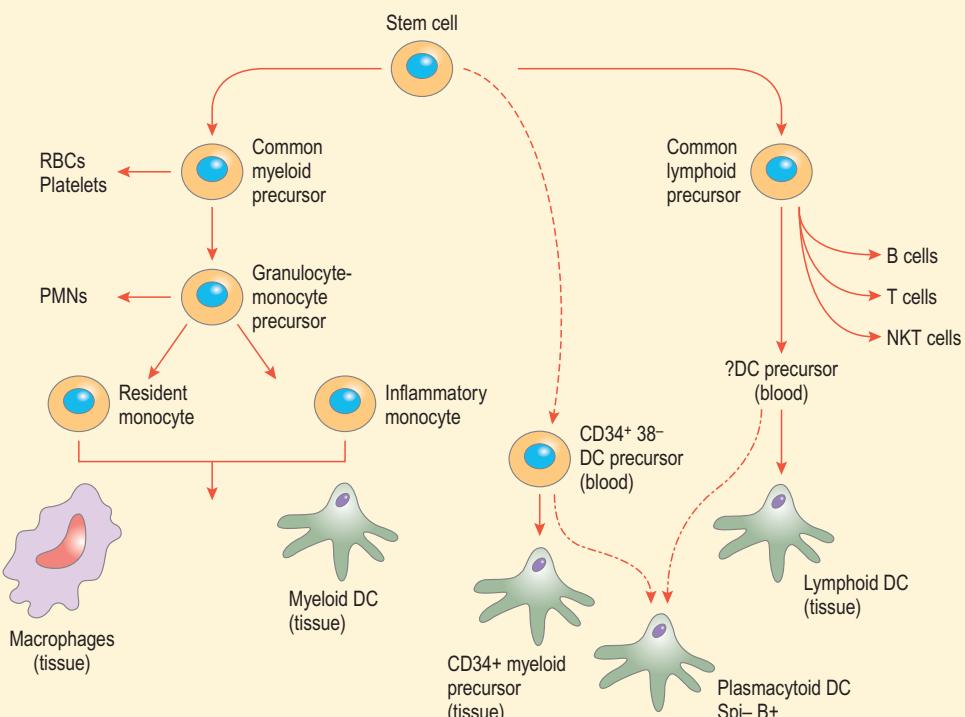
In contrast, the most potent APC in the immune system is the dendritic cell (DC). The DC is the key cell in linking the adaptive and innate immune systems. While the function of most myeloid cells is to engage in innate immune defence functions by marshalling forces against infectious and non-infectious danger signals, the DC takes this one step further: after phagocytosing, killing and digesting an invasive

The functional subtyping of macrophages is of considerable importance to the understanding of disease processes including many ocular diseases such as uveitis, and macular degeneration. The initial classification into M1 classically activated macrophages and M2 alternatively activated macrophages has developed further into a realization that there is greater plasticity in macrophage function than was once thought (eFig. 7-1). Classically activated macrophages remain major players in innate immune defence with the job of removing dangerous foreign and endogenous antigens, clearing necrotic and damaged tissue and setting the scene for repair of tissue. This is

conducted by alternatively activated macrophages involved in such processes as generating new blood vessels to restore blood supply to the tissues and produce factors which rein in the excesses of the inflammatory response. Meanwhile a third type of macrophage differentiates to mediate other aspects of wound healing and repair, i.e. to promote maturation of blood vessels and fibrosis, while a fourth hybrid type of macrophage has features of a regulatory (anti-inflammatory) macrophage, producing the anti-inflammatory cytokine IL-10 if it encounters bacterial factors such as lipopolysaccharide.



**eFIGURE 7-1** Macrophages are classically considered inflammatory cells whose function is to participate in inflammation and get rid of pathogens. However, they are now recognized to be quite heterogeneous in their function: (1) inflammatory macrophages produce interleukin-12 (IL-12) and little IL-10; (2) regulatory macrophages produce IL-10 and little IL-12; (3) alternatively activated macrophages treated with IL-4 ('wound-healing' macrophages) produce little IL-10 or IL-12 but express resistin-like molecule- $\alpha$  (RELM $\alpha$ ); (4) lipopolysaccharide or immune complex treatment of the alternatively activated macrophage produces a mixed macrophage (an ELM $\alpha$ + wound healing-like macrophage) but also produces high levels of IL-10, like a regulatory macrophage. (From Mosser, 2008.)

**BOX 7-3 GENERATION OF MACROPHAGES AND DENDRITIC CELLS FROM MYELOID AND LYMPHOID PRECURSORS**


Note that dendritic cells (DCs) can be derived from both sources. NKT, natural killer T cells; PMNs, polymorphonuclear cells; RBCs, red blood cells.

microorganism the DC, unlike the macrophage or the B cell, or even 'non-professional' APCs, can present the processed antigen to naive T cells and induce an entirely new adaptive immune response with all its features, including memory (see above section). There are several subsets of DCs but, in essence, there are two major groupings: resident, non-migratory DCs which are located in the secondary lymphoid tissues such as the spleen and the lymph node and migratory DCs which arise in the bone marrow and migrate to the tissues where they are available as sentinels to register pathogens and other danger signals and transport antigen to the secondary lymphoid tissues where they activate the adaptive immune system (T and B cells). Tissues at the front line of attack, e.g. lungs, skin, gut mucosa and conjunctiva, are rich in DCs (see eFig 7-2).

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Tissue-resident DCs constantly traffic from the bone marrow to the tissues and then to the secondary lymphoid organs. If they do not detect 'dangerous' antigens, but only self-antigens, they do not activate the adaptive immune system and in fact maintain immune tolerance by sustaining the population of endogenous Tregs, i.e. it seems that, although DCs have the powerful potential to rapidly activate adaptive immunity, e.g. in the case of a viral infection, their homeostatic (and probably main function) is to maintain tolerance.

### Other granulocytes

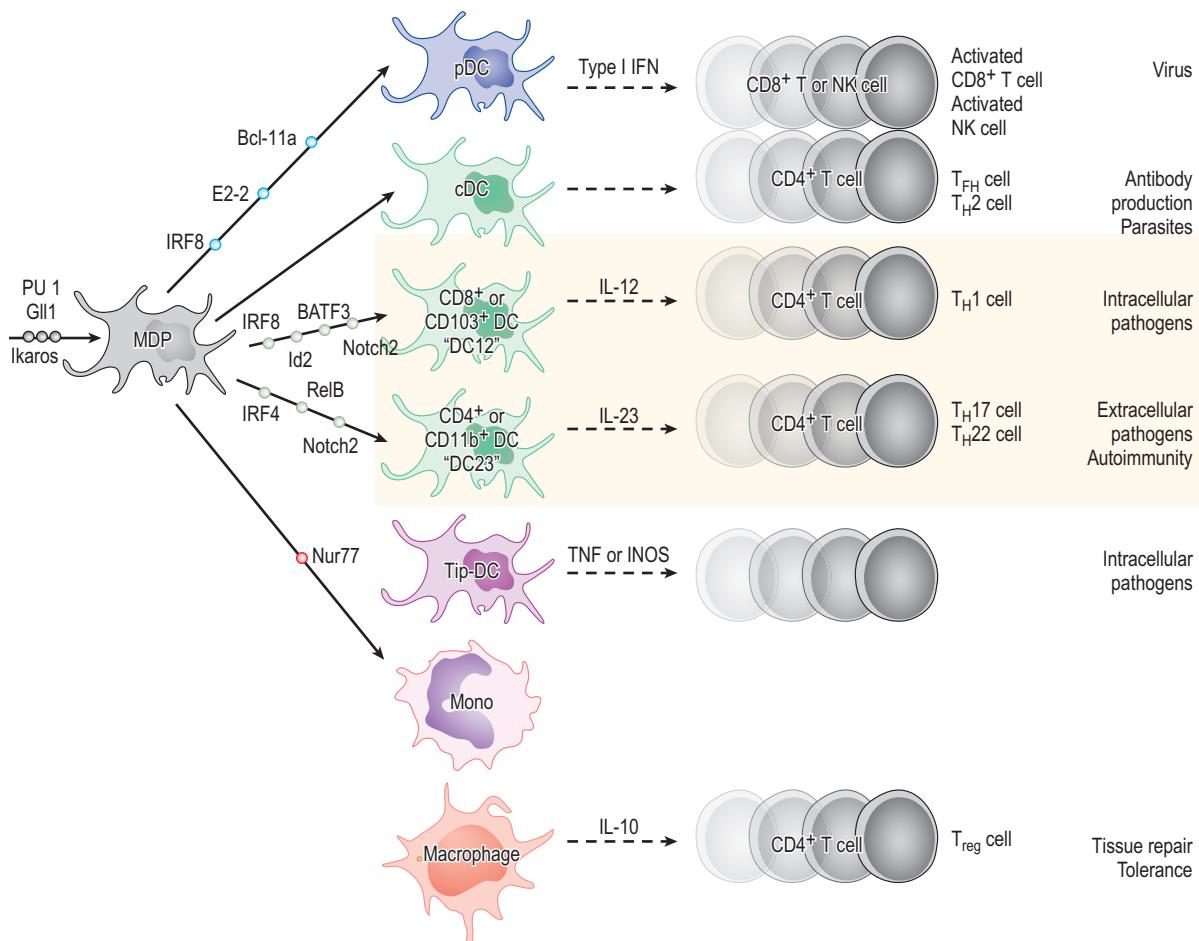
Mast cells/basophils/eosinophils are all part of the granulocyte series of cells. Basophils (life span: 2–3 days) are the circulating equivalent of mast cells (life span: weeks), which occur only in the tissues. However, mast cells and eosinophils may have

## Dendritic cell (DC) subsets

Several types of dendritic cell have been identified on the basis of cell surface markers (CD markers, see p. 381). Two main subsets are recognized: plasmacytoid DC and conventional DC (eFig. 7-2). Both are derived from precursors in the bone marrow, the myeloid-derived precursor cells (MDP), which also generates precursor cells for the macrophage lineage. Such precursors circulate in the blood and seed the tissues in homeostatic conditions where they provide a surveillance function for pathogens and danger signals. They also proliferate and respond rapidly to danger and can become inflammatory DCs. Some of these are derived from monocytes which can become either tissue

inflammatory macrophages (see above) or become Tip DCs (TNF- $\alpha$ , NO-producing DCs) which are active in antigen presentation and generation of the immune adaptive immune response.

Studies on DC lineage and phenotype have mostly been performed in the mouse and human. DC subsets and markers are defined by several different cell surface markers which are variably expressed on different DC subtypes ([www.RnDsystems.com](http://www.RnDsystems.com)). For a summary of how DCs control adaptive immunity, see review by Miriam Merad and colleagues and a summary in the accompanying poster on [www.biologen.com](http://www.biologen.com) (Merad et al., 2008).



**eFIGURE 7-2** Myeloid-derived progenitor (MDP) cells in the bone marrow produce plasmacytoid dendritic cells (pDCs) as well as several types of 'conventional' DC (cDC). CD8<sup>+</sup> cDCs mostly reside in the secondary lymphoid tissue, while CD4<sup>+</sup> cDCs reside in the tissues and form the major population of migratory DCs. MDP also generate monocytes (Mono), which seed the tissues and become tissue-resident macrophages but can also convert to a form of inflammatory DC (TipDC). Each DC subset 'specializes' in a specific immunological function. (From Satpathy et al., 2012.)

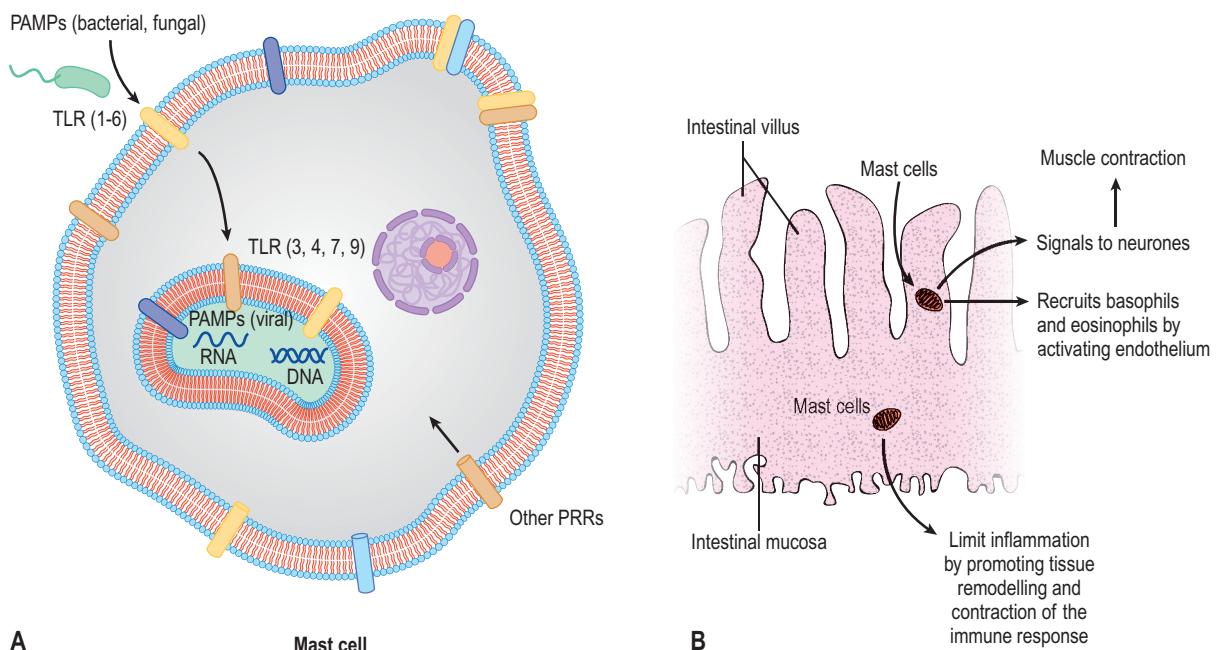
separate lineages and so their ontogeny is not yet decided.

Two types of mast cell are described: the connective tissue mast cells and the mucosal mast cells on the basis of their granule proteases, their susceptibility to degranulation by immunoglobulin E (IgE) and their requirement for different maturation signals (see below). Mucosal mast cells line epithelial and connective tissue mast cells line endothelial (blood vessel) barriers and are thus important innate immune cells, contributing to the initial response to external pathogens or endogenous danger signals by releasing mediators and increasing vascular permeability (Fig. 7-1). Mast cells have a further important role of engineering the contraction of the innate immune response.

Eosinophils account for about 0.1% of circulating leucocytes. Their numbers are elevated in chronic allergic disorders, in both the circulation and the tissues. They are also produced in large numbers in response to parasite and helminth infections and contain a panel of particularly potent parasite-specific

proteases. Like mast cells they have high-affinity IgE receptors, and are probably effectors of tissue damage in allergic disease, including asthma and chronic allergic conjunctival disorders such as atopic keratoconjunctivitis and vernal keratoconjunctivitis (see p. 445). Interestingly, eosinophils are major components of allergic disease, and have been reported to be prominent in fungal antigen-driven allergy. In this respect, eosinophils, like mast cells and basophils, are classical biomarkers of type 2 immune responses (see p. 418) and are associated with alternatively activated (M2) macrophages. As such they promote not only immune homeostasis but also metabolic homeostasis by promoting glucose tolerance and preventing fat deposition.

Natural killer cells are an infrequent but important constituent of innate immune cells, although they are technically lymphoid cells. Their main function is to provide defence against virally infected cells and tumour cells which they perform in an antigen-independent manner. Classically they are considered not to generate immunological memory, which is the



**FIGURE 7-1** Pathogen-recognition receptors (PRRs) occur on many cell types including mast cells, which use them to regulate innate immune responses. In (A) the different types of PRR on mast cells are shown as well as other unique receptors which drive immune responses by the mast cells determined by a level of tissue specificity which act in several ways including cell chemotaxis, vascular permeability increase and mucin production. In (B) location of mast cells in lining of the gut is demonstrated. PAMPs, pathogen-associated molecular patterns. (From St John and Abraham, 2013.)

domain of the adaptive immune system, and so they are not contributors to long-term protective immunity. They express the surface antigen CD56 at both high and low levels. In humans they express the KIR ('killer' inhibitory receptor) family of receptors and in mice a range of Ly-49 antigens which allow subsets of NK cells to be identified and which vary between strains of mice. NK cells use inhibitory receptors to prevent them responding to healthy cells which express MHC class I ('self') antigens (see p. 424) but respond to stressed cells which may have low levels of MHC class I (absent self-antigen) and kill such cells using cytotoxic mechanisms.

NKT cells are a further set of innate immune cells of lymphoid origin which also express a single invariant T-cell receptor which binds CD1 antigens (combined with glycolipid antigens, see p. 412), and thus like NK cells they are essentially involved in innate immune responses to microbial lipid, particularly from Gram-negative microorganisms expressing the glycolipid, glucuronyl ceramides.

### THE LYMPHOID SYSTEM AND ACQUIRED IMMUNITY

The two most important features of the acquired immune system are exquisite antigen specificity and immunological memory. These are properties of lymphocytes. In contrast to cells of the myeloid system, which remove debris and organisms by mechanisms that have limited specificity (thus macrophages will phagocytose broad ranges of organisms using 'pattern recognition receptors' (PRRs) to recognize PAMPs; see p. 389), each clone of lymphoid cell responds to a single antigen. T cells respond to antigen by proliferating and releasing cytokines, while B cells respond by maturing to plasma cells and producing antibodies.

#### T cells

T cells (for thymus-derived) are lymphoid mononuclear cells that recognize antigen in conjunction with self-antigen. T helper (Th) cells respond to antigen in association with MHC class II self-antigen, while T cytotoxic (Tc) cells respond to antigen combined with MHC class I antigen (see p. 424). T cells release cytokines, which are required for T-cell and B-cell proliferation and differentiation, and also for innate immune cell activation. T lymphocytes express surface

markers (molecules detectable by specific monoclonal antibodies) characteristic of their phenotype. Thus Th cells are described as CD4<sup>+</sup> cells and Tc cells are known as CD8<sup>+</sup> cells ([Box 7-4](#)).

This general categorization of T cells (CD4 and CD8, T cells) was found to be insufficient to explain the numerous different functions of T cells. After many years of controversy over whether a T-cell subset existed which suppressed (regulated) the immune response, the discovery of a T cell which was required to prevent spontaneous autoimmune disease indicated that regulatory T cells (Tregs) occurred in normal healthy individuals.

T cells, particularly CD4<sup>+</sup> T cells, are defined by the cytokines they produce and the transcription factors they utilize. Cytokines are multifunctional short-acting, short-range mediators of cellular activities, released by T cells and other immune and non-immune cells (see p. 399). Cytokines are distinguished from other mediators as the molecules of 'intercellular communication'. In addition to CD4<sup>+</sup> Tregs, CD4<sup>+</sup> T cells now include several subsets such as Th1, Th2, Th17, IL22-secreting CD4<sup>+</sup> T cells and it is likely that this T cell specialization will be increasingly recognized ([Fig. 7-2](#)). Subsets of Tc cells and B cells (including B regulatory cells) with defined roles in pathogenesis of disease have been described.

Further subsets of T cells occur, such as the  $\gamma\delta$ T cells (which possess a T-cell receptor (TCR) with a  $\gamma-\delta$  dimer, rather than the  $\alpha\beta$  TCR present on conventional T cells) and NKT cells which combine properties of innate immune NK cells but possess an  $\alpha\beta$  T-cell receptor.

#### B cells

B cells are mononuclear lymphoid cells that are specialized for the secretion of antibody. There are five types (isotypes) of antibody (IgG, IgA, IgM, IgD and IgE) and B cells require 'help' from T cells to function.

During a primary immune response, IgM antibody is initially produced by activated B cells. In secondary immune responses, the B cells switch to producing IgG (isotype switching), often with higher binding capacity (affinity) for the antigen, a process termed affinity maturation. During allergic immune responses, a further isotype switch occurs from IgG to IgE. IgA forms part of the mucosal immune system, being

## BOX 7-4 WHAT ARE CD NUMBERS?

The letters CD mean cluster of differentiation; this term refers to a molecule that has a defined structure, that can be recognized by a group or cluster of monoclonal antibodies, and that identifies a specific lineage or differentiation stage in the cell. CD numbers are continually being defined and the 10th Human Leukocyte Differentiation Antigen (HLDA10) workshop took place in conjunction with the Australasian Society of Immunology in December 2014. At HLDA9 there were 363 CD labelled genes/molecules.

Further details can be obtained through the following link: [www.hcdm.org](http://www.hcdm.org)

Some of the better-known CD numbers are shown below.

CD No.	Cell/molecule	Function/role
1	Thymocytes, Langerhans' cells	
3	T cells	
4	T helper cells	Adhesion
8	T cytotoxic cells	
11a	Leucocytes ( $\alpha L$ chain of LFA-1)	Adhesion
11b	Leucocytes ( $\alpha M$ chain of LFA-1)	Adhesion
11c	Leucocytes ( $\alpha X$ chain of LFA-1)	Adhesion
19	B cells	
25	Activated lymphocytes, IL-2 receptor and macrophages	
28	T cells	Co-stimulation
40	Many cells	Co-stimulation
44	T and B cells	Activated memory cells
45	Common leucocyte antigen	
45RA	Naive T cells	
45RO	Memory T cells	
56	NK cells	
62E,L,P	E, L and P selectins	Adhesion
68	Macrophages	
69	Many cells	Short-term activation
95	Many cells	Fas
120	TNF- $\alpha$ receptor	
142	Tissue factor	Coagulation
152	Cytotoxic T lymphocyte antigen-4	Immunoregulation
169	Sialoadhesin	Macrophages
183	CXCR3	Chemokine
284	Toll-like receptor 4	Myeloid cells
332	FGF receptor	growth factor
363	SIP receptor	lymphocyte homing

FGF, fibroblast growth factor receptor; LFA, leucocyte functional adhesion molecule; IL, interleukin; NK, natural killer; TNF, tumour necrosis factor; SIP, sphingosine-1-phosphate.

present in large amounts in surface secretory fluids including tears. IgA is the most abundant immunoglobulin in the immune system. IgD is present in low amounts in the circulation.

B cells recognize and bind antigen via surface immunoglobulin (sIg) which is in effect the B-cell receptor. Antigen binds to sIg in the afferent phase of the secondary immune response; in contrast, antigen binds to the secreted form of immunoglobulin (antibody) in the effector phase of the response. Secondary afferent (cognitive) interactions are antigen-specific, but effector functions are not (see below).

B-cell subsets also exist such as marginal zone B cells and B1 cells, which like  $\gamma\delta$  T cells and NKT cells lack diversity of  $\alpha\beta$  T cells.

## BLURRING THE MARGINS BETWEEN INNATE AND ADAPTIVE IMMUNITY

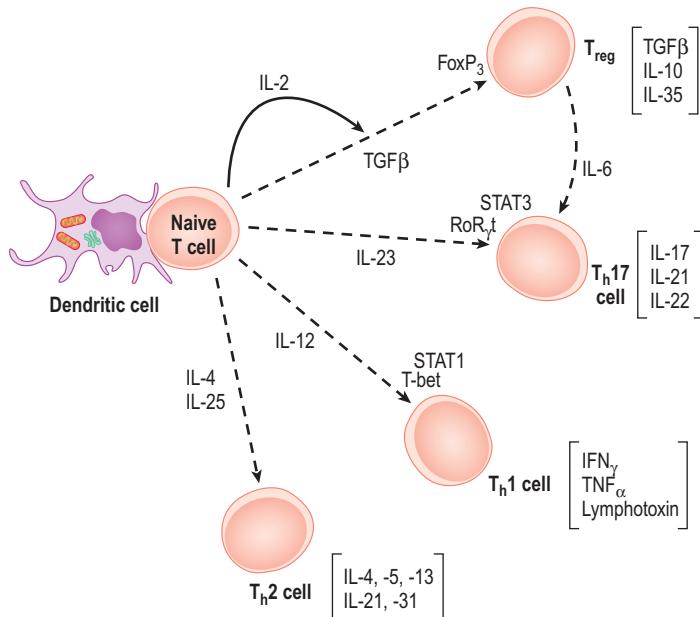
Some cells do not fit into this simple scheme separating innate from adaptive immunity. These includes natural killer (NK) cells, NKT cells and lymphoid tissue inducer (LTi) cells, all of which belong to the recently rebranded set of innate lymphoid cells (ILCs).  $\gamma\delta$ T cells and some of the subsets of B cells described above also probably belong here.

### Natural killer cells

Natural killer (NK) cells are circulating granulocytes but are part of the lymphoid system and are particularly effective against tumour cells and virus-infected cells. Previously called null cells because they lack any of the specific lymphocyte markers including a T-cell receptor, they recognize and kill virus-infected cells when activated for instance by the cytokine IL-2. Similarly, they have receptors for antibody allowing them to kill antibody-coated cells. NK cells are characterized by inhibitory receptors which bind MHC class I, thus preventing them from killing uninfected healthy cells. In contrast, NKT cells are specialized lymphoid cells that only express a single type of TCR which responds to lipid antigen presented via the molecule CD1 on antigen-presenting cells (thus they are known as invariant NKT cells).

### Lymphoid tissue inducer cells

LTis have characteristics of both NK cells and lymphocytes, but are specialized to produce lymphotoxin- $\alpha$



**FIGURE 7-2** T-cell differentiation. The dendritic cell activates naive T cells by presentation of antigen. T cells then differentiate into antigen-specific cells with differing properties depending on the cytokines they also experience in the environment where they are activated by the dendritic cell. Four main types of T cell develop: Th1, Th2, Th17 and T-regulatory cells.

and  $\beta$  (LT $\alpha$ , LT $\beta$ ) which are required for the development of lymph nodes and other secondary lymphoid tissue.

### Innate lymphoid cells

ILCs lack both  $\alpha\beta$  and  $\gamma\delta$  T-cell receptors, a feature which could include them in the myeloid system, but have lymphocyte characteristics. Thus NK cells and LTis can be regarded as one type of ILC. However, three further subsets of ILC have recently been identified which secrete cytokines normally associated with CD4 T cells, such as IFN- $\gamma$  (Th1-like), IL-4 (Th2-like) and IL-22/IL-17 (TH17-like).

## Initial response of the host to injury (the innate immune response)

The acute inflammatory response is the host's initial reaction to challenge. During this response, invasive pathogens are removed by cells of the innate immune system brought to the site of injury by changes in tissue components such as blood vessels and extracellular matrix. Meanwhile antigen from degraded microorganisms is transported to lymphoid tissues to activate the acquired immune system.

## THE ACUTE INFLAMMATORY RESPONSE

**The acute inflammatory response goes through three phases:**

- tissue damage and the acute early response
- the delayed cellular response and phagocytosis
- resolution of the inflammation and tissue remodelling.

The acute early phase has several components:

- tissue damage and release of mediators
- vascular changes
- leucocyte activation and adhesion
- leucocyte emigration.

### Tissue damage and the release of mediators

The response to tissue injury (physical, chemical or mediated by microorganisms) is immediate. Reactions occur at several levels, both locally and systemically. Immediate local reactions include the release of tissue factors and chemoattractants (chemokines, see p. 404) from damaged tissue and microorganisms. Vessels are also damaged, inducing venous stasis and the leakage of plasma components; platelet and leucocyte activation with intravascular clotting occurs; plasma/serum transudation and exudation lead to tissue fibrin

deposition and activation of serum components such as complement (see p. 405).

There are several classes of inflammatory mediators derived from both inflammatory and damaged tissue cells:

- vasoactive amines (e.g. histamine and serotonin)
- cytokines and chemokines
- lipids (e.g. prostaglandins, thromboxane and leukotrienes)
- free radicals (see Ch. 4, Box 4.4-8, p. 193)
- neuropeptides (e.g. substance P, vasoactive intestinal peptide)
- endothelium-derived mediators (endothelin, nitric oxide, prostacyclin, platelet-activating factor, etc.)
- plasma-derived mediators (e.g. complement, kinins and clotting cascade peptides)
- leucocyte-derived mediators (e.g. granule proteases, phospholipase A<sub>2</sub>)
- bacterial products (e.g. endotoxin, proteases and chemotactic factors including formylated peptides).

During the first 20 min to 48 h, there is a progressive increase in polymorphonuclear cell infiltration, initiated by innate γδT cell secretion of IL-17, which tend to accumulate around foci of injury or bacterial proliferation (a process known as 'swarming'). Degranulation of these cells leads to high tissue levels of several proteases, cytokines and cationic proteins. Neutrophils contain some of the most powerful anti-bacterial agents, including defensins, which have similarity to defensins from other species including plants and insects (Table 7-1). Defensins, and the related molecule cathelicidin (LL37), are leucocyte-derived low molecular weight mediators of non-oxidative bacterial killing and are also produced by epithelial cells such as gut and conjunctival mucosal cells. Defensins also direct antiviral activity working on both the virion and the host cell. Among the proteases released by neutrophils during acute inflammation are enzymes that degrade the extracellular matrix, which are also released by activated or injured tissue parenchymal cells and are involved in tissue remodelling. More than 24 of these zinc-dependent endopeptidases, known as matrix metalloproteinases (MMPs) (Table 7-2), have been identified in humans, and

**TABLE 7-1 Neutrophil antibacterial agents**

Class	Agent
Free radicals/gases	Hydrogen peroxide Hypochlorite Chloramine OH radical Nitric oxide
Enzyme	Proteinase 3 Collagenase Elastase Azurocidin Cathepsin G β-glucuronidase Myeloperoxidase
Peptide	Lysozyme Defensin β-lysin
Ion binders	Vasoactive intestinal peptide Lactoferrin Calprotectin

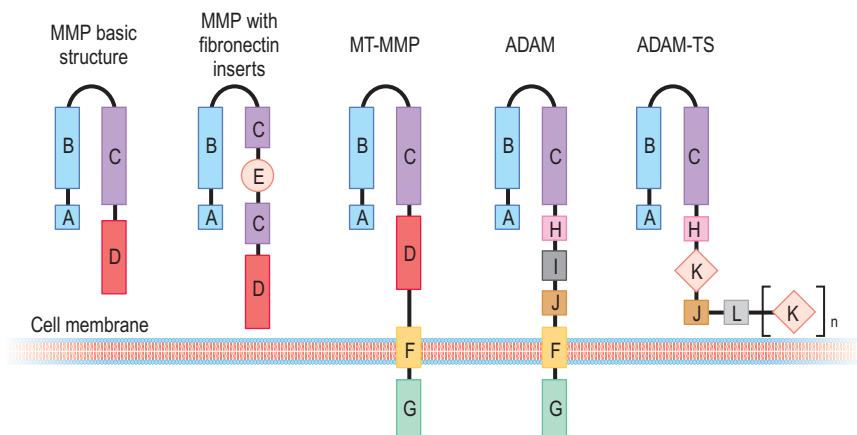
include the transmembrane proteins that contain disintegration and metalloprotease domains (ADAMTs) (Fig. 7-3). The MMPs self-activate and cross-activate each other in a cascade-like fashion, thus permitting maximal tissue degradation as required. Their effects are counteracted by naturally occurring inhibitors, termed tissue inhibitors of matrix metalloproteinases (TIMPs). There are four types of TIMP: 1, 2, 3 and 4, TIMP-4 only occurring in the mouse. MMPs are also inhibited by recognized anti-proteases such as α<sub>2</sub>-macroglobulin, tissue factor pathway inhibitor 2, and a more recently described plasma membrane inhibitor, RECK (reversion-inducing cysteine-rich protein with Kazal motifs) which appears to be downregulated in cancer cells, thus allowing them greater metastatic potential.

However, much of the cell and tissue damage is mediated by free radicals, particularly hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide anions (see Ch. 4, p. 193), which are released as part of the respiratory burst (Box 7-5). Interestingly flavonoids and adenosine, both important molecules in ocular physiology, inhibit the respiratory burst.

Free radicals may also combine with reactive nitrogen species (nitric oxide) released from inflammatory cells (Box 7-6).

TABLE 7-2 Matrix metalloproteinases (MMPs) and their substrates

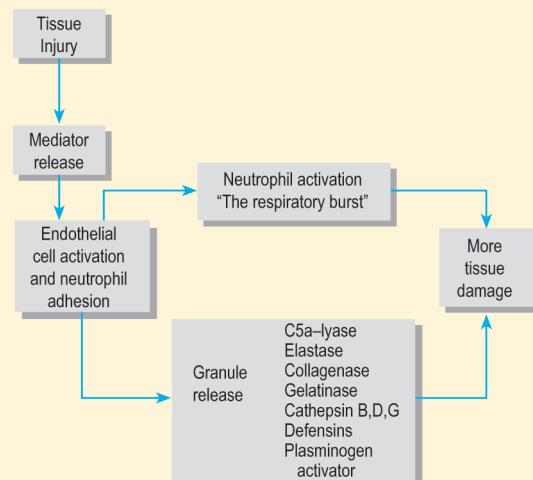
MMP	Interstitial collagens	Basement membrane	Elastin	Other proteins
<b>Collagenases</b>				
MMP-1	Types III, I, II, VII, X	Laminin Entactin		
MMP-8	Types I, III, II	Fibronectin		L-selectin
MMP-13	Types II, I, III	(±) Proteoglycan		
<b>Stromelysin</b>				
MMP-3		Fibronectin Laminin	±	EGF-like proteins
MMP-10		Entactin Proteoglycan	±	Plasminogen
<b>Stromelysin-like</b>				
MMP-7		Fibronectin	+	Plasminogen
MMP-12		Laminin Entactin Proteoglycan	++	↓ Angiostatin; $\alpha_1$ -antitrypsin
<b>Gelatinases</b>				
MMP-2	Types I, VII, X, XI	IV/V Fibronectin	++	
MMP-9		Laminin Entactin Proteoglycan	++	
<b>Membrane-type</b>				
MMP-14	Types I, III, II	Fibronectin		
MMP-15		Laminin		
MMP-16		Entactin		
MMP-17		Proteoglycan		
MMP-24				
<b>Furin-recognition site</b>				
MMP-11				



**FIGURE 7-3** Domain structure of MMPs also known as metzincin proteases. Each of the domains is shown as rectangles identified by letters, as follows: A signal peptide, B prodomain, C catalytic domain, D hemopexin-like domain, E fibronectin type II insert, F transmembrane domain, G cytoplasmic tail, H disintegrin domain, I cysteine-rich domain, J EGF-like domain, K thrombospondin type I-like repeat, L spacer region. (From Klein and Bischoff, 2011.)

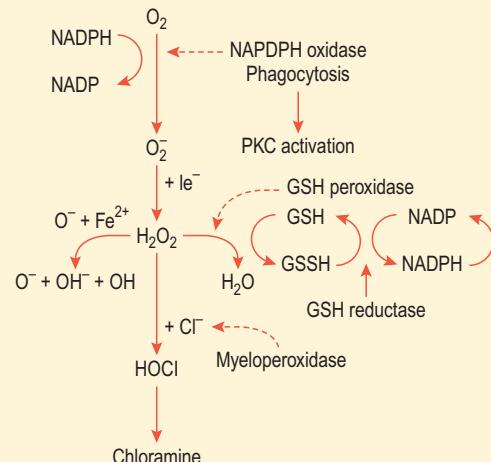
## BOX 7-5 THE NEUTROPHIL RESPIRATORY BURST

The neutrophil respiratory burst describes the activation of neutrophils and their utilization of oxygen during the inflammatory cascade. The stimulus for this response is the release of mediators from injured tissue cells. Tissue damage is caused by free radical release and tissue proteases (see also Ch. 4).



The central component of the respiratory burst is  $H_2O_2$ , which is metabolized through several pathways, some of which cause further tissue damage (e.g. superoxide and chloramine) while others reduce it to water. Tissue damage is therefore dependent on the levels of reduced glutathione

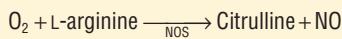
(GSH) in the milieu. In addition, during the respiratory burst, immune cells activate a  $Na^+ / H^+$  membrane exchanger protein which is required to control intracellular pH and cell volume. NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced NADP; PKC, protein kinase C.



Hypochlorous acid (HOCl) is a short-lived, highly reactive oxidant that is lipophilic and membrane permeant. It binds proteins and renders them more susceptible to proteases. Chlorinated proteins are more immunogenic and may provide a link between the innate and the acquired immune systems by acting as 'DAMPs'.

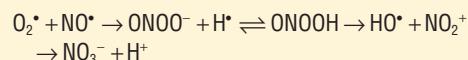
## BOX 7-6 NITRIC OXIDE

Nitric oxide was originally described as endothelium relaxing factor because it was found to be the agent released by the endothelium that was responsible for inducing autocrine vasodilatation in response to insult. Nitric oxide is a gas produced by the activity of the enzyme nitric oxide synthase (NOS) on interaction of the amino acid arginine with oxygen:



There are at least three isoforms of nitric oxide synthase, endothelium-derived (eNOS), neuronal (nNOS) and inducible (iNOS). The iNOS is released from inflammatory and other cells, particularly macrophages, and is involved in both immunoregulation and tissue damage through its interaction with superoxide radicals released from activated neutrophils. In its latter role it may also function as an antibacterial agent by damaging bacterial cell membranes. The prostanoids are co-released with NO through induction of cyclo-oxygenase-2.

NO interacts with superoxide anions to produce peroxynitrite, which is believed to be directly involved in membrane damage (see equation below). NO has direct effects on many other proteins such as the important zinc finger regions of many enzymes where it nitrosylates free cysteine SH groups with ejection of the Zn moiety from the configured protein. These damaging effects to parenchymal cells may also be the basis for T-cell apoptosis (cell death), so leading to downregulation of the immune response.



Thus NO produced constitutively in small amounts has a physiological role involving guanyl cyclase and increases in cGMP, while in large amounts it may be cytotoxic, producing depression of mitochondrial respiration, metal enzyme damage with consumption of both Zn and Fe ions and DNA damage.

## Vascular changes

The immediate cause of inflammation is release of plasma into the extravascular space and the instantaneous coagulation of proteins with activation of inflammatory mediators. Plasma release (vascular leakage) is caused by changes to the blood vessels induced by the inflammatory mediators, particularly reactive nitrogen species (RNS), especially in conditions such as the ischaemia–reperfusion damage of stroke and vascular occlusion. The immediate response of the vascular endothelium is to undergo retraction and this is associated with transient vasoconstriction. The major vascular response, however, is vasodilatation mediated initially by nitric oxide (NO). The initial vasoconstriction is mediated by several locally released compounds, particularly endothelin, which is released by pericytes and smooth muscle cells to act on the endothelium. The later vasodilatation is also mediated by locally released factors, in this case the gas nitric oxide. NO is synthesized by specific enzymes from the amino acid arginine and has widespread physiological and pathological effects, some of which are related to its role as a free radical (see Box 7-6). This is accompanied by an increased blood flow, the opening of capillary channels and the leakage of plasma into the extracellular space. This in turn leads to an increase in the tissue osmotic pressure, thus attracting further fluid build-up in the tissues (oedema). In response to this, there is an increase in lymphatic drainage from the injured site, thus reducing the tissue swelling and at the same time increasing the flow of antigenic material to the draining lymph nodes (see below). These vascular changes vary in degree with the severity of the tissue injury and protective measures are often in place such as the expression of caveolin-1 in vessel walls which mitigates the increased vascular permeability.

The vascular endothelium also undergoes significant functional and morphological changes during the inflammatory response. Whereas the normal endothelium presents a non-adhesive surface to circulating cells such as platelets and leucocytes, during inflammation the endothelium becomes much more adhesive, an effect achieved by the expression of specific adhesion molecules on its surface. There are three major classes of adhesion molecule, the selectins, the integrins and the cell adhesion molecules (CAMs),

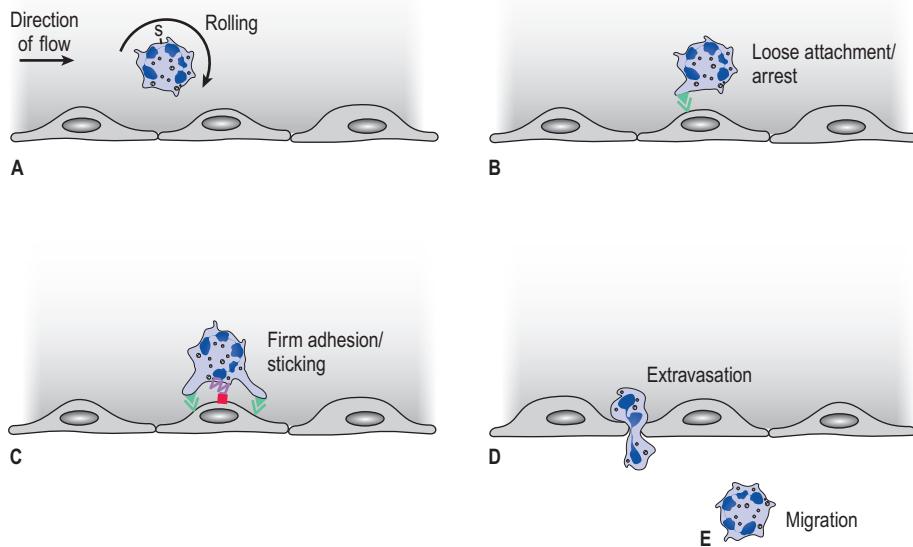
each with different functions (see below) (Fig. 7-4 and Table 7-3). In addition, the endothelium may undergo a marked morphological transformation, changing from a flat resting cell to a large protruding cell with multiple cytoplasmic organelles. These inflammation-associated vascular changes occur almost exclusively in the postcapillary venule and, in this respect, this region resembles the high endothelial venules in the lymph node, which are specialized for leucocyte adhesion (see below).

## Leucocyte activation and adhesion to the endothelium

Neutrophils are attracted to the site of inflammation through a series of discrete events that occur during margination and extravasation of the cell from the vessel. These involve rolling, loose attachment, firm adhesion and then extravasation/migration of the cell through the intercellular junction. Each of these steps is mediated by the reciprocal expression of adhesion molecules and their respective ligands on the surface of leucocytes and the endothelium (see below) (Table 7-3). During the later stages of the response (24–72 h) when other inflammatory cells are involved (monocytes and lymphocytes), similar adhesion mechanisms are involved but with different sets of molecules. Thus, the coordinated expression of adhesion molecules appears to regulate the nature of the inflammatory cell exudate.

## Adhesion of leucocytes to the endothelium thus involves a series of molecular events

- Selectin–ligand (S–L) interactions occur during the initial rolling phase of leucocyte endothelial cell interactions. These are initially low-strength interactions and are enhanced by the upregulation of selectins on the endothelium by inflammatory cytokines such as interleukin-1 (IL-1) and TNF- $\alpha$  or by contact with an activated T cell.
- Leucocyte activation by chemokines is mediated in part through upregulation of specific chemokine receptors (see later) which induces polarization and firm adhesion of the cell to the endothelium.
- Integrin–CAM interactions induce spreading of the leucocyte on the endothelial cell surface and prevent detachment of the leucocyte.



**FIGURE 7-4** Sequence of events in leucocyte adhesion: rolling (**A**), loose attachment (**B**), firm adhesion (**C**), extravasation (**D**), leading eventually to migration (**E**) of the cells within the tissue towards the site of inflammation.

- Extravasation of leucocytes through the endothelium is mediated by expression of PECAM-1 (CD31) on both the leucocyte and the endothelium, possibly through a ‘zipper’ mechanism in which disassembly of intercellular tight (occludin) and adherens junctions occurs with the expression of several junctional adhesion molecules (JAMs; see Table 7-3). CD99, expressed on both leucocytes and endothelium, is also important in transendothelial migration of monocytes.
- Migration of leucocytes through the tissues is the final stage and is induced through binding of chemokines selective for each cell type which activate signalling pathways in the translocating cell activating the motor machinery (actin-myosin cytoskeleton) to propel the cell forward up the chemotactic gradient (Fig. 7-5).

Certain molecules are specific for each type of leucocyte–endothelial cell interaction; for instance, E- and P-selectins mediate the attachment of polymorphonuclear leucocytes to endothelial cells, while vascular cell adhesion molecule (VCAM) preferentially

mediates T lymphocyte–endothelium binding. Both of these interactions have been reported in inflammatory tissue in the eye from cases of sympathetic ophthalmia, a form of autoimmune posterior uveitis.

#### Leucocyte migration into the tissues and chemotaxis

Many of the mediators released in the earliest stages of tissue injury are attractants for inflammatory cells. Both thrombin and the cleavage product fibrinopeptide B from fibrin lead to leucocyte chemotaxis from the onset of vessel leakage and fibrin formation. Prokaryotic peptides released from bacteria, such as formyl-methionine-leucine-phenylalanine, are powerful neutrophil and monocyte chemotactic agents. Activated complement components (see p. 405) have an important role as chemoattractant agents in the neutrophil/monocyte response. Other important chemoattractants include interleukin-8 (IL-8), a cytokine (chemokine) released from tissue cells including the retinal pigment epithelium (RPE), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and platelet release compounds such as platelet-activating factor (PAF), transforming

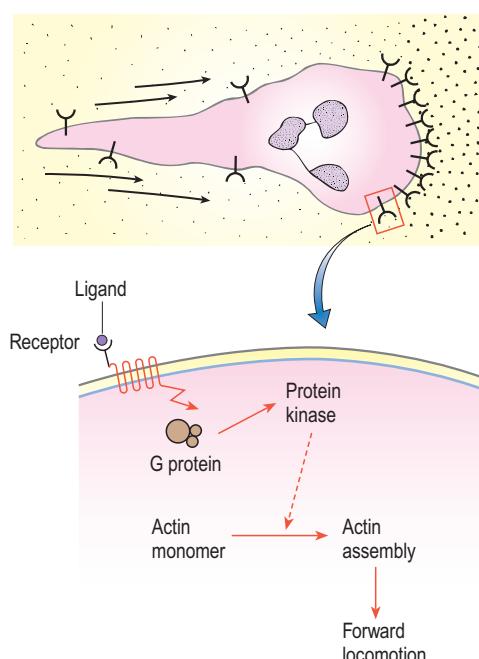
**TABLE 7-3 Endothelial adhesion molecules and their ligands on leucocytes**

	Endothelium	Leucocyte
Loose attachment	P-selectin E-selectin	PSGL-1, PTX3 PSGL-1, ESL, CD44
Slow rolling	PSGL, GlyCAM ICAM-1 E-selectin	L-selectin LFA-1/PSGL-1 PSGL-1, ESL, CD44
Arrest/firm adhesion	ICAM-1	LFA-1
Crawling	VCAM-1 Hyaluronan	VLA-4
Extravasation	ICAM-1 ICAM-1, ICAM-2 VCAM-1 CD99 PECAM-1 JAM-A, -B, -C CD99L2	CD44 MAC-1 LFA-1, MAC-1 VLA-4 CD99 PECAM-1 LFA-1, VLA-4, MAC-1 ?

The cell behavioural sequence of loose attachment, slow rolling, arrest and firm adhesion, crawling along the inner vascular wall, and finally extravasating into the tissues is utilized by neutrophils which preferentially if not exclusively leave from the postcapillary venules.

? indicates unknown or opposing data. CD99L2, CD99 antigen-like protein 2; ESL1, E-selectin ligand 1 (also known as GLG1); GlyCAM, glycosylation-dependent cell adhesion molecule; ICAM, intercellular adhesion molecule; JAM, junctional adhesion molecule; LFA-1, lymphocyte function-associated antigen 1; PECAM-1, platelet/endothelial cell adhesion molecule 1; PSGL-1, P-selectin glycoprotein ligand 1; VCAM-1, vascular cell adhesion protein 1; VE-cadherin, vascular endothelial cadherin; VLA-4, very late antigen 4.

growth factor- $\beta$  (TGF- $\beta$ ), platelet-derived growth factor, platelet-derived endothelial cell growth factor, and many others. Lipid mediators, such as the leukotrienes, are also important neutrophil chemoattractants, while certain cytokines, such as monocyte chemotactic protein (MCP) and the macrophage inflammatory proteins (MIP- $\alpha$ , MIP- $\beta$  and the chemokines; see below) are selective for mononuclear cell chemotaxis. Cytokines that induce inflammatory cell migration are known as chemokines (inducing movement through chemotaxis) and receptors for different



**FIGURE 7-5** Neutrophil migration up a chemical gradient. **(A)** Cells sense the concentration gradient and polarize towards it with a wide leading edge and a trailing tail. Receptors for the chemoattractant cluster in the membrane of the leading edge, increasing the signal at this end of the cell. **(B)** Diagram of the seven-coil transmembrane receptor (the same basic structure as rhodopsin) that mediates the intracellular signal via Gprotein links. This activates a protein, tyrosine kinase, to initiate actin assembly and induce forward movement of the cell.

chemokines are present on different cells, thus regulating not only the numbers of cell that migrate into the tissue but also the type of cells and thus the quality of the inflammatory exudate (see p. 375).

Cells such as neutrophils and monocytes ‘sense’ a chemical gradient of these attractants and migrate up the gradient by using specific cell surface receptors clustered preferentially towards the leading, polarized edge of the cell (Fig. 7-5). These receptors (e.g. the C5a receptor) are composed of seven transmembrane segments (in a manner similar to the transmembrane spanning segments of rhodopsin; see Ch. 4, p. 261.e1) that possess a cytoplasmic connection to a Gprotein-linked second messenger system. This activates the intracellular machinery (actin–myosin motor) required for forward movement. Recent studies have also shown

that activation of G protein receptors occurs in waves, thus enhancing the overall effect of the gradient.

### Phagocytosis and removal of damaged tissue and microorganisms

Recovery from inflammation requires the removal of dead microorganisms and necrotic tissue by phagocytic cells (polymorphonuclear leucocytes and macrophages). Even in the absence of microorganisms, altered (damaged) self-proteins are recognized by cells of the innate immune system and phagocytosed. In the eye this is classically seen with lens-induced uveitis, in which denatured lens crystallin proteins are released into the anterior chamber in cases of traumatic and hypermature cataract. In the latter circumstance, engorged macrophages may block the outflow channels and produce a 'phacolytic glaucoma' (see Ch. 9, p. 512).

Phagocytosis, particularly of bacteria, is facilitated by certain molecules of the innate immune system known as opsonins, which are present in plasma and bind to the surface of microorganisms when they are released into the extracellular space. One of the complement components, C3b (see below), acts as an opsonin. Interestingly, different actin cytoskeletal structures are constructed to phagocytose different types of particle, e.g. IgG-coated versus complement-coated particles. In addition, the role of microbial pathogen receptors such as Toll-like receptors (see next section) on maturation of the phagosome is unclear but appears not to play a significant part.

Molecules of the acquired immune system also promote phagocytosis, particularly antibodies which bind avidly to the foreign antigen by specific interaction of their antigen-binding site (the Fab portion of the molecule) but are non-specifically removed by binding of their Fc portion to the phagocyte cell surface (see section on [antibodies](#) below, p. 396). Cells that express high levels of surface receptors for C3b and Fc are termed 'professional phagocytes', and include neutrophils and macrophages. Studies in genetically targeted knockout mice suggest that the Fc pathway for phagocytic activation is the major one.

### Activation of innate immune cells by microorganisms

Invading microorganisms release factors that attract leucocytes to the site of inflammation and also induce

those same leucocytes to engage in attempted removal of the offending agent. This requires activation of the immune cells. How is this activation of the innate immune response achieved? As indicated in the introduction, this was previously considered to occur by non-specific pathogen–host cell interaction, i.e. there was one general innate immune mechanism whereby the body reacted to many different types of microorganisms. However, it has long been recognized that responses to different organisms vary greatly, some being virulent or lethal while others are harmless, and this is partly due to how different classes of microorganisms activate the initial innate immune cells they encounter. This they do via broadly specific ligands (PAMPs) which bind to pathogen-recognition receptors (PRRs) (see p. 391). There are several classes of both membrane and soluble PRRs, including the Toll-like receptors (TLR) ([Table 7-4](#)), carbohydrate-binding (C)-type lectins, retinoic acid-inducible gene-1 (RIG-1) helicases, nucleotide-binding oligomerization domain protein (NOD)-like receptors (NLRs), the scavenger receptors and some soluble PAMPs such as the collectins (including complement proteins) and acute phase proteins. The components (PAMPs, DAMPs) recognized by the PRRs include pathogen cell wall material, bacterial DNA and proteins, viral DNA and RNA, and lipoproteins from microbes as well as endogenous ligands such as self-DNA (if it is in the wrong place, such as the cytoplasm) and particulate material such as uric acid and cholesterol crystals.

Lipopolysaccharide is a classic microbial product that complexes with a serum protein lipopolysaccharide-binding protein and binds to the microbial co-receptor CD14. This then complexes with TLR4 and initiates the signalling cascade (most involving the adapter protein myeloid differentiation factor 88 (Myd88) via several intermediaries transmitted on a nuclear factor- $\kappa$ B (NF- $\kappa$ B) core to activate the macrophage or dendritic cell ([Fig. 7-6](#)) and subsequently primes T cells through presentation of processed antigen in the presence of the necessary cytokines such as IL-12, IL-23 and IL-17.

TLRs are not so much involved in the phagocytosis and clearance of microorganisms (see section above) as in the maturation of antigen-presenting cells for induction of the adaptive immune response through T-cell activation; for this, different TLRs

**TABLE 7-4 Toll-like receptors – mediators of inflammation with relative selectivity for different classes of microorganisms**

TLR	Localization	Pathogen-derived agonists	Endogenous agonists	Synthetic agonists
TLR1 and TLR2	Extracellular	Bacteria: peptidoglycan, lipoproteins, LTA Fungi: zymosan	–	Pam <sub>3</sub> Cys
TLR2 and TLR6	Extracellular	Bacteria: lipoproteins	Veriscan	MALP2
TLR3	Intracellular	Viruses: dsRNA	mRNA	PolyI:C
TLR4	Extracellular	Bacteria: LPS Viruses: RSV fusion protein Fungi: mannan Protozoa: glycoinositolphospholipids	Saturated fatty acids, β-defensins, oxLDL*, amyloid-β*	Lipid A derivatives
TLR5	Extracellular	Bacteria: flagellin	–	–
TLR7 and TLR8	Intracellular	Viruses: ssRNA	Self-RNA	Imiquimod, R-848
TLR9	Intracellular	Bacteria: CpG DNA Viruses: CpG DNA Protozoa: CpG DNA, haemozoin	Self-DNA	CpG-ODNs
TLR11	Extracellular	Uropathogenic bacteria Protozoa: profilin-like molecule	–	–

CpG-ODNs, CpG-containing oligodeoxynucleotides; dsRNA, double-stranded RNA; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MALP2, mycoplasma macrophage-activating lipopeptide 2; oxLDL, oxidized low-density lipoprotein; polyI:C, polyinosinic-polycytidylic acid; RSV, respiratory syncytial virus; ssRNA, single-stranded RNA. \*Amyloid-β and oxLDL bind to CD36 and a TLR4-TLR6 heterodimer. (From Mills, 2011.)

activate different types of dendritic cell (see eFig. 7-2).

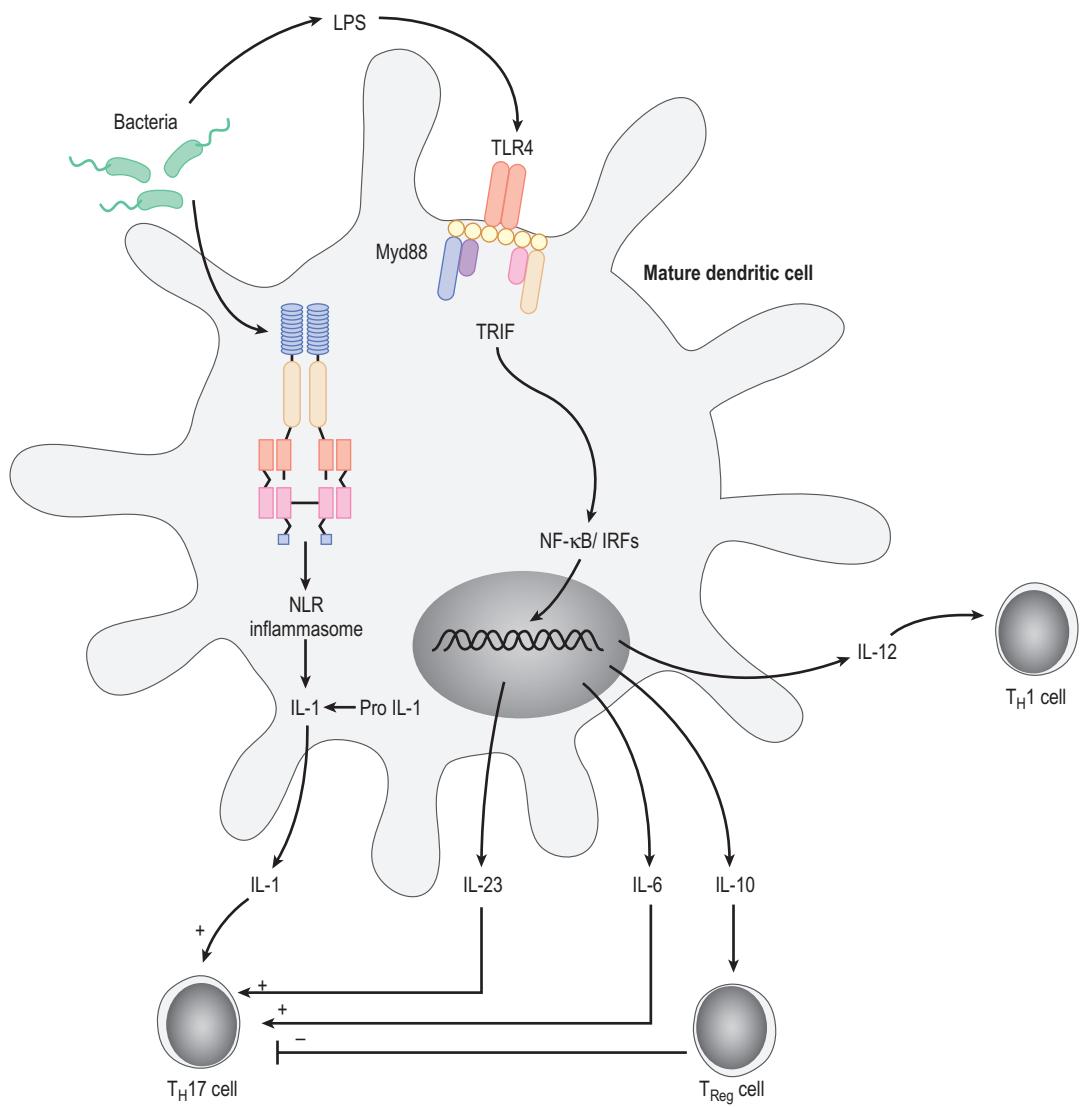
In contrast, macrophages also express other PRRs, including scavenging receptors, which are primarily involved in phagocytosis. Two main classes of scavenging receptors occur in humans: SR-A, which binds molecules on ageing damaged (e.g. oxidized) cells, such as oxidized low-density lipoprotein, as well as some microorganisms; and SR-B, which occurs on other cells as well as macrophages and binds many different types of microorganisms through polyanionic interactions. TLRs are, for the most part, cell surface receptors, although some, such as TLR3 and TLR9, bind intracellular molecules such as viral genomic messenger RNA and nucleotide degradation products such as CpG molecules. In addition, a further set of molecules deals with intracellular pathogen products, the NOD 1 and NOD 2 proteins (nucleotide-binding oligomerization domain family), which like the TLRs have a leucine-rich repeat domain. These proteins are also known as CARD (caspase recruitment domain) proteins and bind unique peptidoglycan bacterial molecules. They are also involved in autoimmunity, especially the inflammatory bowel disease Crohn's disease, which is linked to certain types of ocular

inflammation. Activation of an ‘inflammasome’ complex (see Fig. 7-6) and caspase-1 leads to production of IL-1 and IL-18, which drive the inflammatory process. Interestingly, these molecules have recently been implicated in the pathogenesis of age-related macular degeneration.

### NK, NKT and γδT cells also express PRRs and mediate innate immunity

NK cells recognize self and non-self ligands on virus-infected cells, tumour cells and host cells that have increased levels of stress proteins. MHC class I and MHC class I-like molecules (see below) are involved in these interactions. Using receptors such as Ly49H, natural cytotoxicity receptors and a further receptor termed NKG2D, NK cells recognize a range of virus-infected cells such as cytomegalovirus, myxoviruses and influenza viruses. NKG2D is a receptor for tumour proteins and the MHC class I molecules that they recognize in combination are known as MICA and MICB.

NKT cells, in contrast, express the T-cell receptor (see p. 429) but unlike normal T cells they only express



**FIGURE 7-6** Toll-like receptors (TLRs) act on myeloid and other cells by activation of transcription factors but they do not do this directly. Instead they do so through linker proteins (adaptors), which fit them to their particular pathway. All TLRs except TLR3 use the adaptor Myd88 while other TLRs use more than one adaptor, such as TRIF, TIRAP and TRAF. These generate cytokines through two main pathways, one involving NF $\kappa$ B, which leads to transcription of many pro-inflammatory cytokines and the other IRF3, which predominantly leads to production of INF- $\alpha$ . Both signalling pathways also lead to inflammasome activation with production of the central pro-inflammatory cytokines IL-1 $\beta$  and IL-18. Ultimately, these molecules assist in the activation of T cells, during antigen presentation. (From Mills, 2011.)

one form of the receptor and use this to bind glycolipids on a range of organisms such as *Leishmania donovani*, Gram-negative glycosyl ceramides and similar molecules on organisms such as *Plasmodium* and *Trypanosoma*. Two important ocular microorganisms,

*Pseudomonas* and *Staphylococcus*, may also be detected by NKT cells. NKT cells use a non-classical MHC class I molecule, CD1d, to mediate these interactions. The cytolytic function of NK and NKT cells occurs via the release of granule contents such as perforin, granzyme

and other proteoglycans (see later under cytotoxic T cells).

$\gamma\delta$ T cells colonize the skin and gut epithelium and recognize small alkylamines and pyrophospho-monoesters on microbes and tumour cells. The latter are a major component of mycobacteria. In addition, they recognize heat-shock proteins and have been implicated in inflammatory diseases such as Behçet's disease and the ocular inflammation associated with it.

### Effector cells in the inflammation response

Much of the tissue damage in the early stages of the inflammatory response is caused by release of tissue-degrading enzymes such as MMPs (see Table 7-2) from professional phagocytes and as such they are considered to be important as non-specific effector cells in the inflammatory response. Activation of complement during the early phase of the response also provides the materials for non-antibody-dependent complement-mediated lysis of tissue cells, via the membrane attack complex (MAC) of complement and other cells such as NK cells. Macrophages, recruited by T-cell cytokines, also play a major role as effector cells in the adaptive immune response, in addition to antigen-specific cytolytic roles of T cytotoxic (Tc) cells and NK cells.

Lymphocytes also play a significant part in the initial acute inflammatory response. Even in 'sterile wounds', in which defence against microorganisms does not play a major part, lymphocytes participate in the overall response. Lymphocytes enter into the site of inflammation at the same time as the monocytes, and act as bystanders ready for activation by APCs primed with antigen. This may be from degraded organisms or denatured tissue proteins ('altered self'). In most cases, no detectable adaptive immune response occurs locally unless there is activation of tissue-resident memory T cells (see p. 420), specific for a previous pathogen or antigen.

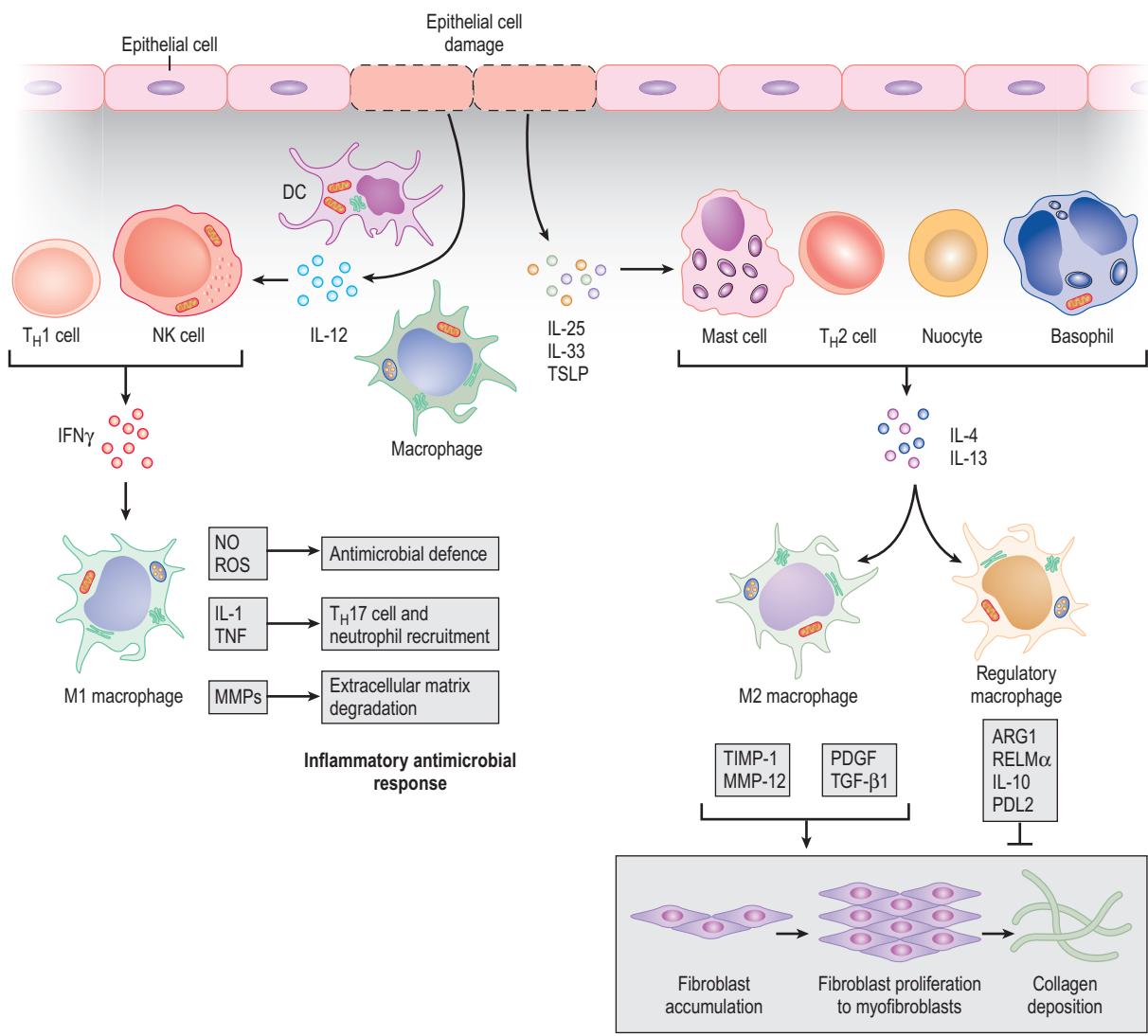
### RESOLUTION OF INFLAMMATION

Resolution of inflammation occurs when the foreign antigens have been completely destroyed and removed ('cleared'), and tissue architecture is restored. Macrophages are critically important in the resolution phase of an inflammatory reaction. Effector pro-inflammatory

M1 macrophages (see p. 377 and eFigs 7-1 and 7-2) which are involved in clearance of microorganisms and in the process generate considerable collateral damage ('friendly fire') are replaced by alternatively activated macrophages which promote healing (M2 macrophages) either by *de novo* recruitment or by re-programming of inflammatory macrophages. Such cells express high levels of arginase-1, characteristic of inhibitory immune cells, such as myeloid suppressor cells (MSCs) with high potency in inhibiting effector T cells.

Resolution of inflammation involves fibrosis, which is also mediated via alternatively activated M2 macrophages and regulatory macrophages through mediators derived from other cells in the inflammatory milieu (Fig. 7-7). In addition, new vessel formation (angiogenesis), restoration of epithelial surfaces by cell migration and proliferation (see Ch. 4, p. 211, cornea), and remodelling of the extracellular matrix by initial deposition of sulphated glycosaminoglycans and hyaluronan deposition, induced by fibroblasts, represent stages in a precisely orchestrated wound-healing process. For instance, angiogenesis is initiated in quiescent endothelial cells by the expression of protease activity on the cell surface, and release of growth factors from surrounding inflammatory cells such as fibroblast growth factor, platelet-derived growth factor platelet-derived endothelial cell growth factor and vascular endothelial growth factor-A (VEGF-A). VEGF is a major player in the overall wound-healing response: it initiates vascular leakage at the onset of inflammation and promotes angiogenesis in the later stages. The VEGF-C isoform of VEGF activates a specific endothelial cell receptor (VEGF-R3) for induction of lymphangiogenesis, essential for transport of soluble and cell-associated antigen to the draining lymph node.

VEGF induction is itself under the control of hypoxia-inducible factor (HIF1 $\alpha$  and  $\beta$ ), a transcription factor generated by cells in hypoxic tissue, as occurs in a wound, but is also produced by both M1 and M2 macrophages in the absence of hypoxia. However, VEGF-A attracts 'immunosuppressive' cells such as CCR2 $^+$  M2 macrophages which secrete the cytokine CCL2, MSCs, and Tregs and Bregs, all of which promote further angiogenesis. Thus wound healing, acute inflammation, innate immunity and adaptive immunity are all part of a coordinated



**FIGURE 7-7** The initial stages the inflammatory response involves many early mediators including clotting factors, fibrinolytic products, cytokines such as IFN- $\gamma$  which programme macrophages towards a pro-inflammatory phenotype (M1). Th2-type cytokines (IL-4, IL-13), released from other cells such as mast cells, generate alternatively activated macrophages as well as regulatory macrophages which promote healing (fibroblasts and collagen deposition) as well as new vessel formation (From Murray and Wynn, 2011.)

response by the organism to remove the offending microorganism and restore tissue homeostasis.

### CHRONIC INFLAMMATION

When the foreign antigen is not completely removed, the inflammatory response enters a chronic state characterized by mononuclear inflammatory cells such as

monocytes and lymphocytes, often arranged in granulomas (see Ch. 9, p. 502). Failure to remove the foreign antigen may occur because of an inadequate initial response or because the antigen effectively evades the immune system. Intracellular bacteria that evoke phagocytic destruction are a prominent stimulus for induction of chronic inflammation. Persistence

of innate immune cells such as neutrophils is a feature of many chronic inflammations and signals a failure to clear the foreign antigen.

However, even if the organisms are cleared, chronic inflammation can ensue in the absence of active processes which lead to the production of specialized pro-resolving mediators (SPMs) such as omega-3 fatty acid-derived lipoxins (see Ch. 4, p. 197), and other more recently described molecules such as resolvins, protectins and maresins. These mediators inhibit leucocyte migrations and suppress effector innate and adaptive immune cells.

In chronic inflammatory disorders, the acquired immune response also participates, but it too appears to be insufficient to remove the foreign antigen. This may be because the antigen has 'fooled' the immune system by parasitizing the inflammatory cells, as in parasitic disease including the worldwide blinding disease *Chlamydia trachomatis* (see Ch. 8). In autoimmune disease (see p. 444), 'altered self' antigen is continually present, acting as a danger signal, and induces persistent inflammation. In certain circumstances a low-grade lymphocytic activation occurs. Such lymphocytes may release cytokines which induce fibroblast activity, such as TGF- $\beta$  and connective tissue-activating peptides (CTAP-1 to CTAP-6). CTAPs are low molecular weight compounds released from leucocyte and platelet granules during inflammation and which themselves undergo partial degradation to produce other pro-inflammatory peptides such as neutrophil-activating peptides (NAP-1 and -2), thereby sustaining the inflammatory response. If this response is excessive, subepithelial fibrosis may occur and produce conditions such as benign mucous membrane pemphigoid and subretinal fibrosis, both extremely debilitating and blinding diseases. CTAPs have been implicated in the fibrosis of Graves' ophthalmopathy through activation of the insulin-like growth factor receptor on orbital fibroblasts.

In many chronic inflammations the balance of competing mediators and cells may decide the outcome of disease. For instance in hepatitis caused by HepB or HepC virus, IL-22 produced by Th17 cells or by iLCs, or even by specialized IL-22-producing T cells, can have either a protective role promoting fibrosis or a pro-inflammatory role leading to liver failure. Thus in chronic inflammation the distinction between innate

and adaptive immune responses becomes blurred and such disorders manifest as a mixture of low-grade inflammatory activity with partial attempts at healing (fibrosis). This is well demonstrated in the subretinal neovascular membranes of chronic posterior uveitis.

### THE SYSTEMIC RESPONSE TO ACUTE INFLAMMATION: THE ACUTE-PHASE REACTION

Although the acute inflammatory response is initiated at the site of tissue injury, systemic effects are produced in proportion to the level of tissue damage and virulence of the organisms. These effects are mediated primarily by cytokines acting in this situation at a distance, and are known as the acute-phase response. These cytokines include the 'alarm' cytokines, IL-1 and TNF- $\alpha$ , released mainly through 'danger' signals, from macrophages activated by mast cell and platelet degranulation and/or directly by bacterial products such as endotoxin, peptidoglycan and degraded nucleotides through their PRRs (see above). During the acute-phase response, adhesion molecule expression on vascular endothelium is induced and initiates further rounds of inflammatory cell accumulation and cytokine release. In addition, changes in vascular tone are caused by release of low molecular weight metabolic products including the prostaglandins PGI<sub>2</sub>, PGE<sub>2</sub>, PGD<sub>2</sub> and PGF<sub>2 $\alpha$</sub>  (vasodilatation), thromboxane A<sub>2</sub> (vasoconstriction), and leukotrienes C<sub>4</sub>, D<sub>4</sub> and E<sub>4</sub> (smooth muscle contraction).

The effect of massive systemic cytokine release (such as occurs in the 'cytokine storm' of acute sepsis) is to induce a fever response by the direct action of IL-1 and IL-6 on the hypothalamic temperature control system, and, second, to induce hepatocyte gene transcription for several 'acute-phase reactants' such as C-reactive protein, serum amyloid components A and P,  $\alpha_1$ -glycoprotein, C3 and collectins (see section on complement below) as well as mannan-binding proteins, and haptoglobin. Fibrinogen,  $\alpha_2$ -macroglobulin and  $\alpha_1$ -antitrypsin are also synthesized.

While many plasma proteins rise in concentration, others such as albumin and transferrin fall. Clinically, the acute-phase response manifests as an elevated erythrocyte sedimentation rate (ESR) caused by more rapid settling of IL-6-mediated, fibrinogen-mediated rouleaux formation of red blood cells.

Many of the acute-phase proteins enhance existing innate defence mechanisms such as C-reactive protein, which acts as an opsonin and binds complement. Others act to inhibit the effect of inflammatory cytokines such as serum amyloid protein and IL-1. The acute-phase reaction has been described as innate immune 'brinkmanship' in that both the pathogen and the host are stressed but the overall outcome aims to limit systemic sepsis while avoiding disseminated intravascular coagulation (DIC), shock, organ failure and death.

## Development of adaptive immunity and immunological memory

Normally, initiation of the adaptive immune response does not take place at the site of injury or penetration by foreign organisms. Instead, antigen is taken up by APCs at the site of inflammation and transported to the regional lymph nodes and/or spleen where it is presented to T and B cells. T and B cells specific for that antigen respond by undergoing a series of activation steps including cytokine production and proliferation (clonal expansion). Such T cells are known as effector T cells which pass into the blood circulation and migrate ('home') back to the site of injury where CD8 T cells (Tc cells) kill infected cells, and CD4 T cells (Th cells) attract other pro-inflammatory phagocytic cells such as activated M1 macrophages. In the lymph node, antigen is also presented to B cells, which differentiate to become antibody-producing plasma cells, some of which migrate to the bone marrow but most of which remain in the draining lymph node follicles and germinal centres, producing high levels of specific antibody, which is released into the circulation (see below).

### ANTIGEN RECOGNITION IS MADE POSSIBLE BY ANTIGEN-PRESENTING CELLS

Foreign antigen is presented to T cells by three types of 'professional' antigen-presenting cell: macrophages, B cells and dendritic cells (DCs). However, antigen is recognized by specific T cells only after it has been processed and made presentable in an appropriate form to the T cell. Some antigens are recognized by T cells without processing, but they are very unusual. Also antigen can be presented by 'non-professional' APCs

such as endothelium but the T cells may not become activated (see p. 441, discussion on tolerance).

There are differences in the type of antigen that each of the three cells can present. Macrophages and B cells usually recognize antigen through the immunoglobulin (Ig) molecule. Thus, macrophages and B cells can initiate an immune response only if the host has already been exposed to that antigen and has the capacity to mount a 'memory' response in the form of IgG. In contrast, DCs can process and present antigen to resting, naive T cells, i.e. cells that have not previously 'seen' the antigen. Accordingly, DCs are considered to be the cells which initiate immune responses to new antigens, while macrophages and B cells may be important in sustaining the response while antigen persists in the tissue.

From the earliest stages of an inflammatory response, DCs at the site of injury (generated from extravasated, circulating monocytes see p. 377) start to migrate in large numbers from the subepithelial layers into afferent lymphatics to the regional lymph nodes. During this phase, they prepare the antigen by combining it with MHC class I and II molecules so that it can be presented as a complex to T cells. T cells will respond only if they possess the specific receptor (the T-cell receptor, TCR) for that antigen and if the antigen is sufficiently immunogenic, i.e. has the capacity to activate innate immune cells via PRRs (see p. 389). In the normal course of events it is likely that the great majority of processed antigens never get as far as initiating a perceptible T-cell response.

### T CELLS RESPOND TO ANTIGEN BY CLONAL EXPANSION

If the antigen is presented to the specific T cell that recognizes it, in a suitable form and in the presence of the correct co-stimulatory signals, the T cell responds by clonally proliferating, i.e. it rapidly divides, producing many daughter cells, which are all exactly the same in their recognition of that antigen alone. This is a very dramatic response and accounts for the enlarged lymph nodes seen for instance after a viral infection. It has been estimated that up to 20% of lymphocytes in an enlarged lymph node are specific for that virus.

Some of the expanded T cells (Th cells) migrate to the B-cell follicles in the lymph nodes (see p. 414) and

release a range of cytokines that 'help' B cells to clonally expand, also in an antigen-specific manner. However, the majority of activated T cells enter the circulation and home to the site of injury where they assist in mounting the antigen-specific effector response that will eliminate the foreign antigen.

## T AND B CELLS PARTICIPATE IN THE EFFECTOR RESPONSE

Effector responses are those that actually mediate the immune response. Activated Th cells release cytokines that activate other cells in addition to B cells. These include:

- cytotoxic T cells that recognize intracellular foreign antigen when it is presented on the surface of tissue cells complexed with MHC class I antigen (see below)
- macrophages that, when activated, remove foreign antigen and perpetuate the immune response by engaging in local antigen presentation at the site of inflammation (if this goes awry, conditions such as lepromatous leprosy and sarcoidosis can develop)
- B cells that are stimulated to full differentiation as plasma cells with considerable local antibody production (see below); soluble antibody is then available to form immune complexes and participate in further local antigen presentation and antibody-mediated cytotoxic reactions via NK cells (see below).

## HOW DOES THE ORGANISM DEAL WITH INTRACELLULAR ANTIGEN?

In the above scenario, we considered how adaptive immunity is triggered when the foreign antigen is extracellular and is a target for phagocytosis. However, what happens if the antigen has already invaded and infected cells, and is persisting as a viable organism intracellularly? This particularly applies to organisms such as viruses and protozoa which infect many cell types and even bacteria such as mycobacteria which evade the immune system by 'hiding' inside cells. Such cells may be killed by NK cells, or by sensitized Tc cells recognizing viral peptide on MHC class I surface antigen as part of a memory response. The apoptotic and dying infected cells containing virus or other microorganisms are then 'cleared' (phagocytosed) by

macrophages and DCs at the site of injury which, after trafficking to the draining lymph node, further activate antigen-specific T cells through presentation of viral antigen (peptide) in conjunction with 'self' MHC class I. Thus Tc can be directly activated on appropriately conditioned DCs without obligatory help from Th cells.

## Effecter mechanisms

The innate immune system has a range of effector cells that remove damaged tissue and dead microorganisms (DCs, macrophages, NK cells,  $\gamma\delta$  T cells). The adaptive immune system also has a variety of effector mechanisms, which it uses to rid the host of specific foreign antigen. These include antibodies and cells, but the adaptive immune system also utilizes non-specific mechanisms that are activated by antigen-specific cells and molecules, including complement and cytokines.

## ANTIBODIES

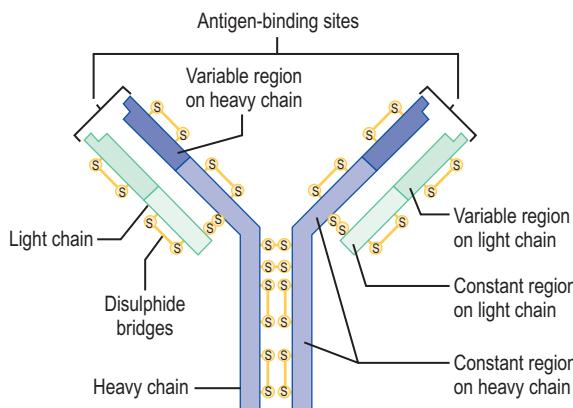
Antibodies are distributed in the endoplasmic reticulum, the Golgi apparatus and the surface of B cells, monocytes, mast cells and NK cells, and in secretory fluids. Each antibody binds uniquely to a single antigen, which is usually a short sequence of an immunogenic molecule and is normally defined as an antigenic epitope. Most antigens are proteins, but carbohydrates and lipid antigens also occur.

### All five antibody isotypes have similar basic structure

Antibodies are Y-shaped molecules composed of light and heavy chains (Fig. 7-8). Two identical heavy (H) chains linked by disulphide bridges to two identical light (L) chains, either a  $\kappa$  chain or a  $\lambda$  chain, form the basic structure of antibodies. Each chain is composed of a series of repeating homologous units, about 110 amino acids in length, comprising discrete immunoglobulin domains. Many other molecules adopt a similar folded structure and are classed together in the immunoglobulin superfamily. Differences exist in the precise geometry of the molecules, as shown by crystallography, which has significance for antigen binding. There are five immunoglobulin isotypes (Box 7-7).

### Special features of H and L chains

Although not directly involved in antigen binding, the framework region determines the folding of the



**FIGURE 7-8** Cartoon of generic immunoglobulin structure showing the light chains, heavy chains and the variable and constant regions with the linking disulphide bridges. (Figure adapted from <http://www.emc.maricopa.edu/faculty/farabee/biobk/BioBookIMMUN.html>. Image used taken from Purves et al., *Life: The Science of Biology*, 4th Edition, by Sinauer Associates [www.sinauer.com](http://www.sinauer.com)) and WH Freeman ([www.whfreeman.com](http://www.whfreeman.com)), used with permission.)

molecules and thus the amount of complementarity-determining region (CDR) that is presented on the surface of the variable sections of the molecule for interaction with antigen (the antibody-binding site) (Fig. 7-8).

The secretory forms of IgM, IgA and IgD have C-terminal extensions (tail pieces) which allow multimer formation by attachment to the J chain, while N-linked oligosaccharides bound via asparagine residues contribute greatly to the differences in the overall conformation. Complement binds to the constant regions of the immunoglobulin molecules.

Limited proteolysis of antibody produces fragments: papain attacks the hinge region, producing single Fab fragments which bind one antigen molecule; pepsin attacks the second CH segment, producing (Fab)<sub>2</sub> fragments which can bind two antigen molecules (eFig. 7-3).

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Immunoglobulin isotypes not only vary on the basis of the number of immunoglobulin chains which make the definitive molecule but also the ends of the heavy chains show considerable and conserved amino acid variations. Similarly, the light chains exist in two isotypes,  $\kappa$  and  $\lambda$ , which vary in proportion (60:40 in humans, 95:5 in mouse). In addition,

membrane-bound immunoglobulin (mIg) is known as the B-cell receptor and contains a transmembrane region and an intracellular cytoplasmic domain.

Antibodies produced in response to the initial encounter with antigen are of the IgM or IgD isotype, while later antibody production (particularly that in response to rechallenge by the same antigen) is of the IgG, IgA or IgE isotypes. This is known as *isotype switching* and is regulated by the enzyme cytidine deaminase, activated through CD40L–CD40 interactions.

### Antigen–antibody binding

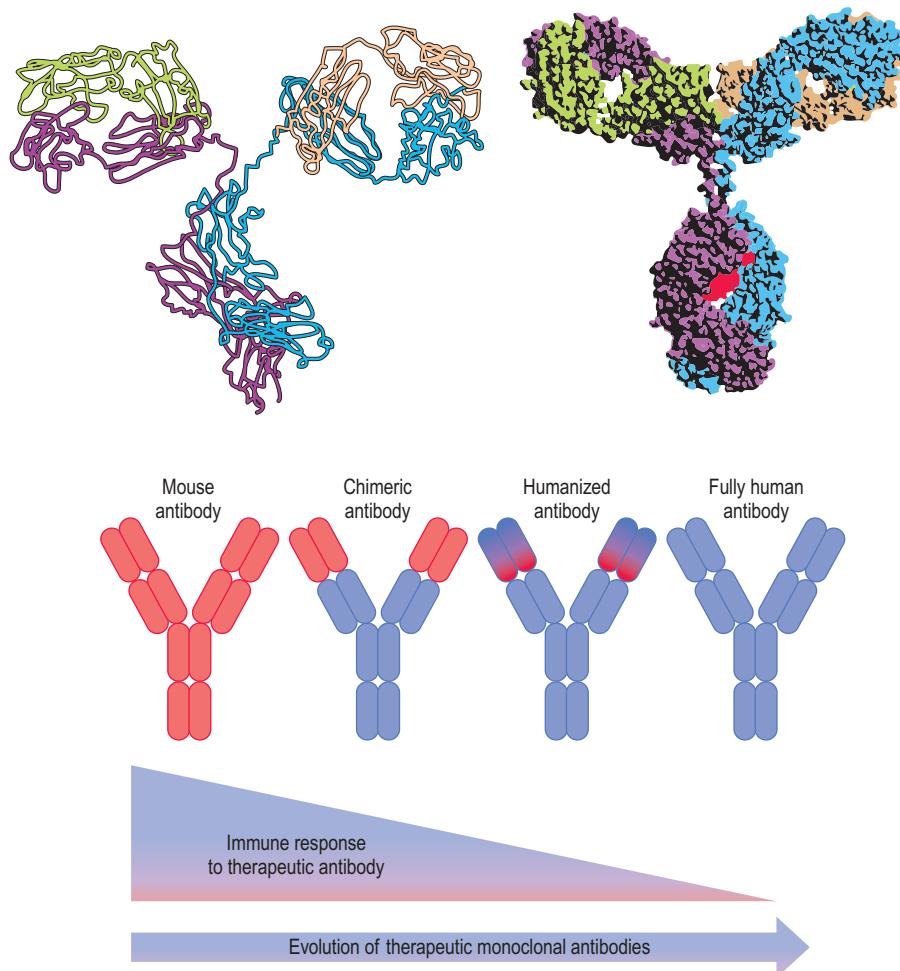
The specific binding site on the antigen is known as the epitope (see p. 421). Antibody binding to carbohydrate or lipid antigens is strictly dependent on the structure of the antigen. Binding to protein antigens may, however, depend on either the linear sequence of amino acids or the three-dimensional conformation of the molecule. This can lead to the appearance of cryptic epitopes or neoepitopes in protein molecules, which are uncovered after changes to the three-dimensional conformation by partial hydrolysis.

Epitopes on protein antigens can be overlapping or non-overlapping. Overlapping epitopes lead to competition for binding; in such circumstances antibodies to an overlapping region might sterically inhibit presentation of a peptide to T cells. This, however, would occur only with MHC class II-peptide complexes where the binding site for the TCR embedded in the MHC groove overlaps with a binding site for antibody. This mechanism has been suggested for the inhibition of experimental uveitis with an antigen-specific monoclonal antibody. Antibodies may also compete by allosteric mechanisms in which binding of the antibody alters the conformation of the molecule so that it does not bind a second antibody.

B cells arise from precursors in the bone marrow as immature B cells expressing IgM, and Ig $\alpha$  and  $\beta$ , and go through a series of transitional stages to become naive mature B cells in the spleen at which stage they express IgD. A small proportion of these cells become marginal zone B cells in the spleen and short-lived IgM-producing plasma cells, while the majority are available for interaction with specialized B-cell follicle (follicular) DCs to initiate antigen-specific immunoglobulin production through the B-cell receptor (BCR). The BCR, similar to the TCR, signals through

There are three components or segments to the CDR (CDR-1, -2 and -3) and the third (CDR-3) in both heavy and light chains is the most variable (Fig. 7-8). The C regions on the heavy chains are globular structures attached to the binding region by a flexible rod-like hinge portion of the molecule (see Fig. 7-8 and eFig. 7-3). The hinge region has both rigidity (conferred by proline residues at the top of the CH rod) and flexibility as the result of a large number of glycine residues. The last CH domain of immunoglobulin has a transmembrane and cytoplasmic portion,

involved in intracellular signalling. Each domain is composed of two layers of  $\beta$ -pleated sheets with three or four strands of anti-parallel polypeptides. Therapeutic monoclonal antibodies are used extensively for many diseases including eye diseases such as age-related macular degeneration (AMD, see Ch. 9, p. 513) in which a humanized (eFig 7-3, lower panel) monoclonal antivascular endothelial growth factor (anti-VEGF) antibody is administered to patients with wet AMD by successive injections of the antibody into the vitreous chamber of the eye.

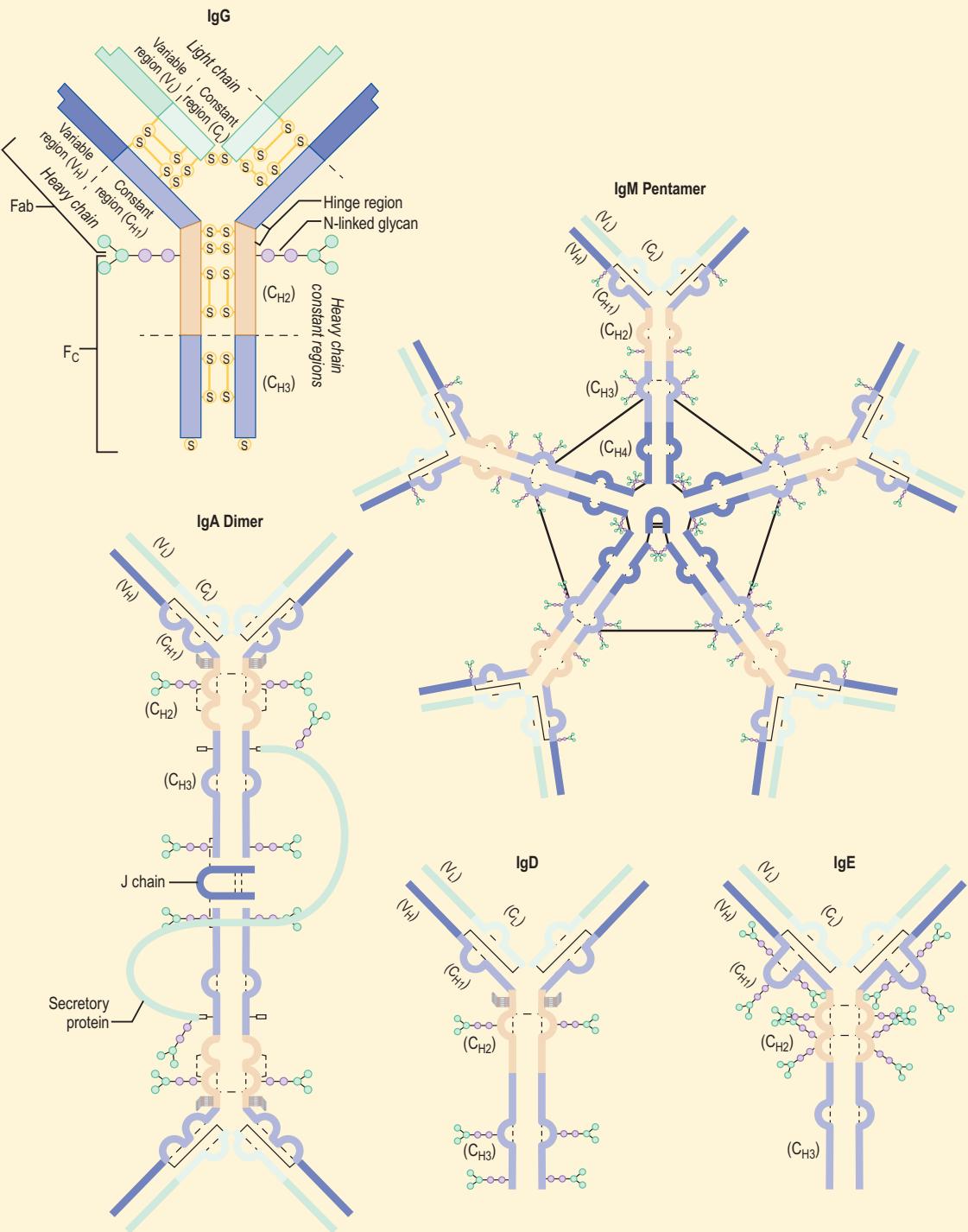


**eFIGURE 7-3** Antibody structure. The globular domains of the Fab and the Fc fragments are shown in the panel on top right and the anti-parallel pleated sheets on the top left. Development of engineered monoclonal fully humanized antibodies is shown on lower panel. [http://www.dsch.univ.trieste.it/~benedetti/antibody\\_catalysis.htm](http://www.dsch.univ.trieste.it/~benedetti/antibody_catalysis.htm) <http://dict.space.4goo.net/dict?q=antibody>. <http://www.chiscientific.cn/service.aspx?ID=30>

## BOX 7-7 ISOTYPES OF IMMUNOGLOBULIN

Five isotypes of immunoglobulin exist, denoted by their heavy chain ( $\alpha$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\mu$ ); there are two light chains ( $\kappa$ ,  $\lambda$ ), which occur in a ratio of 60:40 in humans. The Fc and C3 binding regions are responsible for the effector functions of antibodies (see below). Three complementarity determining regions (CDRs) are nested within framework regions, in the

V and hyper-V regions of the H and L chains, and receive contributions from both chains. Somatic mutations in the CDRs are responsible for the enormous antibody diversity and affinity maturation that occurs on repeated exposure to antigen.





cytoplasmic molecules such as Syk and Lyn, as well as PI3 kinase and SHIP to generate plasmablasts in the B-cell follicle. These cells leave the secondary lymphoid tissue to return to the bone marrow and other tissues, where they function as long-lived antibody-producing plasma cells. Isotype switching, i.e. from IgM to antigen-specific IgG, E and A, occurs while in the germinal centre of the B-cell follicle.

As indicated above, antigen specificity is determined by complementarity between the epitope on the antigen and the CDR. However, this is not exclusive and other neighbouring regions on the antigen (paratopes) and on the IgG molecule outside the hypervariable region may influence the final antigen specificity and avidity. In addition, antibodies as proteins may have other properties relating to their non-ligand (antigen) binding sites, for instance the recombination of the  $V_H$  and  $V_L$  chains may fortuitously produce a site that binds ADP or acts in a catalytic fashion. Such antibodies may therefore have additional functions that may be more closely ascribed to innate rather than adaptive immunity. Such antibodies are termed superantibodies.

### Monoclonal and polyclonal antibodies

Any single antigen, especially large proteins, may have multiple antibody-binding regions (epitopes). Antibody responses may therefore be polyclonal, oligoclonal or monoclonal depending on the immunodominance of the antigenic determinants. Selection of cells from an immunized mouse under special conditions and fusion of those cells with an immortalized cell line is a powerful technique for producing large quantities of antibody to a specific antigenic epitope.

Monoclonal antibodies (Mabs) have revolutionized diagnostic techniques in medicine today, particularly using flow cytometry in which antibodies specific to cell proteins are ‘tagged’ with fluorescent markers which identify even very rare cell types using narrow-wavelength lasers. Mabs are also widely used for therapy of diseases such as cancer and autoimmunity, particularly in ophthalmology where anti-TNF- $\alpha$  therapy is used to treat uveitis and anti-VEGF therapy is used to block blood vessel growth ‘wet’ AMD. Some of these Mabs are chimeric antibodies, i.e. molecules which combine human and mouse antibody segments,

while others are fully ‘humanized’ (see eFig. 7-3). Fragments of antibodies are sometimes as effective as the intact antibody and due to their reduced size they possibly have better penetration into tissues as therapeutic agents (Box 7-8).

## CYTOKINES ARE THE EFFECTOR ELEMENTS RELEASED BY CELLS DURING INNATE AND ADAPTIVE IMMUNE RESPONSES

Almost all of the biological effects of T cells are mediated by cytokines. More importantly, T cells alter the characteristics of an immune response by releasing different cocktails of cytokines. In a feed-forward mechanism dictated by the cytokine milieu of the tissues, naive T cells differentiate into one or more of the several different T-cell types (Fig. 7-2).

Cytokines, in the broadest sense, are produced by a wide variety of leucocytes and tissue cells, particularly monocytes/macrophages, epithelial cells and fibroblasts. The response of tissues to invasion by viruses and bacteria is to produce cytokines; for example, virus-infected cells activate NK cells, which can be induced to release cytokines by innate recognition of viral double-stranded RNA (e.g. IL-1, IL18); bacterial lipid (endotoxin) is recognized by CD14 and TLR4 on monocytes and by complement, all leading to pro-inflammatory cytokine release, e.g. IL-12, and IL-23.

### What makes a cytokine a cytokine?

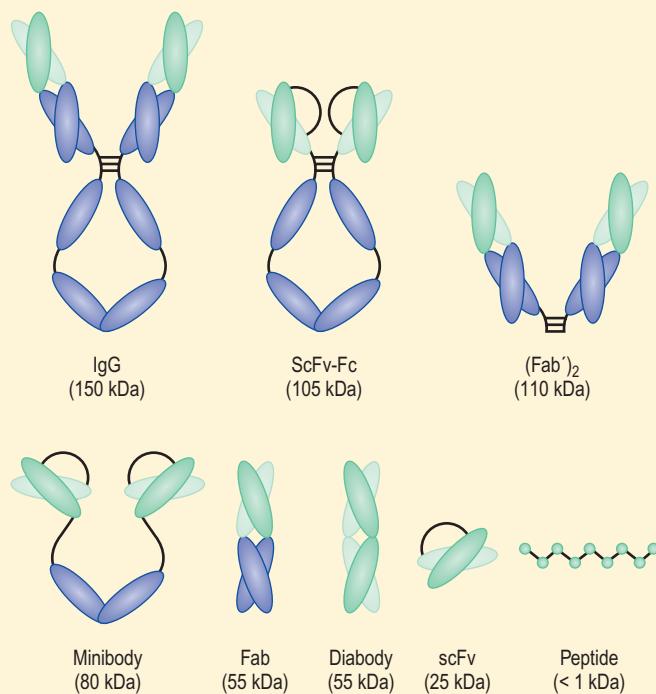
Cytokines have the following properties:

- they are secreted by cells in response to a specific stimulus.
- they are short-lived and short-range molecules, acting on cells within their neighbourhood.
- they may have effects at a distance if they are liberated into the circulation in sufficient concentration.
- they are effective at very low concentration.
- they may secondarily induce cytokine release by the target cell.
- they may act upon many different cell types (pleiotropism) and may have multiple different effects on the same target cell.
- they may be redundant, may induce cytokine synthesis themselves and may alter the effects of other cytokines.

**BOX 7-8 ANTIBODY ENGINEERING FOR THERAPEUTIC USE**

Monoclonal antibodies are produced by the fusion of antibody-secreting B cells with an immortalized myeloma cell line, engineered in such a way that only the B-cell/myeloma fusion cells survive, thus producing an immortalized antibody-producing cell. Some monoclonal antibodies have been produced continuously for decades.

The antibodies themselves can be engineered to produce fragments such as Fab fragments or single chain variable fragments (scFv) or even antibodies which combine a double set of variable fragments (diabodies) (see figure below). In this way the antibodies can be made more selective and potent.



Cytokines have three general effects:

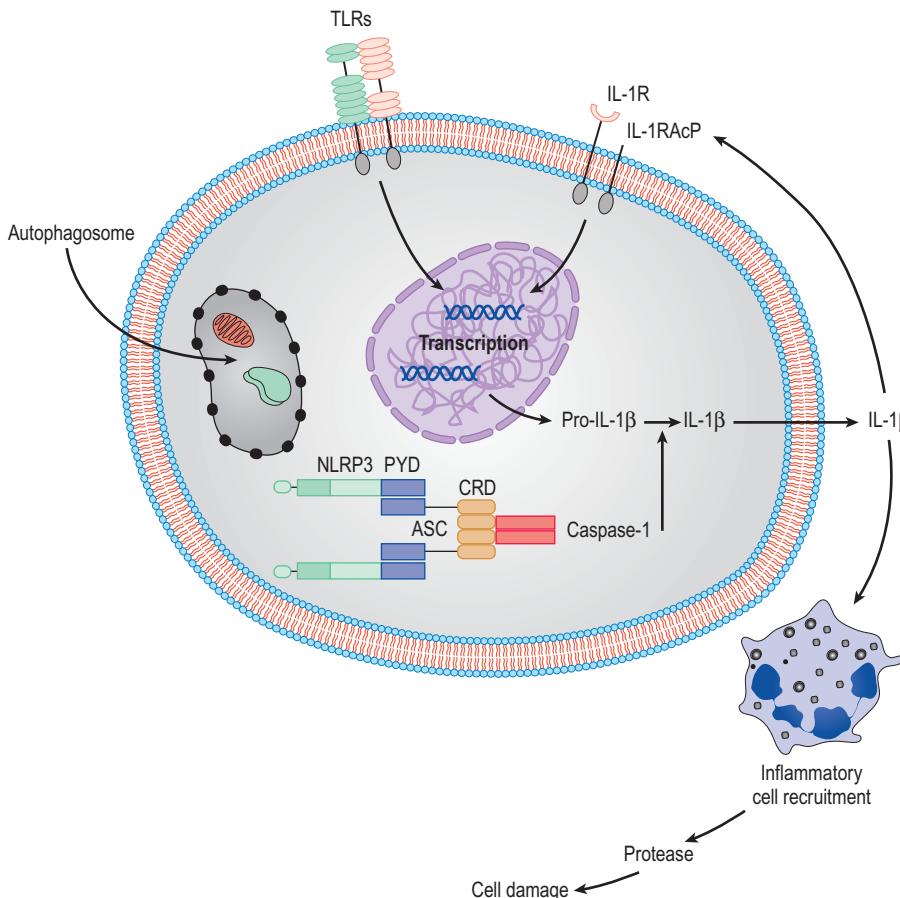
- they regulate the innate immune response.
- they regulate the adaptive immune response.
- they regulate the growth and differentiation of haematopoietic cells.

Cytokines now include several groups of molecules, such as the interleukins, growth factors, colony-stimulating factors, transforming growth factors, interferons, tumour necrosis factors, chemokines and monokines. Considerable functional overlap exists between these groups. At least 39 interleukins have now been described, each with a range of actions. IL-1 is produced by almost all nucleated cells, including ocular cells such as the RPE, and is central to the

initiation of most inflammatory and immune responses. Many of the more recently described interleukins have homology to IL-1, including IL-18, IL-33, IL-35 and IL-39. Some of these have anti-inflammatory effects, unlike IL-1 and IL-18 which are secreted on activation of the inflammasome (Fig. 7-9). IL-1 has extensive homology to fibroblast growth factor and may also be implicated in angiogenesis.

IL-2 is the major T-cell growth factor and initiates release of cytokines from the cells upon which it acts (Fig. 7-2).

Cytokines function in a vast interconnected network of agonist/antagonistic feed-forward and feedback inhibitory and stimulatory loops that ensures fine



**FIGURE 7-9** IL-1 $\beta$  is transcribed through a process involving activation of PRRs such as Toll receptors or via the IL-1 receptor itself, leading to activation of the inflammasome and induction of caspase-1 to generate IL-1 $\beta$  from its precursor pro-IL-1 $\beta$ . The process of autophagy regulates inflammasome activation and IL-1 production. (From van de Veerdonk and Netea, 2013.)

control over the immune response, mostly to avoid the excessive collateral damage that would occur in an over-robust response to a pathogen.

#### Cytokines involved in specific immune reactions

The interleukins, interferons and tumour necrosis factors are central to the immune response, and the character of the immune response is determined by the set of cytokines released. Activation of a naive T cell (a lymphoblast that has recently been presented with antigen) by different cytokines (in the presence of IL-2) released from the APC drives the T cell to differentiate into a Th1, Th2, Th17/Th22 or Treg cell (see Fig. 7-2). Th1 cells secrete interferon- $\gamma$  (IFN- $\gamma$ ,

in response to IL-12 secreted by macrophages or DCs during the initial innate response to antigen. Th1 cells are involved in delayed-type hypersensitivity responses and tissue damage associated with granuloma formation (see Ch. 9, p. 502). This is achieved by activation of macrophages and NK cells, which release reactive oxygen and nitrogen intermediates (free radicals). In addition, release of IL-2 by Th1 cells activates cytotoxic T cells. In contrast, Th2 cells release IL-4, IL-5, IL-6, IL-10 and IL-13, activate B cells and induce antibody production. IL-5 also stimulates eosinophils, which are the effector cells in allergy-associated tissue damage, while IgE avidly binds to mast cells and causes release of the mediators of immediate

hypersensitivity. In mucosal tissues such as the gut lining, IgA is produced with the help of Th2 cytokines.

This simple outline, describing the generation of either Th1 or Th2 responses is considerably modified during the event. For instance, IL-1, IL-2 and IL-4 can also activate macrophages directly, while IFN- $\gamma$  is involved in the production of IgG<sub>2a</sub> (the only immunoglobulin controlled by this cytokine). In contrast, activation of a naive T cell in the presence of the cytokine TGF- $\beta$  leads to the generation of Treg cells which suppress the immune response, unless IL-6 is also present in the milieu, in which case the strongly pro-inflammatory Th17 cell differentiates (see Fig. 7-2) driven by IL-23 production from the APC. IL-6 is produced by many tissue cells, including ocular cells such as the RPE, especially under stress.

### Cytokines involved in lymphomyeloid cell maturation

The bone marrow is the powerhouse of our immune defence system. Several cytokines are involved in the growth and maturation of lymphomyeloid cell populations from stem cell precursors in the bone marrow. These include the colony stimulation factors, granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF). In addition, several other cytokines with pleiotropic effects, including TNF- $\alpha$  and IL-1, have important roles in the maturation of these cells.

### Cytokine receptors and cytokine receptor antagonists

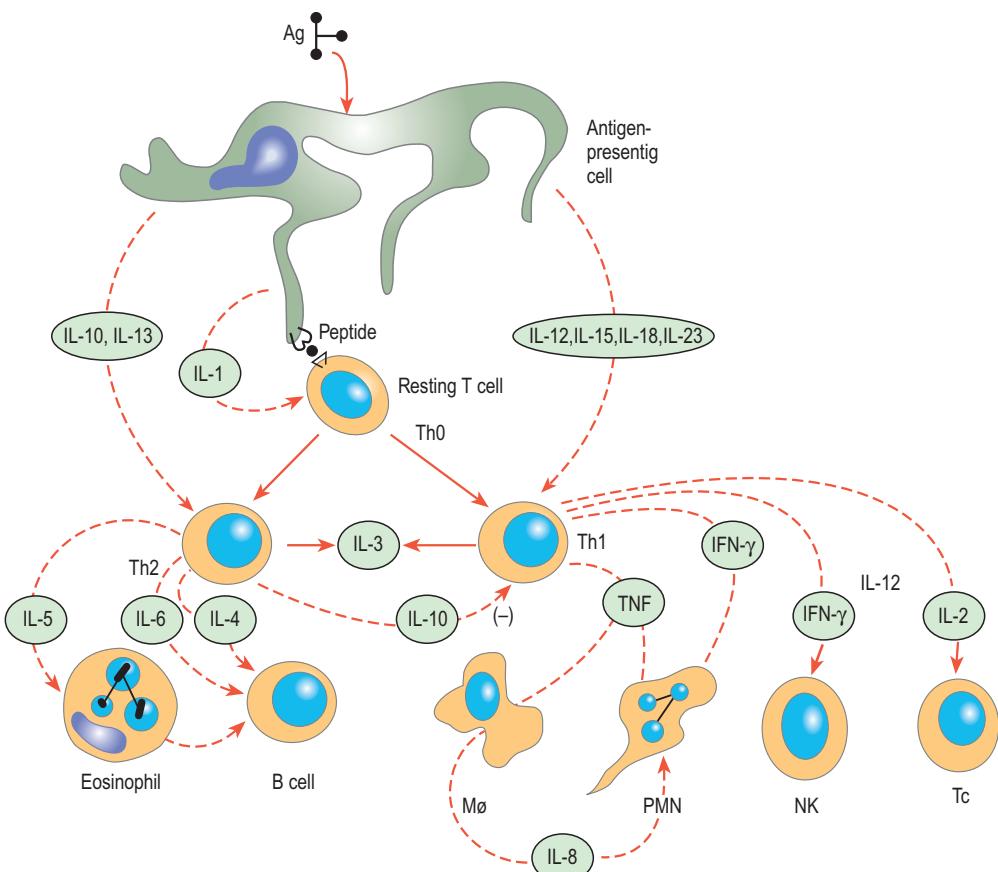
T-cell activation is antigen specific but the cytokines released are not. However, cells targeted by cytokines require the appropriate receptor for that cytokine to respond (Fig. 7.10). In addition, the targeted cells require a cell-signalling mechanism to mediate the response.

Cytokines utilize a common cell-signalling mechanism involving a cytosolic protein NF- $\kappa$ B, which regulates genes encoding cytokines, their receptors and several other genes involved in the acute inflammatory response. NF- $\kappa$ B is released from its inhibitor (I $\kappa$ B) when the cell is stimulated by cytokine and enters the nucleus bound to the transcription factors p65/p50. These initiate the changes in gene transcription with changes in function.

Five families of cytokine receptors are described based on structural motifs in the proteins: (1) the immunoglobulin superfamily (IL-1 and c-kit, IL-1Ra, IL-18, IL-33, IL-35, IL-37, IL-39); (2) a two cysteine/WSXWS or type 1 receptor family (binds IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-13, IL-15, IL-25, IL-27, IL-31, GM-CSF, G-CSF); (3) a type II receptor (binds IFNs, IL-10, IL-9, IL-20, IL-22, IL-24, IL-26); (4) a TNF receptor (part of a larger family of receptors involved in apoptosis including Fas–Fas ligand (FasL) mechanisms, TRADD (TNF receptor-associated death domain), TRAF (TNF receptor-associated factor) and CD40; see eBox 7-1); and (5) a seven transmembrane helix (chemokine) receptor family (see p. 404). Some cytokines have more than one receptor and some of the structural motifs are shared between the receptors: for instance, the common  $\gamma$  chain of the IL-2 and IL-15 receptors. Binding of cytokine to the receptor initiates signal transduction pathways such as the Janus family kinases (Jaks) and the signal transducer and activator of transcription (STATs) proteins which act upstream of NF- $\kappa$ B (Fig. 7-11). Several proteins are included in the Jak–STAT families and these intracellular proteins are prominent targets for drug discovery programmes in attempts to control immune responses.

Cytokines, when released, do not have a free rein in mediating their activities. Cytokine receptor antagonists are well recognized, the prototype in this field being IL-1Ra. This protein is a naturally occurring competitive binding protein for the IL-1 receptor but fails to induce any of the signal transduction events of IL-1. This is important because the uncontrolled activity of IL-1 in large amounts produces severe side-effects similar to the acute-phase response. IL-1Ra recognizes the separate receptors for IL-1 on T and B cells. IL-1 can also bind to a ‘decoy’ receptor, IL-1RII, which fails to transmit the signal.

Receptors may be of low or high affinity in their ability to bind ligands and this encroaches somewhat on receptor specificity, allowing certain cytokines to compete for the same receptor. In addition, for certain multichain receptors, such as the IL-2R, one of the chains may be shared by several cytokines (the common  $\gamma$  chain) and so assembly of the appropriate receptor on the membrane may depend on the precise cytokine milieu presented to the cell. The local concentration of any particular cytokine will therefore



**FIGURE 7-10** Cytokine networks induced by immune responses. Ag, antigen; IL-1, interleukin-1; IFN, Interferon; M $\phi$ , macrophage; NK, natural killer cell; PMN, polymorphonuclear leucocyte; Tc, T cytotoxic cell; TNF, tumour necrosis factor; ● peptide. (Courtesy of A. Abbas.)

have an influence on the final cell response. This cytokine redundancy is seen for instance with GM-CSF, IL-3 and IL-5. GM-CSF and IL-3 have widespread effects, whereas IL-5 is more restricted. By competing for the same receptor on different cell types, the amount and receptor-affinity of any one cytokine can determine the nature of the cellular response.

### Some cytokines play a bigger role in inflammation than others

Lessons learned from treating patients with autoimmune inflammatory disease point towards the importance of certain selected cytokines as major effectors of inflammation. For instance, by selectively targeting TNF- $\alpha$ , remarkable recoveries in function can be achieved in patients blinded by uveitis or crippled with rheumatoid arthritis. Several other cytokines

have primary roles in disease: for instance, IL-1 (and its family members) is involved in virtually every form of inflammation, while IL-2 is very important for T-cell function. Effector T cells can make their own IL-2, but Tregs, which are essential for controlling the inflammatory response, are unable to synthesize IL-2 and depend on IL-2 from other sources, including T effector cells (thus T effector cells initiate their own regulation).

TGF- $\beta$  is also a major regulator of T-cell differentiation (see Fig. 7-2) as well as being much more generally involved in several cell biological processes such as fibrosis and angiogenesis. In addition, it is constitutively present in the anterior chamber where it mediates aspects of immune privilege (see p. 457).

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The number of cytokines being discovered continues to increase and many of these are being shown to play important roles in inflammation and its regulation. The following sections provide brief information on specific cytokines.

### TUMOUR NECROSIS FACTOR

Certain cytokines have a prominent role in tissue damage, one of which is TNF. TNF exists in both soluble and membrane-bound forms and can induce a variety of responses in cells, including activation of polymorphonuclear leucocytes, induction of MHC antigens and adhesion molecule expression, and prostaglandin synthesis. Two forms of TNF exist, TNF- $\alpha$  and TNF- $\beta$ , the latter also known as lymphotoxin; both forms bind to the same cell receptor.

TNF- $\alpha$  is pro-inflammatory, pro-coagulant, cytotoxic and antiviral, and modulates haematopoiesis. Its inflammatory effects act on both the acute-phase response and locally on cells at sites of inflammation. As such it is a mediator of endotoxic shock. TNF- $\alpha$  signals through two receptors (p55 and p75), which are members of a larger TNF receptor family involved in several aspects of the immune response (eBox 7-1). Many are associated with cell death such as Fas-FasL (apoptosis), also important in immune privilege. Each receptor forms a (p55 or p75) homotrimer before ligand binding. TNF- $\alpha$  is produced as a pro-protein with a long signalling sequence. When cleaved off, the molecule trimerizes and binds to the trimeric receptor. TNF receptors can be cleaved from the cell surface and persist in the extracellular space and the bloodstream, where they can act as competitive inhibitors of TNF itself. Humanized fusion proteins using TNF have been used in this way to prevent disease.

TNF is a major mediator of experimental and clinical uveitis, and thus blockade of TNF- $\alpha$  ameliorates disease and preserves sight.

### TRANCE

TRANCE (TNF-related activation-induced cytokine, also known as osteoprotegerin ligand, or OPGL, and receptor activator for NF- $\kappa$ B ligand, or RANKL) was defined initially as an activator of osteoclasts in bone for bone turnover and of innate immune cells, macrophages and dendritic cells for the immune response. Deficiency of TRANCE leads to the severe bone disease osteopetrosis. It is now also recognized as a major ligand for the promotion of immunological tolerance on medullary epithelial cells (mTECs) in the thymus where it binds the receptor RANK and sustains tolerance to self-antigens during adult life once it is established in the fetus.

### INTERLEUKIN-1

Interleukins constitute a subclass of cytokines: their name is derived from their ability to effect communication between leucocytes. However, they are derived from a range of cells and have effects on many cell types, including leucocytes. IL-1 is released on cell death by many cells as part of the injury response and then amplifies this response by signalling via the inflammasome to release more IL-1 and IL-18. In macrophages and endothelial cells (its selective targets) it induces adhesion molecule expression and also promotes prostaglandin synthesis. IL-1 also activates bone cells and accelerates bone turnover, and it induces marrow stromal cells to produce G-CSF and IL-3. Two forms of IL-1 exist, IL-1 $\alpha$  and IL-1 $\beta$ , each with its own receptor, but receptor usage is not highly restricted. Thus, IL-1 $\alpha$  and IL-1 $\beta$  have broadly similar effects on cells. IL-1 is extremely potent and is counteracted by the IL-1 receptor antagonist, which plays a major role in regulating IL-1 activity.

IL-1 activates cells within the eye, particularly the RPE and the retinal vascular endothelium. It has been implicated in the pathogenesis of various forms of uveitis and has been shown to be uveitogenic experimentally and to have significantly greater damaging effects than TNF- $\alpha$ .

IL-1 $\alpha$  and IL-1 $\beta$  are produced as pro-proteins without a secretory signal sequence and must be digested by caspase-1 (IL-1 $\beta$ ) or other proteases (IL-1 $\alpha$ ) before they are activated. IL-1 (and IL-18) signal through Myd88 and the inflammasome.

### INTERLEUKIN-2

IL-2 is the major cytokine involved in T-cell-mediated responses, both Th1 and Th2, and also induces NK cell activity, in both of which it may act in an autocrine manner. IL-2 also initiates activation of CD8 T cells. Activated T cells thus express the IL-2 receptor (CD25), and it has, for instance, been detected on circulating T cells as well as lymphocytes from intraocular samples from patients with endogenous uveitis. Interestingly Treg cells express the highest level of the IL-2 receptor and IL-2 is essential for Treg induction even though Tregs cannot produce this cytokine. Tregs express and require the transcription factor FoxP3 and, conventionally, Tregs are described as CD4 $^+$ CD25 $^+$ FoxP3 $^+$  T cells.

### THE INTERFERONS

IFN- $\alpha$  and IFN- $\beta$  are members of the type I interferons, of which there are many, while IFN- $\gamma$  is the only type II interferon, a classification based on structural differences. IFN- $\alpha$  was the first cytokine to be identified, sequenced,

cloned and introduced to clinical therapeutics. It is used in the treatment of multiple sclerosis, hepatitis C and certain forms of retinal vasculitis. It is produced by many cells in response to virus infection and in this regard is important in promoting viral clearance. The main source of constitutive IFN- $\alpha$  is a rare population of dendritic cells, the plasmacytoid dendritic cells (pDCs). Large quantities of this cytokine are produced by pDCs in response to virus infection, leading to maturation of myeloid dendritic cells with strong induction of antiviral cytolytic activity and B-cell isotype switching. IFN- $\alpha$  can also be produced by activation of DCs via the TNF receptor but with a gradual kinetic as opposed to the massive antiviral response. INF- $\alpha$  produced by TNF receptor stimulation may be immunoregulatory. IFN- $\beta$  is produced mainly by fibroblasts.

Two interleukins (IL-28 and IL-29) have been described which have IFN- $\alpha\beta$ -like activity and can induce antiviral activity in the absence of IFN. These cytokines have now been rebranded as IFN- $\lambda$ , i.e. type III interferons, and like the type 1 interferons they act via the Jak-STAT pathway.

IFN- $\gamma$  is released from virally infected cells and has potent antiviral activity. It is also a major pro-inflammatory cytokine and induces MHC and other antigens on cell surfaces, thus promoting adaptive immunity via antigen presentation. It is the signature cytokine for CD4 $^+$  Th1 cells. It has been shown to induce MHC class II on cells in the retina (which does not normally have many MHC class II $^+$  cells); these may be tissue-specific APCs. It also induces the innate immune response by activating macrophages to produce cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-12, IL-18 and IL-23, and also to engage in cytotoxicity through release of reactive oxygen species and nitric oxide. Like TNF- $\alpha$  and IL-1, IFN- $\gamma$ , has potent uveitogenic activity. In addition, high levels of IFN- $\gamma$  have been detected in aqueous samples from patients with acquired immune deficiency syndrome (AIDS) retinitis.

Production of interferons is under the control of interferon-regulating factors (IRFs), which bind to interferon-stimulated regulatory elements (ISRE) on the promoters of the IFN genes.

### TRANSFORMING GROWTH FACTOR- $\beta$

TGF- $\beta$  is an important immunosuppressive cytokine. There are at least six types of TGF- $\beta$ , and many cell types elaborate this mediator, including cells within the eye such as ciliary body epithelium and RPE. TGF- $\beta$  has been suggested to account for part of the immunosuppressive activity normally found within the eye (see p. 457). TGF- $\beta$  appears to be the main isotype found within the eye and is normally secreted in a latent form; however, it is readily

converted to the active form by enzymes such as plasmin, which would normally be present in an inflammatory exudate.

TGF- $\beta$  promotes T-cell differentiation as well as angiogenesis and chemotaxis of several leucocyte types.

TGF- $\beta$ s have widespread effects on cell adhesion, differentiation, proliferation, migration, maturation, activation and regulation, both within and on cells outside the immune system. *In vitro* studies are greatly affected by small changes in the TGF concentrations, and thus many effects require *in vivo* investigation. TGF- $\beta$ s are essential, however, because deletion of the TGF- $\beta$  gene in mice is lethal for the embryo. Overall, TGF- $\beta$ 1 and TGF- $\beta$ 2 are anti-inflammatory and immunoregulatory, but TGF- $\beta$ 1 is pro-fibrotic. TGF- $\beta$ s are produced by leucocytes and by parenchymal cells in the central nervous system, kidney and eye. TGF- $\beta$  receptors comprise three chains, of which chains I and II bind to form a high-affinity receptor while III binds either of the other two chains in a regulatory, non-signalling role.

### INTERLEUKIN-4

Interleukin-4, -5, and -13 are prototypical cytokines inducing Th2-type responses and are thus major players in allergic diseases including asthma and allergic skin and mucous membrane disorders such as allergic conjunctivitis. In addition, IL-4 and IL-13 are important inducers of alternative macrophage (M2) activation promoting wound healing, fibrosis and angiogenesis.

### INTERLEUKIN-6

IL-6 has pro-inflammatory and fever-inducing activity, but recent evidence suggests that its major role may be to limit tissue damage. It has multiple and wide-ranging effects, and participates in the acute-phase reaction (fever) as well as in haematopoiesis. IL-6 is produced by many cell types, both immune and non-immune, including ocular cells such as RPE cells. IL-6 may be instrumental in promoting a Th2-type response with preferential activation of B cells. However, experimentally, IL-6 appears to have a marked uveitogenic effect, similar in severity to that of endotoxin. In addition, IL-6 has been detected in the aqueous of patients with endogenous and postsurgical uveitis. IL-6 combines with TGF- $\beta$  to promote induction of Th17 effector cells, Th17.

Like many cytokines, IL6 and the IL6 receptor (IL6R) can exist in soluble form and engage in 'trans-signalling'. IL-6, IL-11, IL-30 and IL-31 are grouped together due to similarities in their receptor and signalling pathways. In addition, the immunosuppressive cytokine IL-35

combines the components of IL-6 and IL-12 (<http://www.sciencedirect.com/science/article/pii/S1359610112000160>).

### INTERLEUKIN-8

IL-8 is regarded as a chemokine for neutrophils but also has some level of chemotactic properties for monocytes and lymphocytes. IL-8 is released by immune and non-immune cells but is somewhat more restricted than IL-6. RPE cells, corneal endothelium and stromal cells release IL-8 after appropriate stimulation. IL-8 is less uveitogenic than IL-1, which appears to be the main cytokine in this respect. IL-8 binds to the chemokine receptors CXCR1 and CXCR2.

### INTERLEUKIN-10

IL-10 is an 18 kDa cytokine produced by many cells. It is the prototype cytokine of the IL-10 family of cytokines, which consists of nine members: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 which are also related to IL-28 and IL-29. The IL-10 family are predominantly an ‘immuno-suppressive’ cytokine family and are involved for instance in immunological privilege of the eye and other organs. The effects of IL-10 can override those of many pro-inflammatory cytokines. IL-10 does this by inhibiting synthesis or secretion of TNF- $\alpha$ , IL-1, chemokines and IL-12 by macrophages. It also reduces MHC class II expression on APCs, thus downregulating specific and innate immune responses. Certain viruses such as Epstein–Barr virus release an alternative form of IL-10, raising the possibility of virus-induced immune suppression. The heterodimer IL-10 receptor is expressed mainly on haematopoietic cells.

While IL-10 is mostly produced by all leucocytes, IL-19, IL-20 and IL-24 are produced by myeloid and epithelial cells and are involved in antibacterial responses, wound healing and tissue remodelling. In contrast, IL-22 and IL-26 have similar functions but are produced by T cells, NK and NKT cells, as well as innate immune cells such as those in the gut. IL-28 and IL-29 are predominantly antiviral.

### INTERLEUKINS 12, 17, 23 AND 27

IL-12 rose to prominence as the major mediator and inducer of IFN- $\gamma$ -producing CD4 $^+$  Th1 responses during the time that IL-2 was recognized as having a regulatory (inducer of Tregs) as well as a T-cell-activating role. IL-12 is produced by APCs, particularly mature dendritic cells and activated macrophages, and is strongly pro-inflammatory both for Th1 cells and NK cells. IL-12 synergizes with IL-18 to induce IFN- $\gamma$  production by activated T cells and NK cells. IL-12 is a heterodimer with p35 and p40 chains. It shares the p40 chain with IL-23, in which

the p40 chain combines with a separate p19 chain to form the IL-23 heterodimer. IL-23 is the main pro-inflammatory cytokine, while the p35 chain of IL-12 may have some regulatory activity. IL-23 is responsible for induction of IL-17-producing CD4 $^+$  Th17 cells, which are major players in the pathogenesis of autoimmune disease as well as adaptive immunity to many infectious agents, particularly fungi. Both IL-1 and IL-17 play major roles in recruiting neutrophils to sites of infection, e.g. to *Staphylococcus aureus* skin infections. They may also be involved in tumour immunity. In contrast, IL-27, with p35-like and a p40-like chain, induces the IL-12R $\beta$  chain on Th1 cells during the initiation of Th1 responses, but its main function is to put a brake on inflammatory responses (eFig. 7-4). IL-12, IL-18, IL-23 and IL-27 induce both primary and memory adaptive T-cell responses in a coordinated series of reciprocal interactions between the dendritic cells and T cells.

### INTERLEUKINS 15 AND 21

IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 share the common  $\gamma$ -chain of the IL-2 receptor. NK cells are absolutely dependent on IL-15. Both IL-15 and IL-12 synergize to activate NK cells. IL-15 also activates  $\gamma\delta$  T cells. IL-15 has similar functions to IL-2 on T cells. However, there are striking differences from IL-2; for instance, IL-15 is not produced by T cells but by bone marrow stromal cells. The IL-15 receptor has a unique  $\alpha$  chain but uses the IL-2R  $\beta$  and  $\gamma\delta$  chains. IL-21 also activates NK cells and induces T-cell activation but unlike IL-2 and IL-15 it inhibits B-cell proliferation.

### INTERLEUKIN-18

Both IL-1 and IL-18 are released from pro-peptides in APCs on activation of the inflammasome and caspase-1. IL-18 has similar pro-inflammatory functions to IL-1 and IL-12, and induces production of IFN- $\gamma$  and TNF- $\alpha$  in macrophages. Interestingly, IL-18-deficient mice show marked susceptibility to bacterial infections but have no impairment of response to challenge with ocular antigens. In contrast, IL-18 may have an anti-angiogenic role in diseases such as age-related macular degeneration.

### INTERLEUKIN-32

IL-32 is a pro-inflammatory and a pro-apoptotic cytokine involved in many autoimmune inflammatory conditions and also in viral and mycobacterial infections and cancer in humans. It has not been found in rodents as yet, and there is no clear evidence of a specific receptor, although it does appear to signal via integrins.

**INTERLEUKINS 33 AND 36**

IL-33 and IL-36 are members of the IL-1 family and, like IL-32, have both intracellular and extracellular effects. IL-33 is released on cell form death from nucleus disintegration and mediates both Th2 and anti-Th1/Th17 immunosuppressive effects. IL-36 is associated with a psoriasis-like disease.

**INTERLEUKIN-34**

IL-34 binds to the CSF-1R (M-CSFR) and appears to be necessary for Langerhans cell and microglia development,

differentiation and activation, and may be important in the pathogenesis of neurodegenerative disorders.

**INTERLEUKIN-37**

IL-37 is the newest member of the IL-1 family and is an immunosuppressive cytokine. Like IL-1 $\alpha$  and IL-33, IL-37 binds to the nucleus in a receptor-independent manner to exert its anti-inflammatory effects.

**INTERLEUKIN-38**

IL-38 binds to the IL-36 receptor (also a member of the IL-1 family) and inhibits IL-17 and IL-22 effects.

**eBox 7-1****TNF receptor superfamily**

The tumour necrosis factor receptor (TNFR) superfamily (there are about 50 soluble and membrane-bound members) is a very important class of signal transduction molecules in the immune system. Included in this family are the TNF receptors 1 and 2 (TNFR1 and 2), the low-affinity nerve growth factor receptor (NGFR), CD40, CD30, Fas, and others indicated in the figure. Members of the TNF receptor family are generally transmembrane proteins, but many of them can also be secreted as soluble molecules, derived either by proteolytic cleavage from the membrane or by differential mRNA processing. The TNFR family is characterized by the presence of cysteine-rich motifs of 40 amino acids in the extracellular domain that are involved in ligand binding. The ligands for these receptors are type II transmembrane proteins with a 'jelly-roll'  $\beta$ -sandwich structure, many of which can also be secreted.

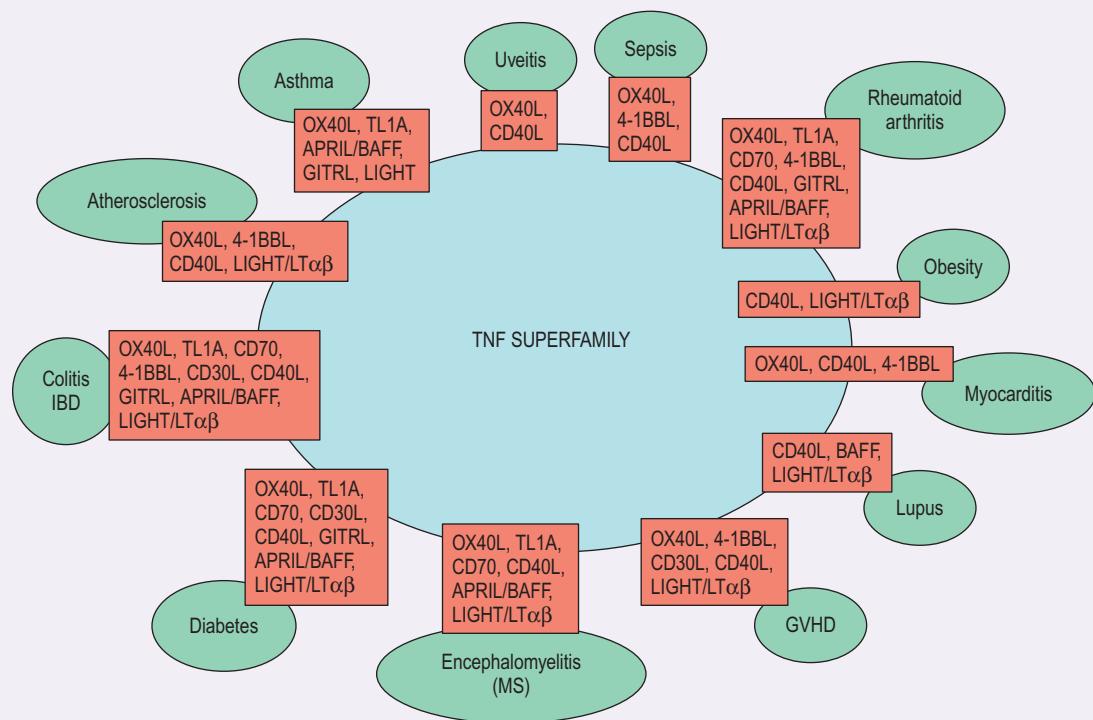
Some TNF receptor family members are associated with additional signal transduction molecules called TNFR-associated factors (TRAFs). These proteins contain zinc-binding domains that are thought to mediate the binding of the protein to DNA, leading to transcriptional activation. Signal transduction is initiated by stimulation of oligomerized receptor complexes upon ligand binding. The ligands, such as TNF, are often found in multimeric form, and this multivalency enhances the induction of signalling. Other members of the TNFR family (most notably, the apoptosis-inducing molecule Fas) contain death domains (DDs) in their cytoplasmic tails. For example, the DD sequence allows the interaction of the membrane-bound

Fas molecule with the signal-transducing protein FADD (Fas-associated death domain), which in turn delivers signals causing the cell to initiate apoptosis. More recently it has been discovered that several proteins containing the DD sequence are not involved in apoptosis at all but actually promote cell survival. In fact, depending on downstream events, engagement of TNFR1 (which contains a DD sequence) can lead either to cell death or to cell survival.

The functional consequences of ligand engagement by members of the TNF receptor superfamily are very diverse. Fas binding induces apoptosis, which is important in the maintenance of immune self-tolerance. CD27, CD30, 41BB, and CD40 signalling enhance the survival, proliferation and activation of B or T lymphocytes, often playing critical roles as co-stimulators or signal modulators. Signalling by other TNFR family members results in the activation of NF- $\kappa$ B in macrophages and the induction of an inflammatory response. The TNF receptor superfamily is thus central to many aspects of both innate and acquired immunity and plays fundamental roles in both cell death and survival. Different TNFR members are involved in different diseases (see figure). A greater understanding of this receptor family, as well as its corresponding family of ligands, holds great potential for the creation of novel drugs and therapeutics that could be used to control many aspects of autoimmunity and other immunopathologies.

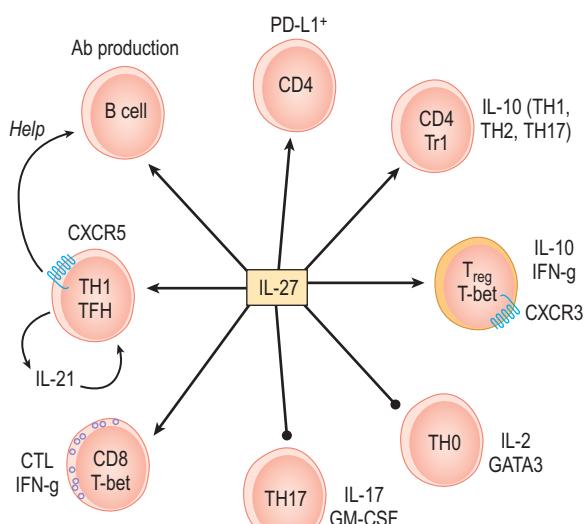
## eBox 7-1

### TNF receptor superfamily—cont'd

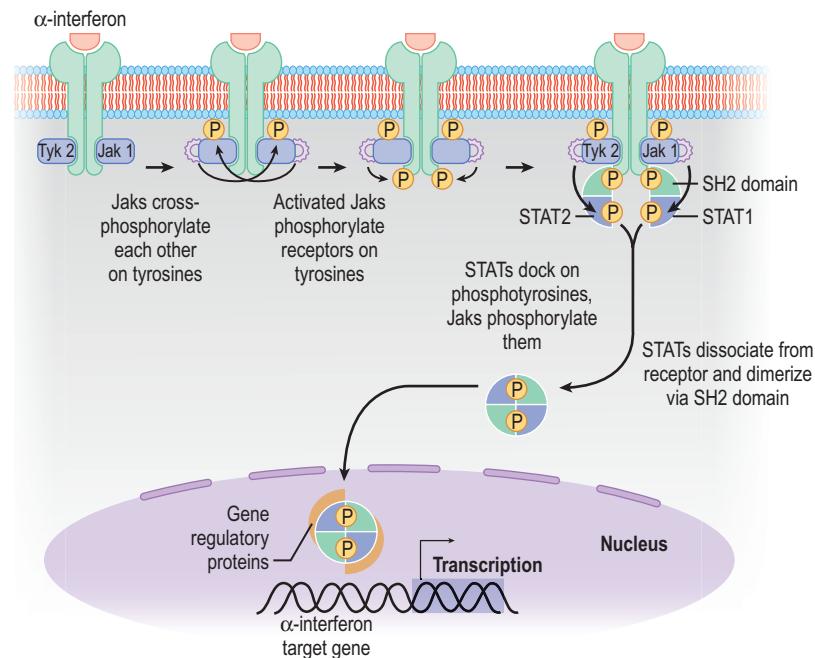


Legend eBox 7.1 Figure: TNF family molecules are implicated in driving inflammatory and autoimmune disease. The figure depicts the TNFSF and TNFRSF molecules that control disease in experimental models in the mouse as a diagram of the TNFR superfamily with identi-

fication of the autoimmune diseases associated with specific molecules. Some molecules, such as OX40/OX40L, are associated generally with organ-specific diseases (such as myocarditis and uveitis) but not with systemic autoimmune diseases such as lupus (Croft et al., 2012).



eFIGURE 7-4 IL-27 is produced by innate immune cells and influences the behaviour of adaptive immune cells.



**FIGURE 7-11** The Jak-STAT pathway is activated by many cytokines, in this example by the cytokine interferon- $\alpha$ . (From <http://www.motifolio.com/5111157.html>.)

### Chemokines and chemokine receptors

Cytokines that are specific for induction of leucocyte migration to the site of inflammation are termed chemokines and include some interleukins as well as other cytokines with additional functions. Chemokines are small peptides (usually 8–15 kDa) and are classified in two main subsets based on a particular amino acid sequence involving two cysteine residues, –C–C– chemokines and –C–X–C– chemokines, in which the latter contains an intervening non-cysteine residue. However, many chemokines were discovered before the current nomenclature came into being, and in many cases the older terminology is still in use (Table 7-5). Some chemokines do not belong to either category, having either only one C residue or having additional intervening amino acids, but they are unusual.

Regulation of inflammatory cell traffic to sites of inflammation is determined by the set of chemokines released in the tissues and the expression of specific

chemokine receptors on different cell types. There is considerable redundancy in the system but, despite this, there is temporal regulation of specific leucocyte recruitment depending on which chemokines and chemokine receptors are active. Chemokines are released by tissue cells at the time of injury or antigen challenge and, due to their low molecular weight, percolate and diffuse through the tissues to line blood vessels and lymphatics, thereby promoting leucocytes to find a pathway to the site of inflammation or, in the case of the lymph node, to the site of T-cell activation. For instance, chemokines such as IL-8 (CXCL8) specifically attract neutrophils through the receptor CXCR1, while CCL19 and 21 attract T cells and dendritic cells using CCR7. In addition there are several other receptors and chemokine-binding agents which act as ‘decoys’ by binding the chemokine but failing to signal, thus effectively ‘confusing’ the cell and preventing effective migration. Such receptors include D6 and DARC (Fig. 7-12).

**TABLE 7-5 Nomenclature for chemokines and their receptors**

Chemokine	Old name	Receptor
CXC family	$\alpha$ family	
CXCL1	Gro $\alpha$	CXCR2
CXCL2	Gro $\beta$	CXCR2
CXCL5	ENA-78	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL9	HuMIG	CXCR3
CXCL10	IP-10	CXCR3
CXCL11	ITAC	CXCR3
CXCL12	SDF-1	CXCR4
CXCL16	SR-PSOX	CXCR6
CX3CL1	Fractalkine	CX3CR1
CC family	$\beta$ family	
CCL2	MCP-1	CCR2
CCL3	MIP-1 $\alpha$	CCR1, CCR5
CCL4	MIP-1 $\beta$	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
CCL7	MCP-3	CCR1, CCR2
CCL19	ELC	CCR7
CCL21	SLC	CCR7
CCL28	MEC	CCR10

(From *Lalor et al., 2007*.)

## COMPLEMENT

The term ‘complement’ was coined to describe an activity in sera required for antibody-mediated lysis of bacteria but that was lost after heating to 56°C. Antibody itself was heat-stable and retained the ability to agglutinate the bacteria but could not kill them. This additional activity therefore ‘complemented’ antibody-mediated cytotoxicity.

### What is complement and what does it do?

Complement is a property of serum derived from sequential zymogen activation of a series of plasma proteins (a zymogen is an enzyme that is activated by a second enzyme, which itself has been activated by proteolytic cleavage, i.e. an enzyme cascade as occurs in kinin formation, coagulation and visual transduction).

Complement is a major component of innate immunity, i.e. the initial response to attack by pathogens, and in this sense can be regarded as a soluble pattern recognition receptor (PRR) as opposed to the

membrane-bound PRRs such as the TLRs (see p. 389). Two enzyme cascades exist, one initiated by antibody combining with antigen, the classical pathway, and a second initiated directly by bacterial surface components, the alternative pathway (Box 7-9). Thus, even at this level, a distinction between innate and acquired immunity exists. The classical complement pathway is in fact the major effector mechanism for humoral immunity (see above).

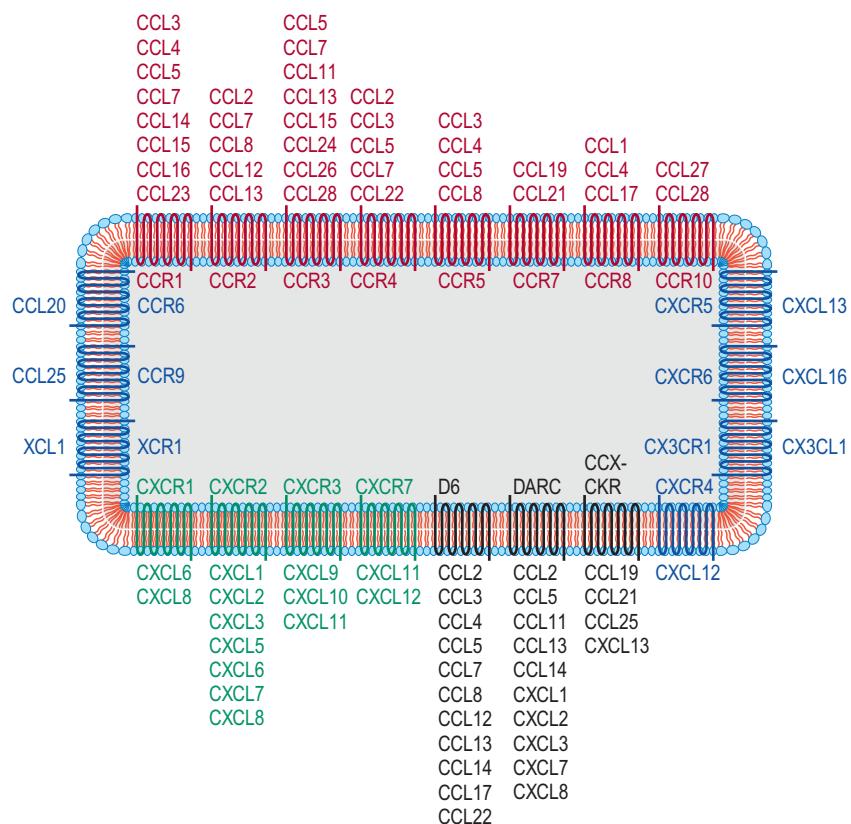
A third mechanism for complement activation has been described. This involves members of a family of molecules called lectins (lectins are non-antibody, non-enzyme carbohydrate-binding proteins, as in the selectin adhesion molecules; see above). Lectins that contain collagen-like domains are known as collectins; these molecules are of considerable importance in innate immune mechanisms against microorganisms. Collectins bind to the same receptor as the C1q component of complement (see below) and are thus able to activate the classical pathway.

A major lectin in this pathway is mannan-binding lectin (MBL), which is linked to serine proteases MASP-1 and MASP-2. These enzymes are responsible for cleaving C4 and C2, respectively.

### Complement has the following effects:

- it is involved in the initiation of the acute inflammatory response by release of certain peptides that act as chemotactic factors and induce vasodilatation with increased permeability (anaphylatoxin).
- it mediates antibody-dependent cytolysis by polymerizing on cell surfaces to form pores in the cell membrane.
- it solubilizes and removes immune complexes from the circulation.
- it induces phagocytosis by acting as an opsonin. Complement proteins in the normal circulation are inactive and are maintained in this state by an elaborate system of inhibitors that not only inhibit activation of the various enzyme systems but also limit the response once activated.

The central axis of the complement pathway is the conversion of C3, activated by C3 convertases, to C5 convertase by the binding of C3b (Box 7-9). This leads to the sequential addition of a series of complement



**FIGURE 7-12** Chemokines and chemokine receptors bind through multiple overlapping, and probably redundant, ligand–receptor interactions. (From Lazzanec and Richmond, 2010.)

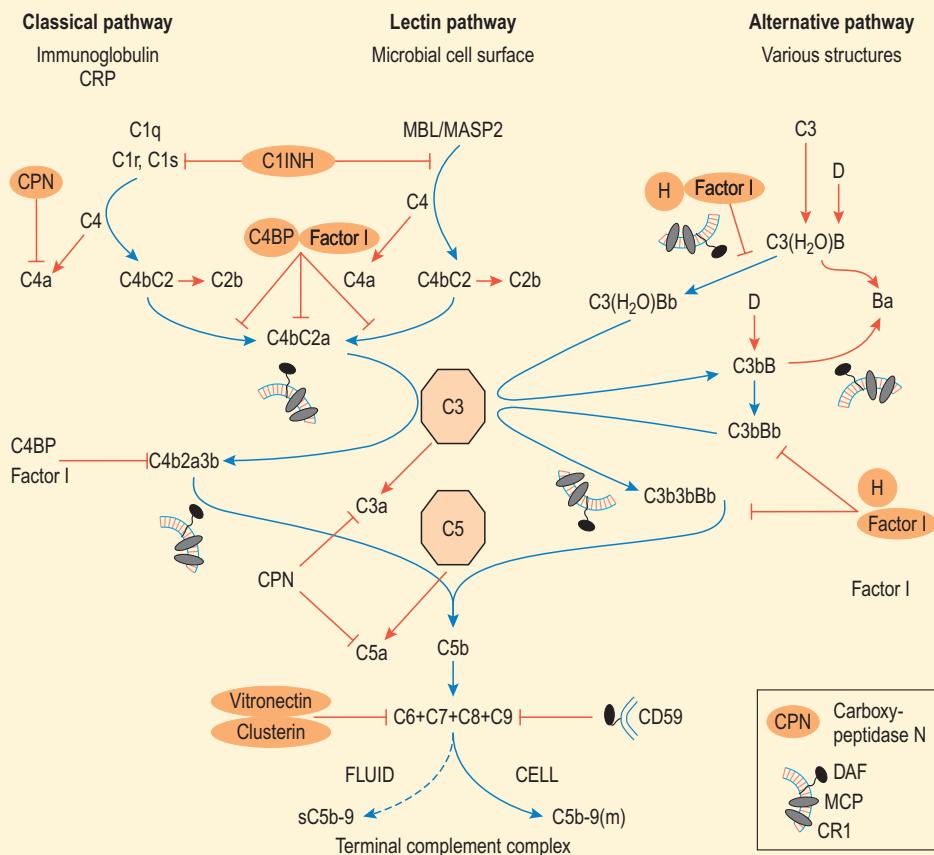
proteins that result in the membrane attack complex (MAC). Complement proteins are synthesized in the liver and in mononuclear phagocytes. In addition, there is some local production by parenchymal tissue cells, as occurs in the retina.

### The classical complement cascade

Activation of C1 is induced by binding to IgM or IgG, but only if the immunoglobulin has bound antigen in the form of an immune complex. Free immunoglobulin does not activate complement. Binding occurs to the C<sub>H</sub>3 domain of IgM or the C<sub>H</sub>2 domain of IgG, and requires at least two immunoglobulin molecules. Thus a single molecule of IgM, which is a pentamer, is able to ‘fix’ complement, while several molecules of IgG, usually aggregated together, are required to achieve the same. Alternatively, cell surface-bound IgG can

suffice. IgM is therefore known as complement-fixing antibody and it is this antibody that plays the major role in the initial response to pathogens as part of innate immunity.

C1 can be activated by antibody-independent mechanisms including contact with retroviruses, mycoplasma, or even polyelectrolytes such as DNA and heparin. These presumably act in a non-specific manner by virtue of their charge. Importantly, the acute-phase proteins, C-reactive protein (CRP) and serum amyloid protein (SAP), can also bind complement non-specifically. C1 is composed of three molecules: C1q, C1r and C1s. C1q is a collagen-like molecule with a triple-helix conformation, while C1r and C1s are serine proteases. The molecular complex comprises a tetramer of C1s and C1r with six or more C1q molecules (Fig. 7-13).

**BOX 7-9 INNATE IMMUNITY: NEW INSIGHTS IN COMPLEMENT AND COLLECTINS**


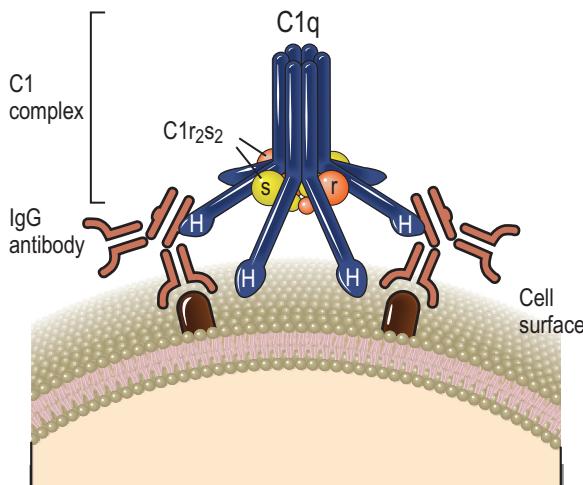
When two or more globular heads of C1q bind IgM or cell-surface bound IgG, C1r is cleaved to C1s<sup>-</sup>, which then cleaves C1s to C1s<sup>-</sup>; this activates C4 to C2 to undergo partial proteolysis and bind to form C4b2a<sup>-</sup>. C4b is unstable because it contains an internal thioester bond and is rapidly inactivated by binding a water molecule to form iC4b. If, however, C4b is formed in close contact with a cell membrane it can covalently bind to the surface and remain in an active state. In contrast, C2 is a single-chain molecule that binds to surface-bound C4a in the presence of Mg<sup>2+</sup> ions. C2b, produced by C1s<sup>-</sup>-induced partial proteolysis of C2, diffuses away while the C2a binds to C4b.

The complex of C4b2a<sup>-</sup> contains the C3 convertase activity. The C4b component binds to the C3 molecule

and brings the C2a moiety into close contact with C3, which it cleaves to C3a and C3b. C3 is a 195 kDa  $\alpha\beta$  heterodimer with an internal thioester bond similar to C4.

When partially cleaved, this molecule is also unstable and is rapidly inactivated to iC3b. However, if in contact with a cell membrane, it binds covalently in conjunction with C4b2a to form C4b2a3b<sup>-</sup>, i.e. C5 convertase (see below).

C3a, C4a and C5a are small cationic peptides also known as anaphylatoxins that bind to specific receptors on basophils and mast cells. C3 cleavage products also have a role in antibody production by interacting with follicular dendritic cells (FDC) in the germinal centre (see p. 414).



**FIGURE 7-13** Diagrammatic representation of the generation of the C1 complex comprising a tetramer of C1s and C1r with six molecules of C1q. (Figure 12-10 from Abbas, A.K. Cellular and Molecular Immunology 7th Edition, p. 381, Elsevier, 2012)

### The alternative pathway

The alternative pathway is triggered by low levels of C3b, which are spontaneously produced *in vivo* by proteolysis, and by C3(H<sub>2</sub>O), which is also formed by spontaneous hydrolysis of C3. On normal cells C3b is rapidly deactivated to iC3b by innate regulatory mechanisms (see below), but on foreign surfaces C3b can remain active. C3b binds to factor B on the surface of the cells and is converted by factor D, a serine protease, to C3bBb. This contains the C3 convertase activity and requires a further protein, properdin, to protect it from proteolysis.

Deposition of C3b on foreign surfaces such as bacteria leads to further production of C3b, i.e. a positive feedback amplification loop occurs, which helps to eradicate foreign particles rapidly. C3bBb is combined to form C3bBb3b, which represents the alternative form of C5 convertase (see Box 7-9).

### Collectin activation of complement

Collectins include several well-characterized proteins, such as conglutinin and MBL, which directly bind to the C1q receptor on cell surfaces and initiate such phenomena as C4-mediated red cell lysis. MBL directly activates the C1r<sub>2</sub>C1s<sub>2</sub> tetramer in the absence of C1q. This may be mediated in association with a serine protease (MBP-associated serine protease, or MASp;

see above). Collectins also have direct opsonin activity (see below, macular degeneration).

### Cytolysis and the membrane attack complex

The membrane attack complex (MAC) is formed by a set of complement proteins inserting themselves into the lipid bilayer and is possible because certain proteins within the complex have a lipophilic core. C5 binds loosely to C5 convertase on the cell membrane, and is split into C5a, which diffuses away, and C5b, which complexes with C6 and then C7. C5b,6,7 is highly lipophilic and burrows into the cell membrane. There it acts as a receptor for C8, an  $\alpha\beta\gamma$  trimer whose  $\gamma$  chain is also lipophilic and similarly inserts into the bilayer. The C5b,6,7,8 complex is weakly cytolytic but becomes considerably more so when it binds C9, a serum protein that polymerizes to form the MAC with 12–15 C9 molecules per C5–9 complex. This forms a ‘pore’ in the cell membrane, similar to the perforin pore of cytotoxic T cells and NK cells. The pore renders the cell permeable to small ions but not to proteins, and is therefore thought to cause cell death by osmotic effects. It is also possible that the large influx of Ca<sup>2+</sup> ions poisons the cell.

The effects of the MAC on the cell are dose dependent. Sublethal doses of MAC may ‘activate’ the cell and induce a protective response against further attack and even cell proliferation. Genes activated in this manner are known as RGCs (response genes to complement) and several have been described.

### Regulation of complement activation

Complement activation is an extremely powerful cytolytic mechanism that can be rapidly activated as a first line of defence against invasion by foreign organisms. It is also extremely effective in memory B-cell responses and antibody-dependent cytotoxicity. It can also swiftly remove potentially toxic immune complexes from the circulation. However, it is potentially extremely hazardous if randomly and uncontrollably activated, and there are therefore several inhibitory mechanisms in place to regulate this system (Box 7-10).

Activation of complement can be induced by many cell types via receptors that exist on their cell surfaces (see Boxes 7-9 and 7-10). Furthermore, some of the biological effects of complement are produced by the cleavage products of complement activation. For

## BOX 7-10 REGULATION OF COMPLEMENT ACTIVATION

Complement proteins comprise around 15% of total serum proteins, much of which is synthesized in the liver, although some local production occurs in the tissues. Inhibitory activities exist for both fluid phase-activated complement and for cell surface-bound complement. Regulation occurs at all stages of activation.

Soluble inhibitors include: factor H, factor HL1 and properdin (alternative pathway); carboxypeptidase N, C4-binding protein, C1q and C1INH (classical pathway); CFHR1, clusterin and vitronectin (terminal complex formation). Surface-bound inhibitors include: CR1, CR2, CR3, CR4, CR-Ig, CD46, CD55 and CD59. Most of these are expressed on leucocytes, particularly myeloid cells, while some, such as CD55 and 59, are expressed on some tissue cells such as renal and retinal pigment epithelium. CD59 is particularly widely expressed.

Some receptors for complement effector proteins also modulate the overall response, such as C3aR and C5aR, C5L2, C1qR and SIGNR (the last on dendritic cells and microglia). Cell surface sialic acid preferentially binds factor H to factor B on material such as mucin, important on many mucosal surfaces including the conjunctiva.

instance, C3a and C5a are potent chemoattractants and anaphylatoxins, and mediate early-phase responses in acute inflammation (see p. 382). Anaphylatoxins act directly via complement receptors on granulocytes, macrophages and mast cells to induce degranulation and the release of vasoactive mediators (Table 7-6).

### Complement activation is usually incomplete: implications for age-related macular degeneration

As shown in Box 7-9, the multistep complement cascade requires the correct conditions for activation of each step and, because of instability in many of the molecular intermediates, it frequently fails to proceed to formation of the full membrane attack complex (MAC), particularly if activated via the collectins. In addition, the process can be blocked by a range of inhibitors at many stages. What happens to these intermediates? Recently, it has been suggested that partial activation of complement may have a physiological role and that not all aspects of complement activation are harmful or even beneficial to the

**TABLE 7-6 Receptors for fragments of C3**

Receptor	Structure	Ligands	Cell distribution	Function
Type 1 complement receptor (CR1, CD35)	160-250 kDa; multiple CCPRs	C3b > C4b > iC3b	Mononuclear phagocytes, neutrophils, B and T cells, erythrocytes, eosinophils, fDCs	Phagocytosis Clearance of immune complexes Promotes dissociation of C3 convertases by acting as cofactor for cleavage of C3b, C4b
Type 2 complement receptor (CR2, CD21)	145 kDa; multiple CCPRs	C3d, C3dg > iC3b	B lymphocytes, fDCs, nasopharyngeal epithelium	Co-receptor for B-cell activation Trapping of antigens in germinal centres Receptor for EBV
Type 3 complement receptor (CR3, Mac-1, CD11bCD18)	Integrin, with 165-kDa $\alpha$ chain and 95-kDa $\beta_2$ chain	iC3b, ICAM-1; also binds microbes	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis Leukocyte adhesion to endothelium (via ICAM-1)
Type 4 complement receptor (CR4, p150,95, CD11cCD18)	Integrin, with 150-kDa $\alpha$ chain and 95-kDa $\beta_2$ chain	iC3b	Mononuclear phagocytes, neutrophils, NK cells	Phagocytosis, cell adhesion?

CCPRs, complement control protein repeats; EBV, Epstein–Barr virus; fDCs, follicular dendritic cells; ICAM-1, intercellular adhesion molecule 1.

(Table 12-8 from Abbas, A.K. *Cellular and Molecular Immunology* 7th Edition, p. 284, Elsevier, 2012)

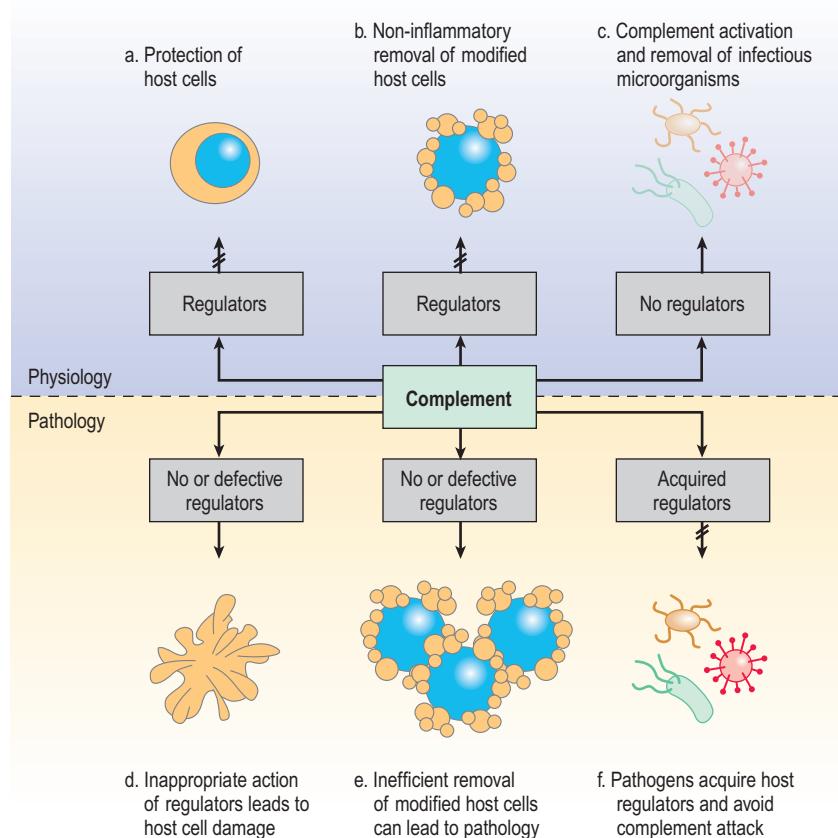
host overall. For instance, partially activated complement molecules may not cause lysis of the target cell; they may coat the cell and promote apoptosis; this is then followed by the silent (i.e. non-inflammatory) removal of the cell debris by scavenger macrophages. Such a mechanism may be occurring at some level of activity during the normal housekeeping actions of the resident macrophages. Indeed, it is likely to take place at sites where there is minimal cell turnover but where removal of cell debris is important, e.g. at the retinal pigment epithelium. Such a fine homeostatic mechanism may be susceptible to dysfunction: indeed, mutations in the complement inhibitory protein,

complement factor H, have been associated with a higher than normal risk of age-related macular degeneration. Factor H provides a check on the complement cascade at two critical points, C3 and C5 induction by their immediate precursors.

In contrast, defective complement activation may fail to properly clear opsonized pathogenic organisms, and continued cell damage may occur to the detriment of the host (Fig. 7-14).

### CELLULAR MECHANISMS OF TISSUE DAMAGE

Tissue damage in cell-mediated immune reactions may be induced by a variety of cell types including



**FIGURE 7-14** Complement is a double-edged sword for the host. It can either have beneficial effects as in the upper panel where it can protect viable cells (a), remove aged or dying cells (b) or remove infectious organisms (c). If these mechanisms fail, healthy cells can be damaged (d), dead cell debris can accumulate and cause inflammation (e) or pathogens can use complement to evade the immune system (f). In the eye, mechanism (d) is thought to account for diseases such as AMD due to genetic faulty complement proteins such as complement factor H. (From Zipfel and Skerka, 2009.)

macrophages, cytotoxic T cells and NK cells. In addition, macrophages are involved in the clearance of cell debris in acute inflammatory reactions and in antibody-dependent cytotoxicity.

### The delayed-type hypersensitivity reaction is the hallmark of cell-mediated immunity

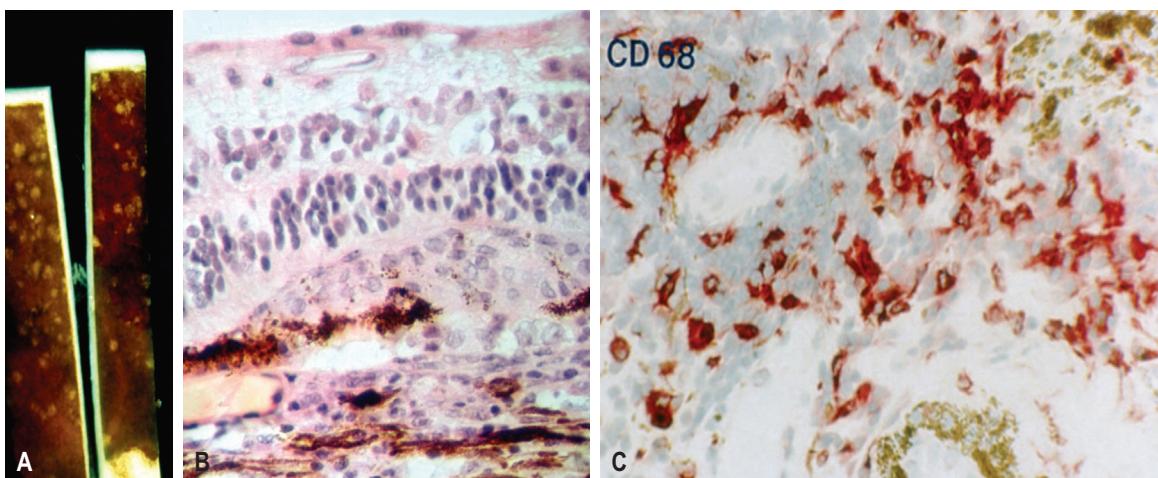
The delayed-type hypersensitivity reaction is a Th1-mediated reaction to foreign and/or autoantigen and is characterized by the presence of granuloma in the tissues. These accumulations of cells contain a central core of macrophages around a vessel with T cells in the surrounding area. Fibrinoid necrotic material may be present in the centre with giant cells (fused macrophages) and epithelioid cells. Such lesions are typical of reactions to mycobacteria and also occur in less well-defined diseases such as sarcoidosis. Similar microgranulomas are typical of sympathetic ophthalmia (Fig. 7-15) and indeed of several chronic posterior uveitis syndromes.

Such granulomas contain many types of T, B and macrophage-like cells, and also have a high content of dendritic cells. It is possible that these cell collections represent small extralymphatic lymphoid follicles where extensive antigen presentation is in progress. Once the antigen has been removed, the granuloma subsides.

### Inflammatory (M1-like) macrophages cause tissue damage; alternatively activated (M2-like) macrophages are more likely to promote healing

Macrophages cause tissue damage by release of reactive intermediate metabolic products and tissue proteases, express high levels of adhesion molecules and chemokine receptors such as CCR5 and CCR2 and are generally recruited *de novo* from circulating monocytes (see p. 404). However, not all macrophages behave in this manner. In the resting state, tissues contain resident macrophages that, in certain tissues, may have an immunoregulatory role (such as alveolar macrophages in the lung). In central nervous system tissue, including the retina, specialized resident tissue macrophages occur (microglia) (see Ch. 1, p. 52). These cells may be induced to express MHC class II antigen during inflammation but are still more likely to promote tissue homeostasis. Such cells express the mannose receptor, arginase and the fractalkine receptor (see p. 404).

Alternatively, activated macrophages are more likely to occur in parasitic infections under control by cytokines such as IL-3, IL-5 and IL-13 and promote a predominantly eosinophilic response. In addition, there is more likely to be fibrosis than tissue damage with angiogenesis.



**FIGURE 7-15** (A) Macroscopic view of a case of sympathetic ophthalmia showing Dalen–Fuchs granulomatous lesions as white ‘excrescences’; (B) macroscopic view of Dalen–Fuchs granuloma; (C) immunohistochemical view of activated (CD68+) myeloid cells in granuloma surrounding an occluded vessel. (Courtesy Prof. W.R. Lee.)

### Do cytotoxic T and NK cells induce cellular damage by making holes in the cell membrane?

Killing by cytotoxic T cells is a multistep process. Initial recognition and binding of a target cell (e.g. an infected or mutated cell) is followed by damage to the cell membrane. This lethal insult induces apoptosis with DNA fragmentation and lysis of the cell. The cytotoxic cell then disengages to attack another target cell.

Membrane damage takes the form of pore formation similar to that induced by the complement membrane attack complex (MAC; see above). In this case, however, it is induced by perforin, a protein released from lysosomes of antigen-activated Tc cells that polymerizes in the cell membrane to form a leaky pore (Fig. 7-16). Perforin is released in association with a granule proteoglycan and serine proteases (granzymes), which are also likely to be involved in the lytic process. However, cell death is not automatically caused by osmotic lysis. Instead prelytic DNA breakup occurs as a result of activation of the apoptosis genes and killing occurs via caspase-3 and -7. Therefore there are important differences between MAC-mediated cell lysis and Tc/NK cell killing.

Alternative mechanisms have also been shown for T-cell cytotoxicity. CD8<sup>+</sup> T cells may recognize non-peptide (e.g. lipid) antigen in the context of CD1. Thus cells containing bacteria are directly recognized by CD8<sup>+</sup> T cells and the bacterial lipid induces the

release of a novel enzyme, granzysin, which directly kills the intracellular bacteria.

### Organization of the immune system

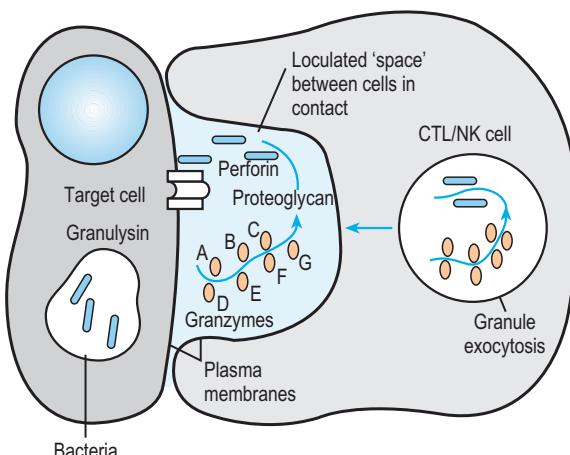
The immune system is designed to provide cells that can circulate freely through the tissues and organs of the body in such a way that they are readily available to mount a defence against foreign organisms at short notice. Immune cells are thus highly motile, normally quiescent cells travelling to and from the lymphoid organs; they can be readily activated if required.

Centralized antigen recognition mechanisms provide the most efficient means of rapid response because all the necessary requirements for cell activation can be concentrated at one site. This takes place in the lymphoid organs (primary and secondary lymphoid organs, SLOs). Some cells carry afferent information concerning possible breaches in the body's defences to the central lymphoid tissues (particularly dendritic cells and other APCs), while effector cells (T and B cells) remove the invading organism (or arrange for this to be done by other cells such as inflammatory macrophages) and restore tissue homeostasis. Trafficking of cells to and from the tissues to the lymphoid organs requires specific receptors on the circulating leucocytes and the vascular endothelium in each tissue (chemokine and adhesion molecule receptors).

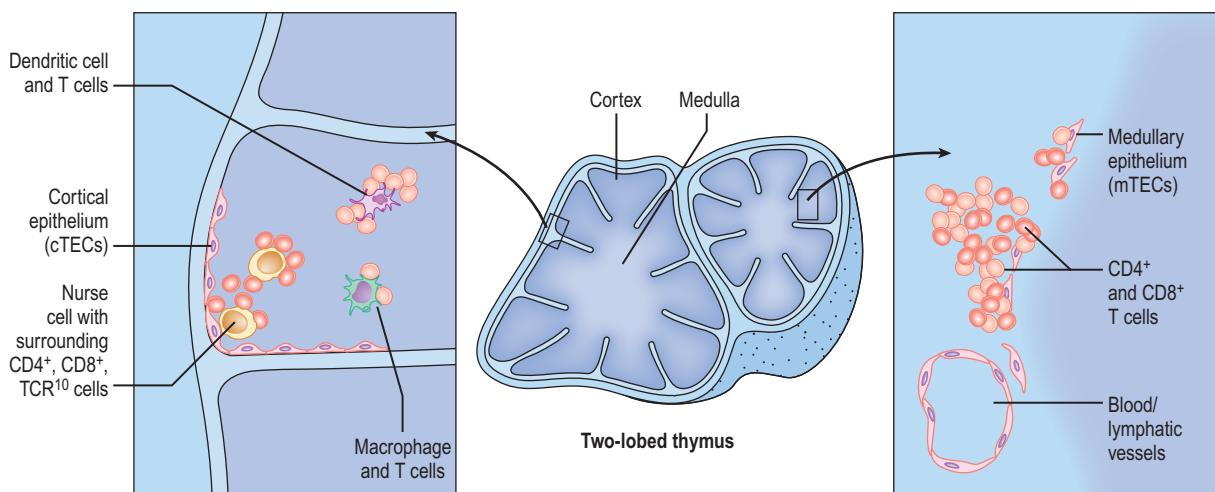
Tertiary outposts of local antigen presentation can be set up in sites where normal lymphoid tissues do not exist (such as the brain and eye) or where persistent antigen generates a chronic inflammatory response (frequent sites are liver and lung). Granulomas, the hallmark of chronic inflammation in the tissues usually forming at sites of small blood venules, represent one type of lymphoid cell accumulation where an antigen processing and presentation 'factory' might develop, but true tertiary lymphoid organs (TLOs) containing discrete T- and B-cell areas with a lymphatic as well as a blood circulation, may also develop *de novo* at sites of persistent chronic inflammation.

### FUNCTIONAL ANATOMY OF LYMPHOID ORGANS

During development, the primary sources of lymphomyeloid cells are the bone marrow and thymus (primary lymphoid organs). Ultimately, all cells derive from stem cells in the bone marrow. Stem cells are



**FIGURE 7-16** Perforin killing.



**FIGURE 7-17** The bi-lobed thymus. The central region shows the cortex and the medulla, while the cortical thymic epithelial cells (cTECs) and medullary medullary thymic epithelial cells (mTECs) are shown to left and right in greater detail.

poorly characterized and their existence is based mainly on evidence for cell differentiation, often from *in vitro* cell culture studies. Haematopoietic stem cells have few lineage markers (i.e. they are Lin<sup>-</sup>) that give rise to common myeloid progenitors and common lymphoid progenitors. These can be induced to give rise to T, B and NK/NKT cells (via IL-7) or myeloid cells (macrophage/dendritic cells/neutrophils (M-CSF, GM-CSF, G-CSF)). B cells mature in the peripheral/secondary lymphoid tissues, particularly Peyer's patches (lymphoid tissue structures in the wall of the small intestine). T-cell precursors from the bone marrow colonize the thymus, where they undergo selection and lineage differentiation before being distributed to the SLOs.

### Bone marrow stem cells produce all blood cells

Blood cells – including red cells and platelets, granulocytes, monocytes and dendritic cells – are produced in the bone marrow and released directly into the circulation. B cells are also released directly into the circulation and circulate between the lymphoid organs and blood.

The marrow is a loose spongy stromal network whose cells, together with local macrophages, release growth factors (cytokines, see above) that initiate differentiation of each cell type. IL-3, G-CSF, GM-CSF and M-CSF are particularly important. IL-1 and IL-6,

released by stromal marrow cells, also participate in T-cell maturation, while IL-7 promotes B-cell development. Stromal marrow cells are therefore essential to the survival of the host.

### The thymus regulates T-cell development and maturation

T-cell precursors enter the thymic cortex (Fig. 7-17). Here they interact with cortical thymic epithelial cells (cTECs or 'nurse' cells), which encapsulate them in large numbers. At this stage they are still immature cells expressing cell surface markers for Th and Tc cells (CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup>: the so-called double-positive cell; see Box 7-4 for explanation of CD numbers). After some time they are released from the nurse/cTEC cells and migrate through the cortex to the medulla, making contact with macrophages and dendritic cells as they go. Many T cells die during this process (clonal deletion and/or apoptosis) but selected cells differentiate to express one or other T-cell phenotypic marker (CD4 or CD8). Maturation in the medulla also involves contact with the medullary thymic epithelial cells (mTECs) and thymic dendritic cells, which express high levels of MHC class II antigen. The T cells then enter the blood vessels and migrate to the lymph nodes and spleen.

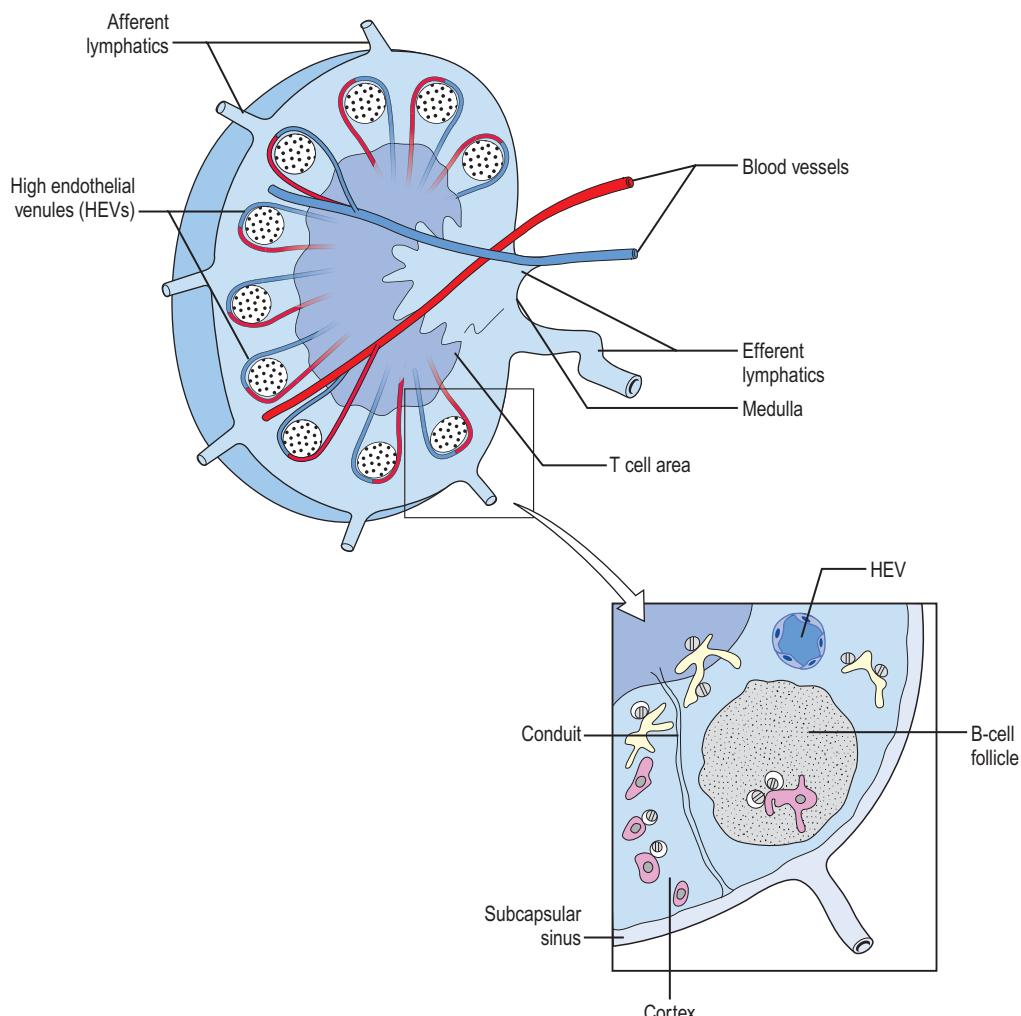
cTECs and mTECs arise from a common TEC progenitor probably located at the interface between the

cortex and medulla. mTECs are important in generation of Tregs as well as deletion of tissue-specific auto-reactive T cells through the autoimmune regulator (AIRE) gene. Defects in this gene causes a condition in humans known as the autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome (APECED) which includes a low-grade form of uveitis due to the failure to delete T cells reactive against IRBP (see p. 459). The medulla of the thymus contains several characteristic whorled bodies (Hassall's cor-

puscles) (Fig. 7-17), which are probably remnants of 'used' nurse epithelial cells. In the adult, the thymus involutes but some T-cell maturation continues into adult life, both in the thymic remnant and extrathymically.

### Lymph nodes are designed for antigen trapping

Lymphocytes and APCs from the tissues enter the cortex of the lymph node via the afferent lymphatics (Fig. 7-18) where they present antigen to T and B cells.



**FIGURE 7-18** Diagram of the lymph node. The T-cell area surrounds the B-cell follicle in the cortex; high endothelial venules (HEVs) are present in the T-cell area, mostly at the junction with the medulla. Conduits allow trafficking antigen-presenting cells to migrate into the node after entry through the subcapsular sinus.

Follicular dendritic cells (fDCs) in germinal centres of the lymph nodes and the spleen present antigen–antibody complexes to B cells in the B-cell areas, while conventional dendritic cells (cDCs) present antigenic peptides to T cells in the T-cell area. cDCs comprise two main types: migratory DCs (CD8 $\alpha$ -) carrying antigen to the lymph node and resident, ‘lymphoid’ CD8 $\alpha^+$  DCs which may sample soluble antigen in the afferent lymph or receive antigen from migratory DCs. The eye (and the brain) connect directly with cervical lymph nodes (see Chs 1 and 4), but lymphocytes and APCs from these tissues also find their way to the spleen through the aqueous veins.

Recent studies have indicated that lymph nodes are organized in chains, the more peripheral node receiving antigen from the tissues and activating (or not as appropriate; see section on tolerance, p. 441) T cells, which leave (egress) via the blood vessels and home to the site of inflammation to clear antigen-infected cells. Naive T cells (i.e. non-activated T cells) meanwhile egress through the efferent lymphatic and traffic to the next more central lymph node in the chain until they reach the thoracic duct and re-enter the circulation to begin another cycle of migration.

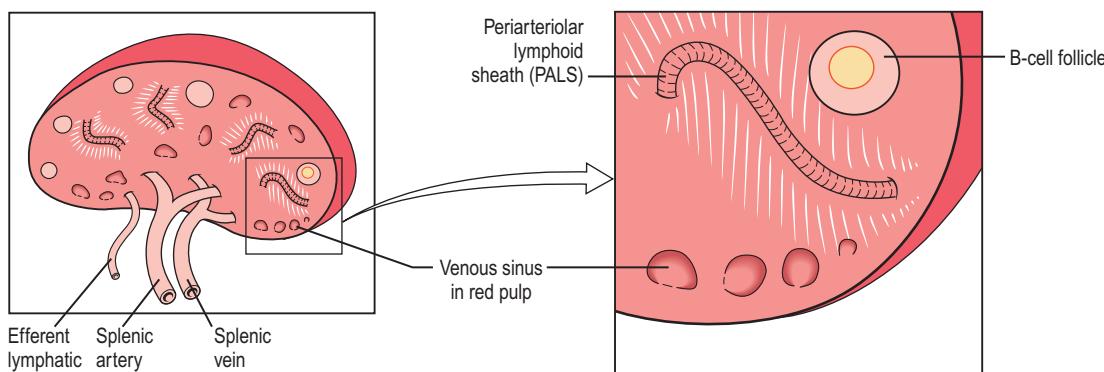
Antigen is carried in soluble form to the lymph node in blind-ended vascular lymphatics from the tissues, and also in cell-associated form inside APCs (dendritic cells and macrophages). APCs migrating from the tissues to the initial peripheral lymph nodes do not leave the node but present antigen to the LN

T and B cells. However, unactivated T and B cells circulate between the lymphatics and the bloodstream as indicated while activated T cells specifically home to sites of inflammation.

### The spleen receives antigen from all sources (lymphatics and blood)

The spleen is a central secondary lymphoid organ at the interface between the blood and lymphatic circulations. The bloodstream communicates with the lymphatic system at the thoracic duct where recirculating lymphocytes enter. The spleen receives antigen-primed APCs from all regions including those that may have bypassed their regional lymph node. For instance, APCs that encounter antigen within the anterior chamber of the eye may pass through the trabecular meshwork and aqueous veins (see Ch. 1, p. 24) to enter the conjunctival veins and eventually drain into the lungs and spleen. However, the spleen has no afferent lymphatics, i.e. it only receives lymphocytes from the bloodstream which are overwhelmingly naive, unactivated T cells.

The spleen is organized like a lymph node with the addition of the red pulp (red cell area) and its venous sinuses (Fig. 7-19). T-cell and B-cell areas are separated, with the T-cell areas (white pulp) being particularly large and aggregated around an arteriole (periarteriolar lymphoid sheath, or PALS) and surrounded by well-defined macrophage/dendritic cell zones (marginal zone).



**FIGURE 7-19** Diagram of the spleen. The white pulp contains the T-cell area surrounded by the B-cell follicles; the red pulp is situated closer to the medulla and contains the venous sinuses with large numbers of red cells and macrophages. There are no afferent lymphatics to the spleen, all cells entering into the spleen stroma via the bloodstream near the periarteriolar lymphatic sheath and leaving via efferent lymphatics.

### Tertiary lymphoid organs

TLOs (see [introduction](#) to this section, p. 412) develop around sites of persistent chronic inflammation, spurred on by lymphoid tissue inducer (LTi) cells, similar to those which establish the development of primary and secondary lymphoid tissues which secrete lymphotoxin  $\beta$  (LT $\beta$ ), the essential cytokine for differentiation of the stromal cells which permit the development of the lymphoid organs. Chemokines such as CXCL13 and cytokines such as IL-17 derived from the inflammatory process provide the conditions for the further differentiation of lymphoid vessels and the T- and B-cell separation ([Fig. 7-20](#)).

## THE MUCOSAL IMMUNE SYSTEM

Initial contact of the host with exogenous antigen takes place at surfaces such as skin and mucous membranes. The skin provides an effective physical barrier to microorganismal contact unless penetration is achieved, as with insect vectors and trauma. However, the mucous membranes are more easily breached by microorganisms and, not surprisingly, considerable lymphoid tissue is concentrated at these surfaces to deal with new antigens as they arrive. This is termed the mucosa-associated lymphoid tissue (MALT) and is specialized to some degree for each tissue, e.g. bronchus-associated lymphoid tissue (BALT), gut-associated lymphoid tissue (GALT) and conjunctiva-associated lymphoid tissue (CALT). In essence it comprises intraepithelial lymphocytes and APCs (especially dendritic cells) and aggregations of lymphoid tissue in the mucosal layers. These are best exemplified by the Peyer's patches of the gastrointestinal mucosa and the tonsils in the oropharynx. Regional specialization occurs in various compartments of the mucosal immune system with specific homing receptors ('addressins', adhesion molecules) directing movement of cells to different mucosal sites (see below) and like TLOs, are induced by innate lymphoid cells (ILCs) (see p. 382), such as LTis as well as a second set of ILCs which are NK-like IL-22 and (possibly) IL-17-secreting cells. In the lamina propria of the gut, CD4 $^+$  Th17 cells as well as CD4 $^+$  Treg cells abound, and are important cells balancing the response of the host to commensal versus pathogenic microorganisms in the microbiome (see p. 374). Similar

immune defence arrangements are likely to occur in all mucosal tissues but are most developed in the gut due to the constant exposure to microorganisms. Disturbance in this regulation accounts for many types of inflammatory bowel disease (IBD), some of which are complicated by systemic involvement including the eye, as in HLA-B27-associated acute anterior uveitis.

As indicated below, MALT is specially adapted for antigen capture and presentation to specific lymphocytes that 'home' to that tissue. In addition, exposure of the host to antigen via the MALT frequently leads to tolerance to that antigen by mechanisms that are as yet poorly understood but involve Tregs (see p. 380).

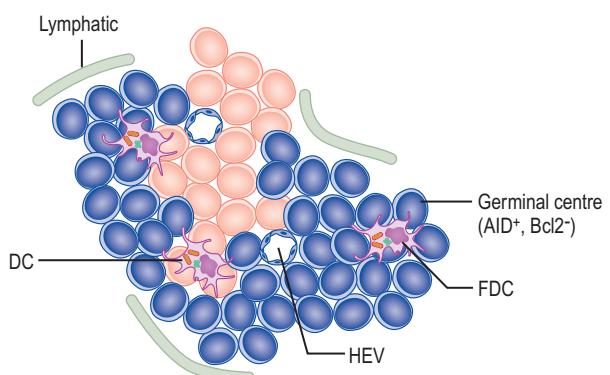
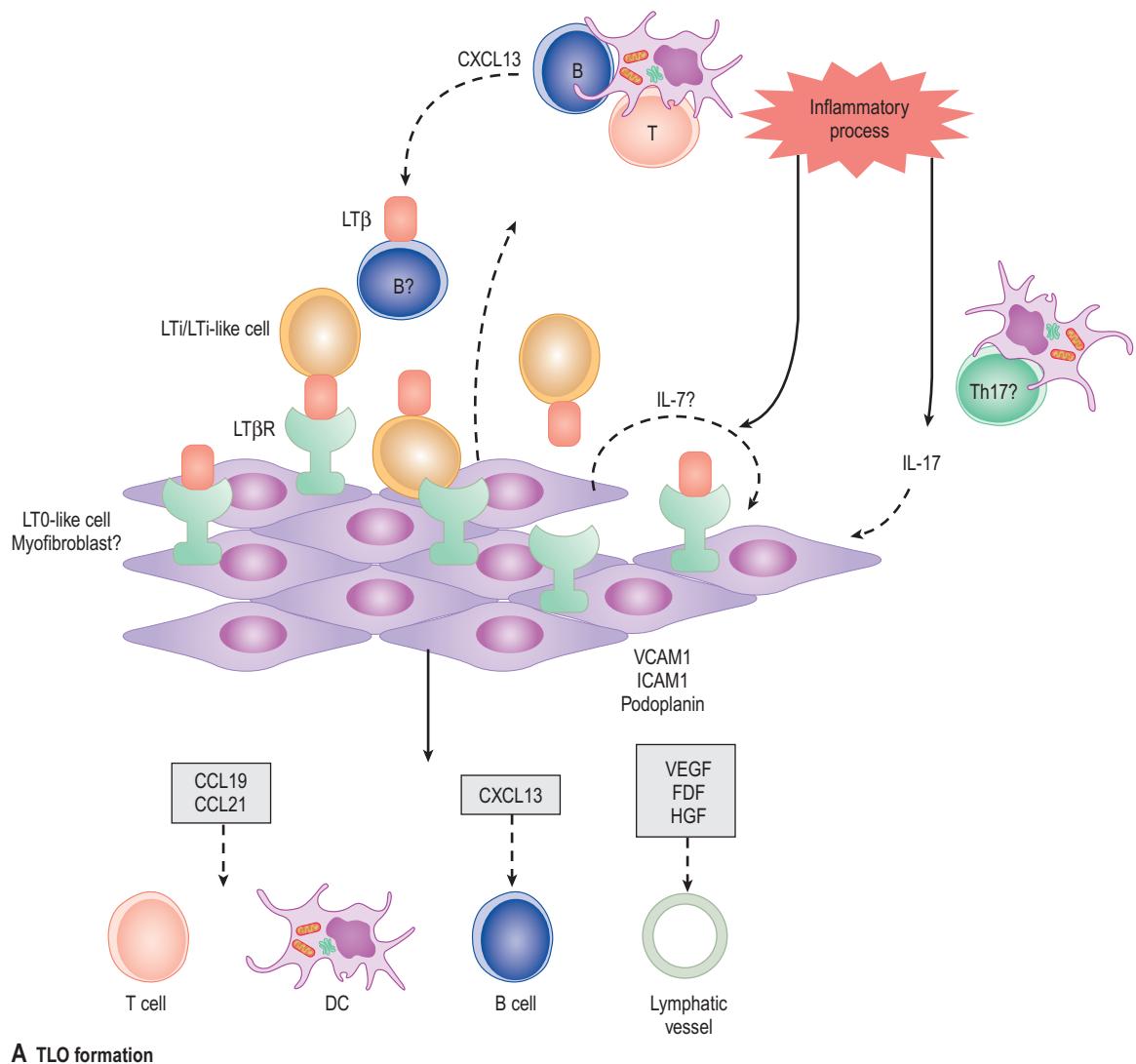
## THE IMMUNE SYSTEM AS A POLICE FORCE

The immune system can be regarded as a surveillance system constantly checking for intruders. Cells of the innate immune system, such as neutrophils and monocytes, undertake part of this function but at a relatively non-specific level. The adaptive immune system has cells that mediate this function in a much more targeted way. The afferent limb of the immune system uses dendritic cells in this role, while the messengers sent out to do the 'dirty work' are the T and B lymphocytes.

### Dendritic cells are the major surveillance cells of the afferent lymphoid system

Dendritic cells are derived from stem cell precursors in the bone marrow and proliferate/differentiate under the influence of cytokines such as GM-CSF, stem cell factor (Flt3 ligand) and TNF- $\alpha$ . They leave the bone marrow and circulate in various shapes and sizes (one of which is as very large 'veiled cells', like large floating jellyfish) in the bloodstream before entering the tissues, where they remain for a few days to weeks. In the skin, conjunctiva and peripheral corneal epithelium, they can be identified as Langerhans' cells; in the stroma of these tissues, they occur as migratory cDCs. They interact closely/intimately with tissue cells such as keratinocytes and epithelial cells. In the uveal tract, there is a rich network of dendritic cells in association with tissue macrophages (see Ch. 1, p. 28).

Dendritic cells from the tissues constitutively migrate in the afferent lymph to the lymph nodes and



**FIGURE 7-20** Tertiary lymphoid structures develop during chronic inflammation and take on a form similar to secondary lymphoid structures such as the lymph node and Peyer's patches. In a sense they are outposts of the lymphoid defence system but often do not have the full characteristics of the structured lymph node. However, they can develop germinal centres and afferent and efferent lymphatics. The granuloma has similarities but is different since it contains a central core of antigen with myeloid cells and a large cuff of T cells with some B cells. (From Neyt et al., 2012.)

spleen (migratory CD8 $\alpha$ -cDC) in response to appropriate stimulation (e.g. TNF- $\alpha$ ); they interact with T cells in the T-cell areas of these tissues (interdigitating dendritic cells). In contrast, cDCs which have directly arrived in the SLOs from the bloodstream without passing through other tissues, become resident CD8 $\alpha$ -cDCs and take up antigen from other APCs which have reached lymphoid organs and undergone apoptosis. Other specialized dendritic cells that possess Fc receptors and serve as antigen traps because they have the ability to bind immune complexes occupy the follicles in lymphoid tissue where they present antigen to B cells (follicular dendritic cells, fDCs). Dendritic cells do not leave the peripheral lymphoid tissue; they are short-lived cells with a half-life of a few days and their numbers are replenished from the bone marrow.

A third set of DCs, termed plasmacytoid DCs (pDCs, so named because they had some morphological similarities to plasma cells), arrive in the SLO from the blood and are programmed to constitutively produce INF- $\alpha$ , which is massively increased in response to viral infections. They may also be involved in tolerance induction (see p. 441).

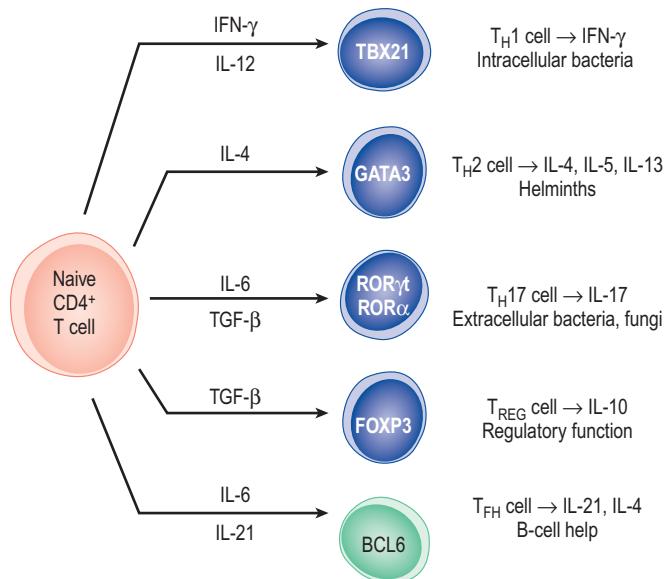
### Trafficking of cells to and from the lymphoid system depends on specific cell surface adhesion molecules

In contrast to DCs, T and B cells recirculate through the lymphoid organs many times. They do this by binding to specific regions of the lymphoid vasculature, the high endothelial venules (HEVs) (see Fig. 7-18), which are specialized for lymphocyte capture by the expression of specific adhesion molecules on their surface. About 25% of the lymphocytes passing through a lymph node will leave the bloodstream and any given naive lymphocyte can traverse each lymph node at least once a day. However, in practice, lymphocyte recirculation follows some patterns: B cells rarely recirculate,  $\gamma\delta$  T cells tend to exist in skin, and most other T lymphocytes recirculate preferentially through their lymph node of origin. If they fail to meet cognate antigen (antigen specific to that T cell's T-cell receptor), they remain unactivated naive cells and pass on to the next lymph node in the chain before finally reaching the thoracic duct. Activated lymphocytes, on the other hand, egress into the bloodstream and 'home' to (more likely are 'captured' by) HEV-like structures which develop in non-lymphoid

vascular endothelium at sites of inflammation (e.g. in retinal vessels during the active phase of retinal vasculitis; see Ch. 9). Activated T cells can circulate many hundreds or thousands of times before they are 'captured' (it has been estimated to take at least 16 hours from time of entry into the circulation to time of entry into the retina in the mouse). Receptors for specific adhesion molecules are reciprocally expressed on activated lymphocytes (and indeed on all classes of inflammatory cells during acute inflammation; see above), which assist in directing them to sites of tissue injury.

Specific adhesion molecules are expressed on HEV in lymph nodes, where they are involved in physiological lymphocyte recirculation. These molecules are therefore sometimes referred to as 'addressins' (one address only!). For each addressin there is a corresponding lymphocyte 'homing receptor'. Circulation through typical lymph nodes is mediated by L-selectin on lymphocytes, which binds to the adhesion molecule GlyCAM-1 on the HEV. In Peyer's patch lymph nodes, HEVs express MAdCAM (mucosal addressin cell adhesion molecule) and VCAM-1 (see Table 7-3), which are the ligands for  $\alpha_4\beta_7$  and  $\alpha_4\beta_1$  integrins of lymphocytic microvilli. It is likely that each tissue has specific addressins that direct the circulation of surveillance lymphocytes through that tissue; in particular, most mucosal lymphoid tissue (MALT, CALT; see above) has addressins that ensure adequate trafficking of lymphocytes through that tissue during health and disease.

Some cytokines and chemokines facilitate the interaction of T cells with activated endothelium, such as macrophage inflammatory protein-1 $\beta$  (CCL4), which has been shown, for instance, to be particularly effective in mediating CD8 $^+$  T-cell adhesion to the endothelium (see section on chemokines above). In addition, certain adhesion molecules are involved not only in endothelial cell interactions but also in mediating the close cell–cell contact required during antigen presentation (see below), particularly ICAM-1/LFA-1, ICAM-1/LFA-3, CD44 and its ligand cell surface hyaluronan, plus several others (see Table 7-3). In addition, chemokines and their receptors are intimately involved in the recirculation and trafficking of T and B lymphocytes and of APCs. In general, however, it is now clear that adhesion of leucocytes, either to



**FIGURE 7-21** The local microenvironment provides signals which permit differentiation of 'newborn' naïve T cells emerging from the thymus. The cytokines signal via specific receptors and activate transcription factors specific for each cell type which sets in motion a protein synthesis programme leading to production of factors specific to that newly differentiated T cell. BCL6, B-cell lymphoma 6; FOXP3, forkhead box protein 3; GATA3, GATA-binding factor 3; ROR $\alpha$ , retinoid-related orphan receptor- $\alpha$ ; ROR $\gamma$ t, retinoid-related orphan receptor- $\gamma$ t; TBX21, T-box transcription factor TBX21; T<sub>FH</sub>, follicular helper T; TGF- $\beta$ , transforming growth factor- $\beta$ ; T<sub>H</sub>1, T-helper-1; T<sub>H</sub>2, T-helper-2; T<sub>H</sub>17, T-helper-17; T<sub>REG</sub>, regulatory T. (From Craft, 2012.)

each other or to the endothelium, during normal recirculation or as part of inflammation, is a complex molecular and tissue-specific process.

### What turns a lymphocyte on?

Most circulating lymphocytes are resting. Most are 'naïve' cells (i.e. have not been activated by interaction with antigen) and have recently been released from the thymus or have passed through the peripheral lymphoid tissues. Others are memory T and B cells that can be very long-lived. Memory cells arise as a residual population after most of the antigen-specific T cells have died once their effector duties are completed (i.e. getting rid of the pathogen). Both CD4<sup>+</sup> and CD8<sup>+</sup> T memory cells occur, some residing long term in the tissues (e.g. the lung parenchyma) where they can rapidly respond to re-challenge with the antigen (e.g. influenza virus). These cells are known as effector memory T cells. Other memory cells recirculate through the lymphoid tissues and are known as central memory cells.

The main stimulus that activates T cells (both naïve and memory cells) is antigen presented in the form of peptide by professional APCs in the lymphoid tissue. Activation of the T cell induces it to release cytokines, particularly IL-2, which initiates the process of clonal

expansion. Depending on the local conditions and the cocktail or 'panel' of cytokines produced by the T cells and by stromal cells in the lymph nodes, further functional diversification is achieved by driving T-cell clonal expansion towards one of several subsets (Fig. 7-21).

Th1 cells, expanded by IL-2 and IFN- $\gamma$ , induce a delayed-type hypersensitivity (DTH) response (type IV hypersensitivity in the classic nomenclature) (Table 7-7), which involves considerable macrophage activation, granuloma formation and tissue damage. It should be noted, however, that memory T cells do not release IFN- $\gamma$ , while release of the chemokine RANTES (CCL5, see Table 7-5) by tissue cells appears to be important in the recruitment of memory T cells and macrophages to the site of granuloma formation. IL-12, IL-23 and IL-27 produced by macrophages and dendritic cells also play a major role in directing Th1/Th17 T-cell responses.

In contrast, Th2 cells, expanded by IL-2 and IL-4, provide B-cell 'help' and lead to the production of antibodies. In addition, IL-4 inhibits Th1 cell activation and the delayed-type hypersensitivity reaction. IL-10 and IL-13 produced locally in tissues and lymph nodes may condition APCs to induce a Th2 response. IL-2 and TGF- $\beta$  combine to promote Treg formation, while IL-6 and TGF- $\beta$  promote Th17 T cells.

TABLE 7-7 Immunopathology of tissue reactions		
Hypersensitivity response type	Immune effector process in tissue	Type of mechanism
I	Allergic reaction	Humoral (IgE)
II	Cytotoxicity	Humoral (IgG/M)*
III	Complement	Humoral (Ag/Ig)
IV	Macrophages	Cellular (T cells) <sup>†</sup>

\*Cytotoxicity in this process is antibody-dependent.

<sup>†</sup>Type IV hypersensitivity is the classically described delayed hypersensitivity response, mediated by macrophages and memory T cells.

Th1/Th17 (DTH) and Th2 cells (allergy, see p. 445) are directed by particular sets of cytokines which determine the nature of the pathological event. Classically, four types are described (types I, II, III and IV hypersensitivity; see Table 7-7) but mixed responses also occur. Th1/Th17 and Th2 cells may initiate reciprocal downregulation of either response via regulated cytokine release. Chemokines and chemokine receptor expression also play a major part in determining whether a Th1/Th17 or a Th2 response is induced (see p. 404).

### Where are memory cells found?

T effector cells that exit the lymph node lose their expression of L-selectin and cannot therefore recirculate through the lymph node. Most of them home to the site of inflammation to promote removal of the antigen and then die *in situ*; as indicated above, a small number of these cells have the potential to survive as memory cells, some of which re-enter the lymph–blood circuit as central memory cells while others remain long term in the tissues as effector memory cells. In contrast, memory B cells do not recirculate but continue to produce antibody of different isotype, with increasing affinity for the antigen, and release it into the blood via the efferent lymphatics. Memory cells for the mucosal lymphoid tissues have been shown to express a specific addressin, LPAM-1 (lymphocyte Peyer's patch adhesion molecule-1), or  $\alpha_4\beta_7$  integrin which binds to MAdCAM-1 in the GALT (see p. 416). IL-7, an essential cytokine for ontogeny of T cells in mice, is also required for induction of memory cells.

### What turns a lymphocyte off?

Most immune reactions do not persist, and it has been presumed that this is the result of effective removal of antigen. Indeed, the corollary is also probably true: that most chronic immunological diseases are the result of persistence of antigen in the tissues in a form that activates lymphocytes, i.e. on the surface of APCs. However, it is now recognized that switching off an immune response is an active process, involving a process known as activation-induced cell death (AICD) as well as a subset of T cells, i.e. T regulatory cells (Tregs).

**T regulatory cells.** Lymphocytes that are not exposed to their cognate antigen in the appropriate form (i.e. on the surface of APCs and in the presence of co-stimulatory support mechanisms) enter a state of anergy and eventually die out. The process of cell death or apoptosis during lymphocyte ontogeny is well established and is associated with the expression of specific ‘suicide’ or ‘death’ genes in the cell (see Box 4.4, p. 168).

Tregs also switch off inflammatory responses. Tregs are the most frequent cell type in the circulation and in the secondary lymphoid organs. Antigen-specific Tregs are induced as part of all-adaptive immune responses to exogenous and endogenous (including self) antigens but these expand with a delayed kinetic compared to effector cells, allowing T effector cells to finish clearing damage-inducing agents (DAMPs, see p. 389). Tregs are characterized by expression of high levels of the IL-2 receptor (CD25) and transcription factor FoxP3 which is essential for their function. Tregs are generated as in the thymus (natural or thymic Tregs) while inducible (iTregs) are also generated in the periphery from naive CD4<sup>+</sup> T cells after presentation of antigen by APCs. Antigen-specific iTregs are more effective than thymic or natural Tregs but are relatively non-specific in their action (i.e. although they are induced by specific antigen, they can suppress T cells which have been activated by other antigens), i.e. they have a broad-spectrum effect. IL-10 and TGF- $\beta$  are important immunosuppressive cytokines secreted by Tregs. They are present in mice and humans and currently there is considerable interest in developing methods to induce them to high levels so as to prevent a range of autoimmune diseases.

CD4<sup>+</sup> CD25<sup>+</sup> T regulatory cells are only one set of ‘suppressive’ T cells that exist; there are also other T cells that produce high levels of IL-10 (Tr1 cells) and TGF- $\beta$  (Th3) cells, some CD8 T regulatory cells, which may be involved in aspects of ocular immune privilege and require NK T cells to mediate their activity (see p. 390), as well as some recently described B regulatory cells (Bregs).

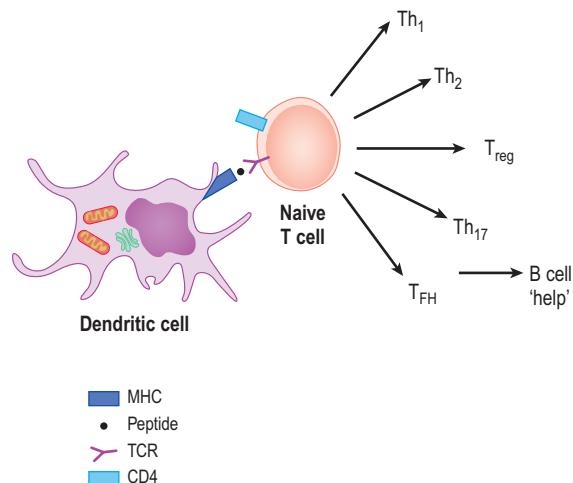
## Antigen recognition

The immune system recognizes antigen through molecular interaction with specific molecules, three of which are antibody (B-cell receptor, sIg), the TCR, and the MHC molecule. MHC molecules are highly polymorphic: i.e. although basically similar in structure, there are many different types (alleles) that occur in each individual, generated by small differences in nucleotide sequences in the MHC genes. Each TCR, antibody and MHC molecule has varying degrees of ligand-binding specificity, the MHC molecule being required to bind to a large variety of antigens, while antibody is specific for only one antigen. The interactions between antigen and antibody, and antigen and the TCR, have, however, many similarities.

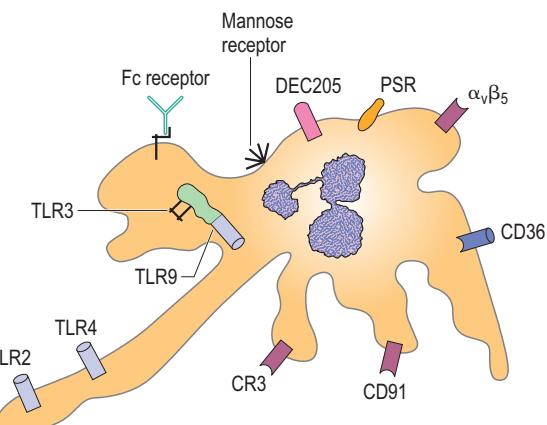
The great majority of immune responses are T-cell-dependent in that the final effector response, including B-cell responses, is achieved through an initial interaction between a resting T cell and an APC (Fig. 7-22).

## THE APC MAKES THE ANTIGEN RECOGNIZABLE

The function of the APC is to capture antigen and to process it into a form that can be recognized by the T cell. The immature (veiled) dendritic cell with its numerous cell surface receptors is ideally designed for this (Fig. 7-23). This it does by partially digesting the antigen into short peptides and complexing the peptide with MHC molecules. Classically, intracellular antigens are complexed with MHC class I molecules for presentation to cytotoxic (CD8<sup>+</sup>) T cells, while extracellular antigens are complexed with MHC class II molecules for presentation to helper (CD4<sup>+</sup>) T cells. However, ‘cross-presentation’ of antigen from either the extracellular or intracellular pathway to pathway can also occur, i.e. both intracellular and extracellular antigens can be presented on either MHC class I or MHC class II (see below).

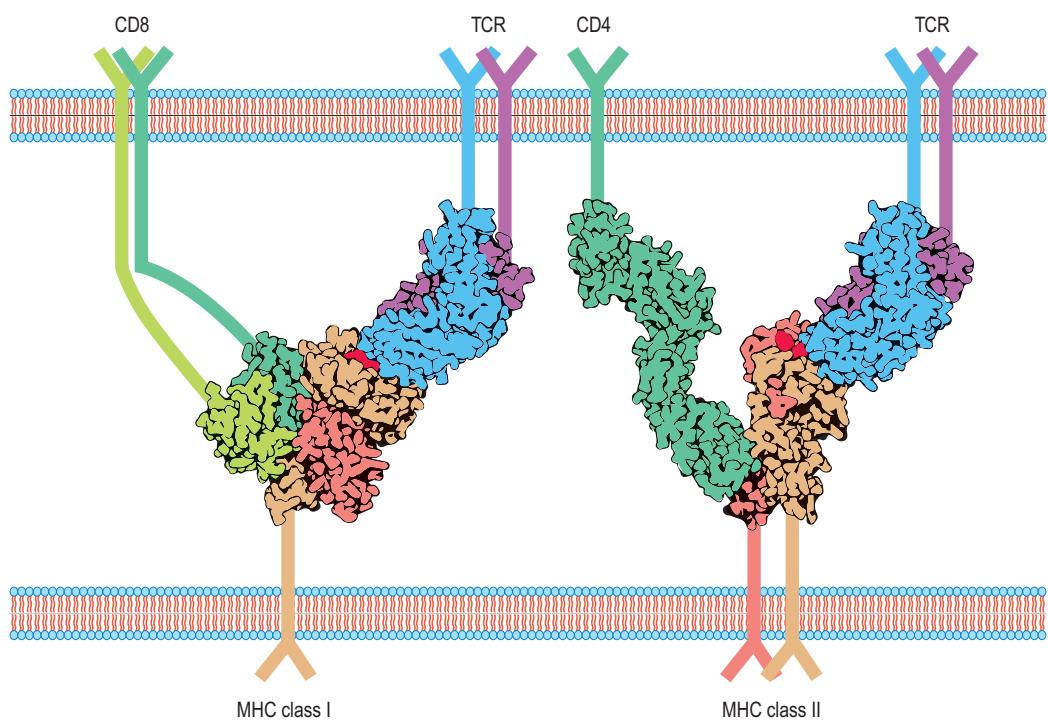


**FIGURE 7-22** Diagram of interaction between dendritic cells presenting peptide on the MHC molecule to a naive T cell prior to T-cell differentiation.



**FIGURE 7-23** Diagram of immature dendritic cell showing some cell surface receptors for innate immune responses. DEC205 (CD205); PSR, phosphatidylserine receptor;  $\alpha_5\beta_5$  integrin; TLR, Toll-like receptor.

Each APC can present hundreds of thousands of MHC-peptide complexes on its surface. In addition, some non-protein lipid antigens, such as those in mycobacterial cell walls, can be presented atypically on MHC class I-like molecules, the CD1 receptor. Most of the antigens expressed on an APC surface are self-antigens, which promote tolerance (see later). Immunogenic peptides occur at a level of 10–100 copies per cell.



**FIGURE 7-24** Models of antigen presentation. The antigen-presenting cell membrane (lower grey band) houses the MHC molecules and presents peptide (small red shapes) embedded in the MHC ‘groove’ to the T cell: the composite MHC-peptide ligand is bound by the T cell receptor (TCR) while the entire structure is stabilized by CD8 molecules on cytotoxic CD8 T cells and CD4 molecules on CD4 helper T cells. (Image courtesy of: <http://www.rcsb.org/pdb/101/motm.do?momID=63> From the World Wide Protein Data bank.)

### Processing by APCs is under tight cellular control

Intracellular antigens that are likely to be processed and bound to MHC class I mostly comprise host cytoplasmic and nuclear proteins, digested during normal ‘housekeeping’ cellular repair work mediated through autophagy. These do not normally induce immunogenic responses. However, intracellular foreign material such as viruses in infected cells is similarly processed via an ATP-dependent structure (the proteasome) and bound to the MHC class I molecule in the endoplasmic reticulum. The proteasome is a constituent of all cells and accepts ubiquinated peptides and proteins for degradation (see Ch. 4, eFig. 4-3); in APCs, the ‘immunoproteasome’ is specialized to degrade antigens into a set of overlapping peptides. For this purpose it contains two MHC-related proteins, LMP-1 and LMP-2. The peptide is bound specifically to a groove formed by the  $\alpha_1$  and  $\alpha_2$  helical domains of the MHC class I molecule, and is anchored at

specific sites to the  $\beta$ -pleated sheets that form the floor of the groove (Fig. 7-24 and eFig. 7-5).

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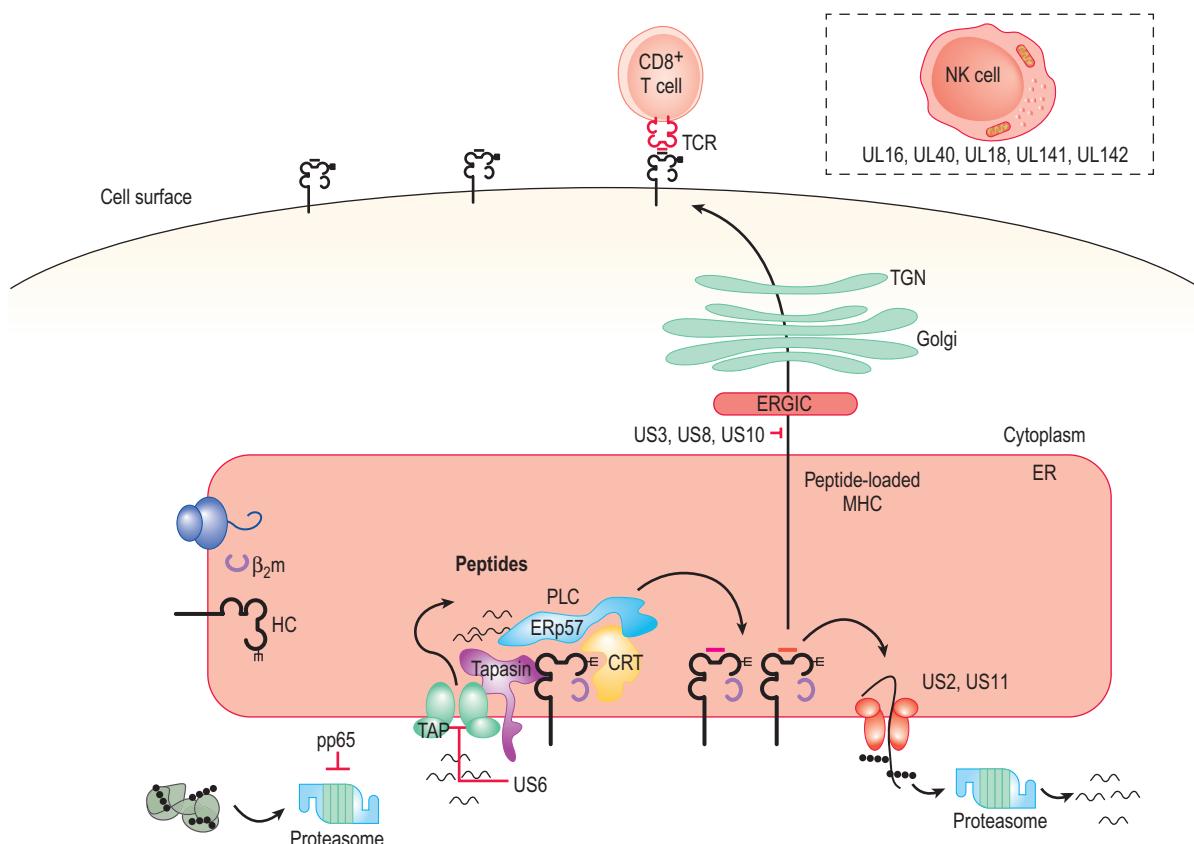
The CD8 and CD4 molecules help to stabilize the TCR:MHC peptide interaction in the MHC class I and MHC class II molecules, respectively. Defects in antigen processing can be associated with disease: for instance, uveitis in certain patients with the joint disease ankylosing spondylitis and severe rheumatoid arthritis is linked to abnormality in the genes coding for the proteasome and in *LMP-2* genes involved in MHC class I processing.

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This illustrates how each step in the antigen-processing pathway (eFig. 7-5) is essential in ensuring correct presentation of ‘normal’ non-immunogenic host peptides rather than peptides that may induce inflammatory disease. This also applies to the other

The proteasome is a general protein processing machine which degrades exogenous and endogenous molecules to peptides and transfers them to other organelles for further processing, in lysosomes finally to amino acids (see Ch. 4, eFig. 4-3). In antigen-presenting cells the proteasome prepares peptides for presentation via MHC molecules to T cells but this process can be disrupted, for instance by infectious organisms such as cytomegalovirus (CMV),

which produces proteins that prevent proper preparation of the peptide. This can occur at several stages, for instance by the viral proteins pp65 and US3-10. These act at various stages either prior to entry of the peptide into the endoplasmic reticulum or before entry of the composite MHC-peptide ligand into the secretory machinery of the cell for ultimate expression on the cell surface (eFig. 7-5).



**eFIGURE 7-5** The proteasome (see Ch. 4) is a 'peptide processing factory' which degrades proteins by ubiquitination and prepares the peptides for incorporation onto the MHC molecules for ultimate presentation on the cell surface. CMV interferes with class I MHC antigen presentation by generating 'immunoevasins' to block CD8 T-cell cytotoxicity. The immunoevasins are the U-labelled proteins and pp65. TCR, T-cell receptor; PLC, peptide-loading complex; TAP, transporter associated with antigen presentation; CRT, calreticulin.

genes that regulate the transport of peptides during their intracellular pathway, such as *TAP1* and *TAP2* (eFig. 7-5).

Loading of peptides via TAP on to the MHC class I molecule is regulated by a further membrane protein, tapasin, while loading of peptide on to the MHC class II molecule is ‘chaperoned’ by the invariant chains.

Although the processing and presentation of intracellular and extracellular antigens have many similarities in detail, there are some fundamental differences. For instance, the term APC refers to cells that express high levels of MHC class II antigen and ‘professionally’ process extracellular antigen. The term is thus restricted to a few cell types such as macrophages, B cells and dendritic cells. In contrast, the presentation of intracellular antigen to CD8<sup>+</sup> T cells on MHC class I has until recently been more difficult to explain. Most cells of the body express low levels of MHC class I and this can be upregulated in cells exposed to cytokines such as IL-1 and IFN-γ. However, effective antigen presentation requires co-stimulatory molecules, which are not normally present on healthy parenchymal cells. When antigen is presented in the absence of these molecules, the effect is to tolerize the T cell to the antigen.

This is fine for self-antigens but defeats the purpose when intracellular foreign antigen has to be dealt with. Ideally, foreign antigen should be presented when co-stimulatory molecules are also expressed in order to ensure an immunogenic, pro-inflammatory response mediated by activated T cells. This is achieved by cross-presentation. Tissue cells infected by virus (or other pathogens) are killed and APCs, especially dendritic cells, phagocytose dead or dying infected tissue cells; the viral or bacterial antigens are then processed within the dendritic cells for presentation on dendritic cell MHC class I antigen in the presence of co-stimulatory molecules (see below). Receptors involved in uptake of dead and dying cells (apoptotic cells) include α<sub>v</sub>β<sub>5</sub> integrin, CD91 and CD36 (scavenger receptors), CR3 (if coated with complement) and phosphatidylserine (see Ch. 4, p. 168, apoptosis pathways). Cross-presentation involves degradation of polypeptides in the proteasome (Fig. 7-25) and may be a major route for activation of CD8 T cells, as well as activation of CD4 T cells on MHC class II antigens. In this way CD8<sup>+</sup> cytotoxic T cells can be directly activated without requiring T-cell help and are

therefore armed to induce killing in other infected tissue cells. Thus, dendritic cells deal with both extracellular and intracellular foreign antigen while the response to self-antigen is minimized.

### Making the antigen presentable: the MHC molecule as candy wrapper

Presentation of the peptide by the MHC molecule to the TCR requires binding of the peptide to the groove in the MHC molecule and has some degree of specificity. Thus, only certain peptides will bind to each MHC allotype, of which there are many (see below). The specificity of the interaction between the MHC molecule and the peptide, however, is orders of magnitude less than that for the TCR, which is in turn considerably less specific than antigen–antibody binding. Preferential binding of certain peptides to different MHC molecules, however, is well established both for peptide length and sequence.

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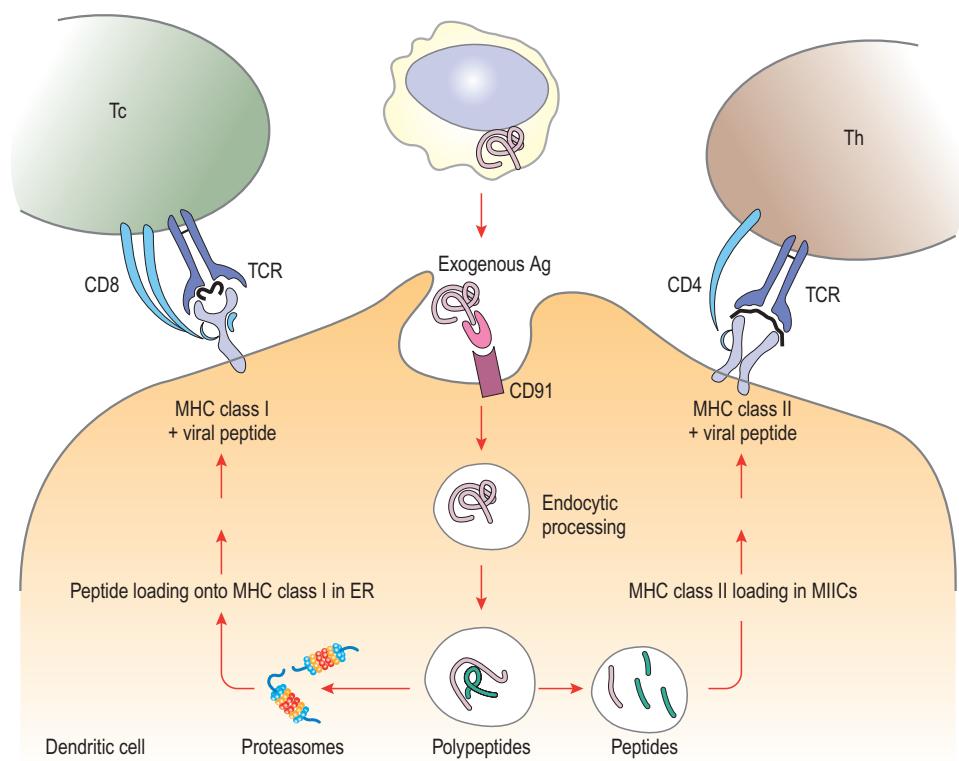
Differences also occur in binding of the TCR to MHC class I and II molecules. For instance, only a single MHC class I complex is required to present peptide to the CD8<sup>+</sup> T cell but two or more MHC class II–peptide complexes combine with two or more TCRs to activate CD4<sup>+</sup> T cells in the immunological synapse (see below). Binding of peptide to the TCR has many resemblances to antigen–antibody binding in terms of specificity but peptide binding affinities vary greatly for the TCR (see section on **T-cell activation**, p. 427). In the SLO, T cells repeatedly ‘sample’ peptides presented on MHC molecules on APCs by making brief interactions which become increasingly prolonged as the TCR:MHC complex ‘finds’ a perfect fit.

In terms of the peptide, this is recognized as ‘immunodominance’. For any given antigen, only one in 2000 peptides is likely to have sufficient binding affinity to make that perfect fit and initiate an immune response. Moreover, it is unclear what represents optimal affinity of the peptide for the receptor: both too little and too much can prevent T-cell activation. This variable peptide affinity is reflected in the TCR, which can bind reciprocally many different peptides: thus, specificity is much less rigorous for the T cell than the B cell. These many factors, i.e. immunodominance of peptides, TCR degeneracy with regard to

The size of the peptide is much more restrictive for MHC class I than for MHC class II. Class I molecules will only bind peptides that are 9 or 10 amino acids long, while class II molecules will bind those of any size, but usually between 16 mer and 30 mer. Class I peptides are anchored to a deep pocket in the groove at the second residue. The C-terminal end of the peptide is also bound to a shallower pocket, normally at the ninth residue. This leaves the peptide essentially free in the middle, apart from some less strong side-pocket binding; as a result, the peptide is usually arched in the middle with its amino acid residues projecting outwards to the TCR (Fig. 7-24). It is these exposed residues that determine the specificity of the reaction with the TCR.

In contrast, peptides in the class II groove overlie the sides of the molecule. Binding to the class II molecule, however, is restricted to the same number of residues as for class I (i.e. 9 or 10), except that they occupy the central portion of the peptide. Anchoring at the second residue is the strongest, frequently mediated by the non-polar residue proline. A further difference is that anchoring of the peptide to the class II molecule occurs at positions 2, 4, 6 and 9, with greater side-pocket interaction. This has the effect of straightening the peptide to take up a twisted, linear conformation.

These differences in class I and II peptide binding can be accounted for by specific amino acid sequences. In addition, the affinity of a peptide for a particular MHC allele is dependent on this sequence and is determined by how well each peptide fits into the pocket in the groove. Clearly, peptides composed of amino acids with large or highly charged side chains will have different requirements for binding than those with smaller amino acids, which might fit easily into the pocket.



**FIGURE 7-25** Cross-presentation. In the upper centre of the figure an endogenously produced antigen (Ag) that eventually ends up in cellular debris has been captured by a scavenger receptor (CD91) on a dendritic cell. The antigen is internalized into the endocytic system and polypeptide fragments are produced. Some of these fragments remain in the exogenous processing pathway (right) and are degraded to peptides that are loaded on to MHC class II in MIICs. These peptides are presented to CD4<sup>+</sup> T cells. However, some of the polypeptide fragments are released from the endosomes into the cytosol (left), where they are taken up by proteasomes and enter the endogenous processing pathway. These peptides are loaded on to MHC class I in the endoplasmic reticulum (ER) and are cross-presented to CD8<sup>+</sup> T cells. (From Mak and Saunders 2006, with permission from Elsevier.)

specificity and the need for repeated TCR activation as well as cross-linking more than one TCR in the immunological synapse before cell signalling can occur, all play a part in determining whether an immune response takes place and indeed what type of T cell is induced.

## The major histocompatibility system

As indicated above, most antigens initiate immune responses by being degraded inside the APC into small peptides, which are then complexed with MHC molecules and presented on the surface of the APC to T cells. So what are MHC molecules? MHC molecules were discovered by Peter Medawar during his studies on rejection of transplants between individuals of the

same species but different genetic make-up, and are now known to determine not only the susceptibility to graft rejection but also to infectious and autoimmune diseases generally.

## WHAT ARE MHC ANTIGENS AND WHERE ARE THEY FOUND?

The MHC gene cluster on human chromosome 6 is a region of highly polymorphic genes whose products are expressed on a variety of cells. Class I genes are termed HLA-A, -B and -C, while those of class II are known as HLA-D. Class III genes lie between the centromeric class II genes and the telomeric class I genes. MHC genes differ from typical germline genes because they are polymorphic and responsible for some of the traits that distinguish one individual from

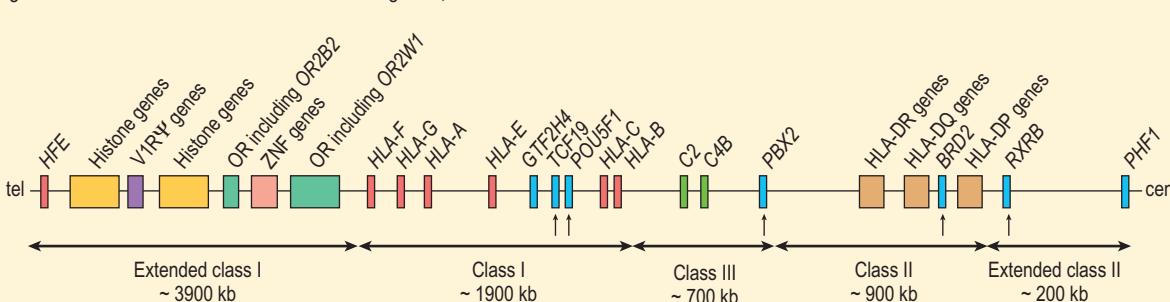
### BOX 7-11 EXTENDED MAP OF THE MHC GENES

The map of the MHC genes is constantly being revised as new information is added (see figure). The HLA-D genes are located towards the centromere and are separated from the class I genes by the complement protein genes (class III). Each of these regions is known to contain multiple genes, which are variably present within each haplotype. For instance the DR4 haplotype contains DRB1, DRB7, DRB8, DRB4 and DRB9; in contrast, the DR1 haplotype contains DRB1, DRB6 and DRB9.

Several associated genes are located close to the MHC genes. These include the TAP1 and TAP2 genes, the DMA

and DMB genes, and the LMP-2 and LMP-7 proteasome genes, all of which are involved in antigen processing (see p. 422e1). The TNF genes are also located in the class III region, as are the RAGE (receptor for advanced glycation end products) genes.

The human MHC gene map has been extended at both the class I region and the class II region to include several additional genes such as histone genes, genes for zinc finger proteins and the important regulatory gene retinoid X receptor gene (RXRB).



another. Most germline genes are by definition non-polymorphic (i.e. identical) within a species. The MHC gene cluster has now been extended to include more than 500 genes (Box 7-11) and has been fully characterized (see eFig. 7-6).

(allele = specific genetic difference belonging to one individual) and have led to subtype identification even within alleles.

Association of allotypes with disease is now performed in genome-wide association studies (GWAS) (see Ch. 3, pp. 143–145), which includes the MHC genes. The long-standing association, for instance, of certain HLA-B27 haplotypes with ankylosing spondylitis and uveitis has now been extended to include some of the genes involved in trimming of the processed peptides for binding to the HLA-B27 peptide groove: these are the *ERAP1* and *ERAP2* (endoplasmic reticulum aminopeptidase) genes, which show haplotype susceptibility at least for the spondylitis component of the disease. Different MHC protein alleles differ in their ability to bind and present different antigenic determinants, and this is probably the basis of each individual's susceptibility to disease. Studies of the association of disease susceptibility and specific HLA alleles have revealed many interesting linkages (see eFig. 7-6).

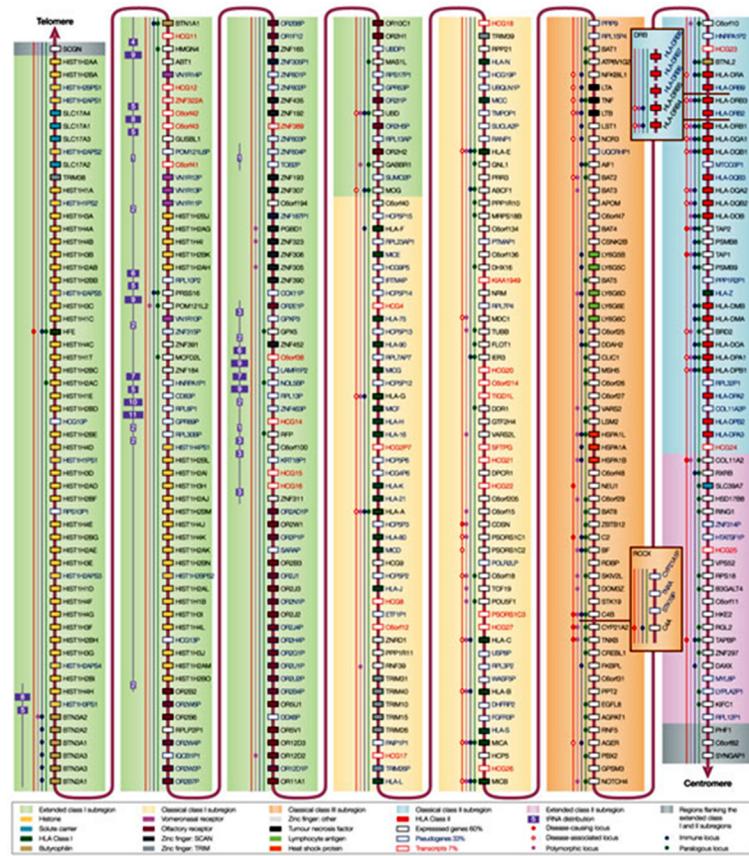
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As indicated above, MHC class I genes were discovered during studies in inbred mice of the genetic control of transplantation rejection phenomena. There are now many HLA class I genes, some with novel functions: for instance, HLA-G genes are expressed in the placenta and may have a role in protecting the fetus from attack by maternal NK cells. MHC class II genes were discovered later in analysis of mixed leucocyte reactions (MLR) in mice, a test that is the basis of tissue typing. The polymorphisms (genetic differences) in MHC gene products that characterize each individual are the antigens responsible for inducing graft rejection, and in humans were originally discovered in pregnancy sera and blood transfusion samples by careful documentation of antibody responses for each allotype (allotype = different type of individual). Currently, direct gene sequencing techniques are used to identify specific alleles

Most disease associations have, however, been detected in MHC class I genes. In particular,

The gene map of the extended major histocompatibility complex (xMHC) is shown from telomere (left) to centromere (right) on the short arm of chromosome 6 I (eFig. 7-6). The five colour-coded sub-regions making up the xMHC span about 7.6 Mb and are defined as: the extended class I sub-region (green block; *HIST1H2AA* to *MOG*; 3.9 Mb), the classical class I sub-region (yellow block; *C6orf40* to *MICB*; 1.9 Mb), the classical class III sub-region (orange block; *PPIP9* to *NOTCH4*; 0.7 Mb), the classical class II sub-region (blue block; *C6orf10* to *HCG24*; 0.9 Mb) and the extended class II sub-region (pink block; *COL11A2* to *RPL12P1*; 0.2 Mb). Regions that flank the extended class

I and II sub-regions are shown as grey blocks. Insets denote the hypervariable RCCX and DRB regions. Numbers and positions of tRNA genes are represented by indigo bars, the length of which is proportional to the gene number between other loci. Vertical lines connect the two main groupings of tRNA genes of 1.6 Mb and 0.5 Mb of the sequence (separated by 0.6 Mb). Circles to the left of each locus indicate disease status, polymorphism, immune status and paralogy as described in the text. The gene map of the xMHC is also available as a poster and online (<http://www.nature.com/nrg/journal/v5/n12/poster/MHCmap>).

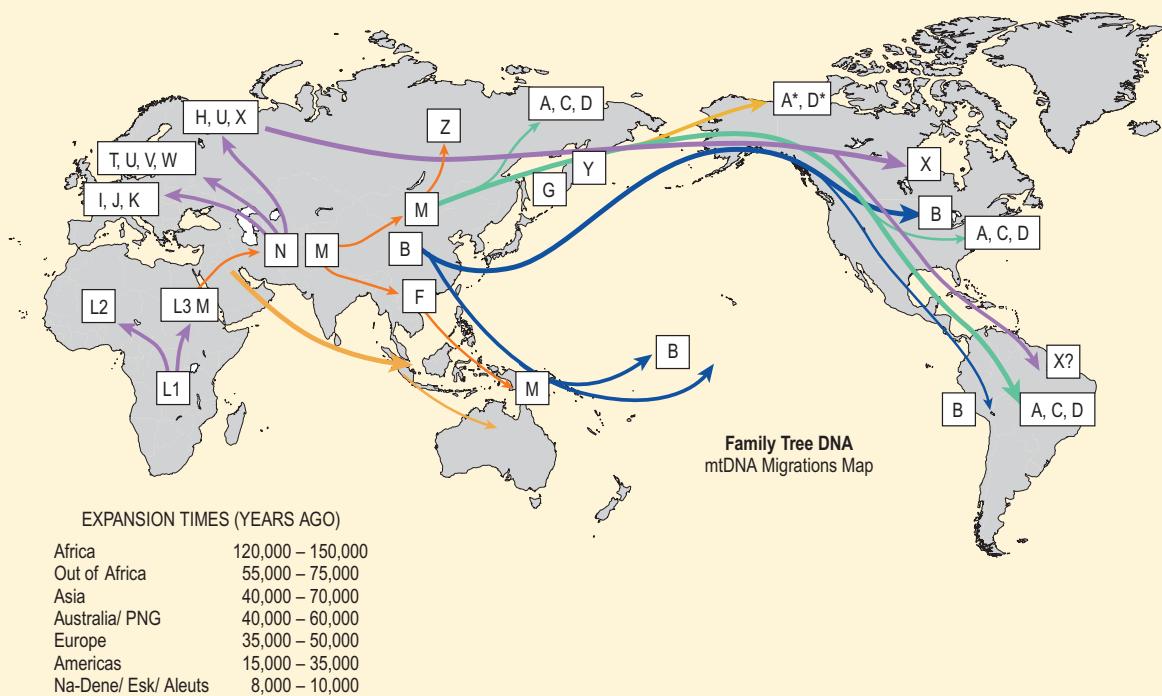


**eFIGURE 7-6** A map of the extended MHC (xMHC) gene region in man. The different regions are colour coded as in the key. Some specific regions are expanded in the inset boxes. (From Horton et al., 2004.)

### BOX 7-12 TRACKING MAP FOR MITOCHONDRIAL DNA

A haplotype is a set of allelic differences (i.e. genes) inherited from a single parent, and has been used in anthropological studies to track the migration of humans over hundreds of thousands of years from the first migration of *Homo sapiens*

out of Africa (see below a tracking map for mitochondrial DNA – further information is available through the link given).



<http://mathildasanthropologyblog.wordpress.com/2008/06/16/mitochondrial-dna-haplotypes-for-dummies/>

the HLA-B27/ankylosing spondylitis/enteric infection/uveitis link is long established. In addition the HLA-B51, but not B52, is linked with Behcet's disease in the Middle East and Japan. Perhaps the strongest known association of any human disease (>90%) is between HLA-A29 and a rare form of endogenous posterior uveitis, birdshot retinochoroidopathy. MHC mapping has greatly informed disease susceptibility among different population groups; and indeed haplotype mapping has allowed the migration of humans during the thousands of years that *Homo sapiens* has populated the earth to be tracked in a similar way (Box 7-12).

### ORGANIZATION OF THE MHC GENES IN THE GENOME

The MHC genes are located on chromosome 6 (see Box 7-11), while the non-polymorphic component of

the class I molecule  $\beta_2$ -microglobulin is on chromosome 15. The MHC region is very large, occupying about 4000 kilobases – as large as the entire genome of certain bacteria.

The class II genes, placed near the centromere, are commonly involved in crossing-over during meiosis. Unlike non-polymorphic genes, both alleles are expressed. Furthermore, there are two or three functioning  $\beta$  chain genes in class II, each of which can combine with the  $\alpha$  chain. This allows some class II alleles to be expressed in more than one allelic form on the same cell. Thus, a heterozygous individual expresses six different class I alleles (two HLA-A, HLA-B and HLA-C from each parent) as separate molecules on each cell. In contrast, for class II, many more than six heterodimers can be inherited (commonly there are 10–20 different class II genes per cell) and

this permits a large range of peptides to be bound on each cell. Furthermore, each MHC molecule can bind many different peptides with differing affinities (see above).

As for other immune genes that involve recombination events such as the immunoglobulin and TCR genes, there is coordinate expression of the HLA genes, e.g. class I on chromosome 6 and  $\beta_2$ -microglobulin on chromosome 13 must be simultaneously activated, and equally so the class II genes during transcription of HLA-DP, -DQ and -DR.

## REGULATION AND TRANSCRIPTION OF THE MHC GENES

Regulatory elements that lie 5' upstream of the MHC class I  $\beta_2$ -microglobulin and MHC class II genes, plus other essential genes such as the invariant chain, tapasin genes, *ERAP1* and *ERAP2* genes and several others, coordinate the expression of MHC genes. These elements include the S, X, Y box and the complex is known as the class II transactivator (CIITA) gene, which regulates MHC class I and II. Mutations in these genes can cause immunodeficiency syndromes.

Cytokines modulate the rate of constitutive transcription and expression of MHC genes. Class I expression is increased by  $\alpha$ ,  $\beta$  and  $\gamma$  interferons, and induction varies with cell type. IFN- $\gamma$  also alters transcription of class II: in macrophages, endothelial cells and some parenchymal cells the change is upwards, but in B cells it is decreased. In contrast, IL-4 has the opposite effect on class II expression of B cells. Some cells, such as neuronal cells, do not respond at all to IFN- $\gamma$ . The interactive responses of immune and tissue cells profoundly affect the final nature and character of the immune response and can convert a tissue-destructive process to a protective response or, depending on the tissue, to an allergic response (see below).

The genetic control of MHC expression and function is also controlled by other genes such as the *TAP1* and *TAP2* genes, which regulate the entry of peptides into the endoplasmic reticulum, the invariant chain gene that regulates stability of the class II complex, the *ERAP* genes that perform final trimming of the peptide in the endoplasmic reticulum and the cal-

nexin gene that regulates the binding of the peptide to MHC class I.

## T-cell activation

Any molecule, large or small, can act as an antigen but only macromolecules can activate lymphocytes and act as immunogens. Small molecules may activate lymphocytes if they are bound to a larger molecule; in this situation the small molecule is called a hapten.

Therefore, although peptides may initiate an immune response, there are several constraints on the induction of a response. For instance, there is a minimum size of peptide for an effective response, which in the case of MHC class I responses is precisely nine amino acids, and between 12 and 30 for class II responses. More importantly, the precise interaction between the T-cell receptor (TCR) and the MHC-peptide complex is under the control of genes regulating both sides of this interaction. The TCR genes have a greater role in determining the specificity of the response than the MHC genes.

## THE TCR AND ANTIGEN BINDING

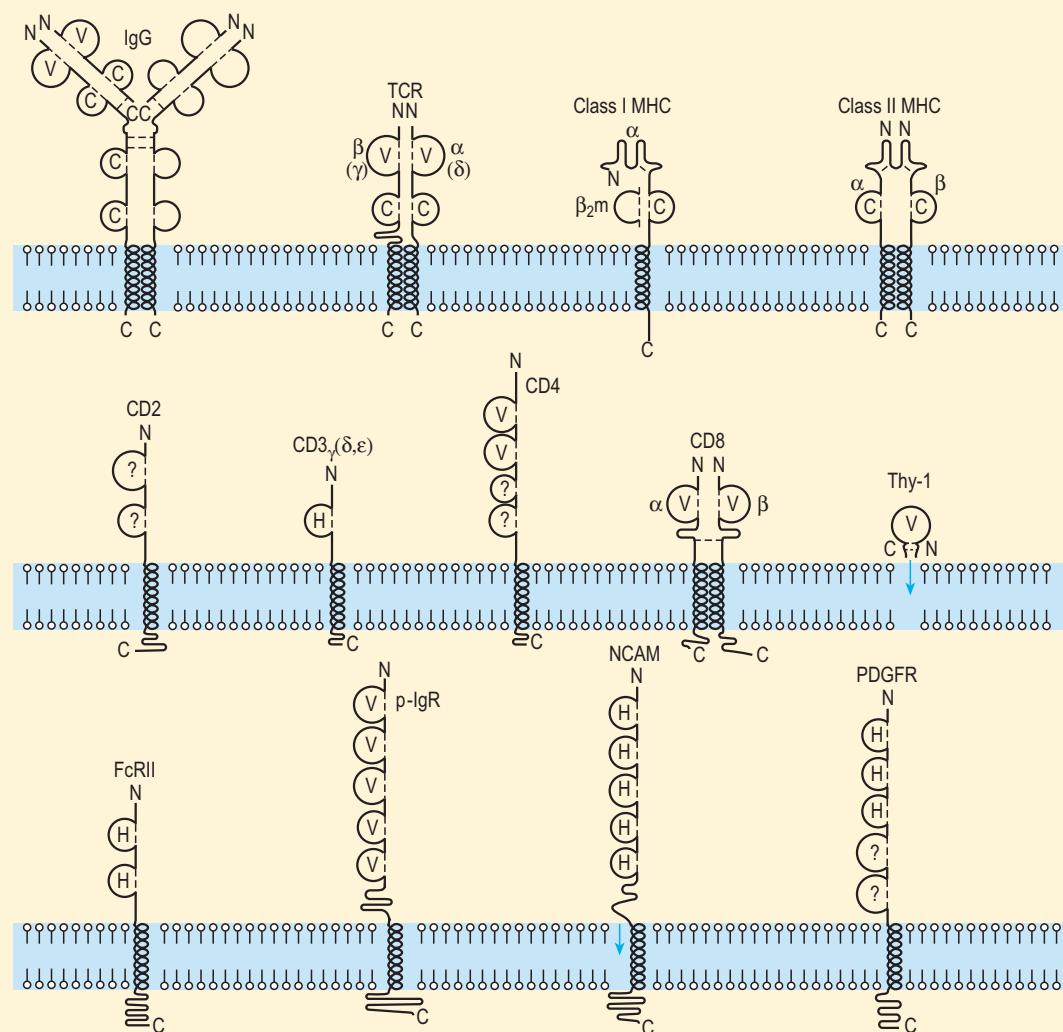
Processing of antigens to peptides in the APC may potentially yield a large number of immunogenic peptides. However, only a small number of these peptides will activate lymphocytes and only one will be specific for a particular cell. This is determined partly by the nature of the antigen but more importantly by the 'TCR repertoire', i.e. the potential range of different TCRs that exist to deal with very large numbers of possible antigens. The immune system has developed a mechanism to deal with this problem, which involves the use of multiple germline genes, each of which undergoes somatic rearrangement on challenge with antigen. The same basic mechanism is used by B cells in their production of antigen-specific antibodies (see below). This is known as genetic recombination and is under the control of enzymes (recombinases), products of specific genes known as recombination activation genes (*RAG-1* and *RAG-2*) which are expressed only in lymphocytes.

TCRs are members of the immunoglobulin gene superfamily (Box 7-13) and are dimeric proteins composed of an  $\alpha$  and a  $\beta$  chain which possess V (variable), D (diverse) and C (constant) regions as for

**BOX 7-13 IMMUNOGLOBULIN SUPERFAMILY OF PROTEINS**

The immunoglobulin superfamily is characterized by the common structural motif, the immunoglobulin domain. This domain may occur singly in small molecules such as Thy-1

or multiply in both chains of dimers such as the T-cell receptor.



immunoglobulin molecules (Figs 7-26 and 7-27). The V region contains the antigen-binding site and interacts with the peptide–MHC complex. At least 20 families of  $V\alpha$  chains are recognized and a similar number of  $V\beta$  families are also known. Several V genes (2–10) exist within each family. A single antigen binds to a single TCR and stimulates antigen-specific clonal expansion. However, as indicated above, several antigens have the

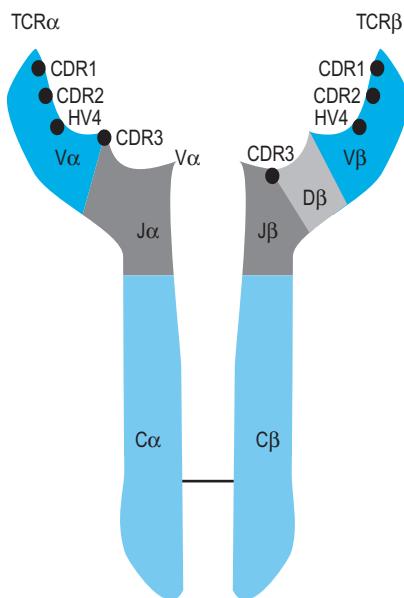
potential to bind to the same TCR, each with different affinities, unlike antigen–antibody interactions.

Databases holding germline gene sequences as a reference resource have been set up, such as the International Immunogenetics information system (IMGT) linked through the *Ensembl* database.

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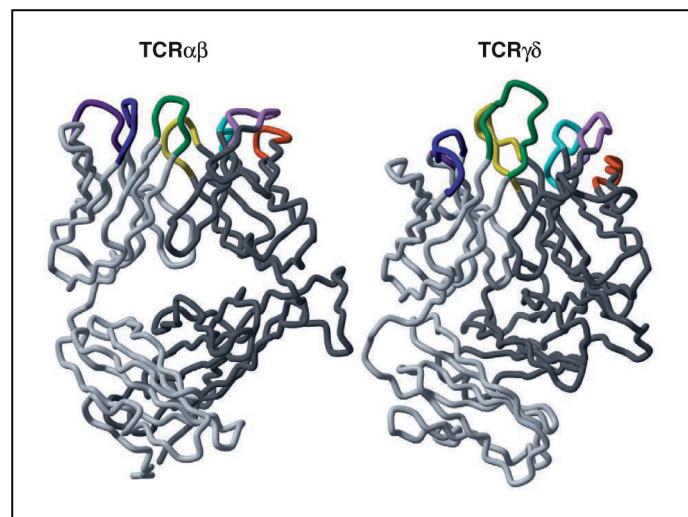
Information on specific TCR and immunoglobulin gene segment data can be obtained through the <http://www.ensembl.org/index.html> database which gives full instructions on how to use the protein ‘gene build’ programs. Gene building for TCRs and immunoglobulins is somewhat specialized due to the recombination events which take place between specific gene segments. This can be investigated using the link to the International Immunogenetics information system (IMGT) (<http://www.imgt.org/>) which can identify the locus on the genome showing the gene segment frequency for the specific Ig sequence of interest, e.g. the human IGH (immunoglobulin heavy chain) locus with specificity for herpes simplex virus type 1 (as of July 2013 there were 4IGHV group, 4IGHJ group, and 4IGHD group TCR gene segments on the IGH locus).



**FIGURE 7-26** Correspondence of TCR hypervariable regions to TCR gene segments. In this schematic example, the areas of the TCR $\alpha$  and TCR $\beta$  proteins derived from the indicated gene segments are shown in different colours. The CDR1, CDR2 and HV4 hypervariable regions are clustered in the variable domains of each chain, while the CDR3 region encompasses the VJ joint in the TCR $\alpha$  chain and the DJ joint in the TCR $\beta$  chain. (From Mak and Saunders 2006, with permission from Elsevier.)

At present there are many allelic variations in all four gene segments, C, D, J and V, for each of the  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  TCR chains, totalling over 200 genes. There are many thousands of possible combinations of the V, J and C genes, which increases the diversity of TCR $\alpha\beta$ -binding capacity enormously. A single V gene encodes a sequence in the TCR, a ‘complementarity determining region’ (CDR) (Fig. 7-26), which binds to that region on the antigenic protein corresponding to the peptide–MHC complex that binds to the TCR. This region of the antigenic protein is known as an epitope. Each protein may contain several epitopes that interact with different TCRs and produce different responses *in vivo* (similar epitopes exist for CDRs on antibodies; see below). Some epitope may even induce anergy in a specific clone of T cells. TCR gene loci to a wide range of antigens from many species can be identified using the database programs.

Immunization with a multideterminant antigen (i.e. an antigen that has several epitopes, which accounts for most protein antigens) will therefore lead to a polyclonal T-cell response in which several T-cell clones are variably activated by each determinant depending on its immunogenicity. Usually one or two epitopes on a molecule are immunodominant.



**FIGURE 7-27** Structures of TCR $\alpha\beta$  and TCR $\gamma\delta$ . Crystal structures showing the carbon backbone of TCR $\alpha\beta$  and TCR $\gamma\delta$  proteins; TCR $\alpha$  and  $\delta$  chains are light grey, TCR $\beta$  and  $\gamma$  chains are dark grey. For the  $\alpha$  and  $\delta$  chains, CDR1 is dark blue, CD2 is magenta and CDR3 is green. For the  $\beta$  and  $\gamma$  chains CDR1 is turquoise, CDR2 is pink, CDR3 is yellow and HV4 is orange. (From Mak and Saunders 2006, with permission from Elsevier.)

Epitope mapping of proteins is therefore possible. It has been shown that only about 30% of most proteins contain sequences that are recognized by lymphocytes. For certain autoantigens, such as retinal S antigen (implicated in the pathogenesis of uveitis, see below), various parts of the molecule have been mapped as regions which bind antibody, regions which are immunogenic (stimulates T cells *in vitro*), and regions which are pathogenic *in vivo* (induces autoimmune uveoretinitis in experimental animals) (peptide band on amino acids from 280 to 364). These sites are different from the rhodopsin-binding sites on S antigen, also known as arrestin (Fig. 7-28), and may represent cryptic epitopes, i.e. epitopes that are revealed only when the molecule is partly degraded.

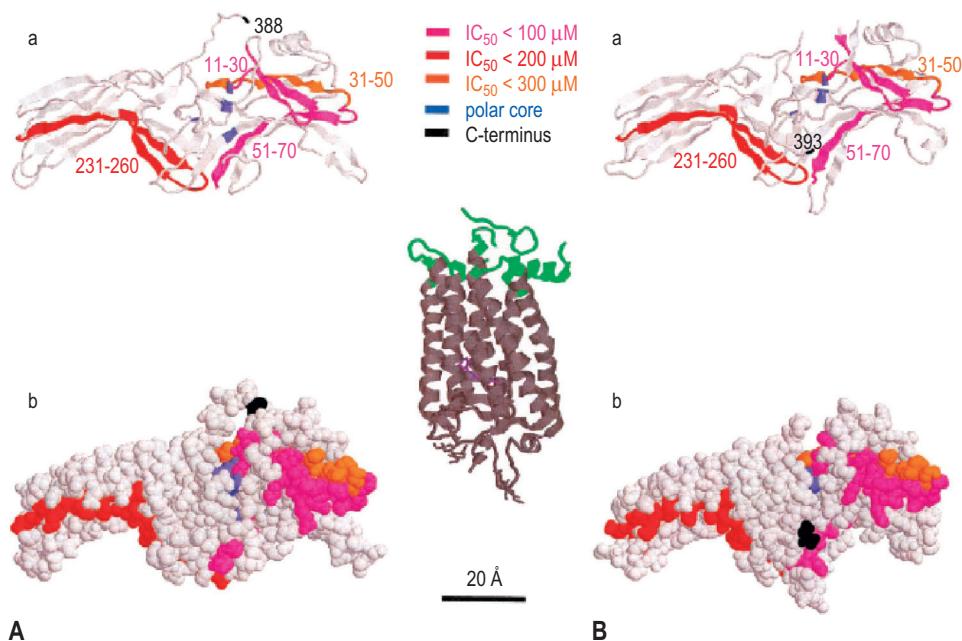
Conversely, preferential usage of certain clones of T cells with particular V $\alpha$  and/or V $\beta$  sequences is recognized in certain diseases, such as rheumatoid arthritis and multiple sclerosis, and has also been described in non-infectious uveitis in humans, where

the (?auto)antigen is suspected but not known. Varicella zoster and *Toxoplasma*-specific V $\alpha$  and V $\beta$  TCRs have been identified in samples from patients with infectious uveitis. Thus, investigation of TCR sequences on these cells may provide clues to the nature of possible autoantigens.

Regulation of TCR gene expression is under the control of several 'gene enhancers' such as activating transcription factor (ATF), cAMP-responsive element binding protein (CREB), T-cell factor (Tcf-1) and lymphoid enhancer factor (Lef-1) as well as specific transcription factors for each T-cell subset. For instance, GATA-3 regulates Th2 cells, while FoxP3 regulates Treg cells. Transcription factors specific for each T cell subset are shown in Figure 7-21.

### The right time and the right place for T-cell activation

One of the long-standing conundrums in T-cell activation has been how to explain the logistics of the interaction between a T cell and an APC. As discussed



**FIGURE 7-28** Rhodopsin-arrestin interaction: binding sites of arrestin (also known as S antigen) from peptide competition. In each case, molecule B of the unit cell is shown in (a) ribbon drawing and (b) space-filling model. The picture in the centre represents the rhodopsin structure in the ground state with the cytoplasmic loops in green and the retinal in purple. (From Pulvermüller et al. 2000, with permission from the American Society for Biochemistry and Molecular Biology.)

Binding of CD21 to C3d-tagged antigens allows the coreceptor to cluster with the antigen receptor coligation of the co-receptor allows receptor-associated kinases to phosphorylate CD19 phosphorylated CD19 binds Src-family tyrosine kinases (e.g. Lyn) and PI3-kinase PI3-kinase initiates a signalling pathway involving the GEF protein Vav

above, antigen traffics either as soluble antigen in the lymph or cell-bound in dendritic cells to the secondary lymphoid tissues (SLOs), usually the peripheral draining lymph node. *In vivo* two-photon studies have shown that T cells entering the lymph node make repeated short interactions with several APCs, and when a T cell decides that one particular antigenic peptide is the specific antigen for that receptor (based on its affinity for binding, i.e. how strongly it is bound), it engages in sustained contact (several hours to 2 days) until, when sufficiently activated (as determined by expression of activation markers such as CD69), it detaches from the APC. If this process takes place with the support of additional molecules which provide co-stimulation (co-stimulatory molecules), the T cell will undergo intense activation, proliferate (clonal expansion) and secrete cytokines. If there is no co-stimulation, then the T cell may proliferate somewhat, but does not become active for effector function but for tolerance (see p. 441). Once the clone of T cells has expanded sufficiently, they will leave (egress) the lymph node essentially *en masse* under the control of a specific receptor ligand interaction (sphingosine-1 receptor) to enter the bloodstream. As more cell-associated antigen arrives from the tissues to activate further clones of T-cells, T-cell egress from the lymph node occurs in waves. As indicated above (see p. 414), unactivated passenger naive T cells and possibly 'tolerized' T cells pass through the lymph node via the efferent lymphatics to the next node in the chain and on to the thoracic duct and the spleen.

Circulating activated effector T cells make the rounds many hundreds of times before entering the site of tissue inflammation since the blood vessels at these sites need to be 'softened up', i.e. start to express appropriate adhesion molecules and chemokines. Once the T cells enter the tissues, they are probably further activated by engaging with antigen, or they may simply continue on their proliferative programme, secrete cytokines, kill infected cells via MHC class I mechanisms (Tc cells, Th killer cells), and recruit inflammatory monocytes/macrophages (M1-like) to clear infected tissues (macrophages), and later alternatively activated macrophages (M2-like) to restore tissue integrity (see eFig. 7-1).

Later, T cells entering the tissues have been programmed as Tregs and probably function *in situ* to promote resolution of the inflammation but it is also

likely that Treg activity in the lymph node is equally important by preventing further activation/proliferation of T cells. This question has not been completely answered as yet nor has the question of whether antigen needs to be completely cleared from the tissues before inflammation can subside, and how this plays out probably determines whether the inflammation persists as chronic disease or whether the infection becomes 'latent' (i.e. ignored by the immune system).

### What are $\gamma\delta$ T cells and what is their role?

$\gamma\delta$ T cells are T cells which express  $\gamma$  and  $\delta$  IgG family chains, almost identical to  $\alpha\beta$  chains of conventional T cells, but they have very limited diversity and recognize a broad range of antigens including self- and non-self-antigens such as heat-shock proteins and lipid antigens; they are, in essence, innate immune cells, becoming involved very early in the inflammatory process. Thus, they populate sites of pathogen entry such as the skin (first line of defence) but also circulate in the blood and respond to 'stress' induced by pathogens or endogenous stimuli (DAMPs).  $\gamma\delta$ T cells do not require classical MHC molecules to recognize antigen but they can respond to unprocessed peptide and other antigens.  $\gamma\delta$ T cells expand 2–10% in response to alkylamines derived from microbes and edible plants and may represent a link between innate and acquired immunity.

$\gamma\delta$ T cells are also implicated in autoimmunity; they appear to recognize heat-shock proteins (proteins expressed in 'stressed cells' and highly conserved across species), which have been suggested to play a role in autoimmune diseases such as rheumatoid arthritis and uveitis associated with Behçet's disease.  $\gamma\delta$ T cells have been reported to be essential in the induction of experimental autoimmune diabetes and are early participants in models of autoimmune uveitis. High levels of  $\gamma\delta$ T cells have also been cultured from the vitreous in a case of sympathetic ophthalmia. Interestingly, as part of the immune response to tumours,  $\gamma\delta$ T cells have been associated with better survival from choroidal malignant melanoma.

### Superantigens

Some diseases induced by organisms are so rapid in their onset and catastrophic in their manifestations that it is difficult to explain their pathogenesis in terms

of an adequate innate or acquired immune response. Examples include the toxic shock syndrome, meningo-coccal meningitis and leptospirosis. These disorders are caused by superantigens derived from bacteria (staphylococci, streptococci, mycobacteria, *Clostridia* and many other organisms release superantigens), viruses (e.g. rabies and ebola viruses) and retroviruses (e.g. mouse mammary tumour virus and human immunodeficiency virus). Superantigens are generated by genetic elements known as 'pathogenicity islands', which are phage-related (a phage = a parasitic virus for bacteria which uses the bacteria's metabolic machinery to replicate) genes (phage-related chromosomal islands, PCRs) which they use for rapid transduction.

Staphylococcal enterotoxins (SE) are a good example of superantigens and are the commonest cause of food poisoning. There are more than 20 SEs, SEA–SEE. They have to bind to the MHC class II antigen to be presented to the T cell but they can bind many polymorphic MHC class II molecules. Superantigens do not require processing by APCs because they activate the T cell by binding to the side of the TCR  $\beta$  chain. They are, therefore, quite promiscuous in that they can polyclonally activate several species of T cell. In superantigen infection, about 10% of the T cells are activated, whereas in immune responses to regular antigens a very restricted set of T cells are activated. Indeed, few antigen-specific T cells can be detected either in the circulation or even in the lesion. The result is the very rapid production of cytokines from activated T cells as well as T-cell-induced macrophages, causing a 'cytokine storm'.

Polyclonal activation in superantigen infection may involve autoreactive T cells and thereby initiate an autoimmune disease, which may persist even if recovery from the infection occurs. Studies of preferential V $\beta$  TCR gene usage by lymphocytes infiltrating the tissues may shed light on the nature of the autoantigens in conditions such as uveitis and rheumatoid arthritis and help to identify sequence similarities between autoantigens and superantigens.

### Antigens can be presented by other MHC and MHC-like molecules

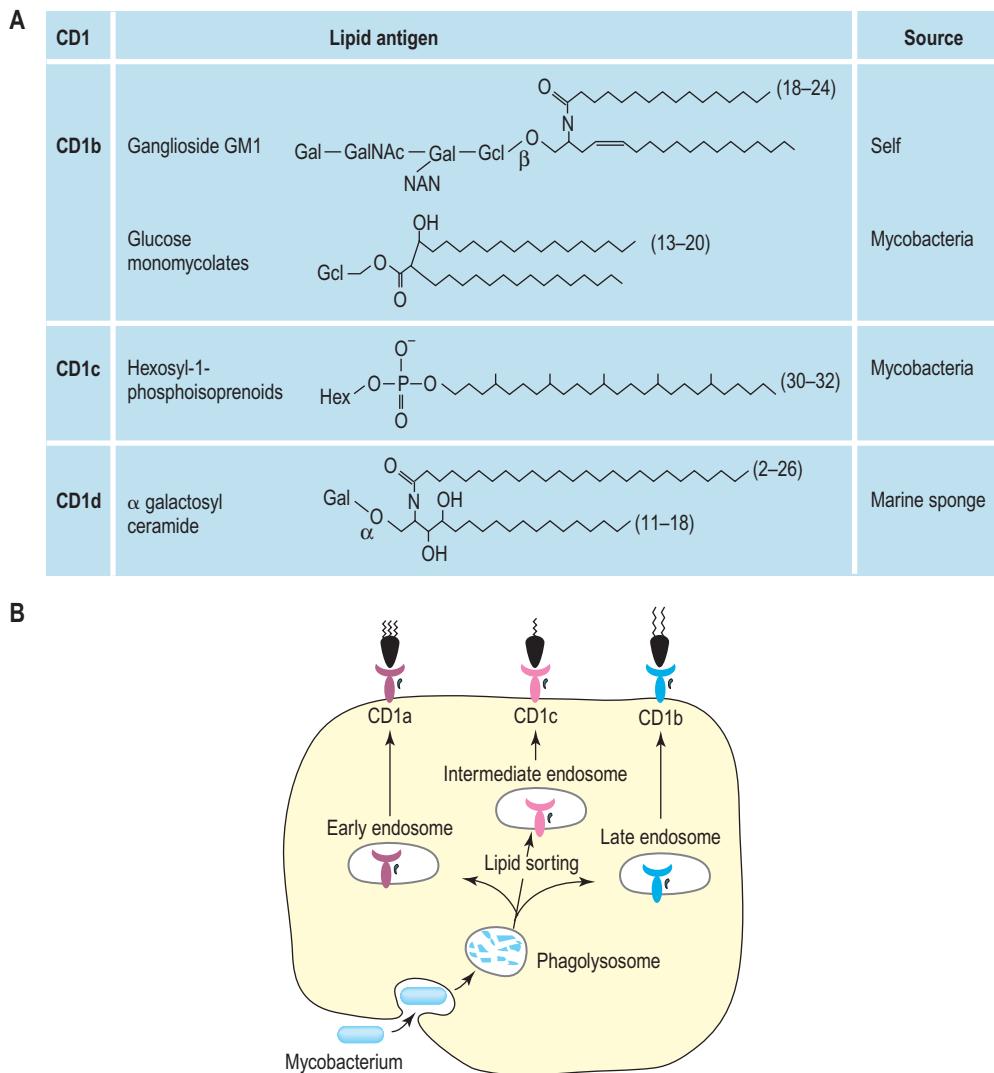
Antigens presented through the canonical MHC class I and II routes are exclusively small peptides. However, it is well known that other molecules such as sugars

and lipids can evoke T-cell and antibody responses. MHC class Ib molecules include human leucocyte antigen (HLA) -E, -F, -G and in the mouse molecules such as Qa-1. Although these molecules have some diversity, the range of peptides they can present is restricted, e.g. Qa-1 presents only a small set of microbial peptides, while HLA-G is involved in maternofetal antigen presentation.

The MHC-like molecule CD1, which does not contain an  $\alpha_2$ -microglobulin protein, has a highly hydrophobic groove with two deep pockets that neatly binds the fatty acid chains of glycolipid antigens while the sugar moieties bind the TCR (Fig. 7-29). Recent studies suggest that both innate and adaptive immunity may be involved in age-related macular degeneration (AMD) and that modification of self-antigens by products of lipid peroxidation such as carboxyethyl-pyrrole (CEP) may act as autoantigens in causing disease. Lipidated antigens may be presented via CD1 molecules; for instance, CD1d binds NK T cells via  $\alpha$ Gal-ceramide, while CD1c binds  $\gamma\delta$  T cells via an unknown antigen.

### CO-STIMULATION: PRESENTATION OF ANTIGEN REQUIRES 'HELP' FROM OTHER MOLECULES

As discussed in the previous sections, MHC:peptide complexes act as ligands for binding to the TCR. The outcome of ligand binding is dependent on several factors: in particular, the cytokine milieu decides which type of T cell is induced (see Figs 7-2, 7-22 and 7-23). The cytokine milieu is itself dependent on whether the antigen is presented by an APC which has been activated by a DAMP (see p. 389). For instance, activation of a TLR or NALP3 with consequent induction of the inflammasome will release pro-inflammatory cytokines such as IL-1 $\beta$ , IL-12, IL-23 and TNF- $\alpha$ , all of which provide a suitable environment for activation of a Th1 or Th17 CD4 $^+$  T cell; in contrast, if IL-10 or TGF- $\beta$  are released, then Tregs are more likely to be induced. In both cases the TCR is triggered but different transcriptional pathways are induced. Activation of Th1 or Th17 cells via the TCR not only requires the appropriate cytokine milieu but also requires several TCRs to be simultaneously engaged in a membrane structure known as the immunological synapse. This comprises the aggregated TCRs as well as several surrounding co-stimulatory molecules. In contrast, less is



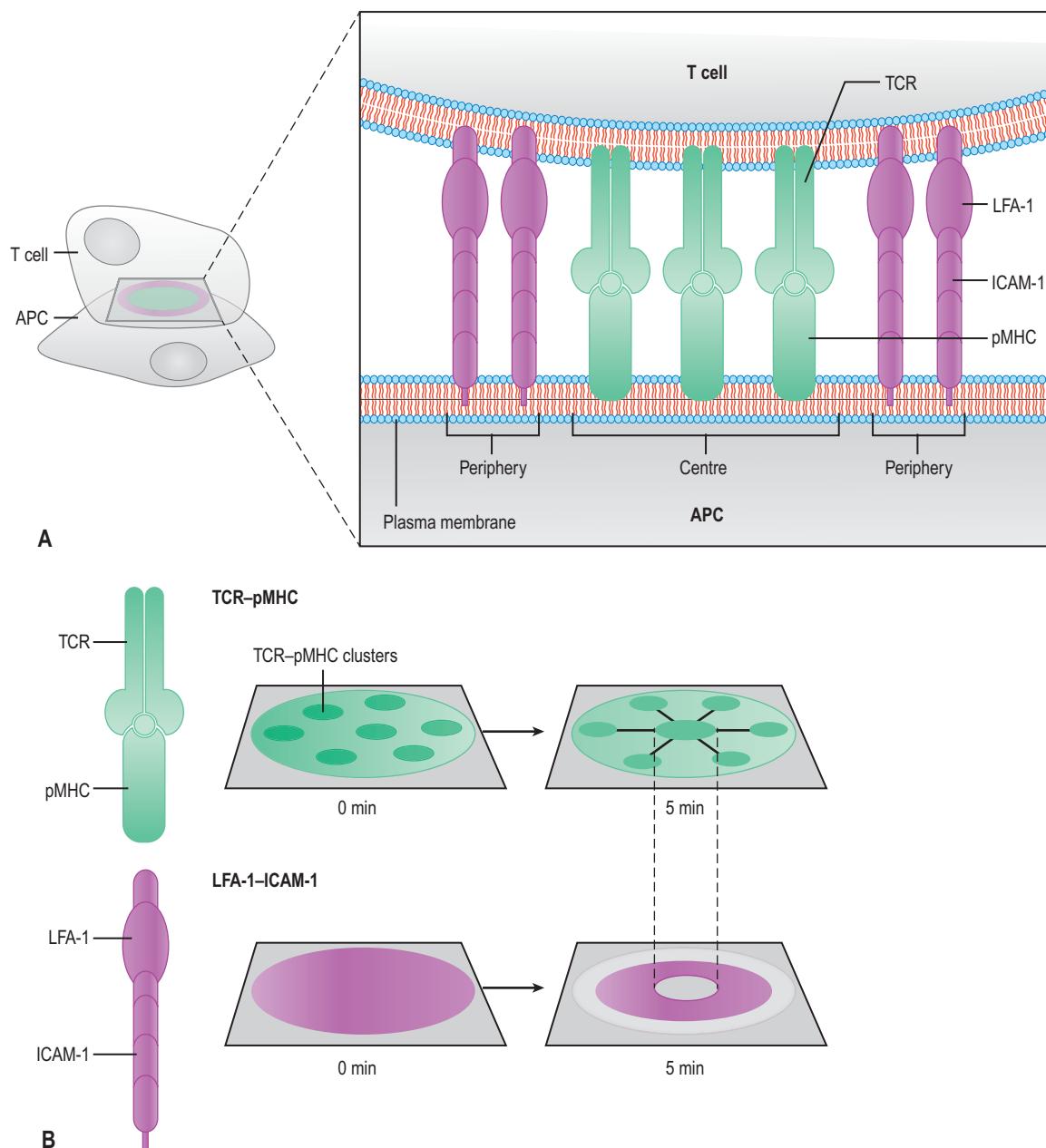
**FIGURE 7-29** Lipid antigen presentation by CD1 molecules. (A) Structures of lipid antigens presented by CD1 molecules. The precise structure of the CD1a antigen is unknown. (B) Sorting of mycobacterial lipids. At the bottom left of the figure a mycobacterium has been phagocytosed and degraded. The mycobacterial lipids are sorted by structure into different endosomal compartments where they are loaded on to different CD1 molecules for presentation to T cells. (From Mak and Saunders 2006, with permission from Elsevier.)

known about induction of Tregs, or even engagement of naive T cells to induce anergy (i.e. non-responsiveness), but several ‘co-inhibitory’ molecules have now been discovered.

### Co-stimulatory molecules

Activation of the T cell is achieved by the clustering of several ligand–receptor pairs between it and the APC. Clustering of these ligands at the point of contact

between the cells helps to strengthen and prolong the contact between the cells and thus facilitate the presentation of peptide to the TCR (Fig. 7-30). Mobilization of the various sets of molecules to the site of TCR–pMHC interaction occurs within localized membranous patches (lipid rafts, see Ch. 4, pp. 161–162) and the various ligand–receptor pairs participate in the formation of the immunological synapse (Fig. 7-30). Some of the molecules involved either in T-cell



**FIGURE 7-30** The immunological synapse. This term has been used to describe the binding between the T cell and the antigen-presenting cell because of its structural similarity to the neurological synapse. There is a centre containing the ligand receptor pair (MHC peptide:TCR) surrounded by a ring of adhesion and other accessory molecules which are progressively recruited to the synapse as initial non-T-cell activating binding is converted to a firm prolonged contact necessary to activate the T cell. **(A)** A side and enhanced view of the synapse. **(B)** An *en face* view. Over a period of about 5 min, many TCRs are recruited to the centre of the synapse while further adhesion and accessory molecules firm up the outer ring. The synapse is generated in a membrane lipid raft (see text). (From Manz and Groves, 2010.)

co-stimulation or co-inhibition including adhesion molecules such as ICAM-1/LFA-1 (see Table 7.3), co-stimulatory molecular pairs such as CD40-CD40L, ICOS-ICOSL and CD80-CD28, as well as inhibitory molecular pairs such as PD1-PDL1, and CD28-CTLA4 (reviewed by Chen et.al., 2013).

Two important ligand-receptor pairs during CD4<sup>+</sup> T-cell activation (not shown in Fig. 7-3) are the B7:CD28 interaction and CD40:CD40 ligand (CD40L). Interaction between these molecules is a prerequisite for T-cell activation and, indeed, in the absence of this 'second signal' presentation of peptide to the TCR is likely to lead to anergy rather than stimulation. B7 is a 45 kDa to 60 kDa cell surface glycoprotein expressed on activated B cells, dendritic cells and macrophages, which binds to CD28, a 44 kDa homodimeric molecule expressed constitutively on the T cell, and leads to the proliferation of, and IL-2 secretion by, T cells. B7 can also bind to a second receptor, cytotoxic T-lymphocyte antigen 4 (CTLA-4), which is slowly induced in T cells and is an inhibitory molecule with greater affinity for B7; as such this ligand pair is involved in downregulation of T-cell activation in the later stages of the response. CTLA-4 is also expressed at high level in Tregs, thus mediating part of their suppressive function.

CD40-CD40L interactions are also very important in T-cell activation, and in reciprocal dendritic cell conditioning there is a two-way signalling interaction via this ligand pair. CD40 is present on APCs and can be upregulated. In addition, it is present on many non-APCs such as endothelial cells. CD40L is only present on activated T cells and even then transiently, so that the time of CD40L expression in part regulates the overall duration of the immune response. CD40L and a further molecule, Lag3 (CD223), may be involved in the concept of APC 'licensing', in relation to activation of cytotoxic T cells (see later, [T-cell activation](#)).

Several other accessory molecules are involved in the T-cell/APC interaction leading to the immunological synapse to maximize the T-cell/APC contact. Activation of the T cell leads to the expression of integrin adhesion molecules on the cell surface, including LFA-1 and VLA-4 ( $\alpha_4\beta_1$ ) (see Table 7-3). Reciprocal expression on cells promotes binding. VLA-4 also binds to extracellular matrix molecules, as does CD44

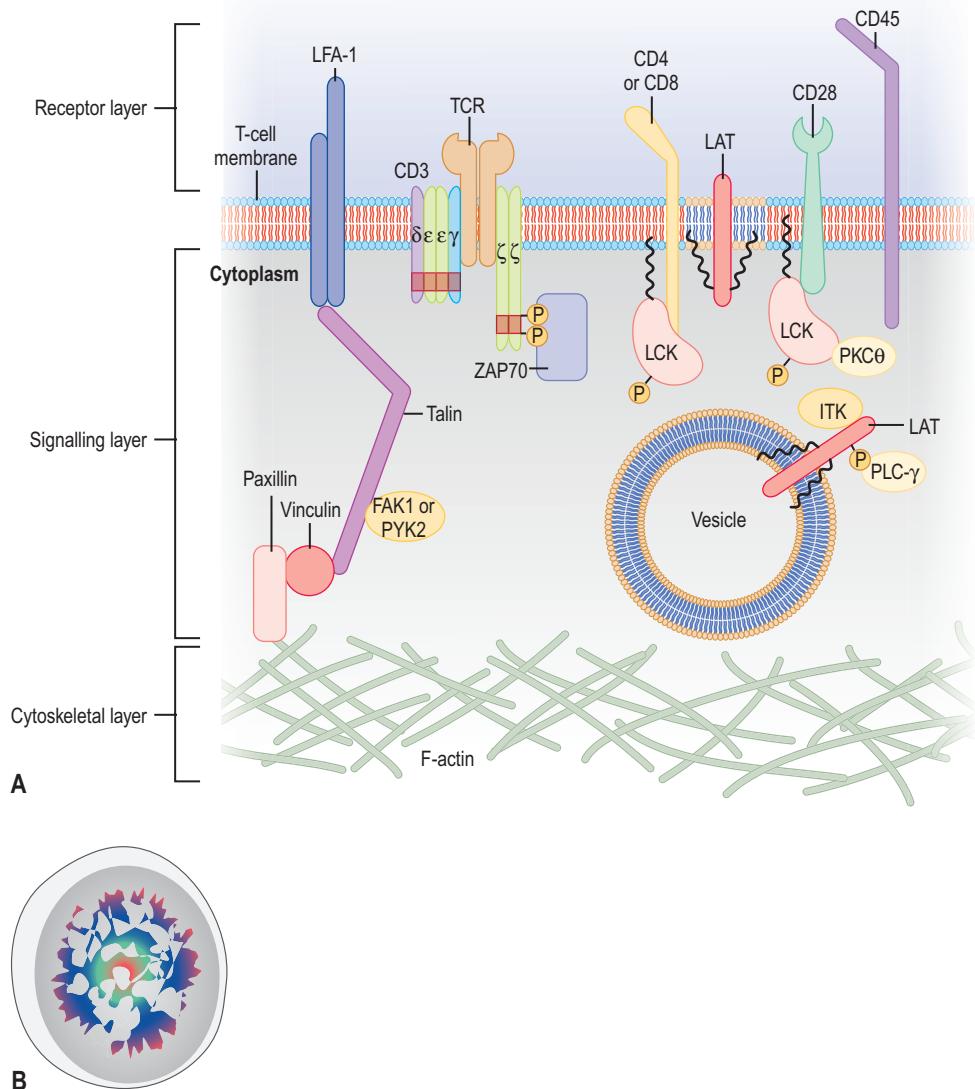
– the hyaluronan receptor. Some molecules provide co-stimulation for specific T-cell activities, such as OX40 and ICOS, while others such as PDL-PDL1 are negative regulators of T-cell activation.

The process of antigen presentation for the generation of an active immune response (immunity) therefore involves multiple molecular interactions and requires co-stimulatory activity for induction of positive adaptive immunity and co-inhibitory molecular interaction for downregulation and anergy induction. It is not clear, however, whether all of the above interactions are required to initiate T-cell clonal expansion or whether immunogenicity is a function not just of peptide/TCR specificity but also depends on which and how many of the accessory interactions are entrained.

### Getting the message across: cell signalling through the immunological synapse

Cells activated through cell surface receptors alter cell function through second messenger systems in the cytoplasm. These are likewise linked to events in the nucleus that mediate protein transcription and ultimately to changes in cell function (see cell signalling, Ch. 4, p. 159). The immunological synapse (also known as the supramolecular activation complex, SMAC) provides this function for the T cell. The initial contact between the T cell and the APC mediated through reciprocal LFA-1 and ICAM-1 interactions and also probably by CD45, stabilized by the CD4 and CD8 molecules in the appropriate T cells, induces changes in the actin cytoskeleton that draw several molecules on both sides in lipid rafts into a stable adhesive complex (Fig. 7-31).

The effect is dependent on the signalling molecule VAV. In the centre of the synapse lies the CD3/CD4 (or CD8)/CD45/TCR-pMHC complex surrounded by an inner ring composed of CD2-LFA-3 paired molecules, itself surrounded by an outer ring of ICAM-1-LFA-1 molecules linked to talin and thus to the cytoskeleton (Fig. 7-31). The TCR is linked to its second messenger system through a complex of transmembrane dimeric proteins, the CD3 complex which links intracellularly to cytoplasmic vesicles via an adapter protein, linker for activation of T cells (LAT), and to a signal transduction complex, which belongs to a group of molecules known as immunoreceptor



**FIGURE 7-31** Molecular details of the immunological synapses on the T-cell receptor side. The TCR linked to the adhesion molecule LFA-1, the CD4 T-cell-specific anchoring molecule CD4, the accessory activation molecules CD28 and CD45, activates the signalling machinery (VAV, ZAP-70, LCK, PKC $\theta$ , and LAT) as well as the T-cell cytoskeletal motor (vinculin, F-actin, etc.) which sets the T cell on a cytokine secretion programme, and migration towards chemotactic stimuli. The side view is shown in (A) and the *en face* view on the T cell in (B). (Dustin and Depoil, 2011.)

tyrosine-based activation motifs (ITAMs). The T-cell ITAM (known as p59<sup>fyn</sup>) acts in conjunction with a second molecule (p56<sup>lck</sup>) (LCK) as the major tyrosine phosphorylating mechanism in T cells.

The four TCR–CD3 complexes may each initiate discrete signalling events, the summation of which

produces an effective stimulus for T-cell activation (similar to events in neural cells; see Ch. 4). A progressive accumulation of signal thus develops through the sustained presentation of antigen mediated by recruitment of co-stimulatory and accessory molecules into the membrane surrounding the

immunological synapse. In contrast, short-lived contacts between APCs and T cells may fail to initiate responses in the T cells and probably do not even involve the TCR. Downstream signalling from the Src family of tyrosine kinases is another set of protein tyrosine kinases with docking sites that bind SH-2 molecules. One of these is a protein known as ZAP-70 (associated with the  $\zeta$  protein of CD3) (Fig. 7-31), which plays a major role in sustained TCR signalling. In fact, the interactions between the various signalling molecules through the TCR are a good example of how signalling networks function.

### Action of drugs on cell activation

Certain immunosuppressive drugs, used clinically in transplantation and autoimmune diseases, are known to act at various stages in T-cell activation. These include steroids, cyclosporin A, tacrolimus, rapamycin and mycophenolate mofetil. Cyclosporin, tacrolimus and mycophenolate mofetil are particularly relevant to ophthalmology because they are used in a variety of conditions such as sight-threatening uveitis, severe scleritis and corneal graft rejection.

Cyclosporin and tacrolimus specifically inhibit the transcription of the IL-2 gene in CD4 T cells by binding to intracellular proteins (immunophilins) that subsequently bind a  $\text{Ca}^{2+}$ -regulated phosphatase, calcineurin. This enzyme is activated by  $\text{Ca}^{2+}$  influx during T-cell activation and is required for assembly of the nuclear transcription factor (NF-AT) involved in IL-2 secretion. Cyclosporin may also act via induction of TGF- $\beta$  release, a cytokine involved both in Treg cell function and in induction of Th17 cells (see Fig. 7-2).

### B-cell activation

Until now we have considered only cellular interactions with antigens. However, the first evidence for the existence of an adaptive immune system came through the discovery of antibodies, circulating proteins in the globulin fraction of the serum. Free antibodies are produced by plasma cells, the fully differentiated version of the B cell. B cells are derived from stem cell precursors in the bone marrow and are released into the circulation as  $\text{CD45}^+$   $\text{sIgM}^+$   $\text{CD19}^+$   $\text{CD20}^+$  MHC class II $^+$  immature cells which migrate to the B-cell follicle regions of the SLOs. B cells comprise 5–10% of the circulating lymphocyte pool. B cells are

important not only as antibody producers but also as APCs in their own right.

## ANTIGENS, B CELLS, AND T CELLS: WHICH DOES WHAT?

### Antigen recognition by B cells

The rules governing the size of peptide antigen required to induce an immune response apply to T and B cells. However, most T cells are restricted in that they can respond only to peptides. B cells can also respond to carbohydrate and glycolipid antigens, producing T-independent B-cell responses. This occurs for instance with blood group antigens.

B-cell responses to peptide antigens require T-cell help, usually provided by cytokines such as IL-2, IL-4, IL-5 and IL-6 released from activated Th2 cells. B cells acquire antigen from specialized follicular dendritic cells (fDCs) which present antigen as immune complexes bound to Fc and C3 receptors generating an activated germinal centre B cell. This cell undergoes isotype switching (see pp. 396–398), somatic hypermutation and clonal expansion (proliferation) to produce memory B cells and long-lived plasma cells, each of which secretes a specific monoclonal antibody. B cells also present processed antigen on MHC class II molecules to T follicular helper cells which reciprocally assist in the presentation of antigen to B cells by fDCs. This is mediated by co-stimulation via CD40–CD40L interactions. This form of T-cell and B-cell recognition of the same peptide antigen is known as mature B-cell (plasma cell)-linked recognition. Other co-receptor molecules are involved in the formation of the contact site of the B-T-cell interface, e.g. CD30:CD30L and B-lymphocyte stimulator (Bly5) and its receptor TAC1.

B cells arriving from the bone marrow to the secondary lymphoid tissues traverse the T-cell area on their way to the B-cell follicles. However, if they meet an antigen-specific Tfh cell, they become trapped in the marginal T-cell zone and start proliferating and producing antibody (a primary lymphoid focus). Some of these B cells mature to antibody-producing plasma cells, which leave the T-cell area and return to the bloodstream and ultimately the bone marrow. In contrast, the majority enter the B-cell area where they form a B-cell follicle, and ultimately form the B-cell germinal centre, an antibody-producing mini-factory, where extensive isotype switching takes place.

The B cells then migrate to the medulla of the SLO, and out into the circulation where they home to the bone marrow and differentiate into mature antibody-secreting plasma cells. They may also populate sites of inflammation and develop into plasma cells. Three checkpoints exist therefore for B-cell expansion: the first is at the stage of T-cell help, regulated by CD40L; the second is at the stage of selection in the germinal centre by the follicular dendritic cell delivering anti-apoptosis signals; and the third is at the stage of migration of the mature B cell (plasma cell) to bone marrow and tissues generally. However, most B cells remain in the follicle/germinal centre and secrete antibody directly into the bloodstream via the efferent lymphatics and thoracic duct.

### B cells as antigen-presenting cells

Endocytosis of antigen via its sIg receptor activates the B cell to process and present peptide fragments in association with MHC class II on its surface (see above). B cells are efficient APCs but can be activated

to present antigen only via the sIg receptor. Therefore, in this regard they act as memory cells.

Although B cells are activated in an antigen-specific manner and their effector molecules (antibodies) are also antigen specific, they induce their effects in an antigen-non-specific way, predominantly via complement. B-cell responses are also greatly enhanced by activation of the B-cell co-receptor complex (CD19:CD21:CD81). CD21 is the receptor for complement component C3d, thus permitting antibody–antigen–complement interaction.

### B-CELL DIFFERENTIATION

As indicated above, B cells are derived from stem cell precursors in the bone marrow (Fig. 7-32). Immature B cells home to the SLOs. However, if they do not encounter specific antigen, they undergo apoptosis and are removed within 3–4 days. In contrast, when activated, they develop into lymphoblasts and ultimately plasma cells (see above). Some activated cells develop into long-lived memory cells.

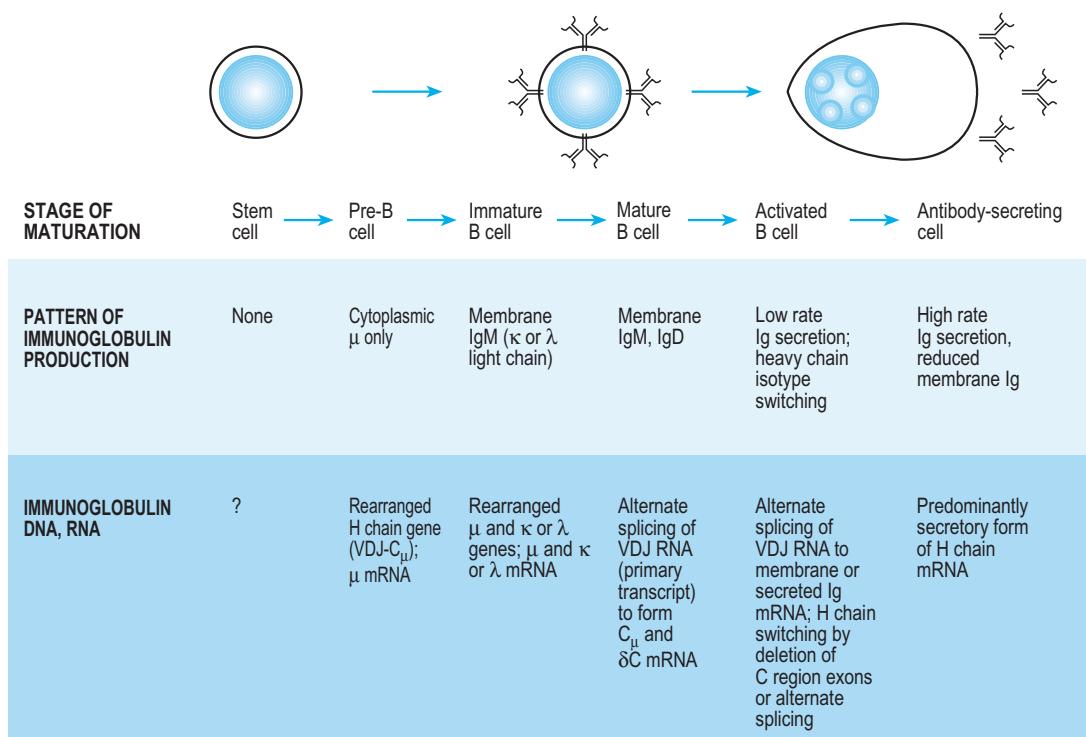


FIGURE 7-32 B-cell maturation. (Courtesy of A. Abbas.)

Pre-B cells lack membrane-bound IgM and immature B cells may fail to respond to antigen despite the presence of sIgM, owing to lack of accessory molecules. Anergy or tolerance may instead be induced under these conditions. Immature and mature B cells express surface IgM and IgD, whereas activated B cells express IgG after heavy-chain isotype switching. At this stage most of the immunoglobulin is still membrane bound. After some rounds of activation, the cell switches to high secretion of antibody and becomes a plasma cell.

### Antibody generation during B-cell ontogeny

The immune system utilizes the same general mechanism to generate an almost infinite range of specific antibodies as it does for TCRs, via recombination of the heavy and light chains of the C (constant) regions, and somatic mutations in the V (variable) regions of the respective antibody and TCR molecules under control of the *RAG* genes (see p. 427). The genes encoding antibody structure are located on chromosomes 14, 2 and 22 in humans (Fig. 7-33).

In memory B cells particularly, somatic mutations that occur during progression of the B cell through isotype switching to the plasma cells permit the antibody to be 'shaped' to fit the antigen better. This is known as 'affinity maturation' of the B cell and its immunoglobulin molecules, a process that describes the stronger affinity of antibodies for an antigen on repeated exposure (Fig. 7-34). Thus, antibodies produced after repeated exposure to the antigen have a much higher binding capacity for the antigen and are more effective in dealing with its removal. This is the basis of various immunization protocols. Isotype switching is under the control of cytokines and varies for different immunoglobulins; thus IL-4 induces switching to IgG1 and IgE while IFN- $\gamma$  induces IgG3 and IgG2a and TGF- $\beta$  induces IgG2b and IgA switching.

Under normal bone marrow conditions of continual cell proliferation and maturation, with unique mutations being produced in each cell at each cell division, a very large range of possible permutations of antigen specificity is met within a short time but initially with low affinity. When the correct match between a particular antigen and receptor occurs, antigen-specific clonal expansion ensues. However,

this happens only rarely; most cells expressing a particular sIg do not meet the corresponding antigen and are eliminated.

### Somatic mutations occur in the CDR of the V genes

Antigen-binding sites are located in the complementarity determining regions (CDRs) of the V segments of the H and L chains (see eFig. 7-3, particularly in the hypervariable region). However, some overlapping binding to the conserved regions of these proteins may also occur. Mutations that increase the affinity of the antigen–antibody reaction occur in the hypervariable regions and become increasingly important with each exposure to the antigen (affinity maturation). Mutations occur in all three CDRs in both chains and may also occur in some intervening regions of the encoded sequence. Antibody diversity is therefore due to several factors (Box 7-14).

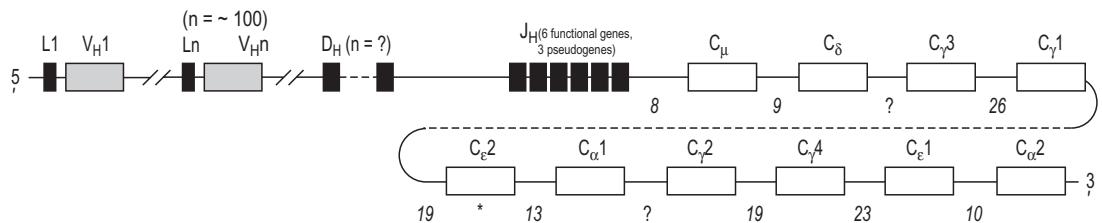
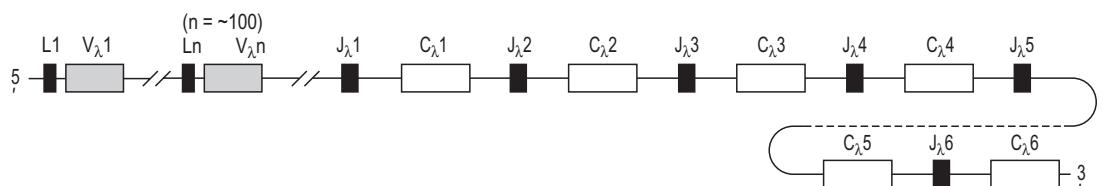
Some microbial antigens (superantigens) have the ability to induce a polyclonal response in B cells in the absence of T cells, similar to T-cell superantigens (see p. 431). Superantigens bind to the majority of VH gene families, especially VH3<sup>+</sup> IgM. They also bind to conserved regions of the antibody outside the CDR at FR3 (framework region 3). Antibodies are very important to immunity and protection from microorganisms, especially for memory responses to viruses. In such situations the paracrystalline repeated structural epitopes on the virus surface may induce a B-cell response without T-cell help.

### Genetic control of antibody production

In addition to the normal TATA boxes of V region promoters, immunoglobulin genes contain regions modulated by trans-acting nuclear factors that regulate the promoters and enhancers. Trans-acting nuclear factors are DNA-binding proteins, some of which are specific to B cells. Others occur in a number of cells that have to respond rapidly and quantitatively, e.g. NF- $\kappa$ B (also involved in IL-2 transcription) and NF-AT (the target for cyclosporin and FK506).

### Immunological tolerance and autoimmunity

Many diseases involve the immune system in the absence of clear evidence for a direct causation by a

**H chain locus (chromosome 14)****κ chain locus (chromosome 2)****λ chain locus (chromosome 22)**

**FIGURE 7-33** The heavy (H) and light (L) chain genes are located in the sequence in which they are transcribed. There are over 100 V genes, each of around 300 base pairs in length and grouped in six or seven families on the basis of > 80% homology. Each V gene is preceded by a leader sequence characteristic of secreted and transmembrane proteins. There are an unknown number of D genes and five or six J genes. The J and D genes code for the C-terminal region of the V gene including the third complementarity-determining region (CDR) (see below). Each C gene is composed of several exons represented by the single box. The D<sub>H</sub> gene is the main genetic determinant of D-H-J diversity. The amino acid composition of the IgH CD3 region is the driving force for selection. Hydrophilic/aromatic amino acid groups are more likely to be selected than hydrophobic groups.

Recombination of the gene segments to generate a mature immunoglobulin is achieved by excision of the intervening DNA and ligation of the immunoglobulin genes. This ‘looping out’ is facilitated by specific enzymes (recombinases) that act during the earliest stages of B-cell maturation. Segments of DNA, 3' of each V gene and 5' of each J gene in the non-coding intervening DNA of the light chain and similarly in the VDJ genes of the heavy chain, are excised and re-annealed. Recombinases themselves are under genetic control via recombination activation genes (*RAG* genes).

Recombination occurs in a precise sequence. V<sub>H</sub> genes join to the DJ gene, at which point the cell is committed to becoming a B cell. The VDJ sequence then binds to C<sub>μ</sub> and a poly-AAA tail is added. The L chain follows the same sequence and the expressed L chain then assembles with the ‘μ only’ H chain in the endoplasmic reticulum. When this pre-B cell expresses the IgM on its cell surface, it becomes an immature B cell.

The μ chain regulates somatic rearrangement by allelic exclusion (activation of genes on one set of chromosomes suppresses activation of the other chromosome) and by initiating L chain rearrangement (the κ chain is activated first; if this is non-productive, λ chain genes are activated). (Courtesy of A Abbas.)

foreign infective organism. In many of these diseases, antibodies and T-cell-mediated immune responses to ‘self-antigens’ can be demonstrated and these disorders are considered to be ‘autoimmune diseases’. The notion that immune responses to self-antigens are unusual implies that immunological non-responsiveness to

self-antigens is the normal situation; this state is known as *tolerance*. Therefore, autoimmune diseases are considered to be the result of a breakdown in the normal immunological machinery that permits tolerance to self-antigens, resulting in pathology, i.e. actual tissue damage. The detection of B (antibodies) or T-cell

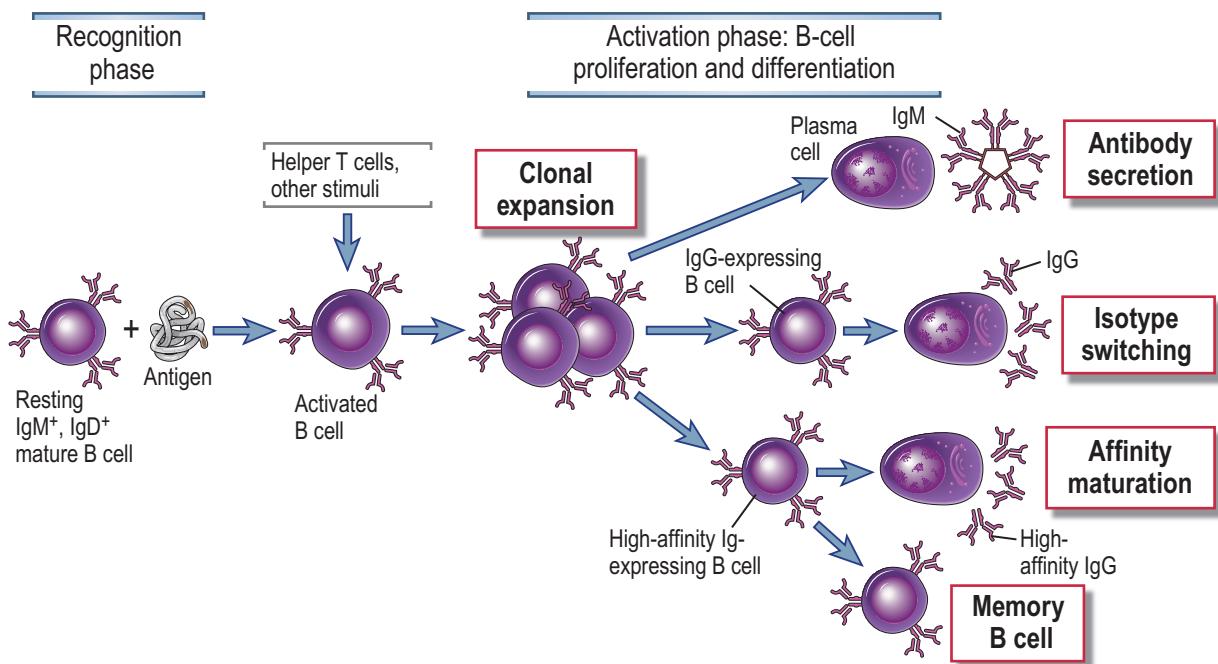


FIGURE 7-34 Abbas, A.K. Figure 11-1 Cellular and Molecular Immunology

#### BOX 7-14 FACTORS CONTROLLING ANTIBODY DIVERSITY

- Multiple germline genes
- Somatic combinatorial diversity
- Junctional diversity
- H and L chains contributing to the antibody combining site
- Somatic mutations

In the mouse it has been estimated that the potential antibody repertoire due to recombination mechanisms is  $10^9$ – $10^{11}$  (i.e. before antigen has initiated a response).

responses in the absence of pathology is not sufficient for the diagnosis of autoimmune disease since these occur in normal individuals also and are a measure of normal regulated autoimmunity.

Thus, in a broader sense, the immune system has evolved mechanisms that downregulate (switch off) the immune response after it has been activated because it would clearly be autodestructive to have a continuing inflammatory response. The mechanisms that invoke tolerance and those that switch off immune responses may be the same.

#### WHAT IS TOLERANCE?

Tolerance may be defined as antigen-induced inhibition of the development, growth, or differentiation of antigen-specific lymphocytes, i.e. adaptive immunity. Tolerance has the following properties:

- it is antigen-specific – individuals who are tolerant to one antigen are not necessarily tolerant to all antigens or even a second antigen
- tolerance to autoantigen is acquired during development – immature lymphocytes develop tolerance more easily than adult ones
- maintenance of tolerance requires persistence of (auto)antigen throughout the life of the individual
- tolerance to foreign antigens can be induced if the conditions are right.

#### SITES OF TOLERANCE INDUCTION

Tolerance classically has been described to occur in two main ‘sites’: centrally in the thymus (central tolerance) and peripherally in the secondary lymphoid organs (SLOs) such as the spleen and the lymph nodes. According to the thymic deletion concept of

Burnett (see below), most of the autoreactive T cells which would be generated by random re-arrangements of the TCR permitting reactivity to self-antigens by chance, are deleted as part of central tolerance. However this is not a fail-safe mechanism and some autoreactive T cells 'escape' to the periphery where they are also deleted, anergized or regulated (see below): this is peripheral tolerance.

Recently, as an extension of Matzinger's concept that the organism does not truly distinguish between self- and non-self-antigens but between immunogenic and non-immunogenic antigens (the Danger hypothesis, see p. 389) and that self-antigens themselves can become 'dangerous' under appropriate conditions, the notion has arisen that the immune response may be modified by the tissues and the 'self' antigens (or cells and mediators) they contain (tissue-based tolerance).

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Thus, immune responses to the same antigen or microorganism may differ depending on the tissues: this in fact is not a new concept but an elaboration of the notion of *immune privilege* (see below), which is particularly well expressed in the eye and the brain and is a means whereby ocular and brain tissues modify (downregulate/dampen) immune and inflammatory responses generally.

The same cellular mechanisms are utilized to induce central, peripheral and tissue-based tolerance.

## MECHANISMS OF TOLERANCE INDUCTION

Tolerance (better described as immunological non-responsiveness, although this is rather unwieldy) is considered under various forms, e.g. neonatal versus adult, central versus peripheral, innate versus acquired, etc. In fact, tolerance is always acquired; the differences arise when the acquisition of tolerance occurs in the thymus during development (central) or in the peripheral lymphoid tissues during adulthood (peripheral).

Several mechanisms of tolerance have been suggested.

### Clonal deletion

This mechanism was originally proposed by Burnett to explain the antigen-induced destruction of self-reactive lymphocytes that occurs in the thymus during

development (negative selection) in relation to tissue-specific antigens expressed in the thymus under the control of genes such as *Aire*. This is a form of activation-induced cell death (AICD) that is a characteristic of peripheral tolerance as well as central tolerance.

It has been postulated that removal of all autoreactive cells occurs in the thymus, both for the large pool of thymic antigens and for tissue-specific antigens, which are transported to the thymus on circulating dendritic cells. This has been shown for certain brain (myelin basic protein) and retinal (interphotoreceptor binding protein and retinal S antigen) proteins under the control of *Aire* expressed in thymic medullary epithelial cells (Fig. 7-17).

However, this mechanism does not account for the induction of tolerance to all tissue-specific (non-thymic) antigens, and peripheral tolerance mechanisms are also necessary, some of which also include some level of T-cell deletion.

### Anergy

Anergy describes T- and B-cell non-responsiveness to specific antigens but in cells with the capability to respond to non-specific mitogenic stimuli, i.e. the cells are in a sort of 'suspended animation'. Anergy in lymphocytes is thought to arise via lack of co-stimulation required for antigen presentation to Th1/Th2/Th17 cells (the B7:CD28 and the CD40:CD40L interactions are considered particularly important; see pp. 432–434) and has been proposed to account for the non-responsiveness of lymphocytes to peripheral autoantigens. It is therefore considered to be a major mechanism of tolerance induction in the adult.

However, it is likely that clonal deletion and anergy involve the same processes. Thymic education of lymphocytes involves a discrete series of events in arming the T cell to develop towards a CD4<sup>+</sup> or a CD8<sup>+</sup> phenotype (see Fig. 7-17) and antigen specificity. Each of these steps requires more than one signal and, at any point, lack of a particular signal might induce anergy. If anergy persists, the cell is driven down a pathway towards apoptosis (i.e. deletion). Similar mechanisms exist in the bone marrow and in the germinal centres of lymph nodes and spleen. Apoptotic B cells are removed from germinal centres by the well-recognized 'tingible body' macrophages.

Matzinger has suggested a third mechanism for immunological tolerance. In addition to the two canonical mechanisms of central (thymic) and peripheral tolerance, she has suggested that the immune response is modulated by the tissue in which the reaction takes place. Thus, for instance, certain tissues constitutively secrete mediators which influence the nature (e.g. Th1 vs Th2), the strength (a cytokine storm or a chronic low-level inflammation), or even the outcome (complete healing of the tissue with resolution of the inflammation or permanent tissue damage with continued dysfunction). For instance, mediators such as immunosuppressive cytokines, TGF- $\beta$  and vasoactive intestinal peptide (VIP) are common to both the eye and the gut. The ocular cells also secrete  $\alpha$  melanocyte stimulating hormone ( $\alpha$ MSH), while the gut produces thymic stromal lymphopoitin (TSLP), which ultimately promotes IgA production. IgA is the immunoglobulin generated by mucosal tissue to provide mucosal immunity. Matzinger (2011) has gone as far as to suggest that the tissues are 'in control' of the immune response (see [Further reading](#)).

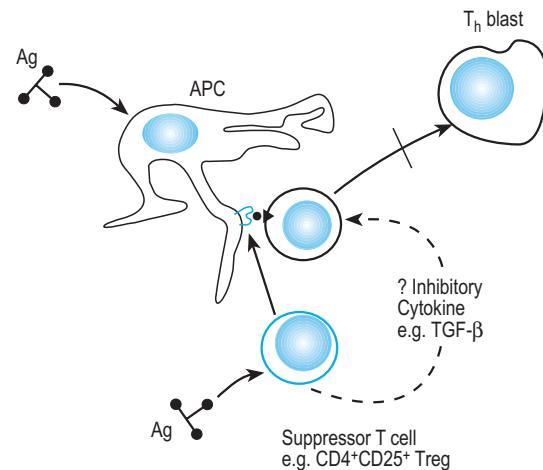
Thus the same mechanisms for removal of autoreactive cells, involving anergy and cell death, probably occur in the periphery and the central lymphoid tissues, although there is a fine balance between deletion and anergy in the induction of tolerance.

### Active suppression by specific lymphocytes: T-regulatory cells

Experimental studies in animals have shown that tolerance, which can be induced to autoantigens and foreign antigens alike using specific techniques (see below), can be adoptively transferred via an infusion of 'tolerized' lymphocytes to naive animals that have not previously been exposed to the antigen. This suggested that tolerance is mediated by cells that inhibit or regulate the immune response, probably at the level of antigen presentation ('suppressor or regulatory cells'). Such regulatory cells have been difficult to identify, mainly because of their lack of responsiveness to specific antigen *in vitro* (anergy). However, seminal work by Sakaguchi in Japan showed that depleting mice of a subset of CD4<sup>+</sup> T cells expressing high levels of the IL-2 receptor (CD25) lead to the development of spontaneous autoimmune disease with severe pathology particularly affecting the bowel (inflammatory bowel disease). These cells, known as T regulatory cells (Tregs, see p. 380), are defined by the transcription factor FoxP3 (see Figs 7-2, 7-21 and 7-22), and are required for prevention of autoimmune disease in humans as well as mice. Tregs are decreased in various forms of ocular inflammation such as Vogt–Kayanagi–Harada (VKH) disease. Other Tregs have also been discovered and the important mediators of immune suppression are inhibitory cytokines. Such cells include IL-10-producing Tr1 cells, the TGF-β-producing Th3 cells, CD8<sup>+</sup> suppressor T cells which mediate their action through macrophages and NKT cells, B regulatory cells, and γδ T cells. It is not clear how Tregs induce the suppression of T effector cells but the effect is believed to be via dendritic cells and not directly through Treg:T effector interactions (Fig. 7-35).

### T-CELL TOLERANCE IS DIFFERENT FROM B-CELL TOLERANCE

The Th cell is the critical control element for adaptive immune responses. Therefore inhibition of Th responses should lead to inhibition of all self-reactivity.



**FIGURE 7-35** Mode of action of regulatory cells.

T cells can be rendered unresponsive by very low doses of antigen, while B cells usually require larger doses. Most self-antigen T-cell tolerance is induced in the thymus by clonal anergy/deletion and is therefore antigen-specific and MHC restricted. In addition, natural/thymic (nTregs, tTregs, both terms used reciprocally) are generated in the thymus and released to the periphery. In the periphery, Tregs constitute the main regulator of autoimmunity (see above) and include both natural / thymic (n/t) Tregs and antigen-specific induced (iTregs) derived from naive T cells during the progression of all adaptive immune responses (i.e. to both foreign and auto-antigens). Anergic T cells and Tregs may also be induced by presentation of antigen by aberrantly expressed MHC class II antigen on parenchymal cells in the absence of co-stimulation.

B-cell tolerance occurs particularly to antigens that are 'T-independent'. This includes carbohydrate and glycolipid antigens such as the ABO blood groups, and thus has direct clinical relevance. Tolerance is induced by anergy/deletion of antigen-specific B cells in the marrow by mechanisms similar to those described for T cells in the thymus. Studies using transgenic mice in which genes for neoantigens and the corresponding antibody were inserted into the genome have elegantly demonstrated that B-cell anergy in the marrow occurs by maturation arrest and failure of these autoreactive B cells to enter the peripheral lymphoid organs. After some time these cells underwent

apoptosis (i.e. were deleted). Interestingly, some of these B cells could render the autoantigen non-anergy-inducing by switching the  $\kappa/\lambda$  chain. B cells that had undergone 'receptor editing' of this nature appeared in the periphery (i.e. were not deleted).

### FAILED TOLERANCE

A failure to develop or maintain tolerance to autoantigens leads to the development of autoimmune disease. To some degree, tolerance is incomplete and 'natural autoimmunity' to autoantigens is the norm. This has been shown for most autoantigens including several ocular/retinal antigens. However, there are immunological mechanisms in place that inhibit excessive expression of natural autoimmunity.

### Autoimmune disease is the dysfunction or damage of tissue caused by immune responses to autoantigens

Autoimmune diseases take many forms, from organ and even cell-specific antibody-mediated diseases such as myasthenia gravis (where the antigens are located at the neuromuscular junction) to widespread systemic diseases such as systemic lupus erythematosus, where the antigen is distributed in all tissues (DNA).

Tissue damage in autoimmune disease can be induced by any of the accepted forms of immunopathological mechanisms (types I–IV; Table 7–7). However, as for most immune mechanisms, autoimmune diseases are usually initiated by CD4<sup>+</sup> T cells. Several mechanisms have been proposed to account for this process:

- Molecular mimicry between foreign and autoantigen sequence homologies – as might be expected with the wide range of antigenic peptides occurring in infective organisms – occur with predictable frequency. Processing of foreign antigenic peptide might thus lead to activation of autoreactive T cells if the foreign antigen is 'mistaken' for self. This has been shown for several retinal antigens that have amino acid sequences similar to bacterial and viral antigens, including Gram-negative bacteria such as *Escherichia coli* and parasites such as *Onchocerca*, which causes endemic blindness in certain regions of Africa ('river blindness').
- Bystander activation – the initiation of autoimmune disease may also occur by bystander acti-

vation, for instance during infection. Upregulation of co-stimulatory molecules on APCs in the lymphoid tissues in the vicinity of anergized but not deleted autoreactive T and/or B cells may lead to autoimmune disease. Anergy may require expression of CTLA-4, which, if absent, may also allow self-autoreactivity.

- Failure of Treg activity – tolerance is mediated by Tregs (see above) that homeostatically inhibit autoreactive T cells, possibly by direct cell contact or release of cytokine. Tregs constitute a major proportion of circulating CD4<sup>+</sup> T cells. Reduced Treg activity is a feature of most autoimmune diseases.
- Failure of deletion (activation induced self-death) – T-cell deletion and apoptosis is mediated by cell surface molecules such as Fas–FasL and TNF-receptor apoptosis inducing ligand (TRAIL) – death receptor (DR4/DR5). Failure of the apoptotic machinery might lead to autoimmunity. TRAIL and Fas have both been shown to play a role in immune privilege.
- Polyclonal B-cell activation – certain compounds such as endotoxin and bacterial glycolipids can activate B cells directly, either to produce cytotoxic antibody or to act as APCs and thus present autoantigen to responding T cells.
- Superantigen – simultaneous activation of several subsets of T cell by superantigens, which do not require processing because they link the T cell and MHC antigen directly, may also lead to activation of autoreactive T cells. Preferential usage of certain TCRs by superantigens may enhance the risk of autoimmune responses.

### Parasitic infections persist by giving false signals to the immune system

Diseases caused by parasites are of major medical and economic importance worldwide. Parasites invade the tissues and enter into a symbiotic relationship, sometimes within the cytoplasm of the cell, particularly macrophages. Extracellular parasitic infections are even more frequent, such as those involving nematodes. Examples include filariasis, schistosomiasis and toxocariasis, the first and last of which can infect the eye. Ocular toxocariasis causes blindness in children,

presenting as one cause of the white (cat's eye) pupillary reflex.

Parasites present the immune system with enormous problems, partly because they go through a life cycle in which they present different antigens at different times; the host therefore has difficulty in generating an adequate response and may enter a chronic state of inflammation. The parasite achieves this by manipulating, evading or diverting the immune system. Although initially a Th1 response may be induced after infection by the parasite, the response is later diverted to become Th2-mediated with secretion of IL-2, IL-4 and IL-10. Several explanations have been proposed for this change, including defects in the presentation of parasite antigen, inappropriate production of regulatory cytokines such as IL-10 and IL-13, or the induction of a form of tolerance via prolonged binding of the antigen to the TCR. However, rather than being an anergic or impaired type of immune response, the switch from Th1 to Th2 may be quite pronounced. In some respects it is similar to the response in allergic disease because the Th1/Th2 immune response system becomes similarly dysregulated and can lead to excessive mast cell infiltration and eosinophilia, particularly with helminth infestations. Cytokines play a significant role in protection from parasites. In many infections, induction of a strong INF- $\gamma$ -mediated Th1 response through APC production of IL-12, IL-23 and IL-27 eliminates most of the parasites. This occurs in primary toxoplasmic infections, but some parasites escape detection inside circulating monocytes, which then 'hide' in sites of immune privilege such as the eye, brain and liver.

The parasite thus remains undetected until it kills its host cell, and the newly exposed antigens are released into the tissues to induce an immune response. Toxoplasmic retinochoroiditis is thought to occur by this mechanism. This condition is a relatively common cause of posterior uveitis, characterized by one or more large chorioretinal scars that undergo spontaneous reactivation, producing recurrent attacks of uveitis as the organisms spread.

## Allergy and immediate hypersensitivity

The immune system's attempts to rid itself of harmful foreign elements can sometimes be so exaggerated or

inappropriate that the host is damaged in the process. Such a situation arises in type I hypersensitivity (see Table 7-7) involving IgE responses to foreign antigens (allergens), thereby causing allergy. However, not all allergic responses are IgE mediated.

## ATOPIY, ASTHMA AND ALLERGIC EYE DISEASE

Allergic reactions are usually effective, short-lived immune responses which clear the allergen. Allergic disease is where this fails.

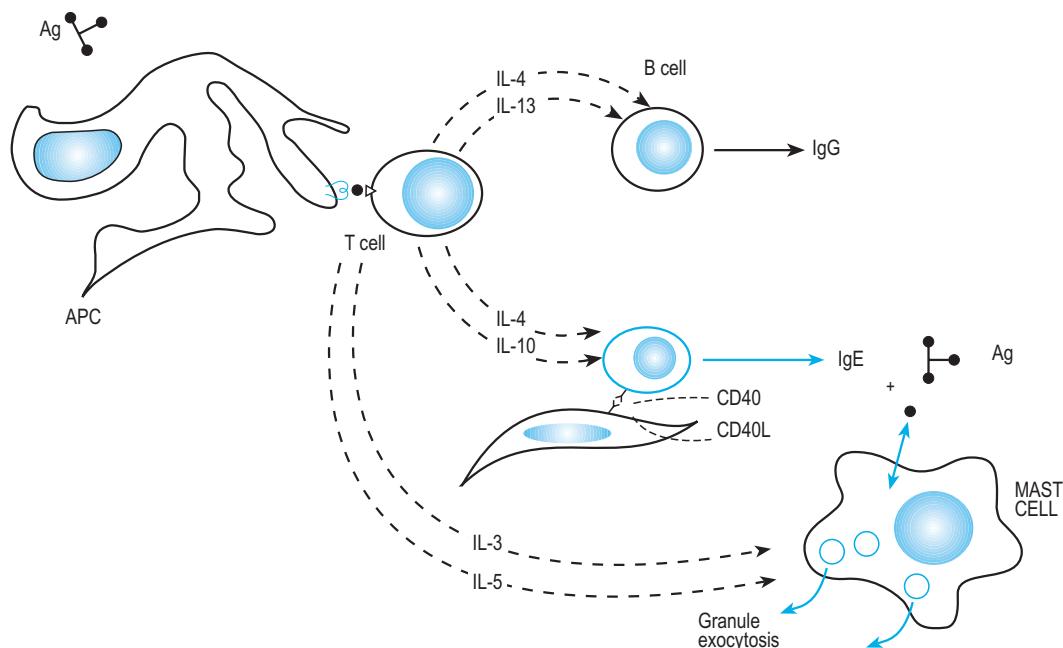
Allergic reactions have the following features:

- allergen-specific Th2 cell activation and production of IgE by B cells
- binding of IgE to mast cells with mast cell degranulation
- very wide range of 'allergens' including animal dander, chemicals (haptens), house dust mite proteins, commensals including fungal proteins, plant and other environmental proteins
- symptoms of immediate hypersensitivity due to cytokines.

Allergic disease occurs when the allergic reaction (i.e. the immune response) is exaggerated and the symptoms are the result of this exaggerated reaction. This can be very severe and acute, even life-threatening (as in acute asthmatic attacks). The allergen may have been cleared or may persist and induce the development of chronic disease (e.g. eczema, atopy, chronic asthmatic wheeze).

The prototype allergic disease is asthma, in which reversible obstruction of the airways occurs. This is often the acute manifestation of a chronic inflammatory process in the mucous membranes and skin. Asthma frequently coincides with chronic dermatitis (eczema), rhinitis and sinusitis, and conjunctivitis. When there is a strong genetic predisposition to synthesize IgE specific for certain external antigens, the disease is known as atopy. However, patients with asthma are not necessarily atopic. Other forms of asthma in non-atopic individuals are recognized (intrinsic versus extrinsic asthma). Similarly, all patients with severe allergic conjunctivitis are not always atopic. These concepts are important clinically as seen by the selective efficacy of monoclonal antibody anti-IgE therapy in the treatment of refractory asthma.

Allergic conjunctivitis induced by specific antigens such as pollens or house dust mite may be chronic



**FIGURE 7-36** Mast cell activation is dependent on antigen-specific activation of T cells (Ag) which, in concert with concomitant engagement of the CD40 membrane protein by the CD40 ligand (CD40L), induces an isotype switch in the presence of Th2-type cytokines to direct the B cell to produce IgE. IgE–antigen complexes are then available to activate the mast cell.

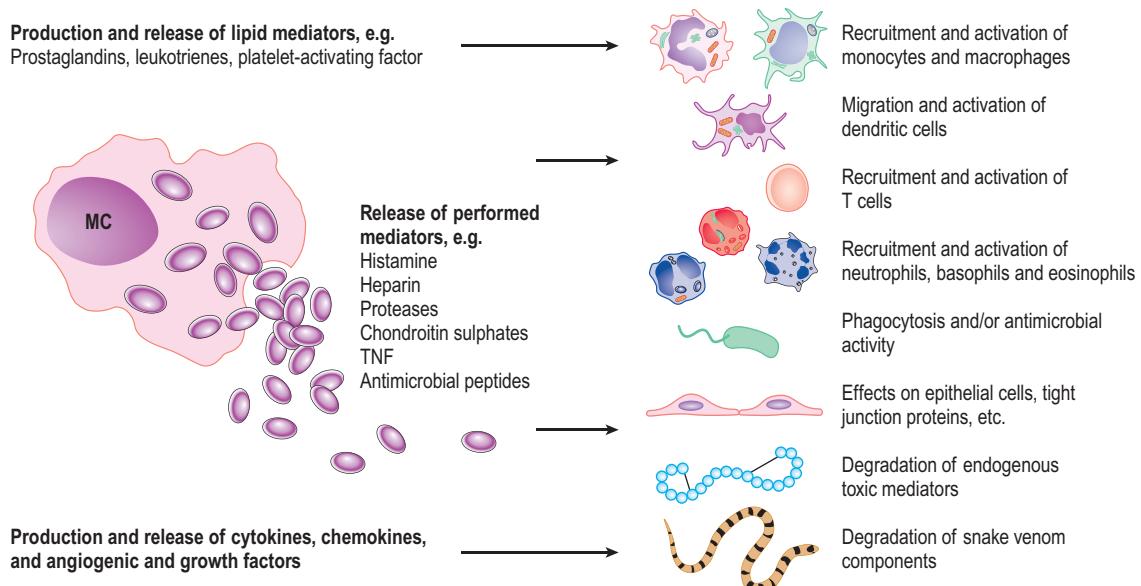
(perennial allergic conjunctivitis) or intermittent (seasonal allergic conjunctivitis), and usually produces mild symptoms. Chronic conjunctivitis in patients with atopy is more damaging, often with involvement of the cornea (atopic keratoconjunctivitis; see next section). A distinct clinical entity with massive follicular conjunctivitis and corneal opacification is known as vernal keratoconjunctivitis. In addition, contact lens wear is associated with a significant amount of allergic eye disease, producing giant papillary conjunctivitis.

In spite of the clinical differentiation between various forms of allergy, it is likely that the underlying mechanisms are similar. Activation of Th2 cells is mediated by migratory dendritic cells which capture allergen, activate Th cells in the LN to become Th2 cells which secrete IL-4, IL-5, IL-13 and Tfh cells (T follicular helper cells which activate B cells in the B-cell follicle). Mast cells have long been recognized as effector cells in allergic disease. However, mast cells are dependent on IL-3 and IL-5 production by T cells, which is ultimately induced by antigen presentation

to T cells (Fig. 7-36). In this regard, conjunctival dendritic cells have been considered to be major contributors to allergen capture and presentation to T cells locally in the tissues. In severe chronic asthma and ocular surface (conjunctival) disease there is massive thickening of the subepithelial mucosa with fibrosis and infiltration of other pro-inflammatory granulocytes such as eosinophils, which are important in tissue damage.

### Mast cells and mast cell degranulation

Mast cells are derived from precursors in the marrow and mature into one of two phenotypes depending on the microenvironment in which they reside. Stem (c-kit) cell factor is an important mast cell growth factor. In humans, mucosal mast cells contain tryptase ( $M_T$ ), histamine and heparin in their secretory granules, while connective tissue mast cells also contain chymase ( $M_{TC}$ ). In the normal conjunctiva and choroid,  $M_{TC}$  cells predominate. However, in allergic conjunctivitis,  $M_T$  cells appear to increase in number in association with the expression of adhesion molecules



**FIGURE 7-37** Mast cells have preformed granules ready to be released instantly on activation. They can also produce massive amounts of mediators *de novo* and are thus very potent cells with diverse effects on a wide range of cells as indicated in the figure. (From Metz and Maurer, 2007.)

such as E-selectin and ICAM-1. These changes are likely to be important in the pathogenesis of disease (see below). The significance of mast cell heterogeneity is not yet clear but it would appear that M<sub>T</sub> are involved in the active stages of inflammation.

Mast cell degranulation is mediated both by IgE or by non-IgE mechanisms (see below). The role of antigen-specific IgE in this response can be demonstrated *in vivo* experimentally by the passive cutaneous anaphylaxis test. However, IgE is neither sufficient nor essential to induce mast cell-related allergic disease. Also involved are chemokines, mediating not only leucocyte recruitment but also mast cell activation through mediators such as eotaxin 1 (CCL11) and MIP-1 $\alpha$ .

Mast cell degranulation leads to the release of preformed mediators including histamine and serotonin, which bind to a variety of receptors and induce second signalling events with release of a great variety of secondary agents depending on the cell type. This includes NO $^\bullet$ , prostacyclin, smooth muscle relaxants and many others. Several receptors exist for histamine (H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, etc.), which can be distinguished by

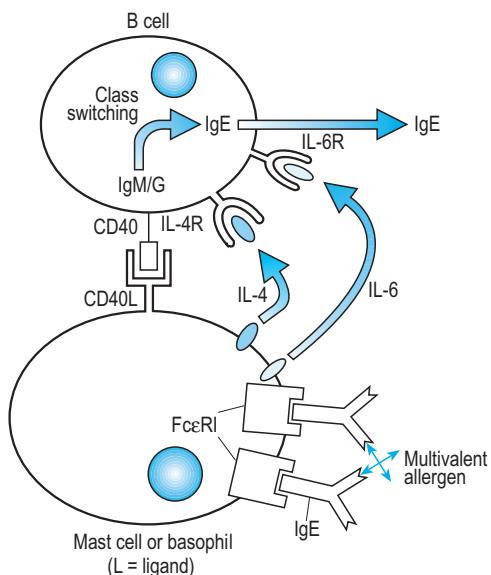
pharmacological agents (Fig. 7-37). Mast cells also synthesize and release newly formed materials after stimulation. These include prostaglandin D<sub>2</sub> (vasodilator and bronchoconstrictor), leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub>, previously known as slow-releasing substance A; cause prolonged bronchoconstriction), and platelet-activating factor (PAF).

Cytokine release by mast cells is the major inflammatory pathway. TNF- $\alpha$ , IL-1, IL-4, IL-5, IL-6, IL-13 and several colony-stimulating factors are released and help to recruit cells to the site of antigen exposure on the mucous membrane.

## IMMUNOGLOBULIN AND HELPER T CELLS

There are two types of IgE receptor: Fc $\epsilon$ RI, which is present in mast cells, basophils and activated eosinophils, and Fc $\epsilon$ RII (CD23), a low-affinity receptor present on many cell types including B, T and dendritic cells, monocytes and some thymic epithelial cells. Fc $\epsilon$ RII is important for initial antigen capture by dendritic cells.

IgE-mediated degranulation of mast cells occurs via high-affinity receptors (Fc $\epsilon$ RI). Signalling via the IgE



**FIGURE 7-38** Mast cells can themselves initiate isotype switching via CD40 because they express the CD40L antigen when they combine with allergen–IgE complexes. In this way the allergic response is amplified and perpetuated.

receptor is inducible only by IgE–antigen complexes and requires local production of IgE by infiltrating B cells.

Isotype switching in B cells from IgG to IgE requires binding of the cell surface antigen CD40 on the B cell to a ligand on the T cell (gp39). This can also be induced by mast cells that express the CD40 ligand and promote local production of IgE in the tissue under cytokine stimulation (Fig. 7-38). Thus the disease can be perpetuated locally and enter a phase of chronicity, as occurs in asthma.

### MECHANISM OF DISEASE PRODUCTION IN ALLERGIC DISEASE

Exposure of atopic individuals to allergen is accompanied by an immediate response mediated by mast cell degranulation and a later response occurring after 4–6 hours. The later response is induced by cytokine release from mast cells, which causes recruitment of M<sub>T</sub> cells and basophils to the tissue and the expression of adhesion molecules which promote eosinophil accumulation. In addition, release of the chemokine eotaxin by mast cells is probably the main stimulus

for eosinophil recruitment. Basophils are circulating granulocytes that are similar in many respects to tissue mast cells in their expression of FcεRI and release of cytokines. However, they are considered to be of a different lineage in the bone marrow (see pp. 376 and 378). T cells are not involved in this stage of the disease.

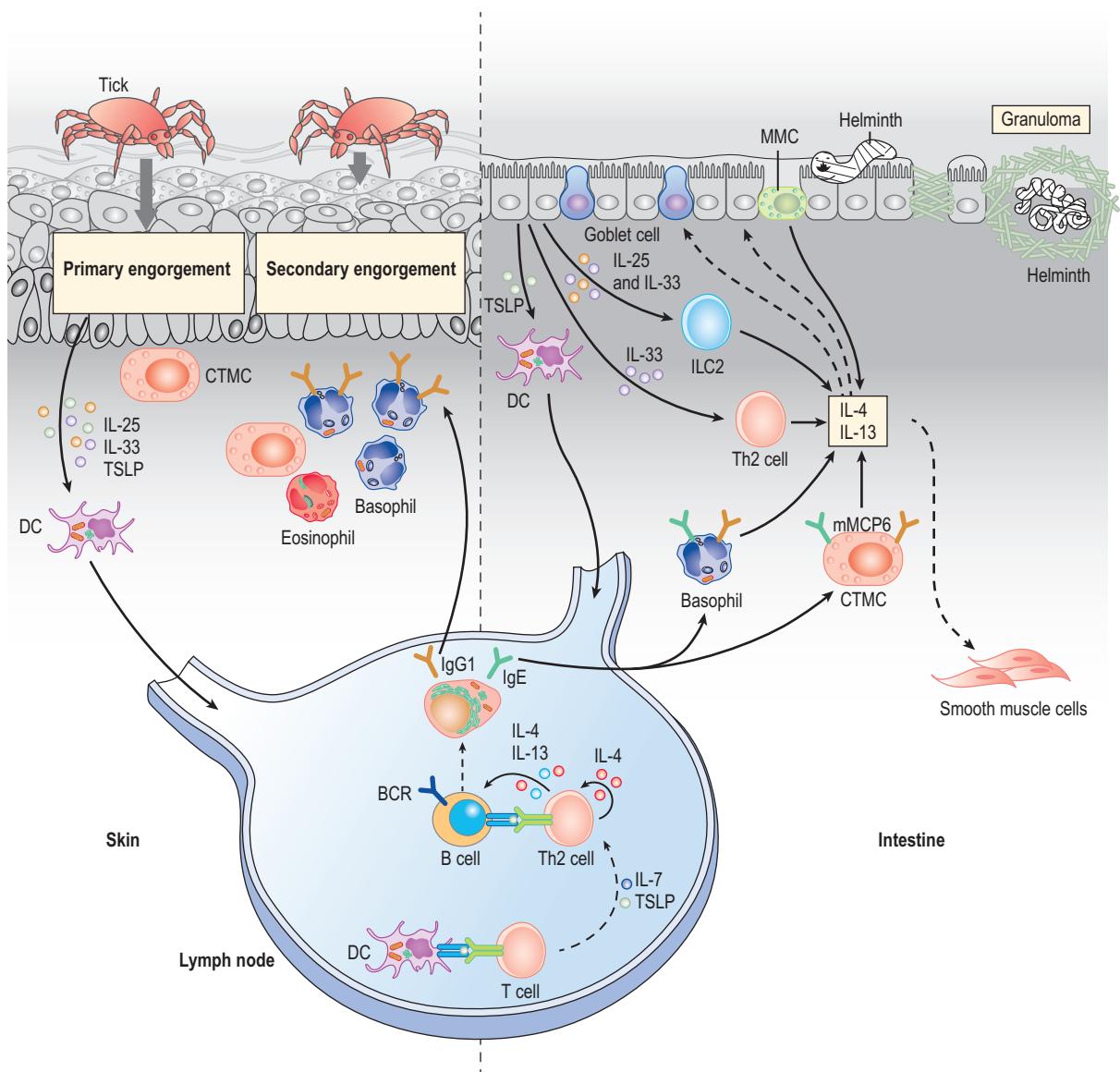
Eosinophils are strictly regulated because of their potential toxicity. In the absence of infection, very few eosinophils are produced. Even in the presence of an eosinophil, entry to the tissues is tightly controlled by eotaxins (via CCR3) and by other chemokines such as MCP-3, MCP-4 and RANTES.

Release of granule content by eosinophils, particularly erythropoietin (EPO), eosinophil major basic protein (MBP) and eosinophil cationic protein (ECP), causes much of the tissue damage. Eosinophils are attracted to the site of inflammation by specific adhesion molecules, particularly VCAM-1, on the endothelial cells. Induction of VCAM expression on endothelial cells is mediated by cytokine release from other inflammatory cells such as macrophages and T cells, and thus T cells are intricately involved in destructive tissue damage during the progression of allergy from a reaction to a disease. In addition, neutrophils are attracted to the site as a result of upregulated adhesion molecule expression, particularly of E-selectin and ICAM-1 in the acute stage. Neutrophils are themselves responsible for considerable tissue injury in certain forms of allergic disease and characterize a subtype of refractory asthma. The risk of superadded infection in these cases is unclear.

In the later stages of the disease, increased numbers of T cells may be recruited as part of the continuing inflammatory response because of persistent adhesion molecule expression on endothelial cells, especially VCAM. T cells are then available to interact with APCs, particularly activated B cells, causing further release of IL-3, IL-5, IL-13 and GM-CSF, which perpetuates the disease.

### MAST CELLS ALSO HAVE A PROTECTIVE ROLE

Mast cells may also have a role in the initiation of the immune response as effectors of innate immunity mediating adaptive immunity, particularly to organisms inducing Th2 responses such as helminths and ticks (Fig. 7-39). Mast cells express MHC class I and



**FIGURE 7-39** Parasites, like any antigen, are taken up by phagocytes such as dendritic cells, which, selectively, through particular Toll-like receptors, activate signals which polarize T helper cells towards Th2 through activation of STAT5. IL-4, IL-5 and IL-13 produced by the DCs themselves or by neighbouring tissue and other cells (e.g. mast cells) help to drive the response down a Th2 route. The process is amplified by continued production of Th2 cytokine, the formation of a parasite-enclosed granuloma and other cytokines such as IL-33, released from damaged and dying cells. (From Voehringer, 2013.)

class II antigens, and presentation of exogenous helminth antigen to T cells leads to activation reciprocally of the mast cell with release of chemokines and cytokines. These direct the overall response towards a Th2-type response.

## Organ and tissue transplantation

Transplants or grafts are described as syngeneic when they are between genetically identical individuals, allogeneic when they are between individuals of the same species and xenogeneic when they are between individuals of different species. Autologous grafts refer to the transplantation of tissue within the same individual, as for instance when the cornea from one eye is transplanted to the fellow eye. Allogeneic and xenogeneic grafts are normally not accepted unless the immune system is suppressed (usually by immunosuppressant drugs) and rejection is described as acute, chronic or accelerated.

The discovery of HLA antigens came about as a result of studies that attempted to explain how organ and tissue grafts, especially skin grafts, between members of the same species were rejected. Only in the case of genetically identical individuals were grafts accepted. For instance, in skin grafts, not only was there rejection of the initial graft between two non-identical individuals but also subsequent attempts to graft donor skin between the same two individuals led to an even more rapid rate of rejection, indicating that there was some sort of memory response to the antigens in the original graft.

Although potentially any antigen in the donor graft could act as a target for rejection ('danger' signal similar to an altered autoantigen), the strongest candidate antigens are those where there are the greatest frequency of polymorphisms (differences between individuals or alloantigens) and top of the list are MHC antigens. Cells with the strongest expression of MHC antigens are leucocytes and so rejection of grafts is considered to be the result of the presence of alloantigens on 'passenger leucocytes' in the donor graft. For solid organs and bone marrow grafts, tissue matching on the basis of both blood group and HLA antigens are therefore routinely performed and the closest 'match' attempted in order to ensure graft survival. However, even in these circumstances

immunosuppressive drugs are often necessary and usually for the lifetime of the patient.

Since in any one individual leucocytes express a large number of MHC alloantigens (see p. 425), a wide range of allopeptides will be presented to the host, thus leading to a polyclonal T-cell expansion. Damage to the graft is the result of alloantigen-specific CD8<sup>+</sup> cytotoxic T cells recognizing MHC antigens on the donor tissue. The number of mismatches on the MHC antigens between the donor and host determines the rapidity and acuteness of the graft rejection.

There are two main mechanisms whereby MHC antigens in the donor graft induce graft rejection. Passenger leucocytes in the donor graft can act as APCs and present antigen directly to host T cells (direct allorecognition) or passenger leucocytes can be killed by host immune cells, the dead cell material phagocytosed by host APCs, and the donor MHC antigens processed and presented on host APCs to host T cells (indirect allorecognition). Grafts are therefore more likely to be accepted if the MHC antigens between donor and host are closely matched and this is clearly the case in identical twins.

Donor non-MHC antigens (minor antigens) can also generate a host immune response and induce graft rejection, but the intensity of the response is considerably less. Mismatching at the minor alloantigen loci is a cause of chronic rejection, while accelerated rejection occurs predominantly between xenogeneic grafts (where differences exist in non-protein antigens such as carbohydrate antigens) or in individuals who have been sensitized to the donor, e.g. via previous blood transfusion. Disparities in MHC genetic loci have classically been determined by the mixed leucocyte response (MLR), an *in vitro* test in which leucocytes derived from one individual (donor) are mixed with those from another individual (host) and an immune response determined by the proliferation of the host leucocytes (the donor leucocytes are irradiated to prevent them from proliferating so that any response is the result of antigen presentation to host T cells). If the number of MHC disparities between the host and the donor is large, there is a correspondingly high proliferative response. However, molecular typing of genetic loci is now more common.

Most donor solid organ, vascularized and bone marrow transplants induce a strong early direct

alloimmune response which requires long-term therapeutic immunosuppression to be controlled if the graft is to survive. Graft rejection is mediated by CD8<sup>+</sup> T cytotoxic T cells in this circumstance. Simultaneously, as donor leucocytes die, an indirect alloimmune response develops which is slower but more insidious and is mediated by host CD4<sup>+</sup> T cells activating innate immune cells such as macrophages and other cells. Many types of chronic rejection, for instance of kidney allografts, are the result of the indirect route of antigen presentation and CD4<sup>+</sup> T-cell activity.

Corneal transplantation differs from other forms of skin and solid organ transplantation in the number of donor present in the graft, since there are no leukocyte-rich blood vessels in the central cornea, and even leukocytes that are resident in the corneal stroma are generally not equipped to activate the host T cells directly (i.e. in the quiescent cornea they do not express high levels of MHC antigen).

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Rejection of corneal grafts is thus mainly through indirect allorecognition. However, if rejection does occur, regrafting with a second donor who expresses some MHC antigens that are similar to the first donor will induce a strong CD4<sup>+</sup> T-cell memory response and cause rapid rejection of the second graft. For this reason alone there is a strong case for matching corneal grafts, although this practice is not currently routine due to the assumed high success rate of unmatched corneal grafts.

The role of other cells in the immune response to allografts is increasingly recognized. Th cells, both Th1 and Th17 cells, almost certainly have a role via CD40–CD40L interactions and may perhaps involve cross-presentation of antigen. In addition, ‘danger signals’ which activate cells of the innate immune system such as macrophages play a significant role. Surgical technique influences the acceptance or failure of grafts, both vascularized grafts, such as kidneys, and even more so corneal grafts. Donor-specific anti-HLA antibodies have the potential to activate complement, for instance in living-donor kidney allografts. In apparently accepted clear corneal grafts, rejection can be triggered by an unrelated event such as herpes simplex virus infection in the graft or even by a simple procedure such as corneal suture removal.

Presumably, activation of innate immune cells such as macrophages and dendritic cells via the inflammasome and antigen presentation with co-stimulation is sufficient to lead to activation of previously primed or memory T cells leading to rejection.

Tregs also play an important role in the overall immune response to a foreign allograft. In the initial inflammation associated with the surgical trauma, both naive T cells and Tregs extravasate into the tissues. In addition, APCs migrating to the draining lymph node activate both Tregs and non-Tregs which home with different kinetics to the graft. The balance between T-effector cells (Th1/Th17) and Tregs determines the survival of the graft.

Finally, rejection of xenografts (the first ever corneal graft performed was a xenograft) is mediated by pre-formed antibody and complement and generates hyperacute rejection, induced through failure of complement regulatory protein to act across species barriers.

## Tumours induce immune responses

Clinicopathological studies have long indicated that tumours induce some form of immune response in the host. This is based mainly on observations of T-cell, macrophage and NK-cell infiltration of tumours independent of whether there is any inflammatory response or tissue necrosis. Experimental studies of chemically induced tumours have also shown that transplantation of resected tumours to the original host, or to a host previously sensitized to tumour antigens, leads to rapid rejection of the tumour, but not when the tumour is transplanted to a syngeneic naïve host. Rejection is tumour-specific. Thus, tumours possess specific antigens and these induce an MHC class I-restricted T-cell cytotoxic response.

The concept that cancer cell mutations occur very frequently but are held in check from developing into full-blown tumours is long-standing and indeed NK cells are specially equipped to kill and delete malignantly transformed cells as well as infected cells (see pp. 389 and 390). Antigen-specific cytotoxic T cells (mostly CD8<sup>+</sup> but also CD4<sup>+</sup> killer cells) may also play this role.

Tumour antigens may be tumour-specific, i.e. only expressed on that tumour, or they may be

Corneal grafting is unusual. Successful corneal allografts between unrelated donors and recipients have been performed since the early part of the 20th century, long before HLA antigens were discovered. This was attributed to immune privilege of the eye, and specifically to the lack of 'passenger' leucocytes in the normal cornea. However, it is now accepted that the apparent success rate of corneal graft applies only to 'low-risk' recipients, namely patients with opaque corneas without significant chronic inflammation or infiltration of the cornea with abnormal blood vessels. Work from Australia has re-examined the question of the privileged status of the cornea in protection against rejection when used as an unmatched allograft and without concomitant immunosuppression. In fact the 5-year survival of unmatched corneas in high-risk recipients, i.e. those patients who have ongoing low-grade inflammation or blood vessel infiltration as in herpes simplex keratitis (see Ch. 9, p. 495), is less than for renal allografts, where steady improvements in survival of allografts has continued ([Williams and Koster, 2007](#)). Recent experimental studies of high-risk graft rejection have shown that sensitization, even to a single corneal antigen, in high-risk grafting (particularly in regraft procedures where the first graft failed) can be highly immunogenic and leave a strong immunological memory. To reduce the risk of rejection due to memory T cells sensitized to the antigens present in the first graft, HLA matching of all grafts should be performed so that a second graft has the fewest HLA matches to the first donor and thus maximizes the chances of acceptance of the regraft ([Vitova et al., 2013](#)).

tumour-associated, in which case they are also expressed on normal cells. Most tumour antigens are identified in transplantation-type experiments such as that described above in which cloned cytotoxic T cells are generated. This allows identification of the tumour-derived peptides and the genes that regulate their expression in the tumour. Tumour antigens are believed to be recognizable by cytotoxic T cells in this manner, owing to antigenic mutation that converts the normal self-protein, which would induce tolerance, to a non-self-protein, which is recognized as foreign and induces immunity. Thus some tumour antigens are products of normal cells such as tyrosinase in melanoma cells. However, many tumour antigens are produced by oncogenes, genes which are implicated in the cell cycle and in cell differentiation. These include *p21 ras*, *HER-2/neu*, the very important *p53* gene and others not normally expressed on cells, such as the MAGE series of proteins expressed by melanoma cells including cells of the choroid. Several viral genes may also be expressed in tumours, including those for the SV40 T antigen, the human papillomavirus E6 gene and the EBV antigen. Certain B-cell tumours are indirectly linked to EBV genes, particularly in the presence of immunodeficiency, and appear to involve the translocation of the *myc* oncogene to the immunoglobulin locus. Retroviruses also have the potential to induce malignant transformation of normal cells including the *src*, the *myc* and the *k-ras* gene. The human T-cell lymphotropic virus-I gene is also implicated in certain aggressive T-cell tumours and interestingly is also involved in some forms of intraocular inflammation (uveitis).

Certain tumour antigens are recognized by antibodies as well as by cytotoxic T-cell lines. These include the oncofetal antigens, carcinoembryonic antigen and  $\alpha$ -fetoprotein. Other tumour-associated antigens are linked to tumours such as the surface glycoprotein MUC-1 in breast cancer, S-100 in neural crest cell tumours and malignant melanoma, and cytokeratins in epithelial cell tumours.

Immune cells infiltrating tumour are considered to be effector cells against the tumour at least in some instances. Indeed the process of immune surveillance in which T cells and antigen-presenting cells migrate through the tissues detecting altered self-antigens applies particularly to tumours and is necessary for

the initiation of the anti-tumour response. Tumour-infiltrating lymphocytes (TIL) include both CD4 $^{+}$  and CD8 $^{+}$  cytotoxic cells and the former probably supply essential cytokines to the latter to promote tumour-killing ability. Some tumours aberrantly express MHC class II antigens plus co-stimulatory molecules; they may directly present tumour antigens to CD4 $^{+}$  T cells and initiate the immune response *in situ*.

NK cells may also be important in killing tumours (as well as removing individual transformed cells as they arise), particularly those that have been induced by viruses. IL-2-activated NK (lymphokine-activated killer, LAK) cells have a markedly enhanced ability to lyse tumour cells and they are in trials as immunotherapeutic agents. Macrophages are also important in tumour killing, usually via release of TNF- $\alpha$ , a cytokine that was first identified by its ability to induce necrosis in tumours. Tumour cells appear to be unable to synthesize superoxide dismutase, which is required to protect cells from the TNF- $\alpha$ -induced release of cytotoxic superoxide free radical.

Despite the variety of mechanisms for tumour killing, many tumours evade death. Tumours utilize a variety of strategies: e.g. they downregulate MHC class I, thus inhibiting cytotoxic T-cell killing; they may not express co-stimulatory molecules necessary for T-cell activation; they may secrete immunosuppressive cytokines such as TGF- $\beta$ ; tolerance to tumour cells may occur if the tumour antigen is expressed during the neonatal period; antigenic modulation of the tumour may occur if the antigen binds non-complement binding antibody; or the tumour antigen may be prevented from gaining access to the immune system by a dense glycocalyx on the cell surface. The immune response to tumours is more likely to be modulated by any or some of these mechanisms, thus explaining why tumour immunity is imperfect and cancer remains a major cause of death.

Tumours may evade the immune system by promoting tolerance rather than immunity. In particular, Tregs comprise a significant component of tumour infiltrating lymphocytes (TILs) and prevent development of an effective antigen-specific anti-tumour response. In addition, the recently identified Gr1+Lys6C-myeloid derived suppressor cell (MDSC) was first isolated from within tumour tissue and is now recognized to have a significant immunosuppressive effect, not

only against tumours but also against any immune response. MDSCs are thus being proposed as therapy for autoimmune and other immune-mediated disease. In contrast, other cell-based therapies are in the vanguard of 'cell vaccination' therapies for cancer treatments: these are based on the use of activated, pro-inflammatory dendritic cells loaded with tumour-specific antigens to enhance strong anti-tumour responses and have been introduced for certain forms of prostate cancer. The main difficulty with this approach is identifying peptides specific for the tumour that are sufficiently immunogenic.

## The eye and the immune system

The eye participates in all aspects of immune responses like any other tissue, but the immune response is modulated by the cells and tissues of the eye. In this respect, the eye (and the brain) are regarded as 'immunologically privileged', a concept which has now been broadened to be included as a third mechanism of tolerance induction. Both the innate and acquired immune systems function in ocular defence mechanisms.

### THE INNATE IMMUNE SYSTEM AND THE EYE

Reference has already been made to the several physical and chemical barriers to ocular infection included in the blink reflex, the lids, and the components in the tears such as lysozyme, lactoferrin and complement (see also Ch. 4, p. 198). Lysozyme is effective against Gram-negative bacteria and certain fungi but is ineffective against Gram-positive organisms such as *Staphylococcus aureus*. Lactoferrin and transferrin, however, are more effective in defence against Gram-positive bacteria because they bind iron, an essential cofactor for eukaryotic as well as prokaryotic cell growth. In addition, tears have specific anti-adhesive properties for bacteria and therefore inhibit bacterial attachment and invasion of the ocular surface, and are incidentally important for prevention of contact lens contamination.

Tears also contain polymorphonuclear leucocytes (PMNs), which increase in number when the lids are closed for prolonged periods, e.g. during sleep. The anti-adhesive properties of tears extend to these cells, ensuring that they pass through the lacrimal

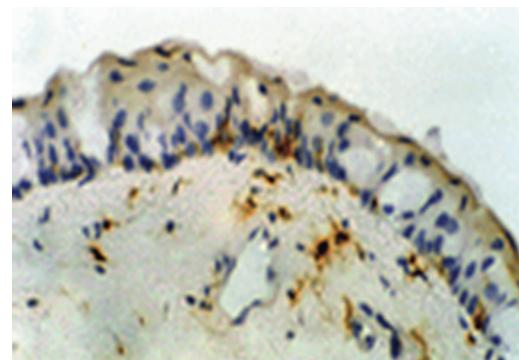
passages and do not penetrate the corneal surface. Many of these leucocytes pass directly from the conjunctival vessels through the epithelial layers into the tear film. PMNs contain numerous antibacterial protein enzymes, including proteinase 3, myeloperoxidase (which generates free radicals), calprotectin,  $\beta$ -lysin and the cathepsins.

Tear lipid also has an antibacterial effect. This applies to both short- and long-chain fatty acids, the former affecting surface properties of the bacterial cell membrane and the latter having a direct effect on metabolism. In addition cationic peptides, such as the defensins, as well as surfactant protein-D are part of the conjunctiva-associated lymphoid system (CALT, see p. 416).

The cells of the ocular surface also express, or can be induced to express, many of the innate immunoreceptors such as TLRs and are involved in many of the cornea and conjunctival innate immune responses to infectious agents (see Ch. 8, p. 469). More importantly, the conjunctiva contains numerous dendritic cells (similar to Langerhans' cells in the skin) which act as APCs in the draining lymph nodes in the afferent limb of the immune response. It is thus possible to become sensitized to environmental antigens and allergens via the conjunctiva (Fig. 7-40).

### THE ADAPTIVE IMMUNE SYSTEM AND THE FIRST LINE OF DEFENCE IN THE EYE

Tears contain immunoglobulins such as IgA (see Ch. 4) and occasional specific immune cells such as



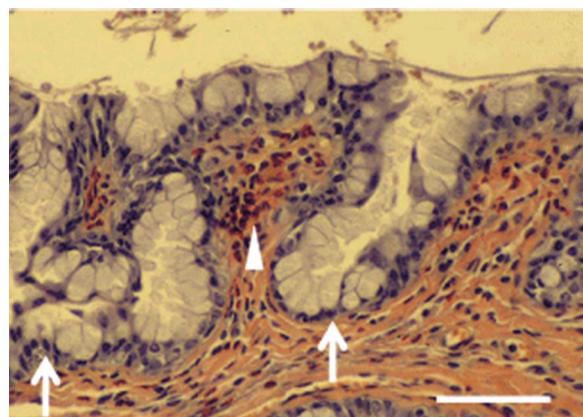
**FIGURE 7-40** Conjunctival DCs in the rat. Both intraepithelial and stromal DCs are shown expressing MHC class II (immunoperoxidase, dark brown stain). DCs, dendritic cells; MHC, major histocompatibility complex.

lymphocytes. IgA is produced by B cells in the lacrimal gland and secreted as sIgA in the tears. In addition, the lacrimal gland produces other immunosuppressive cytokines such as TGF- $\beta$ . The TGF- $\beta$ -deficient mouse exhibits extensive ocular surface pathology, indicating the importance of this cytokine in tears.

Conjunctival sensitization to environmental antigens is best demonstrated by the allergic (Th2) type response. Common antigens include pollens, house dust mite and animal dander (particularly cat dander). Although the effect is produced locally, the initial sensitization is a systemic one via activation of Th2 cells in the draining lymph nodes (see pp. 414–415). This results in local conjunctival mast cells becoming loaded with antigen-specific IgE, which renders these cells acutely sensitive to re-exposure to antigen. The chemokine CCL2 appears to be partly involved in this process of mast cell degranulation in giant papillary conjunctivitis, while the receptor CCR7 is active in directing conjunctival dendritic cell migration during this process.

If the sensitization is long-lived and recurrently activated with frequent mast cell degranulation episodes, it can lead to chronic inflammation. Thus seasonal allergic conjunctivitis can progress to perennial conjunctivitis and, in atopic individuals, may manifest as severe atopic conjunctivitis and/or vernal keratoconjunctivitis. Much of the damage in vernal keratoconjunctivitis is thought to be induced by eosinophils, because eosinophil cationic protein levels in tears are greatly increased in both atopic and vernal keratoconjunctivitis (Fig. 7-41). In addition, different types of allergic conjunctivitis are associated with different patterns of cytokine production and Th-cell patterns. Th2-like profiles are linked to vernal keratoconjunctivitis, while Th1 is associated with atopic keratoconjunctivitis. There may also be a defect in histaminase function in allergic eye disease causing prolonged histamine effects after mast cell degranulation.

The corneal surface also contains populations of intraepithelial and stromal leucocytes, some of which have the characteristics of dendritic APCs (see Ch. 1, p. 18). The role of these cells in protective immune responses is not clear but there is evidence that, like the conjunctival cells, they can capture antigen and transport it to the draining lymph node. In addition, they are likely to respond to cytokines produced by surrounding epithelial cells in response to TLR



**FIGURE 7-41** Histopathology in allergic conjunctivitis in an experimental model. Note the pseudotubular structure formations in conjunctival epithelium (arrows) and infiltration of eosinophilic cells in the subepithelial stroma (arrowhead). Bar = 100  $\mu\text{m}$ . (From Hara et al., 2012.)

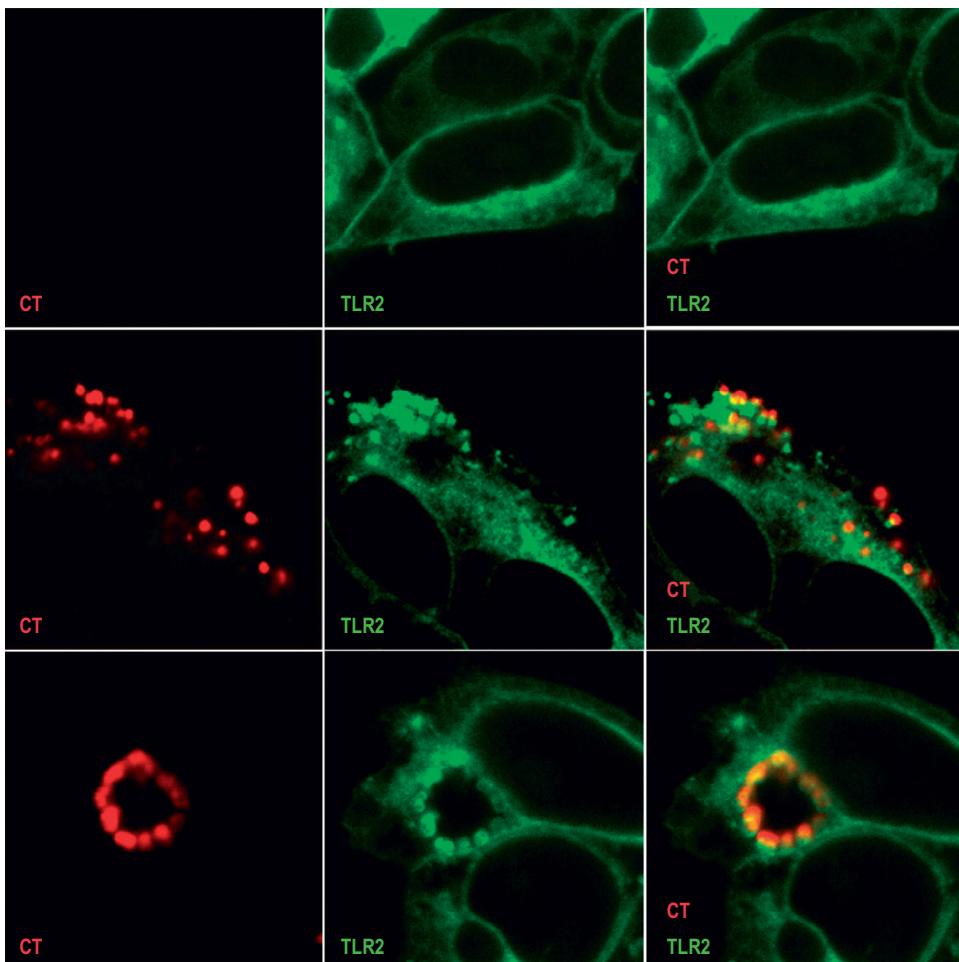
engagement by common invading microorganisms such as herpes viruses and bacterial pathogens. This may be a direct effect of the pathogen; for instance, the obligate intracellular parasite *Chlamydia*, which is the cause of the worldwide blinding disease trachoma, signals through TLR2 inside epithelial cells (Fig. 7-42). Indeed the possibility of regulating host-pathogen interactions has been tested in an experimental model of *Pseudomonas* keratitis in which ‘silencing’ of expression of TLR9 was achieved using small interfering RNA for this receptor.

## PROGRESSIVE OCULAR SURFACE DISEASE

Certain other ocular surface diseases occur that do not appear to be allergic in nature but are considered to be immune-mediated if not autoimmune in their pathogenesis. These include cicatrizing conjunctival disorders, various forms of ‘melting’ corneal disorders and a number of scleral and orbital inflammations.

### Cicatrizing disease of the conjunctiva

Subconjunctival fibrosis occurs in certain rare disorders such as benign mucous membrane pemphigoid and the Stevens–Johnson syndrome. Both are considered to be autoimmune in nature by virtue of the detection of antibodies to basement membrane components such as integrins. Pemphigoid is characterized



**FIGURE 7-42** TLR2 co-localizes with intracellular *Chlamydia trachomatis*. HEK293 cells stably expressing CFP-tagged TLR2 (green) were infected with *C. trachomatis*. Uninfected cells (top row) and infected cells at 16 hours (middle row) and 24 hours (bottom row) post-infection were stained using a monoclonal antibody against *Chlamydia* lipopolysaccharide. (From O'Connell et al., 2006, with permission from the American Society for Biochemistry and Molecular Biology.)

by progressive cicatrization of the conjunctiva stroma leading to severe shallowing of the fornices (see Ch. 9, p. 501). In this condition, specific autoantibodies against conjunctival epithelial  $\beta_4$  integrin, a component of the hemidesmosome (see Ch. 4, eBox 4-2) have been identified. This is in contrast to bullous pemphigoid, a skin disorder that does not affect the conjunctiva but is characterized by widespread areas of epithelial detachment, and in which antibodies against other proteins such as desmoglein and plectin may be detected. Dysregulation of TGF- $\beta$  in conjunctival cells has been reported in ocular pemphigoid.

The Stevens–Johnson syndrome has similar appearances but is much more acute, although self-limiting. This condition is normally associated with drug administration in which the drug is considered to act as a hapten. The severity of the condition is limited in its effects by the degree and duration of exposure to the drug.

#### Keratitis and ‘melting’ corneal ulcers

Many forms of keratitis are considered to be immune-mediated, including postherpetic disciform keratitis

(see Ch. 9, p. 501) in which residual herpes simplex virus antigen may play a role. In addition, certain debilitating corneal diseases characterized by peripheral corneal thinning and ulceration, sometimes leading to perforation, may be autoimmune or at least immune-mediated, mainly because they represent 'vasculitic' complications of 'classic' autoimmune diseases such as rheumatoid arthritis and also because of the absence of overt infection. In this condition, reductions in Tregs cells have been reported as well as increases in B cells and antiorneal epithelial antibodies. Some forms of peripheral ulcer such as Mooren's ulcer have been linked to a cornea-associated antigen, calgranulin, also found in peripheral blood neutrophils and filarial nematodes. These disorders occur just distal to the source of corneal epithelial stem cells at the limbus, which suggests a defect at this level.

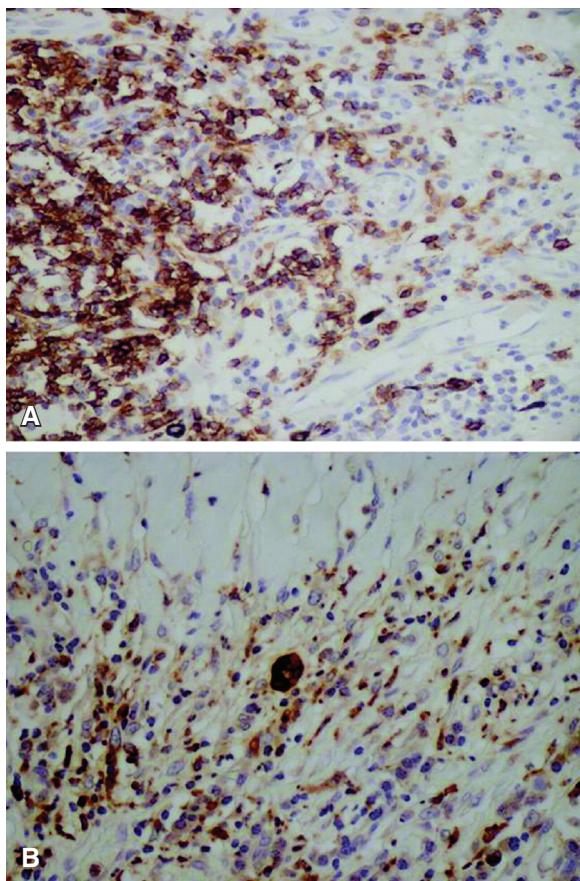
### Inflammatory disorders of the orbit and sclera

While many inflammatory conditions of the sclera (scleritis) are considered to be autoimmune, or at least immune-mediated, it is important to consider infectious aetiologies such as syphilis and other bacterial causes. Spontaneous (autoimmune) inflammatory disorders of the episcleral tissue and the sclera (episcleritis and scleritis) represent a type IV immunopathological disease with close association with rheumatoid arthritis and a similar pathogenesis. Scleritis occurs in rheumatoid arthritis patients who have vasculitis. Rare histological studies have shown activated CD4<sup>+</sup> T cells in the lesion in a perivascular location, in addition to macrophages. CD8<sup>+</sup> T cells have also been demonstrated, but in fewer numbers. There is a prominent vasculitic component to the disease with extensive necrosis of the scleral layers. Typical granulomatous lesions are a feature of this condition (nodular scleritis). However, not all of these lesions are T-cell-dominated, because B-cell 'follicles' have been identified in some cases, suggesting the development of tertiary lymphoid structures (see p. 417). In addition, increased matrix metalloproteinase activity has been detected in these lesions (e.g. collagenase and stromelysin) (Fig. 7-43). Although the antigen(s) for scleral inflammation has not been identified, it is presumed to be a component of the extracellular matrix such as dermatan sulphate proteoglycan

or type 1 collagen. Scleritis most commonly affects the anterior sclera but if it affects the posterior sclera it is more difficult to diagnose. In addition, it may be mistaken for a less well-defined group of orbital inflammatory disorders known as pseudotumour of the orbit, for which the aetiology remains obscure but which responds to systemic steroid therapy. A specific form of pseudotumour known as orbital myositis, in which an acute inflammatory swelling of a single ocular muscle occurs, is particularly responsive to steroid therapy. The autoantigen in this disorder is assumed to be a component of the ocular muscle.

Swelling of the orbital muscles also occurs in dysthyroid eye disease, causing proptosis and exophthalmos, but in this condition all four muscles are variably involved. This disorder is closely linked to Graves' disease of the thyroid in which thyroid autoantigens such as thyroglobulin and the thyroid-stimulating hormone receptor are implicated. Patients with dysthyroid eye disease have circulating lymphocytes that react with ocular muscle cell membrane antigens. However, thyroglobulin does not appear to be the important antigen for ocular muscle damage, and some other antigen such as the thyroid-stimulating hormone receptor (TSHr) may be involved. Models of thyroid ophthalmopathy using T cells sensitized to the thyroid-stimulating hormone receptor have been reported. Immunological studies have shown that the T-cell infiltrate is almost exclusively Th1 in type, with secretion of IL-2 and IFN- $\gamma$ .

Inflammatory disease of the lacrimal gland may be primary (autoimmune), as in Sjögren syndrome, or secondary, as in sarcoidosis, in which the aetiology is unknown. In both disorders there is a deficiency of tear secretion that produces a secondary keratoconjunctivitis (keratoconjunctivitis sicca or the dry eye syndrome), common in the elderly. Primary Sjögren syndrome involves other secretory glands such as the salivary glands and is characterized by specific autoantibodies against ribonucleoproteins (antiRo and antiLa) whose role in the pathogenesis of the condition is not clear. Biopsies of salivary gland tissue have shown a predominant T-cell infiltrate, but with little evidence of T-cell activation (as evidenced by the lack of IL-2 receptor (CD25) expression). In contrast, conjunctival biopsies from patients with Sjögren syndrome have shown significant T-cell infiltrates with activation



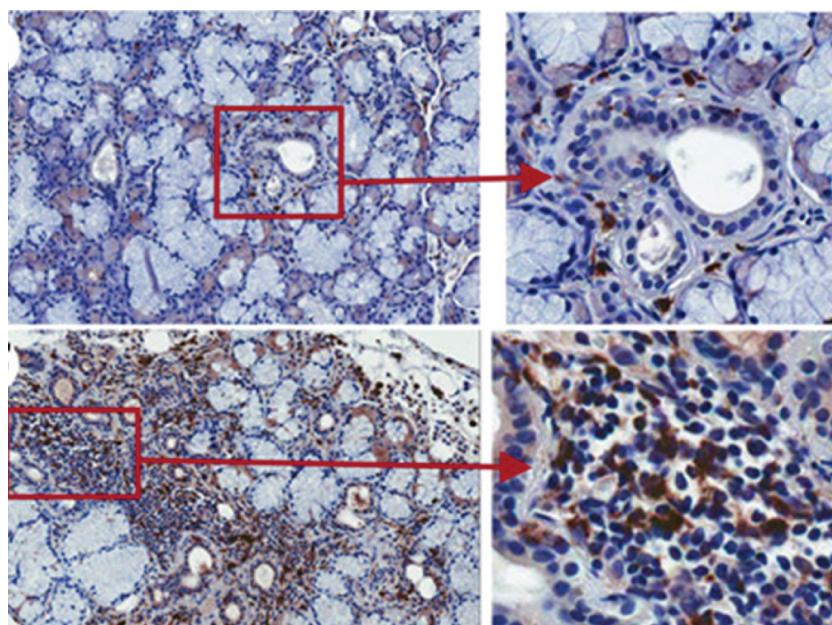
**FIGURE 7-43** Immunopathology of scleritis. (A) Autoimmune scleritis. Infiltration of CD20-positive B cells in the autoimmune group (original magnification  $\times 100$ ). (B) Necrotizing scleritis. Large numbers of CD68-positive macrophages in the idiopathic necrotizing scleritis groups (original magnification  $\times 80$ ). (From Usui et al., 2008.)

markers. In addition, extensive adhesion molecule expression has been detected in the lacrimal gland, not only on endothelial cells but also on the acinar epithelial cells. Both VCAM-1 and E-selectin are upregulated on the endothelium, indicating that this chronic disease is in a state of persistent activation. Eventually these glands undergo involutionary atrophy. Recent extensive microarray studies have shown that mammalian chitinases, innate immune products of macrophages with specificity against chitin-containing pathogens are highly active in severe acinar gland destruction of Sjögren syndrome (Fig. 7-44).

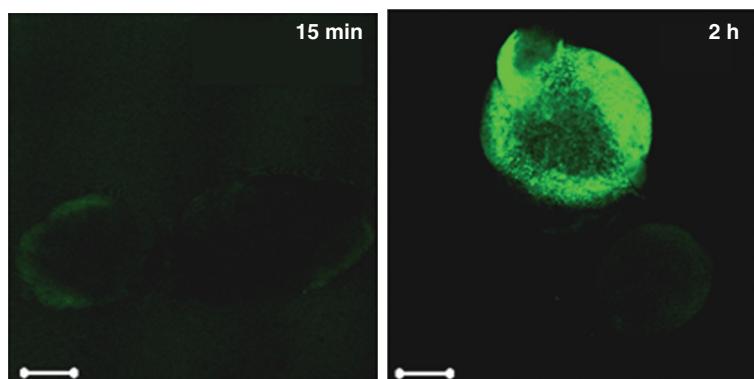
### The eye as a privileged site: what does this mean for the survival of corneal grafts?

The eye has been considered immunologically ‘different’ since the first corneal graft in a human was performed more than 100 years ago, and shown not to be rejected. However, the eye as an immunologically privileged site was not formally recognized until 1945 when Peter Medawar in his seminal studies on alloreactivity in rejection of skin grafts, showed that skin grafts placed in the anterior chamber of the eye or the brain were not rejected: i.e. these tissues demonstrated tolerance (see p. 441) to foreign antigens. The basis for this phenomenon was attributed to the avascularity of the cornea and/or to the lack of lymphatic drainage for intraocular structures. However, alloreactive T cells specific for corneal antigens can be detected in the circulation and appear to be generated in the secondary lymphoid tissues (SLO), indicating that despite the apparent lack of ocular lymphatics, foreign antigens in the cornea (and indeed from other ocular structures such as the retina) are clearly transported to the SLO. Most recently, antigen tracking to the submandibular draining lymph node has been detected using green fluorescent-labelled proteins applied to the corneal surface (Fig. 7-45).

Despite this, immunological ‘privilege’ (IP) in the eye is a real phenomenon and a property of the intraocular compartments, because antigens and cells including some xenogeneic tumour cells appear to be well tolerated in the anterior chamber of the eye. In addition, after acceptance of grafts in the eye, second grafts from the same individual are accepted at sites where they would previously have been rejected, e.g. the skin. This indicates that the tolerance induced by the graft placed in the eye is a systemic phenomenon which was previously considered a ‘deviation’ from normal immune responses; it was thus termed anterior chamber-associated immune deviation (ACAID). Interestingly, ACAID requires an intact spleen and is mediated by a range of immunosuppressive factors in the eye, particularly TGF- $\beta$ , as well as by immuno-suppressive ligand receptor pairs such as Fas–FasL, PD1–PDL1 and TRAIL–D5 (see p. 433). Importantly, Tregs (both CD4 $^{+}$  and CD8 $^{+}$  Tregs) play a significant role in sustaining IP, both of which are generated in the SLOs, particularly the spleen.



**FIGURE 7-44** Upper panels: normal mouse salivary gland has only a few resident macrophages (immunoperoxidase staining for CD68) (original magnification  $\times 20$ ,  $\times 40$  right panel). Lower panels: diseased salivary gland is heavily infiltrated with CD68 $^{+}$  macrophages (magnification also  $\times 20$ ,  $\times 40$ ). (From Greenwell-Wild et al., 2011.)



**FIGURE 7-45** Immunofluorescence of submandibular draining lymph node from mouse after application of a green fluorescent-labelled protein to the surface of the mouse cornea. Two hours after application, the protein was readily detectable in the lymph node. (From Dang et al., 2013.)

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Ocular IP (and its secondary phenomenon ACAID) is a manifestation of *tissue-based immune tolerance* (see online text referring to Matzinger in this Chapter). Most compartments and tissues of the eye have tissue-based ‘tolerizing’ properties, as do many other tissues such as the brain, testis, pancreas and several acinar tissues. This contrasts with tissues closer to

the external environment, where there is greater likelihood of encountering pathogenic foreign antigens, and the default response in these tissues (e.g. skin, conjunctiva, lung and gut) is more likely to be an inflammatory response aimed at clearing the pathogen. However, this response is still modulated by powerful regulatory mechanisms, particularly by Tregs in the GI tract, the absence of which leads to severe autoimmune disease.

Recently it has been recognized that ocular immune privilege comes with risks. While it is important for the preservation of sight that immune responses within the eye do not get out of control when inflammation occurs, those modified immune defences which the eye has may not be enough to prevent an exaggerated response. Thus, certain organisms can proliferate or reactivate with less of a trigger than if they were activated in other sites. For instance, toxoplasmosis is a common cause of ocular inflammation but is still relatively infrequent given that almost a third of the population have been infected, mainly through eating infected meat. This indicates that for most of the population toxoplasmosis is controlled and indeed in the initial infection in the gut, the organism is rapidly cleared and cleared completely. Some organisms (tachyzoites) escape into the bloodstream carried within infected monocytes and find their way to sites of immune privilege where they can lie dormant and never cause a reaction. However, if the immune defences are diminished, as occurs in HIV-positive patients or patients taking immunosuppressant drugs, the *Toxoplasma* organisms can escape immune control and reactivate, causing a severe retina-destructive inflammation. Similar pathogenesis occurs with other organisms such as cytomegalovirus and *Mycobacterium tuberculosis* (Forrester and Xu, 2012).

Tissue-based immune tolerance (ACAIID-like mechanisms) may extend to innate immunity and have implications for the ability of the eye to counteract microorganisms. Thus the eye appears to be the preferred site for certain parasites such as *Toxoplasma* and *Toxocara* (see Ch. 8) and intravenously injected fungi such as *Candida* may find a 'tolerant' environment within the retina and vitreous with disastrous effects on vision. In addition, certain viruses such as cytomegalovirus and herpes simplex can proliferate unchecked within the retina. While there is no direct evidence that this is a result of the less than optimal ('privileged') immune microenvironment within the eye, it is a possibility that remains to be tested. It seems therefore that IP may come at a cost.

Despite these properties of the intraocular compartments, bacterial infection during intraocular surgery is remarkably infrequent. This has been attributed in part to the direct bacteriostatic properties of the aqueous, which have been shown to inhibit bacterial growth *in vitro*. The nature of this activity is not known but several antibacterial proteins are present in the aqueous, including complement, immunoglobulin, defensins and  $\beta$ -lysin. In addition, it is dependent on the size of the bacterial inoculum and the virulence of the organism (see Ch. 8, p. 473).

### Intraocular inflammation

A common cause of visual impairment is intraocular inflammation, which includes the many forms of uveitis as well as conditions such as retinitis, and even optic neuritis. Intraocular inflammation may be exogenous, in which the organism is clearly evident, as in bacterial endophthalmitis (see Ch. 9, p. 494) or cytomegalovirus retinitis, or there may be no obvious infectious agent. Most such cases are described as idiopathic or non-infectious uveitis and may affect the anterior segment (iritic cyclitis, inflammation of the iris and ciliary body) or posterior segment (posterior uveitis). Posterior uveitis may take many clinical forms such as multifocal choroiditis and retinal vasculitis and formal definition and classification of the numerous entities is work in progress.

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Non-infectious intraocular inflammation is thought to be autoimmune or at least immune-mediated. Around 50% of cases of acute anterior uveitis have a strong association with HLA-B27 alloantigen and are linked to ankylosing spondylitis and low-grade Gram-negative enteric infection with organisms such as *Yersinia*, *E. coli* and *Klebsiella*. Posterior uveitis is much less closely linked to MHC class I antigens, except for certain well-defined syndromes such as birdshot retinochoroidopathy (HLA-A29) and Behcet's retinal vasculitis (HLA-B51 in oriental and Middle Eastern people). Vogt-Koyanagi-Harada disease has been closely linked to HLA-DR4 (subtype DRB1 0405; see Box 7-11) and there is a similar association with sympathetic ophthalmia.

Posterior uveitis, however, has close similarity to certain experimental CD4 $^{+}$  T-cell-mediated uveoretinal inflammations (experimental autoimmune uveoretinitis, EAU) in which the autoantigens have been well defined. Most of these are derived from the outer retinal layers and include the visual protein rhodopsin. The relationship between EAU and non-infectious posterior uveitis is, however, tantalizingly tenuous because patients with these diseases do not have significantly raised levels of antibodies to retinal antigens, although some patients manifest T-cell responses to these antigens. The most compelling evidence has recently come from a transgenic humanized mouse model in which mice demonstrated autoimmunity to human retinal S antigen, the commonest autoantigen in uveitis patients.

Clinical studies of aqueous and vitreous samples have also been relatively uninformative to date, although in certain diseases such as Fuchs heterochromic cyclitis high levels of CD8 T cells have been found. In addition, high concentrations of IL-6 and IL-8 have been detected in ocular fluid samples from patients with uveitis, and Fas-FasL interactions have been shown to be active in patients with acute anterior uveitis.

A strong hint that idiopathic non-infectious posterior uveitis is immune-mediated is its clinical response to immunosuppressive agents such as cyclosporin A and, currently, several immunological approaches, including anti-TNF- $\alpha$  humanized monoclonal antibodies, and oral tolerization schedules, are being evaluated for their efficacy in this condition. Other

Classification of the many forms of uveitis/intraocular inflammation as diagnostic entities has lagged behind many other diseases, mainly because of difficulties in reaching agreement about what constitutes each entity. For instance, diseases such as presumed ocular histoplasmosis syndrome are accepted entities in the USA but the same clinical disease in Europe is termed punctate inner choroidopathy. In addition, many of the conditions present with creamy-white lesions on ophthalmoscopic examination, but these 'white dot' syndromes comprise many different entities, both infectious and non-infectious. Accordingly, a large international consortium of experts has gathered together many of the different clinical examples and has been cataloguing and characterizing the different uveitis syndromes, both in terms of general levels of activity/severity and risk of threat to sight as well as the phenotypic characteristics which define each disease. This effort is named the Standardization of Uveitis Nomenclature (SUN) project and the group is continuing to publish its findings. These will be of great value to clinicians generally, as well as to the conduct of clinical trials of new drugs ([Trusko et al., 2013](#)).

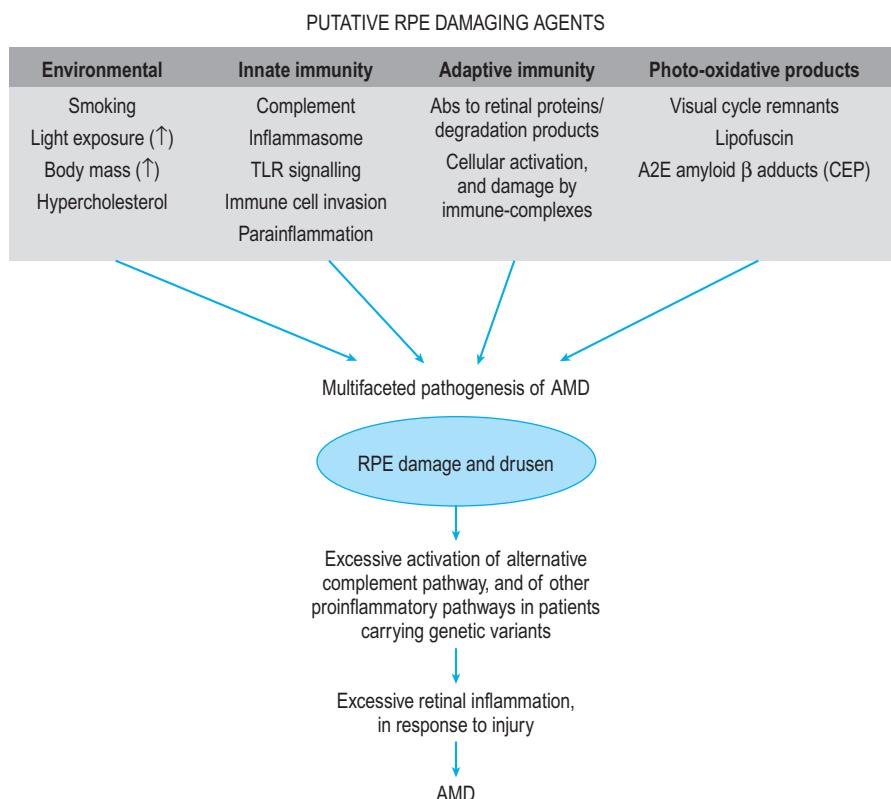
immunosuppressive agents are now being used in a range of ocular inflammatory diseases including mycophenolate mofetil and FK506 (see Ch. 6, p. 364).

### The role of the immune system in ageing and degeneration in the eye

The recent understanding of the innate immune system, its inducers and receptors and its close link with the adaptive immune system, has revealed the role of immune processes in several ocular and non-ocular disease which were previously considered genetic, developmental, metabolic, age-related/degenerative and even cancer. This includes many conditions such as diabetes and its complications, obesity, neurodegenerations such as Alzheimer's and classical genetic disease such as retinitis pigmentosa. Inflammation induced via PAMPs, DAMPS and PRRs (see p. 391) with activation of inflammasomes and secretion

of IL-1 and its many family members is now known to play a significant part in the pathogenesis of many of these diseases, initiated by the original abnormality, be it developmental, viral induced, genetic or other. For instance, diabetic retinopathy is now recognized to be the result of leucocyte activation, in part via upregulation of the CCR5 receptor in response to high glucose levels. Succinate, generated in excess via the glycolytic pathway, is a central ligand for inducing inflammation in diabetes, ageing, cancer and obesity.

In the eye the most prominent example of the involvement of innate immune mechanisms and inflammation is in age-related macular degeneration (AMD). The risk of developing AMD, the commonest cause of blindness in developed nations, is directly linked to mutation in a number of innate immune genes, particularly complement factor H (see p. 405) and the risk increases with accumulation of mutations.



**FIGURE 7-46** Flow diagram outlining the main checkpoints in the development of age-related macular degeneration (AMD). (From Whitcup et al., 2013.)

The identification of associations of genetic mutations in complement proteins with AMD has opened a rich area of research into the possible role of inflammation in both wet and dry AMD. Wet AMD is characterized by the growth of abnormal vessels from the choroid through Bruch's membrane into the subretinal space where bleeding and vessel leakage cause an acute loss of vision if the lesion is at the fovea. In dry AMD there is progressive atrophy of the retinal pigment epithelium, with loss of photoreceptors and progressive choroidal avascularity. In both conditions macrophages have been implicated in wet AMD, activated macrophages release VEGF, which promotes new vessel formation, while in dry AMD, macrophages are involved in progressive cell death of the RPE layer. Macrophages are switched on by many mediators, in particular complement components (see p. 405), and complement inhibitors are in place to prevent excessive complement activation. Mutations in complement proteins, such as complement factor H (CFH), CFH-related proteins 1 and 3, factor B/C2, C3 and factor I, are linked to increased risk of AMD and for the inhibitory proteins evidence has been produced to show that proteins such as complement factor H are less effective in controlling C activation if they contain one or more of the mutations. The pathogenetic role of complement factors in AMD is, however, complex. Partial activation of complement can produce factors which promote removal of tissue debris and thus prevent accumulation of material which is presumed to lead to overt AMD such as drusen. However, defective inhibitors clearly promote overactivation of complement. Although most of the complement components are produced in the liver, several can be generated locally within the retina and other parts of the eye, and thus locally generated inflammatory responses are possible given the correct triggers.

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Macrophages and dendritic cells are directly implicated in the pathology of AMD, classical inflammatory macrophages in the early stages and alternative (angiogenic) macrophages when active choroidal neovascularization occurs in the wet form of the disease. In the atrophic form of AMD, inflammasome products IL-1 and IL-18 appear to be important in causing progressive atrophy of the RPE, while in the wet form of the disease IL-18 has been proposed to be anti-angiogenic. However, there are many different aspects to AMD pathogenesis which involve more than complement and induction of the inflammasome and AMD truly reflects a multifactorial disease (Fig. 7-46).

Much is still to be learned concerning the role of the innate immune system, and possibly the adaptive immune system in AMD pathogenesis, as well as in other diseases such as glaucoma and intraocular tumours, not to mention more obvious inflammatory diseases such as uveitis, but a rich harvest of potential

therapeutic targets using immune modulators has yet to be reaped.

## Conclusion

The eye and its several tissues may be involved in any of the immune responses described in this chapter, either as a primary target of attack (e.g. in disciform herpetic keratitis or toxoplasmic choroiditis) or as part of a generalized immune disorder such as in Wegener's granulomatosis or sarcoidosis. The pathological processes and the mechanisms of initiation of immune responses are fundamentally similar from tissue to tissue. However, as stated in the introduction, each tissue has its unique microenvironment and this undoubtedly plays a part in the final expression of the immune response.

## FURTHER READING

A full reading list is available online at <https://expertconsult.inkling.com/>.

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