

9

Innate and acquired immunity

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KEY POINTS

- The cells of the immune system are divided into lymphoid and myeloid lineages. The former include T lymphocytes and their subsets identified by CD markers, B lymphocytes and natural killer (NK) cells. The myeloid lineage includes the neutrophils, eosinophils and basophils as well as the monocyte/macrophage series and platelets.
- Innate immunity depends on physical, physiological and chemical barriers to infection, on the response to injury and on detection of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). Phagocytic cells and the enzyme cascade known as *complement* are key effectors responding to PAMPs and components of *acute inflammation*.
- Acquired immunity depends on specific recognition of antigens either directly by antibodies on the surface of B cells or through presentation of processed antigens in the context of MHC molecules by host cells to T cells. In contrast to innate immunity, on re-exposure the responses are faster, more vigorous and more specific.
- Acquired immune responses are driven by the availability of antigen. As they mature, only cells with high-affinity receptors for the antigen are stimulated to divide. The expanded clones of antigen-specific cells are said to have resulted from *clonal selection*.
- Lymphocytes are activated by antigen and the appropriate combination of *cytokines*, signalling molecules secreted by other lymphocytes and by macrophages.
- Humoral acquired immunity leads to antigen-antibody complexes that neutralize key aspects of microbial activity either directly or through the activation of complement, *opsonization* and directed cytotoxicity.
- Cell-mediated immunity generates cytotoxic T lymphocytes (CD8⁺), which directly kill cells containing intracellular pathogens, and helper T cells (CD4⁺), which secrete lymphokines that stimulate other effector aspects of immunity.
- Inherited and acquired defects in the immune system lead to immunodeficiencies that make individuals more susceptible to certain infections.
- Damage due to immune reactions may reflect attempts to eliminate micro-organisms or self antigen-directed (*autoimmune*) reactions.

The environment contains a vast number of potentially infectious organisms – viruses, bacteria, fungi, protozoa and worms. Any of these can cause damage if they multiply unchecked, and many could kill the host. However, the majority of infections in the normal individual are of limited duration and leave little permanent damage. This fortunate outcome is due largely to the *immune system*.

The immune system is split into two functional divisions. *Innate immunity* is the first line of defence

against infectious agents, and most potential pathogens are checked before they establish an overt infection. If these defences are breached, the acquired immune system is called into play. *Acquired immunity* produces a specific response to each infectious agent, and the effector mechanisms generated normally eradicate the offending material. Furthermore, the adaptive immune system remembers the particular infectious agent and can prevent it causing disease later.



THE IMMUNE SYSTEM

The immune system consists of a number of organs and several different cell types. All cells of the immune system – tissue cells and white blood cells or *leucocytes* – develop from pluripotent stem cells in the bone marrow. These haemopoietic stem cells also give rise to the red blood cells or *erythrocytes*. The production of leucocytes is through two main pathways of differentiation (Fig. 9.1). The *lymphoid* lineage produces T lymphocytes and B lymphocytes. Natural killer (NK) cells, also known as large granular lymphocytes, also develop from lymphoid progenitors. The *myeloid* pathway gives rise to mononuclear phagocytes, monocytes and macrophages, and granulocytes, basophils, eosinophils and neutrophils, as well as platelets and mast cells. Platelets are involved in blood clotting and inflammation, whereas mast cells are similar to basophils but are found in tissues.

Lymphoid cells

Lymphocytes make up about 20% of the white blood cells present in the adult circulation. Mature lymphoid cells are long lived and may survive for many years as memory cells. These mononuclear cells are heterogeneous in size and morphology. The typical small lymphocytes comprise the T and B cell populations. The larger and less numerous cells, sometimes

referred to as large granular lymphocytes, contain the population of NK cells. Cells within this population are able to kill certain tumour and virally infected cells (natural killing) and destroy cells coated with immunoglobulin (antibody-dependent cell-mediated cytotoxicity).

Morphologically it is quite difficult to distinguish between the different lymphoid cells and impossible to differentiate the subclasses of T cell. As these cells carry out different processes, they possess molecules on their surface unique to that functional requirement. These molecules, referred to as cell markers, can be used to distinguish between different cell types and also to identify cells at different stages of differentiation. The different cell surface molecules have been systematically named by the CD (cluster of differentiation) system; some of those expressed by different T cell populations are shown in Table 9.1. These CD markers are identified using specific monoclonal antibodies (see p. ••). The presence of these specific antibodies on the cell surface is then visualized using labelled antibodies that recognize the first antibody. 1

Myeloid cells

The second pathway of development gives rise to a variety of cell types of different morphology and function.

Mononuclear phagocytes

The common myeloid progenitor in the bone marrow gives rise to *monocytes*, which circulate in the blood and migrate into organs and tissues to become *macrophages*. The human blood monocyte is larger than a lymphocyte and usually has a kidney-shaped nucleus. This actively phagocytic cell has a ruffled membrane and many cytoplasmic granules. These *lysosomes* contain enzymes and molecules that are

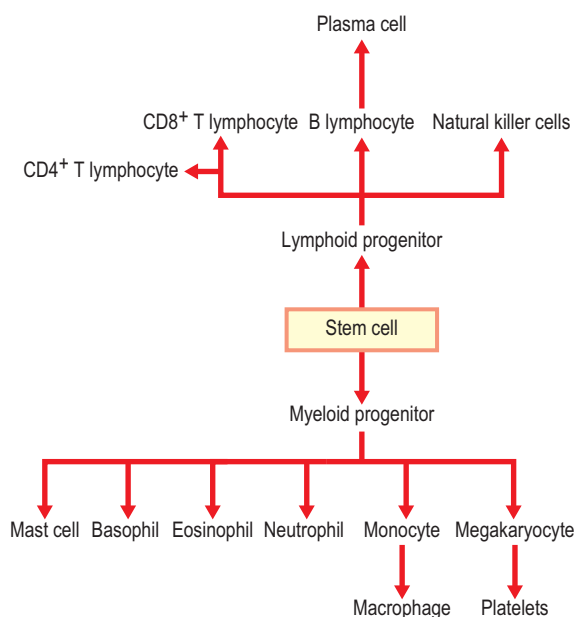


Fig. 9.1 Cells of the immune system.

Table 9.1 Major T lymphocyte markers

| Marker | Distribution | Proposed function |
|------------------------------------------------------------------------|------------------------------------|-----------------------------------------|
| CD2 | All T cells | Adherence to target cell |
| CD3 | All T cells | Part of T cell antigen-receptor complex |
| CD4 | Helper subset (T _H) | MHC class II-restricted recognition |
| CD7 | All T cells | Unknown |
| CD8 | Cytotoxic subset (T _C) | MHC class I-restricted recognition |
| CD, cluster of differentiation; MHC, major histocompatibility complex. | | |

involved in the killing of microorganisms. Mononuclear phagocytes adhere strongly to surfaces and have various cell membrane receptors to aid the binding and ingestion of foreign material. Their activities can be enhanced by molecules produced by T lymphocytes, called *lymphokines*. Macrophages and monocytes are capable of producing various complement components, prostaglandins, interferons and *monokines* such as interleukin (IL)-1 and tumour necrosis factor. Lymphokines and monokines are collectively known as *cytokines*.

Granulocytes

Granulocytes are short-lived cells, days, compared to macrophages, which may survive for months or years. They are classified as *neutrophils*, *eosinophils* and *basophils* on the basis of their histochemical staining. The mature forms have a multilobed nucleus and many granules. Neutrophils constitute 60–70% of the leucocytes, but also migrate into tissues in response to injury or infection.

Neutrophils. These are the most abundant circulating granulocyte. Their granules contain numerous microbicidal molecules and the cells enter the tissues when a chemotactic factor is produced, as the result of infection or injury.

Eosinophils. Eosinophils are also phagocytic cells, although they appear to be less efficient than neutrophils. They are present in low numbers in a healthy individual (1–2% of leucocytes), but their numbers rise in certain allergic conditions. The granule contents can be released by the appropriate signal, and the cytotoxic molecules can then kill parasites that are too large to be phagocytosed.

Basophils. These cells are found in extremely small numbers in the circulation (<0.2%) and have certain characteristics in common with tissue *mast cells*. Both cell types have receptors on their surface for the Fc portion of immunoglobulin (Ig) E, and cross-linking of this immunoglobulin by antigen leads to the release of various pharmacological mediators. These molecules stimulate an inflammatory response. There are two types of mast cell: one is found in connective tissue and the other is mucosa associated. Mast cells and basophils are both derived from bone marrow, but their developmental relationship is not clear.

Platelets

Platelets are also derived from myeloid progenitors. In addition to their role in clotting, they are involved in inflammation.

Table 9.2 Characteristics and determinants of innate and acquired immunity

| Innate immunity | Acquired immunity |
|------------------------------------------------------------------------------------------------------------------------|--------------------|
| Broad specificity No change with repeat exposure Mechanical barriers Bactericidal substances Natural flora | Specific Memory |
| Humoral Acute-phase proteins Interferons Lysozyme Complement | Antibody |
| Cell-mediated Natural killer cells Phagocytes | T lymphocytes |

INNATE IMMUNITY

The healthy individual is protected from potentially harmful micro-organisms in the environment by a number of effective mechanisms, present from birth, that do not depend upon prior exposure to any particular microorganism. The innate defence mechanisms show broad specificity in the sense that they are effective against a wide range of potentially infectious agents. The characteristics and constituents of innate and acquired immunity are shown in Table 9.2.

FEATURES OF INNATE IMMUNITY

The components of the innate immune system recognize structures that are unique to microbes. These include complex lipids and carbohydrates such as peptidoglycan of bacteria, lipopolysaccharides of Gram-negative bacteria, lipoteichoic acid in Gram-positive bacteria and mannose-containing oligosaccharides found in many microbial molecules. Other microbial specific molecules include double-stranded RNA found in replicating viruses and unmethylated CpG sequences in bacteria. Therefore, the innate immune system is able to recognize non-self structures and react appropriately but does not recognize self structure, so the potential of autoimmunity is avoided. The microbial products recognized by the innate immune system, known as pathogen-associated molecular patterns (PAMPs), are essential for survival of the micro-organisms and cannot easily be discarded or mutated. Different classes of micro-organism express different PAMPs that are recognized by different

**Table 9.3** Examples of pathogen-associated molecular patterns (PAMPs) and pattern recognition receptors (PRRs) in innate immunity

| PAMP | Source | PRR | Response |
|------------------------------------------|-----------------------------------------|-----------------------------------------------------------------------|--------------------------------------------------------------|
| Sugars (mannose) | Microbial glycoproteins and glycolipids | Mannose receptors Mannose-binding protein Lectin-like receptors | Phagocytosis Complement activation Phagocytosis |
| <i>N</i> -formylmethionyl peptides | Bacterial protein synthesis | <i>N</i> -formylmethionyl peptides receptors | Chemotaxis and phagocyte activation Complement activation |
| Phosphorylcholine | Microbial membranes | C-reactive protein | } Macrophage activation Cytokine production |
| Lipoarabinomannan | Yeast cell wall | Toll-like receptor 2 | |
| Lipoteichoic acid | Gram-positive bacterial cell wall | Toll-like receptor 2 | |
| Lipopolysaccharide | Gram-negative bacterial cell wall | Toll-like receptors 4 and 2 | |
| Unmethylated CpG nucleotides | Bacterial DNA | Toll-like receptor 9 | |
| dsRNA | Replicating viruses | Toll-like receptor 3 | Type 1 interferon production |
| dsRNA, double-stranded ribonucleic acid. | | | |

pattern recognition receptors (PRRs) on host cells and circulating molecules (Table 9.3). One group of PRRs that are still being characterized are the Toll-like receptors. Mammalian Toll-like receptors are expressed on different cell types that are components of the innate defences, including macrophages, dendritic cells, neutrophils, mucosal epithelial cells and endothelial cells. Recognition of microbial components by these receptors leads to a variety of outcomes, including cytokine release, inflammation and cell activation.

Innate defences act as the initial response to microbial challenge and can eliminate the micro-organism from the host. However, many microbes have evolved strategies to overcome innate defences, and in this situation the more potent and specialized acquired immune response is required to eliminate the pathogen. The innate immune system plays a critical role in the generation of an efficient and effective acquired immune response. Cytokines produced by the innate immune system signal that infectious agents are present and influence the type of acquired immune response that develops.

DETERMINANTS OF INNATE IMMUNITY

Species and strains

Marked differences exist in the susceptibility of different species to infective agents. The rat is strikingly resistant to diphtheria, whereas the guinea-pig and man are highly susceptible. The rabbit is particularly susceptible to myxomatosis, and human beings to syphilis, leprosy and meningococcal meningitis. Susceptibility to an infection does not always imply a lack

of resistance to disease caused by the micro-organism. For example, although man is highly susceptible to the common cold, the infection is overcome within a few days. In some diseases, it may be difficult to initiate the infection, but once established the disease can progress rapidly, implying a lack of resistance. For example, rabies occurs in both human beings and dogs but is not readily established as the virus does not ordinarily penetrate healthy skin. Once infected, however, both species are unable to overcome the disease. Marked variations in resistance to infection have been noted between different strains of mice, and it is possible to breed, by selection, rabbits of low, intermediate and high resistance to experimental tuberculosis.

Individual differences and influence of age

The role of heredity in determining resistance to infection is well illustrated by studies on tuberculosis in twins. If one homozygous twin develops tuberculosis, the other twin has a three in one chance of developing the disease, compared with a one in three chance if the twins are heterozygous. Sometimes genetically controlled abnormalities are an advantage to the individual in resisting infection as, for example, in a hereditary abnormality of the red blood cells (sickling). These red blood cells cannot be parasitized by *Plasmodium falciparum*, thus conferring a degree of resistance to malaria in affected individuals.

Infectious diseases are often more severe in early childhood and in young animals; this higher susceptibility of the young appears to be associated with immaturity of the immunological mechanisms affecting the ability of the lymphoid system to deal with and react to foreign antigens. This is also the time when

infectious agents are encountered for the first time (primary exposure), and a memory-acquired immune response cannot be called upon to aid elimination. In certain viral infections (e.g. polio and chickenpox), the clinical illness is more severe in adults than in children. This may be due to a more active immune response producing greater tissue damage. In the elderly, besides a general waning of the activities of the immune system, physical abnormalities (e.g. prostatic enlargement leading to stasis of urine) or long-term exposure to environmental factors (e.g. smoking) are common causes of increased susceptibility to infection.

Hormonal influences and sex

There is decreased resistance to infection in those with diseases such as diabetes mellitus, hypothyroidism and adrenal dysfunction. The reasons for this decrease have not yet been clarified but may be related to enzyme or hormone activities. It is known that glucocorticoids are anti-inflammatory agents, decreasing the ability of phagocytes to ingest material. They also have beneficial effects by interfering in some way with the toxic effects of bacterial products such as endotoxins.

There are no marked differences in susceptibility to infections between the sexes. Although the overall incidence and death rate from infectious disease are greater in males than in females, both infectious hepatitis and whooping cough have a higher morbidity and mortality in females.

Nutritional factors

The adverse effects of poor nutrition on susceptibility to certain infectious agents are not now seriously questioned. Experimental evidence in animals has shown repeatedly that inadequate diet may be correlated with increased susceptibility to a variety of bacterial diseases, associated with decreased phagocytic activity and leucopenia. In the case of viruses, which are intracellular parasites, malnutrition may have an effect on virus production, but the usual outcome is enhanced disease as a result of impaired immune responses, especially the cytotoxic responses.

MECHANISMS OF INNATE IMMUNITY

Mechanical barriers and surface secretions

The intact skin and mucous membranes of the body afford a high degree of protection against pathogens.

In conditions where the skin is damaged, such as in patients with burns and after traumatic injury or surgery, infection can be a serious problem. The skin is a resistant barrier because of its outer horny layer consisting mainly of keratin, which is indigestible by most micro-organisms, and thus shields the living cells of the epidermis from micro-organisms and their toxins. The relatively dry condition of the skin and the high concentration of salt in drying sweat are inhibitory or lethal to many microorganisms.

The sebaceous secretions and sweat of the skin contain bactericidal and fungicidal fatty acids, which constitute an effective protective mechanism against many potential pathogens. The protective ability of these secretions varies at different stages of life, and some fungal 'ringworm' infections of children disappear at puberty with the marked increase of sebaceous secretions.

The sticky mucus covering the respiratory tract acts as a trapping mechanism for inhaled particles. The action of cilia sweeps the secretions, containing the foreign material, towards the oropharynx so that they are swallowed; in the stomach the acidic secretions destroy most of the micro-organisms present. Nasal secretions and saliva contain mucopolysaccharides capable of blocking some viruses.

The washing action of tears and flushing of urine are effective in stopping invasion by micro-organisms. The commensal micro-organisms that make up the natural bacterial flora covering epithelial surfaces are protective in a number of ways:

- Their very presence uses up a niche that cannot be used by a pathogen.
- They compete for nutrients.
- They produce byproducts that can inhibit the growth of other organisms.

It is important not to disturb the relationship between the host and its indigenous flora.

Commensal organisms from the gut or bacteria normally present on the skin can cause problems if they gain access to an area that they do not normally populate. An example of this is urinary tract infection resulting from the introduction of *Escherichia coli*, a gut commensal, by means of a urinary catheter. Some commensal organisms possessing low virulence (see p. 22) that are provided with the circumstances by which to cause infections may be referred to as *opportunistic pathogens*. Infections with these opportunists are quite widespread, often appearing as a result of medical or surgical treatment that breaches the innate defences or reduces the host's ability to respond.



Humoral defence mechanisms

A number of microbicidal substances are present in the tissue and body fluids. Some of these molecules are produced constitutively (e.g. lysozyme), and others are produced in response to infection (e.g. acute-phase proteins and interferon). These molecules all show the characteristics of innate immunity: there is no recognition specific to the micro-organism beyond the distribution of the molecule(s) detected, and the response is not enhanced on re-exposure to the same antigen.

Lysozyme

This is a basic protein of low molecular weight found in relatively high concentrations in neutrophils as well as in most tissue fluids, except cerebrospinal fluid, sweat and urine. It functions as a mucolytic enzyme, splitting sugars off the structural peptidoglycan of the cell wall of many Gram-positive bacteria and thus causing their lysis. It seems likely that lysozyme may also play a role in the intracellular destruction of some Gram-negative bacteria. In many pathogenic bacteria the peptidoglycan of the cell wall appears to be protected from the access of lysozyme by other wall components (e.g. lipopolysaccharide). The action of other enzymes from phagocytes or of complement may be needed to remove this protection and expose the peptidoglycan to the action of lysozyme.

Basic polypeptides

A variety of basic proteins, derived from tissues and blood cells, have some antibacterial properties. This group includes the basic proteins called spermine and spermidine, which can kill tubercle bacilli and some staphylococci. Other toxic compounds are the arginine- and lysine-containing proteins protamine and histone. The bactericidal activity of basic polypeptides probably depends on their ability to react non-specifically with acid polysaccharides at the bacterial cell surface.

Acute-phase proteins

The concentration of acute-phase proteins rises dramatically during an infection. Microbial products such as endotoxin can stimulate macrophages to release IL-1, which stimulates the liver to produce increased amounts of various acute-phase proteins, the concentrations of which can rise over 1000-fold. One of the best characterized acute-phase proteins is *C-reactive protein*, which binds to phosphorylcholine residues in the cell wall of certain micro-organisms. This complex is very effective at activating the

classical complement pathway. Also included in this group of molecules are α_1 -antitrypsin, α_2 -macroglobulin, fibrinogen and serum amyloid A protein, all of which act to limit the spread of the infectious agent or stimulate the host response.

Interferon

The observation that cell cultures infected with one virus resist infection by a second virus (viral interference) led to the identification of the family of antiviral agents known as *interferons*. A number of molecules have been identified; α - and β -interferons (see Ch. 5) are part of innate immunity, and γ -interferon is produced by T cells as part of the acquired immune response (see Ch. 10).

Complement

The existence of a heat-labile serum component with the ability to lyse red blood cells and destroy Gram-negative bacteria has been known since the 1930s. The chemical complexity of the phenomenon was not appreciated by early workers, who ascribed the activity to a single component, called complement. Complement is in fact composed of a large number of different serum proteins present in low concentration in normal serum. These molecules are present in an inactive form but can be activated to form an enzyme cascade: the product of the first reaction is the catalyst of the next and so on.

Approximately 30 proteins are involved in the complement system, some of which are enzymes, some are control molecules and others are structural proteins with no enzymatic activity. A number of the molecules involved are split into two components (a and b fragments) by the product of the previous step. There are two main pathways of complement activation, the alternative and classical, that lead to the same physiological consequences:

- opsonization
- cellular activation
- lysis.

The two pathways use different initiation processes. Component C3 forms the connection between the two pathways, and the binding of this molecule to a surface is the key process in complement activation.

Classical pathway

The classical pathway of activation leading to the cleavage of C3 is initiated by the binding of two or more of the globular domains of the C1q component

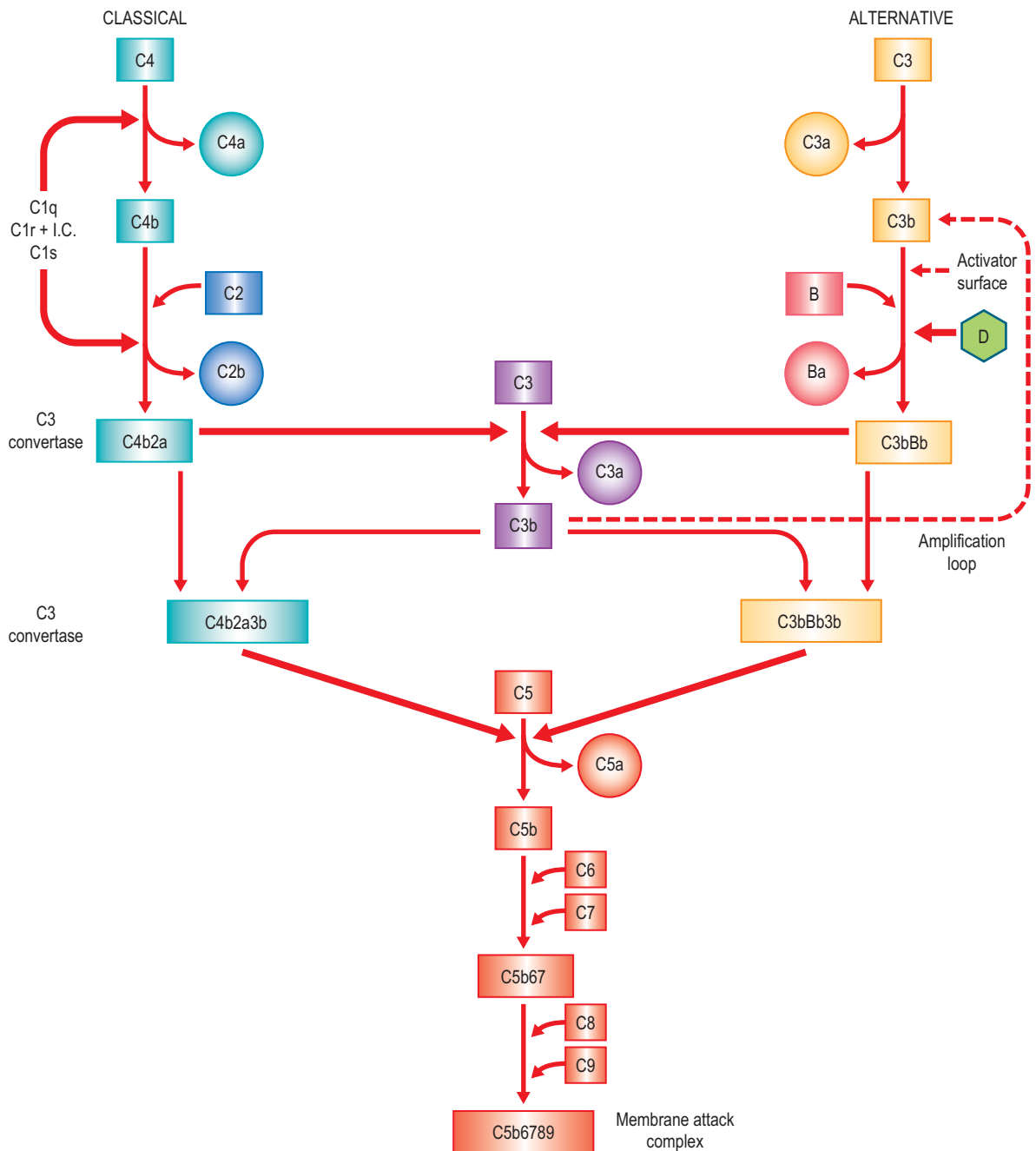


Fig. 9.2 Complement activation: classical and alternative pathways. Enzymatic reactions are indicated by thick arrows. I.C., immune complex.

of C1 to its ligand: immune complexes containing IgG or IgM and certain micro-organisms and their products. This causes a conformational change in the C1 complex that leads to the auto-activation of C1r. The enzyme C1r then converts C1s into an active serine esterase that acts on the thioester-containing molecule

C4 to produce C4a and a reactive C4b (Fig. 9.2). C4a is released and some of the C4b becomes attached to a surface. C2 binds to the surface-bound C4b, becomes a substrate for the activated C1 complex, and is split into C2a and C2b. The C2b is released, leaving C4b2a – the classical pathway C3 convertase. This active



enzyme then generates C3a and the unstable C3b from C3. A small amount of the C3b generated binds to the activating surface and acts as a focus for further complement activation. Activation of the classical pathway is regulated by C1 inhibitor and by a number of molecules that limit the production of the 'C3 convertase'.

The so-called *lectin pathway* is initiated by mannose-binding lectin (a secreted PRR) attaching to the surface of a micro-organism. This leads to the production of C4b2a and the generation of C3b on the activating surface.

Alternative pathway

Intrinsically, C3 undergoes a low level of hydrolysis of an internal thioester bond to generate C3b. This molecule complexes, in the presence of Mg^{2+} ions, with factor B, which is then acted on by factor D to produce C3bBb. This is a 'C3 convertase', which is capable of splitting more C3 to C3b, some of which will become membrane bound.

The initial binding of C3b generated by either the classical or the alternative pathway leads to an amplification loop that results in the binding of many more C3b molecules to the same surface. Factor B binds to the surface-bound C3b to form C3bB, the substrate for factor D – a serine esterase – which is present in very low concentrations in an already active form. The cleavage of factor B results in the formation of the C3 convertase, C3bBb, which dissociates rapidly unless it is stabilized by the binding of properdin (P), forming the complex C3bBbP. This convertase can cleave many more C3 molecules, some of which become surface bound. This amplification loop is a positive feedback system that will cycle until all the C3 is used up unless it is regulated carefully.

Regulation

The nature of the surface to which the C3b is bound regulates the outcome. Self cell membranes contain a number of regulatory molecules that promote the binding of factor H rather than factor B to C3b. This results in the inhibition of the activation process. On non-self structures the C3b is protected, as regulatory proteins are not present, and factor B has a higher affinity for C3b than factor H at these sites.

Thus the surface of many micro-organisms can stabilize the C3bBb by protecting it from factor H. In addition, another molecule, properdin, stabilizes the complex. The deposition of a few molecules of C3b on to these surfaces is followed by the formation of the relatively stable C3bBbP complex. This C3

convertase will lead to more C3b deposition. Immune complexes composed of certain immunoglobulins (e.g. IgA and IgE) also function as protected sites for C3b and activate complement by the alternative pathway. Poor activation surfaces are made more susceptible to deposition by the presence of antibody that generates C3b by the classical pathway.

Membrane attack complex

The next step after the formation of C3b is the cleavage of C5 (Fig. 9.3). The 'C5 convertases' are generated from C4b2a of the classical pathway and C3bBb of the alternative pathway by the addition of another C3b molecule. These membrane-bound trimolecular complexes selectively bind C5 and cleave it to give fluid-phase C5a and membrane-bound C5b. The formation of the rest of the membrane attack complex is non-enzymatic. C6 binds to C5b, and this joint complex is released from the C5 convertase. The formation of C5b67 generates a hydrophobic complex that inserts into the lipid bilayer in the vicinity of the initial activation site. Usually this is on the same cell surface as the initial trigger, but occasionally other cells may be involved. Therefore 'bystander' lysis can take place, giving rise to damage to surrounding tissue. There are a number of proteins present in body fluids to limit this potentially dangerous process by binding to fluid-phase C5b67. C8 and C9 bind to the membrane-inserted complex in sequence, resulting in the formation of a lytic polymeric complex containing up to 20 C9 monomers. A small amount of lysis can occur when C8 binds to C5b67, but it is the polymerized C9 that causes the most damage.

Functions

The activation of complement by either pathway gives rise to C3b and the generation of a number of factors that can aid in the elimination of foreign material.

The complete insertion of the membrane attack complex into a cell will lead to membrane damage and lysis, probably by osmotic swelling. Some thin-walled pathogens, such as trypanosomes and malaria parasites, are killed by complement-mediated lysis. Some Gram-negative bacteria can be killed by complement in conjunction with lysozyme. However, complement-mediated lysis is of limited importance as a bactericidal mechanism compared with phagocyte destruction of bacteria. Inherited deficiencies of the terminal components are associated with infection by gonococci and meningococci, which can survive inside neutrophils and for which complement-mediated killing is important.

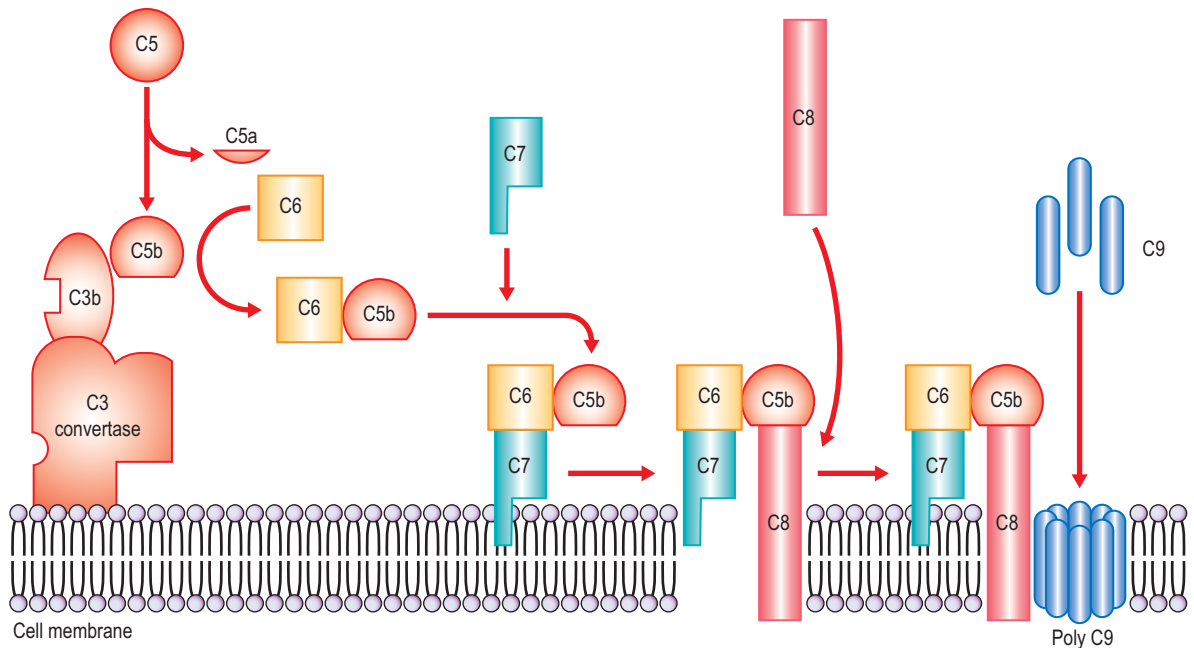


Fig. 9.3 Membrane attack complex.

Phagocytic cells have receptors for certain complement components that facilitate the adherence of complement-coated particles. Therefore, complement is an *opsonin*, and in certain circumstances this attachment may lead to phagocytosis.

Two of the molecules released during the complement cascade, C3a and C5a, have potent biological activities. These molecules, known as *anaphylatoxins*, trigger mast cells and basophils to release mediators of inflammation (see below). They also stimulate neutrophils to produce reactive oxygen intermediates, whereas C5a on its own is a chemo-attractant and acts directly on vascular endothelium to cause vasodilation and increased vascular permeability.

Cells

Phagocytes

Micro-organisms entering the tissue fluids or bloodstream are rapidly engulfed by *neutrophils* and *mononuclear phagocytes*. In the blood the latter are known as *monocytes*, whereas in the tissues they differentiate into *macrophages*. In connective tissue they are known as *histiocytes*, in kidney as *mesangial cells*, in liver as *Kupffer cells*, in bone as *osteoclasts*, in brain as *microglia*, and in the spleen, lymph node and thymus as the *sinus-lining macrophages*.

The essential features of these cells are that they:

- are actively phagocytic
- contain digestive enzymes to degrade ingested material
- are an important link between the innate and acquired immune mechanisms.

Part of their role in regard to acquired immunity is that they can process and present antigens, and produce molecules that stimulate lymphocyte differentiation into effector cells.

The role of the phagocyte in innate immunity is to engulf particles (phagocytosis) or soluble material (pinocytosis), and digest them intracellularly within specialized vacuoles. The macrophages present in the walls of capillaries and vascular sinuses in spleen, liver, lungs and bone marrow serve an important role in clearing the bloodstream of foreign particulate material such as bacteria. So efficient is this process that the repeated finding, generally by sensitive broth culture, of a few bacteria or yeasts in the bloodstream usually indicates that there is a continuing release of micro-organisms from an active focus such as an abscess or the heart valve vegetations found in bacterial endocarditis.

The ability of macrophages to ingest and destroy micro-organisms can be impaired or enhanced by depression or stimulation of the phagocyte system.



Some microorganisms, such as mycobacteria and brucellae, can resist intracellular digestion by normal macrophages, though they may be digested by 'activated' ones.

Chemotaxis

For phagocytic cells to be effective, they must be attracted to the site of infection. Once they have passed through the capillary walls they move through the tissues in response to a concentration gradient of molecules produced at the site of damage. These chemotactic factors include:

- products of injured tissue
- factors from the blood (C5a)
- substances produced by neutrophils and mast cells (leukotrienes and histamine)
- bacterial products (formyl-methionine peptides).

Neutrophils respond first and move faster than monocytes.

Phagocytosis

Phagocytosis involves:

- recognition and binding
- ingestion
- digestion.

Phagocytosis may occur in the absence of antibody, especially on surfaces such as those of the lung alveoli and when inert particles are involved. Cell membranes carry a net negative charge that keeps them apart and stops autophagocytosis. The hydrophilic nature of certain bacterial cell wall components stops them passing through the hydrophobic membrane. To overcome these difficulties the phagocytes have receptors on their surface that mediate the attachment of particles coated with the correct ligand. Phagocytes have receptors for the Fc portion of certain immunoglobulin isotypes and for some components of the complement cascade. The presence of these molecules, or *opsonins*, on the particle surface markedly enhances the ingestion process and, in some cases, digestion. Whether mediated by specific receptors or not, the foreign particle is surrounded by the cell membrane, which then invaginates and produces an *endosome* or *phagosome* within the cell (Fig. 9.4).

The microbicidal machinery of the phagocyte is contained within organelles known as *lysosomes*. This compartmentalization of potentially toxic molecules is necessary to protect the cell from self-destruction and produce an environment where the molecules can function efficiently. The phagosome and lysosome

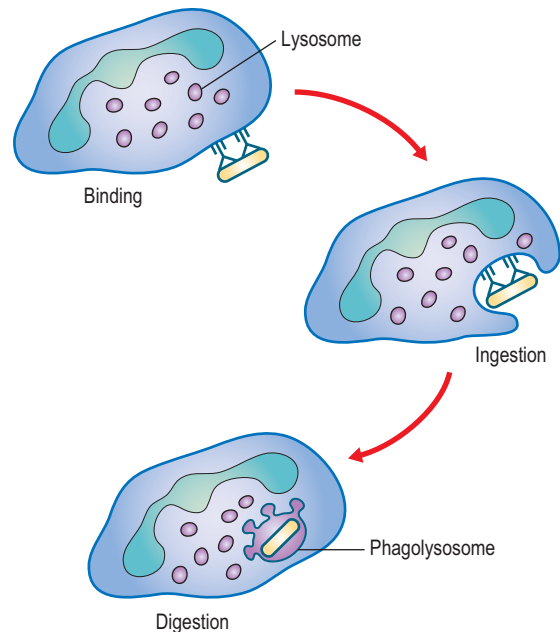


Fig. 9.4 Stages in phagocytosis.

fuse to form a *phagolysosome* in which the ingested material is killed and digested by various enzyme systems.

Ingestion is accompanied by enhanced glycolysis and an increase in the synthesis of proteins and membrane phospholipids in the phagocyte. After phagocytosis there is a respiratory burst consisting of a steep rise in oxygen consumption. This is accompanied by an increase in the activity of a number of enzymes and leads to the reduction of molecular oxygen to various highly reactive intermediates, such as the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), singlet oxygen (O^{\cdot}) and the hydroxyl radical (OH^{\cdot}). All of these chemical species have microbicidal activity and are termed oxygen-dependent killing mechanisms. The superoxide anion is a free radical produced by the one-electron reduction of molecular oxygen; it is very reactive and highly damaging to animal cells, as well as to micro-organisms. It is also the substrate for superoxide dismutase, which generates hydrogen peroxide for subsequent use in microbial killing. Myeloperoxidase uses hydrogen peroxide and halide ions, such as iodide or chloride, to produce at least two bactericidal systems. In one, halogenation (incorporation of iodine or chlorine) of the bacterial cell wall leads to death of the organism. In the second mechanism, myeloperoxidase and hydrogen peroxide damage the cell wall by converting amino acids into aldehydes that have antimicrobial activity.

Within phagocytes there are several oxygen-independent mechanisms that can destroy ingested material. Some of these enzymes can damage membranes. For example, *lysozyme* and *elastase* attack peptidoglycan of the bacterial cell wall, and then hydrolases are responsible for the complete digestion of the killed organism. The cationic proteins of lysosomes bind to and damage bacterial cell walls and enveloped viruses, such as herpes simplex virus. The iron-binding protein *lactoferrin* has antimicrobial properties. It complexes with iron, rendering it unavailable to bacteria that require iron for growth. The high acidity within phagolysosomes (pH 3.5–4.0) may have bactericidal effects, probably resulting from lactic acid production in glycolysis. In addition, many lysosomal enzymes, such as acid hydrolases, have acid pH optima. There are significant differences between macrophages and neutrophils in the killing of microorganisms. Although macrophage lysosomes contain a variety of enzymes, including lysozyme, they lack cationic proteins and lactoferrin. Tissue macrophages do not have myeloperoxidase but probably use catalase to generate the hydrogen peroxide system. Normal macrophages are less efficient killers of certain pathogens, such as fungi, than neutrophils. The microbicidal activity of macrophages can, however, be greatly improved after contact with products of lymphocytes, known as lymphokines.

Once killed, most micro-organisms are digested and solubilized by lysosomal enzymes. The degradation products are then released to the exterior.

Natural killer (NK) cells

NK cells recognize changes on virus-infected cells and destroy them by an extracellular killing mechanism. They recognize changes in the level of MHC class I molecules on cell membranes of cells infected with certain viruses. If the NK cell binds to an uninfected host cell, the presence of normal levels of MHC class I molecules leads to inhibition of the killing mechanisms. However, certain viruses, such as herpesviruses, evade the adaptive immune system by interfering with the production of MHC class I molecules. This leads to a reduced level of MHC class I molecules on the infected cell membrane and no inhibition of the killing mechanisms which involve the NK cell producing molecules that damage the membrane of the infected cell leading to its destruction.

Natural killing is a function of several different cell types. This activity is performed by cells described as large granular lymphocytes and also by cells with T cell markers, macrophage markers and others that do not have the characteristics of any of the main cells of

the immune system. Natural killing is present without previous exposure to the infectious agent and shows all the characteristics of an innate defence mechanism. NK cells have also been implicated in host defence against cancers by a mechanism similar to that used to combat virus infection. Natural killing is enhanced by interferons that appear to stimulate the production of NK cells and also increase the rate at which they kill the target cells.

Eosinophils

Eosinophils are granulocytes with a characteristic bi-lobed nucleus and cytoplasmic granules. They are present in the blood of normal individuals at very low levels (<1%), but their numbers increase in patients with parasitic infections and allergies. They are not efficient phagocytic cells, although their granules contain molecules that are toxic to parasites. Large parasites such as helminths cannot be internalized by phagocytes and therefore must be killed extracellularly. Eosinophil granules contain an array of enzymes and toxic molecules active against parasitic worms. The release of these molecules must be controlled so that tissue damage is avoided. The eosinophils have specific receptors, including Fc and complement receptors, that bind the labelled target (i.e. antibody or complement-coated parasites). The granule contents are then released into the space between the cell and the parasite, thus targeting the toxic molecules onto the parasite membrane.

Temperature

The temperature preference of many micro-organisms is well known, and it is therefore apparent that temperature is an important factor in determining the innate immunity of an animal to some infectious agents. It seems likely that the pyrexia that follows so many different types of infection can function as a protective response against the infecting micro-organism. The febrile response in many cases is controlled by IL-1 produced by macrophages as part of the immune response.

Inflammation

A number of the above factors are responsible for the process of *acute inflammation*. This is the reaction of the body to injury, such as invasion by an infectious agent, exposure to a noxious chemical or physical trauma. The signs of inflammation are redness, heat, swelling, pain and loss of function. The molecular and

**Table 9.4** Mediators of inflammation

| Mediator | Main source | Function |
|---------------------------------------|------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| Histamine ^a | Mast cells, basophils | Vasodilatation, increased vascular permeability, contraction of smooth muscle |
| Kinins (e.g. bradykinin) | Plasma | Vasodilatation, increased vascular permeability, contraction of smooth muscle, pain |
| Prostaglandins | Neutrophils, eosinophils, monocytes, platelets | Vasodilatation, increased vascular permeability, pain |
| Leukotrienes | Neutrophils, mast cells, basophils | Vasodilatation, increased vascular permeability, contraction of smooth muscle, induction of cell adherence and chemotaxis |
| Complement components (e.g. C3a, C5a) | Plasma | Cause mast cells to release inflammatory mediators; C5a is a chemotactic factor |
| Plasmin | Plasma | Breaks down fibrin, kinin formation |
| Cytokines | Lymphocytes, macrophages | Chemotactic factors, colony-stimulating factors, macrophage activation |

^aIn rodents, 5-hydroxytryptamine (serotonin) is present in mast cells and basophils.

cellular events that occur during an inflammatory reaction are:

- vasodilatation
- increased vascular permeability
- cellular infiltration.

These changes are brought about mainly by chemical mediators (Table 9.4), which are widely distributed in a sequestered or inactive form throughout the body and are released or activated locally at the site of inflammation. After release they tend to be inactivated rapidly, to ensure control of the inflammatory process.

There is increased blood supply to the affected area owing to the action of vasoactive amines, such as histamine and 5-hydroxytryptamine, and other mediators stored within mast cells. These molecules are released:

- as a consequence of the production of the anaphylatoxins (C3a and C5a) that trigger specific receptors on mast cells
- following interaction of antigen with IgE on the surface of mast cells
- by direct physical damage to the cells.

Other mediators, such as bradykinins and prostaglandins, are produced locally or released by platelets. The vasodilatation causes increased blood supply to the area, giving rise to redness and heat. The result is an increased supply of the molecules and cells that can combat the agent responsible for the initial trigger.

The same molecules, vasoactive amines, prostaglandins and kinins, increase vascular permeability, allowing plasma and plasma proteins to traverse the endothelial lining. The plasma proteins include immunoglobulins and molecules of the clotting and complement cascades. This leaking of fluid causes swelling (oedema), which in turn leads to increased tissue tension and pain. Some of the molecules themselves, for example prostaglandins and histamine, stimulate the pain responses directly. The inflammatory exudate has several important functions. Bacteria often produce tissue-damaging toxins that are diluted by the exudate. The presence of clotting factors results in the deposition of fibrin, creating a physical obstruction to the spread of bacteria. The exudate is drained continuously by the lymphatic vessels, and antigens, such as bacteria and their toxins, are carried to the draining lymph node where immune responses can be generated.

The production of chemotactic factors, including C5a, histamine, leukotrienes and molecules specific for certain cell types, attracts phagocytic cells to the site. The increased vascular permeability allows easier access for neutrophils and monocytes, and the vasodilatation means that more cells are in the vicinity. The neutrophils arrive first and begin to destroy or remove the offending agent. Most are successful but a few die, releasing their tissue-damaging contents to increase the inflammatory process. Mononuclear phagocytes arrive on the scene to finish off the removal of the residual debris and stimulate tissue repair.

When the swelling is severe there may be loss of function to the affected area. If the offending agent is quickly removed, the tissue will soon be repaired. The inflammatory process continues until the conditions responsible for its initiation have been resolved. In most circumstances this occurs fairly rapidly, with an acute inflammatory reaction lasting for a matter of hours or days. If, however, the causative agent is not easily removed or is reintroduced continuously, chronic inflammation will ensue with the possibility of tissue destruction and complete loss of function.

ACQUIRED IMMUNITY

Micro-organisms that overcome or circumvent the innate non-specific defence mechanisms or are administered deliberately (i.e. active immunization) come up against the host's second line of defence: *acquired immunity*. To give expression to this acquired form of immunity it is necessary that the antigens of the invading microorganism come into contact with cells of the immune system (macrophages and lymphocytes) and thereby initiate an immune response specific for the foreign material. The cells that respond are pre-committed, because of their surface receptors, to respond to a particular epitope on the antigen. This response takes two forms, *humoral* and *cell mediated*, which usually develop in parallel. The part played by each depends on a number of factors, including the nature of the antigen, the route of entry and the individual who is infected.

Humoral immunity depends on the appearance in the blood of antibodies produced by plasma cells.

The term 'cell-mediated immunity' was originally coined to describe localized reactions to organisms mediated by T lymphocytes and phagocytes rather than by antibody. It is now used to describe any response in which antibody plays a subordinate role. Cell-mediated immunity depends mainly on the development of T cells that are specifically responsive to the inducing agent, and is generally active against intracellular organisms.

Specific immunity may be acquired in two main ways:

1. induced by overt clinical infection or inapparent clinical infection
2. deliberate artificial immunization.

This is *active acquired immunity*, and contrasts with *passive acquired immunity*, which is the transfer of preformed antibodies to a non-immune individual by means of blood, serum components or lymphoid cells.

Actively acquired immunity is long lasting, although it may be circumvented by antigenic change in the infecting micro-organism. Passively acquired immunity provides only temporary protection. Passive immunity may be transferred to the fetus by the passage of maternal antibodies across the placenta.

TISSUES INVOLVED IN IMMUNE REACTIONS

For the generation of an immune response, antigen must interact with and activate a number of different cells. In addition, these cells must interact with one another. The cells involved in immune responses are organized into tissues and organs in order that these complex cellular interactions can occur most effectively. These structures are collectively referred to as the *lymphoid system*, which comprises lymphocytes, epithelial and stromal cells arranged into discrete capsulated organs or accumulations of diffuse lymphoid tissue. Lymphoid organs contain lymphocytes at various stages of development and are classified into primary and secondary lymphoid organs.

The primary lymphoid organs are the major sites of lymphopoiesis. Here, lymphoid progenitor cells develop into mature lymphocytes by a process of proliferation and differentiation. In mammals, T lymphocytes develop in the thymus, and B lymphocytes in the bone marrow and fetal liver. It is within the primary lymphoid organs that the lymphocytes acquire their repertoire of specific antigen receptors in order to cope with the antigenic challenges that the individual receives during its life. It is also within these tissues that self-reactive lymphocytes are eliminated to protect against autoimmune disease.

The secondary lymphoid organs create the environment in which lymphocytes can interact with one another and with antigen, and then disseminate the effector cells and molecules generated. Secondary lymphoid organs include lymph nodes, spleen and mucosa-associated lymphoid tissue (e.g. tonsils and Peyer's patches of the gut). These organs have a characteristic structure that relates to the function they carry out, with areas composed of mainly B cells or T cells.

DEVELOPMENT OF THE IMMUNE SYSTEM

In man, lymphoid tissue appears first in the thymus at about 8 weeks of gestation. Peyer's patches are distinguishable by the fifth month, and immunoglobulin-secreting cells appear in the spleen and lymph nodes at about 20 weeks. From this time onwards, IgM and

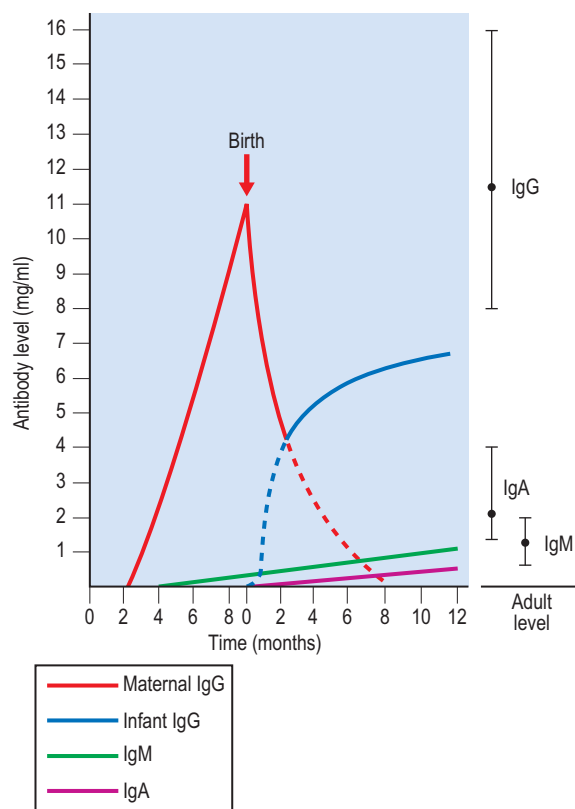


Fig. 9.5 Immunoglobulin levels in the fetus and neonate. Adult levels of the major isotypes are shown as normal ranges with mean serum levels.

IgD are synthesized by the fetus (Fig. 9.5). At birth the infant has a blood concentration of IgG comparable to that of the maternal circulation, having received IgG but not IgM via the placenta. The rate of synthesis of IgM in the infant increases rapidly within the first few days of life but does not reach adult levels until about a year. Serum IgG does not reach adult levels until after the second year, and IgA takes even longer. There is an actual drop in the level of IgG from birth due to the decay of maternal antibody, with the lowest levels of total IgG at around 3 months of age. This corresponds to an age of marked susceptibility to a number of infections. Cell-mediated immunity can be stimulated at birth, but these reactions may not be as powerful as in the adult.

LYMPHOCYTE TRAFFICKING

Lymphocytes differentiate and mature in the primary lymphoid organs and then enter the blood lymphocyte pool. B cells are produced in the bone marrow and

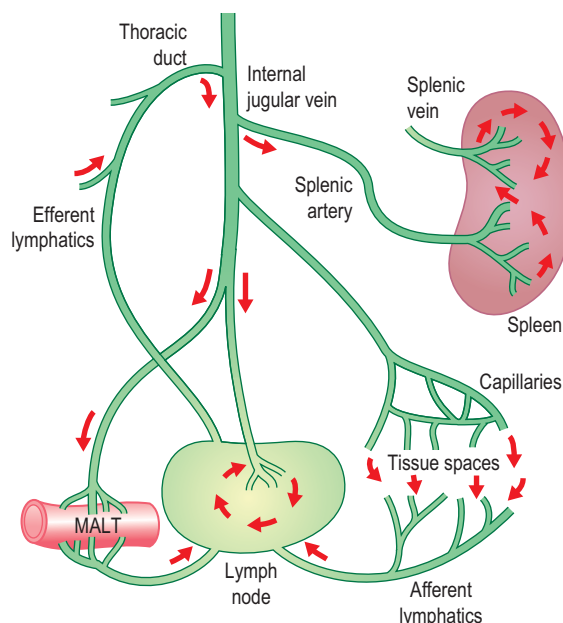


Fig. 9.6 Lymphocyte recirculation. MALT, mucosa-associated lymphoid tissue.

mature there before proceeding via the circulation to the secondary lymphoid organs. T cell precursors leave the bone marrow and mature in the thymus before migrating to the secondary lymphoid organs. Once in the secondary lymphoid tissues, the lymphocytes do not remain there but move from one lymphoid organ to another through the blood and lymphatics (Fig. 9.6). One of the main advantages of this *lymphocyte recirculation* is that during the course of a natural infection the continual trafficking of lymphocytes enables many different lymphocytes to have access to the antigen.

Only a very small number of the lymphocytes will recognize a particular antigen. Pathogens can enter the body by many routes, but must be carried from the site of infection to the secondary lymphoid tissues where they are localized and concentrated on the dendritic processes of macrophages or on the surface of antigen-presenting cells. If the infection is in the tissues, antigen is carried in the lymphatics to the draining lymph node. Under normal conditions there is a continuous active flow of lymphocytes through lymph nodes, but when antigen and antigen-reactive cells enter there is a temporary shut-down of the exit. Thus, antigen-specific cells are preferentially retained in the node draining the source of the antigen. This is partly responsible for the swollen glands (lymph nodes) that can sometimes be found during an infection. Microbes present on mucosal surfaces are taken

up by specialized cells known as M cells, and are then delivered to the mucosa-associated lymphoid tissues such as the tonsils and Peyer's patches. Blood-borne antigens are trapped in the spleen. The passage of lymphocytes through an area where antigen has been localized facilitates the induction of an immune response. Lymphocytes with appropriate receptors bind to the antigen and become activated. Once activated, the lymphocytes mature into effector cells. In the case of B lymphocytes they become plasma cells and secrete antibody. T lymphocytes leave the secondary lymphoid tissue and return to the site of infection to destroy the infectious agent.

There is evidence for non-random migration of lymphocytes to particular lymphoid compartments. For example, lymphocytes that home to the gut are selectively transported across endothelial cells of venules in the intestine. It appears that lymphocytes have specific molecules on their surface that preferentially interact with endothelial cells in different anatomical sites. A lymphocyte that was initially stimulated by antigen in a Peyer's patch will migrate to the draining lymph node, respond, and memory cells will be produced. It is important that these memory cells migrate back to the area where the same pathogen might be encountered again. Therefore, they are found preferentially in the mucosa-associated lymphoid tissue.

CLONAL SELECTION

During their development in the primary lymphoid tissues both T and B lymphocytes acquire specific cell surface receptors that commit them to a single antigenic specificity. For T cells this receptor remains the same for its life, but the surface immunoglobulin on B cells can be modified as a result of somatic mutations. In the B cell this is mirrored in the modification of the antibody produced by the cell on exposure to its specific antigen. The lymphocytes are activated when they bind specific antigen and then proliferate, differentiate and mature into effector cells.

The lymphocytes reactive to any particular antigen are only a small proportion of the total pool. Therefore, antigen binds to the small number of cells that can recognize it and selects them to proliferate and mature so that sufficient cells are formed to mount an adequate immune response. A cell that responds to an antigenic trigger and proliferates will give rise to cells with a genetically identical makeup (i.e. *clones*). This phenomenon is therefore known as *clonal selection*.

Lymphocyte receptors, generated in the primary lymphoid tissues, are created in a random fashion, so

there is no reason why some could not recognize 'self' molecules. An obviously important attribute of the immune system is that it is able to discriminate between 'self' and 'non-self'. During development, any lymphocyte with a receptor that binds strongly to self molecules is eliminated.

CELLULAR ACTIVATION

When an individual is exposed to foreign material, selected lymphocytes respond. B lymphocytes proliferate and differentiate into antibody-producing plasma cells and memory cells. T lymphocytes are stimulated to become effector cells that can directly eliminate the foreign material or produce molecules that help other cells to destroy the pathogen. The type (immunity or tolerance) and magnitude of the response, if generated, depends on a number of factors, including the nature, dose and route of entry of the antigen, and the individual's genetic make-up and previous exposure to the antigen.

The first stage in the production of effector cells and molecules is activation of the resting cells. This involves various cellular interactions with maturation of the response, leading to a co-ordinated, efficient production of effector T cells, immunoglobulin and memory cells.

Cross-linking of the B cell antigen receptor, surface immunoglobulin, is the initial trigger for activation. When this happens, a number of biochemical changes are instigated. These changes probably act through protein kinases that cause the synthesis of RNA and ultimately immunoglobulin production. In some cases this is all that is required to stimulate antibody production. However, for the majority of antigens this initial cross-linking is not enough, and molecules produced by T cells are also required.

Thymus-independent antigens

A number of antigens will stimulate specific immunoglobulin production directly. These T-independent antigens are of two types: *mitogens* and certain large molecules.

Mitogens are substances that cause cells, particularly lymphocytes, to undergo cell division (i.e. proliferation). Certain glycoproteins, called lectins, have mitogenic activity. These molecules have specificity for sugars; they bind to the cell surface and activate all responsive cells. The response to the mitogens is therefore polyclonal, as lymphocytes of many different specificities are activated. However, at low

**Table 9.5** Antigen-presenting cells in the lymph nodes

| Area | Antigen-presenting cell | Antigen |
|----------------------------|---------------------------------|----------------------------|
| Subcapsular marginal sinus | Marginal zone macrophage | T-independent antigens |
| Follicles and B cell areas | Follicular dendritic cells | Antigen-antibody complexes |
| Medulla | Classical macrophages | Most antigens |
| T cell areas | Interdigitating dendritic cells | Most antigens |

concentrations these mitogens do not cause polyclonal activation but can lead to the stimulation of specific B cells. Lipopolysaccharide is an example of a B cell mitogen.

Some large molecules with regularly repeating epitopes, for instance polymers of D-amino acids and simple sugars such as pneumococcal polysaccharide and dextran, can interact directly with the B cell surface immunoglobulin. They may also be held on the surface of specialized macrophages in secondary lymphoid tissues, and the B cells interact with them there. The multiple repeats of the epitope interact with a large number of surface immunoglobulin molecules; the signal that is generated is sufficient to stimulate antibody production.

The immune response generated to these antigens tends to be similar on each exposure, that is, IgM is the main antibody and the response shows little memory. This suggests that class switch and memory production require additional factors (products of T lymphocytes).

Thymus-dependent antigens

Many antigens do not stimulate antibody production without the help of T lymphocytes. These antigens first bind to the B cell, which must then be exposed to T cell-derived lymphokines (helper factors) before antibody can be produced. For the second activation signal (i.e. help) to be targeted effectively at the B cell, the T and B cells must be in direct contact. For this to happen, the B and T cell epitopes must be linked physically. However, T cells only recognize antigen that has been processed and presented in association with products of the major histocompatibility complex (MHC), so it is impossible for native antigen to form a bridge between surface immunoglobulin and the T cell receptor. The B cell binds to its epitope on free antigen, but there is no site on this molecule to which the T cell can bind, because it requires antigen associated with MHC products. The answer to this problem can be seen when the requirements for antigen presentation in T cell recognition are considered.

Antigen processing and presentation

The development of an antibody response to a T-dependent antigen requires that the antigen becomes associated with MHC class II molecules (i.e. *processed*) and expressed on the cell surface (i.e. *presented*) in a form that helper T cells can recognize.

All cells express MHC class I molecules, but class II molecules are confined to cells of the immune system – the antigen-presenting cells. These cells present antigen to MHC class II-restricted T cells (the CD4-positive [CD4⁺] population) and therefore play a key role in the induction and development of immune responses. Within lymph nodes, different antigen-presenting cells are found in each of the main areas (Table 9.5).

There are a large number of antigen-presenting cells in the body, most of which constitutively express MHC class II molecules. Other cells, such as T lymphocytes and endothelium, can be induced to express MHC class II molecules by suitable stimuli such as lymphokines. The relative importance of each type depends on whether a primary or secondary response is being stimulated and on the location. The most studied antigen-presenting cells are the macrophages and dendritic cells. However, it is now apparent that in certain situations B cells may be important antigen-presenting cells. The relative importance of B cells becomes greatest during secondary responses, especially when the antigen concentration is low. Here the B cells can specifically engulf antigen via their surface immunoglobulin. In a primary response, specific B cells are at a low frequency and their receptors are of low affinity; in this situation macrophages and dendritic cells are probably most important.

The key feature of all antigen-presenting cells is that they can ingest antigen, degrade it and present it, in the context of MHC class II molecules, to T cells. The antigen is taken into the antigen-presenting cells and enters the endocytic pathway. Before it is destroyed completely, peptide fragments are taken to a structure called the *compartment for peptide loading*. MHC class II molecules are synthesized within the endoplasmic reticulum and are also transported to the compartment for peptide loading, where they

associate with the processed antigen. The MHC class II molecule with the bound peptide is then transported to the cell surface.

T cell activation

The activation of resting CD4⁺ T cells requires two signals. The first is antigen in association with MHC class II molecules, and the second is the *co-stimulatory signal*. The generation of the first of these signals has just been discussed (i.e. antigen presentation). The second signal is delivered by the same antigen-presenting cell that gave the first signal. The co-stimulatory signal is mediated by the interaction of a molecule on the antigen-presenting cell engaging with its receptor on the T cell. The best characterized pairing is B7 on the antigen-presenting cell and CD28 on the T cell.

When both of these signals are generated, biochemical changes occur within the T cell, leading to RNA and protein synthesis. The responsive cells progress through the cell cycle from the G₀ to the G₁ phase. The cells start to express IL-2 receptors and produce IL-2, a T cell growth factor that causes the expansion of the responsive T cell population. IL-2 was originally thought to be the only T cell growth factor, but it is now known that IL-4 and IL-1 can support T cell growth, although they are not as potent. After about 2 days, IL-2 synthesis stops, whereas IL-2 receptors remain for up to a week if the cell is not reactivated. Therefore, there is a built-in limitation on T cell growth and clonal expansion. When stimulated, T cells secrete IL-2, which interacts with IL-2 receptors to mediate growth. This can be in an '*autocrine*' fashion if the same cell that released the IL-2 is stimulated. If the responding cell is in the vicinity of the producer, the stimulation is in a '*paracrine*' manner. IL-2 is not present at detectable levels in the blood; therefore no '*endocrine*' activity is involved (i.e. action at a distant site). The end-result is the production of a large number of activated CD4⁺ T lymphocytes.

The other main type of T lymphocyte is the CD8⁺ T cell. Antigen recognition by these cells is restricted by MHC class I molecules. Again, these cells require two signals to be activated: (1) antigen fragment in association with MHC class I and (2) the co-stimulatory signal. A cell that 'sees' both of these signals responds by clonal expansion and differentiation into a fully active effector T cell.

B cell activation

Mitogens and T-independent antigens have an inherent ability to drive B cells into division and differentiation. T-dependent responses rely on T cells and their

products to control the antibody class, affinity and memory. The first cells to be activated are CD4⁺ T cells that recognize the antigen in association with MHC class II molecules (see above). These cells respond to the signal of the antigen fragment–MHC complex, and produce a variety of lymphokines that act on B cells.

As far as B cell development is concerned, the antigen-stimulated cells develop under the influence of IL-4 (previously known as B cell stimulation factor), which is produced by closely adherent T cells. IL-5 and IL-6 then bring the cells to a state of full activation with terminal differentiation into an immunoglobulin-producing plasma cell. All this happens within a germinal centre of a lymph node secondary follicle that has evolved to facilitate the necessary cellular and molecular interactions.

Therefore, for both B and T cell activation, two stimuli are required:

1. The recognition of antigen makes sure that only those cells that will be effective against the foreign material are recruited.
2. The provision of the co-stimulatory signal has evolved to control the process and aid discrimination of 'self' and 'non-self'.

HUMORAL IMMUNITY

Synthesis of antibody

On exposure to antigen, antibody production follows a characteristic pattern (Fig. 9.7). There is a lag phase during which antibody cannot be detected. This is the time taken for the interactions described above to take place and for antibody to reach a level that can be measured. There is then an exponential rise in the antibody level or titre. This log phase is followed by

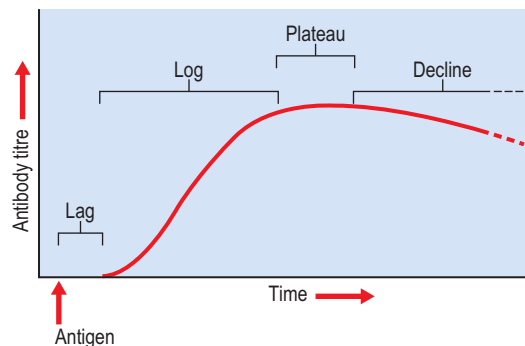


Fig. 9.7 Pattern of antibody production following antigen exposure.

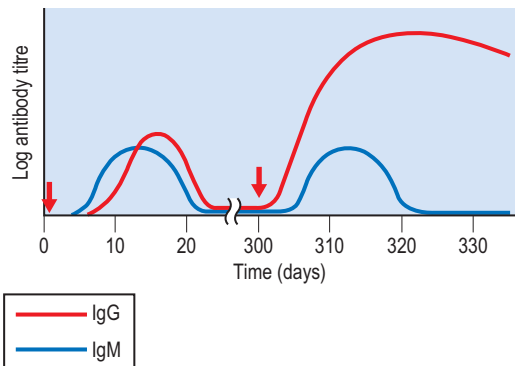


Fig. 9.8 Primary and secondary antibody response. The level of serum IgM and IgG detected with time after primary immunization (day 0) and challenge (day 300) with the same antigen.

a plateau with a constant level of antibody, when the amount produced equals the amount removed. The amount of antibody then declines, owing to the clearing of antigen–antibody complexes and the natural catabolism of the immunoglobulin.

If the response is to a T-dependent antigen, the B cells can switch to the production of another isotype; for example, in a primary response IgM gives way to IgG production. This process is under the control of T cells, as the class of antibody produced depends on signals from the T cell. At some point, again under the control of T cells, a proportion of the antigen-reactive cells develop into memory cells. These cells react if the epitope is encountered again.

There are a number of differences in the reaction profile on second and subsequent exposures to an antigen compared with the primary response (Fig. 9.8). There is a shortened lag and an extended plateau and decline. The level and affinity of antibody produced are much increased, and antibody is mostly of the IgG isotype. Some IgM is generated, but it will follow the same pattern as in the primary response.

When first introduced, the antigen selects the cells that can react with it. However, before antibody is produced the B cell must differentiate into a plasma cell, involving the interactions already described. The B cells that are stimulated in the primary response synthesize IgM. With time, class switch will occur in some of the B cells, leading to the production of other isotypes. Somatic mutations occur, giving rise to *affinity maturation* through selection of cells bearing high-affinity receptors as the amount of antigen in the system falls. Memory cells are also produced. An equilibrium is reached whereby there is a balance between the amount of antibody synthesized and the amount used. Various mechanisms then come into

play to turn off the response when it is no longer needed (see below). The simplest is the removal of the stimulant (antigen). Thus the production of antibody is stopped and there is a natural decline in antibody levels.

On subsequent exposure the responding cells (i.e. memory cells) are at a different level of activation and are present at an increased frequency. Therefore, there is a shorter lag before antibody can be detected; the main isotype is IgG. The level of antibody produced is ten or more times greater than during the primary response. The antibody is present for an extended period and has a higher affinity for antigen due to affinity maturation. As is seen in Figure 9.8, some IgM is also produced during a secondary response. This immunoglobulin is produced by the activation of B cells that were not present in the lymphocyte pool on the previous exposure but have developed since. The development of these cells follows the characteristics of a primary response, and they will give rise to a secondary response if the antigen is encountered again.

Monoclonal antibodies

When an antigen is introduced into the lymphoid system of a mouse, all the B cells that recognize epitopes on the antigen are stimulated to produce antibody. The serum of the immunized animal is known as a polyclonal antiserum, as it is the product of many clonally derived B cells. Even when highly purified antigen is used, the antiserum produced will contain a number of antibodies that react to the antigen and others that interact with antigens encountered naturally by the animal during this time. It is extremely difficult to purify the antibodies of interest from this complex mixture, but it is possible to fuse single plasma cells with a myeloma (a tumour) cell line to form a hybridoma that will grow in tissue culture. These cells will all be identical and therefore secrete the same antibody, a *monoclonal antibody*.

Human monoclonal antibodies are potentially of value in patient treatment. As starting material, peripheral blood or secondary lymphoid tissue such as tonsils have been used. It is impossible, for ethical reasons, to expose human subjects to most of the antigenic material that would be required to induce useful antibodies. Therefore, cells are only available from patients with certain diseases, such as tumours, infections and autoimmune diseases, or from individuals who have received immunizations.

All the molecules in a monoclonal antibody preparation have the same isotype, specificity and affinity, in contrast to the polyclonal antiserum produced by

the inoculation of antigen into an experimental animal. In addition, the same polyclonal antiserum can never be reproduced, not even when using the same animal. However, monoclonal antibodies are defined reagents that can be produced indefinitely and on a large scale. They provide a standard material that can be used in studies ranging from the identification and enumeration of different cell types to blood typing and diagnosis of disease. They are also used increasingly in attempts to treat and prevent disease.

CELL-MEDIATED IMMUNITY

Specific cell-mediated responses are mediated by two different types of T lymphocyte. T cells that have the CD8 molecule on their surface recognize antigen fragments in association with MHC class I molecules on a target cell and cause cell lysis. MHC class II-restricted recognition is seen with T cells that have the CD4 marker. These cells secrete lymphokines when stimulated by the antigen–MHC class II complex. CD4⁺ T cells are involved in two main activities:

1. Cell-mediated reactions, as the lymphokines can aid in the elimination of foreign material by recruiting and activating other leucocytes and promoting an inflammatory response.
2. The generation and control of an immune response, as some of the lymphokines produced are growth and differentiation factors for T and B cells.

The other cell types, NK cells and phagocytes, that can participate in cell-mediated defence mechanisms have been described.

Cell-mediated cytotoxicity

Certain subpopulations of lymphoid and myeloid cells can destroy target cells to which they are closely bound. The stages and processes involved are similar for the different cell types, although the molecules that mediate the recognition of the target by the effector differ.

Cytotoxic T lymphocytes

Cytotoxic T cells (Tc cells) are small T lymphocytes derived from stem cells in the bone marrow. These cells mature in the thymus. Most cells that mediate MHC-restricted cytotoxicity are CD8⁺, and therefore recognize antigen in association with MHC class I antigens. Some are CD4⁺, and therefore MHC class II restricted.

MHC-unrestricted cytotoxic cells

A number of partially overlapping cell populations are able to carry out MHC-unrestricted killing. These include NK cells, lymphokine-activated killer (LAK) cells and killer (K) cells.

Most cells that have the capacity to perform natural killing have the morphology of large granular lymphocytes and a broad target range. Receptors on the NK cell recognise structures on host cells and will kill the host cell unless inhibitory receptors are engaged by MHC class I molecules. Therefore NK cells are involved in the destruction of host cells with low levels of MHC class I molecules, e.g. cells infected with certain viruses and some cancer cells. NK cells have been shown to produce a number of cytokines, including γ -interferon.

Several types of cell are able to destroy foreign material by antibody-dependent cell-mediated cytotoxicity. The cells that carry out this activity have a receptor for the Fc portion of immunoglobulin and are therefore able to bind to antibody-coated targets.

Lytic mechanism

Three distinct phases have been described in cell-mediated cytotoxicity (Fig. 9.9):

- binding to target
- rearrangement of cytoplasmic granules and release of their contents
- target cell death.

Once the effector–target conjugate has been formed, the cytoplasmic granules appear to become rearranged and concentrated at the side of the cell adjacent to the target. The granule contents are then released into the space between the two cells. There are at least three different types of molecule stored within the granules that can cause cell death. T cells and NK cells contain perforin, which is a monomeric protein related to the complement component C9. In the presence of Ca²⁺ ions the monomers bind to the target cell membrane and polymerize to form a trans-membrane pore. This upsets the osmotic balance of the cell and leads to cell death. The granules also contain at least two serine esterases that may play a role in destroying the target cell. Several other toxic molecules are produced by cytotoxic cells, including tumour necrosis factor (TNF)- α , lymphotoxin (TNF- β), γ -interferon and NK cytotoxic factor. The process is unidirectional, with only the target cell being destroyed. The effector cell can then move on and eliminate another target cell.

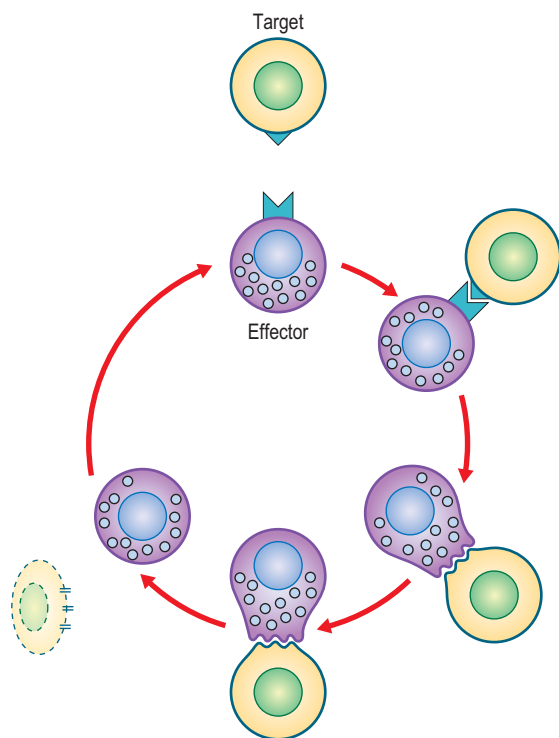


Fig. 9.9 Mechanism of cell-mediated cytotoxicity. The effector cell has a receptor that is able to bind to a target cell that possesses the appropriate ligand (▲).

Lymphokine production

The other arm of cell-mediated immunity is dependent on the production of lymphokines from antigen-activated T lymphocytes. These molecules, produced in an antigen-specific fashion, can act in an antigen-non-specific manner to recruit, activate and regulate effector cells with the potential to combat infectious agents.

The first documented reference to the production of lymphokines is credited to Robert Koch in 1880. Injection of purified antigen (tuberculin) into the skin of immune individuals produced a reaction that peaked within 24–72 h. The response was characterized by reddening and swelling, and accompanied by the accumulation of lymphocytes, monocytes and basophils. Because of the time course of the reaction, this response has become known as *delayed-type hypersensitivity* (DTH), and the cells responsible were called delayed-type hypersensitivity T lymphocytes (T_{DTH} or T_D cells). These cells are identical to the helper T (T_H) cell subset as far as antigen recognition is concerned. $CD4^+$ T cells, usually still referred to as T_H cells, are therefore capable of mediating both

helper activities and so-called delayed hypersensitivity reactions by producing lymphokines. Although the term ‘delayed hypersensitivity’ suggests a disease process, the production of lymphokines has a physiological function, and only in some situations do pathological consequences occur.

Cytokines are biologically active molecules released by specific cells that elicit a particular response from other cells on which they act. A number of these regulatory molecules produced by lymphocytes (lymphokines) and monocytes (monokines) are shown in Table 9.6. The responses caused by these substances are varied and interrelated. In general, cytokines control the growth, mobility and differentiation of lymphocytes, but they also exert a similar effect on other leucocytes and some non-immune cells.

The exact signals and mechanisms controlling the activation of T cells and the release of lymphokines are not known. The balance between the different lymphokines produced determines the response generated. $CD4^+$ T cells can be divided into two main types depending on the profile of lymphokines they secrete. The T_H2 subset produces IL-4 and IL-5, which act on responsive B cells with antibody production as the main feature of the response. The T_H1 subset secretes mainly IL-2 and γ -interferon. The production of IL-2 stimulates T cell growth, whereas γ -interferon has multiple effects, including macrophage activation.

Role of macrophages

Macrophages are able to carry out a remarkable array of different functions (Fig. 9.10). They play a key role in several aspects of cell-mediated immunity, being involved at the initiation of the response, as antigen-presenting cells, and as effector cells having microbicidal and tumoricidal activities. They also produce a number of cytokines (or more precisely monokines) that function as regulatory molecules. These monokines contribute to inflammation and fever, and affect the functioning of other cells. Macrophages can also produce various enzymes and factors that are involved in reorganization and repair following tissue damage. However, as they contain many important biological molecules, they can themselves cause damage if these enzymes and factors are released inappropriately.

Many of these activities are enhanced in macrophages that have been ‘activated’ by exposure to lymphokines, such as γ -interferon produced by T cells. Macrophage activation is a complex process that probably occurs in stages, with different effector functions being expressed at different stages. Macrophages from different sites in the body show different

Table 9.6 Examples of some cytokines that are of importance in the immune system

| Cytokine | Main source | Target | Main effects |
|-------------------------------------|------------------------------------------------------------------|--------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|
| IL-1 | Macrophages Endothelial cells Some epithelial cells | T lymphocytes Tissue cells | Fever Inflammation T cell activation Macrophage activation Stimulates acute-phase protein production |
| IL-2 | T lymphocytes | T lymphocytes NK cells B lymphocytes | T cell proliferation |
| IL-4 | T _H 2 cells Mast cells | B lymphocytes T lymphocytes Mast cells | Stimulates proliferation, differentiation and class switch in B cells Differentiation and proliferation of T _H 2 cells Mast cell growth |
| IL-8 | Macrophages Endothelial cells | Neutrophils | Chemotaxis |
| IL-13 | T _H 2 cells | Macrophages | Inhibits macrophage activation and activities |
| TNF- α | Macrophages T lymphocytes | Macrophages Tissue cells | Fever Inflammation Macrophage activation Stimulates acute-phase protein production Kills certain tumour cells |
| Type I IFN (α and β) | Virus-infected cells | Tissue cells | Antiviral effect Induction of MHC class I Antiproliferative effects Activation of NK cells |
| IFN- γ | T lymphocytes (T _H 1 and T _D) NK cells | Leucocytes and tissue cells | Macrophage activation Induction of MHC class I and II Antibody class switch Antiviral effect |
| GM-CSF | T lymphocytes Macrophages Endothelial cells Fibroblasts | Immature and committed progenitor cells in bone marrow | Stimulates growth and differentiation of myelomonocytic cells Macrophage activation |

Many of the molecules detailed above act synergistically to produce their biological effects.

GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IFN, interferon; MHC, major histocompatibility complex; NK, natural killer; TNF, tumour necrosis factor.

characteristics; they are heterogeneous and have different activation requirements.

γ -Interferon is a powerful macrophage-activating molecule that increases the uptake of antigens by an enhanced expression of Fc and complement receptors; the activities of intracellular enzymes involved in killing are also raised. As γ -interferon causes an increase in MHC class II expression, there will be an enhanced presentation of antigen to CD4⁺ T cells. This leads to the production of more lymphokines and more effective elimination of the offending material.

CD4⁺ T cells secrete the lymphokines that activate macrophages. Therefore, the presentation of antigen by an antigen-presenting cell leads to the production of lymphokines by T_H1 cells *specific* for the antigen involved. The lymphokines produced then activate any responsive macrophage in the vicinity of the responding cells. The activation process appears to depend on the presence of a number of lymphokines that act synergistically to induce activation. For example, pure IL-2, IL-4 or γ -interferon is unable to induce resistance to infection, but if γ -interferon is

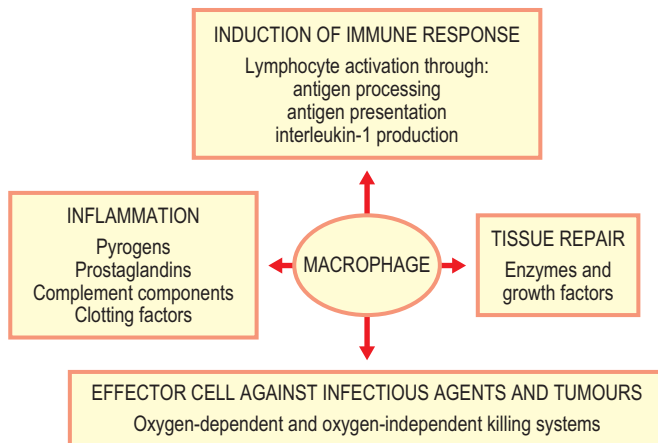


Fig. 9.10 The central role of macrophages.

combined with any of the others then resistance is observed.

Macrophages and monocytes themselves are capable of producing a number of important cytokines. These monokines include:

- IL-1
- IL-6
- various colony-stimulating factors
- TNF- α .

TNF- α and IL-1, acting independently and together, have effects on many leucocytes and tissues. TNF- α is responsible for the tumoricidal activity of macrophages but is also implicated in the elimination of certain bacteria and parasites. It has a synergistic effect with γ -interferon on resistance to a number of viral infections.

GENERATION OF IMMUNE RESPONSES

As discussed above, the generation of humoral and cell-mediated responses requires the recognition of antigen, by the responding cell, as the first signal and a co-stimulation second signal. T_H cells, as has been emphasized, recognize antigen fragments only when in association with MHC class II molecules. The distribution of MHC class II molecules is limited, in normal situations, to certain cells of the immune system: the antigen-presenting cells. In certain circumstances non-lymphoid cells can present antigens if they are induced to express MHC class II molecules. To stimulate a T_H cell the antigen must be taken into the cell and re-expressed on the surface in association with MHC class II molecules. As the T cell antigen receptor recognizes antigen fragments bound to the MHC molecules, the antigen-presenting cell must also

be able to process the antigen. MHC class I- and class II-restricted recognition by $CD8^+$ and $CD4^+$ T cells requires antigen processing. The pathways that lead to association of an antigen fragment with a particular restriction element are not fully understood.

Immune responses are generated in secondary lymphoid tissues, such as lymph nodes. As a number of cells and molecules must all interact, the architecture of the secondary lymphoid tissue has evolved for the efficient induction of an immune response. In a secondary immune response the cells involved are at a different stage of activation: they are memory cells, having already been exposed to antigen. Therefore, the growth factor signals may not be so critical, although antigen in association with MHC class II molecules is still required. In this situation B cells are important as antigen-presenting cells.

$CD8^+$ T cells, as we have seen, recognize antigen fragments associated with MHC class I molecules. All cells have MHC class I molecules on their surface and are therefore expected to be capable of presenting antigen fragments to cytotoxic T cells, which are, for the most part, MHC class I restricted. The antigen fragments derived from endogenously synthesized molecules, for instance from a virus, are produced at a site distinct from the endocytic vesicles where exogenous antigens are processed.

At or around the site of protein synthesis, endogenously produced antigen fragments become associated with the newly produced MHC class I molecules. The MHC class II molecule picks up internalized antigen within the compartment for peptide loading (CPL), as it moves to the cell surface. In this compartment, antigen fragments cannot bind to the MHC class I molecules because these molecules have already associated with endogenously produced antigenic fragments within the endoplasmic reticulum and

never go to the CPL. Thus the site where the antigen is processed determines whether it will associate with MHC class I or class II molecules. This separation of processing pathways explains why CD4⁺ and CD8⁺ T cells are involved in the destruction of exogenous and endogenous antigens, respectively.

If a particular antigen does not become associated with either MHC class I or class II molecules, no T-dependent immune response will be directed against that antigen. As MHC class II molecules are involved in the initiation of immune responses by presenting antigen fragments to T_H cells, they can control whether or not a response takes place. It has been clearly shown that the level of an immune response to a particular antigen is controlled by the MHC class II molecules. The genes that code for these molecules (MHC class II genes) have therefore been referred to as *immune response genes*.

It should be obvious that if an antigen cannot associate with the MHC class II molecules of an individual then no immune response will be generated. As the MHC molecules are polymorphic, the cells of some individuals will present, and therefore respond to, certain antigen fragments, whereas cells from other individuals will not. Fortunately, more than one antigenic fragment can be generated from each pathogen; otherwise individuals who did not respond to the particular sequence would be vulnerable to that micro-organism. In addition, individuals have at least six different MHC class II genes and therefore an increased chance that some fragments will bind to at least one of their MHC class II molecules. Variations in the levels and specificity of response occur in individuals who have different MHC class II molecules and have therefore produced different MHC–antigen complexes on their cells.

Immune response gene effects can also be controlled at the level of the T cell receptor. If an individual does not have a T cell with a receptor that recognizes a particular antigen–MHC complex, no response will be generated. The T cell receptor repertoire is generated in the thymus, where the genes of the immature T cells are rearranged to give rise to a functioning receptor. T cells that cross-react too strongly with self molecules are deleted, as are cells whose receptors do not interact with self MHC molecules. Therefore, T cells that interact weakly with MHC molecules are selected to mature and leave the thymus. When these cells later come across an antigen–MHC complex, the presence of antigen strengthens the weak T cell receptor–MHC interaction, leading to a stimulatory signal being transmitted to the T cell. If, for some reason, T cells that respond to a particular MHC–antigen configuration have been deleted, suppressed

or not formed, no immune response will be generated to that antigen. There is what is known as a ‘hole’ in the T cell repertoire.

CONTROL OF IMMUNE RESPONSES

An antigen can induce two types of response: immunity or tolerance. Tolerance is the acquisition of non-reactivity towards a particular antigen. The generation of immunity or tolerance depends largely on the way in which the immune system first encounters the antigen. Once the immune system has been stimulated, the cells involved proliferate and produce a response that eliminates the offending agent. It is then important to dampen down the reacting cells; various feedback mechanisms operate to bring this about.

Role of antigen

The primary regulator of an immune response is the antigen itself. This makes sense, as it is important to initiate a response when antigen enters the host and once it has been eliminated it is wasteful, and in some cases dangerous, to continue to produce effector mechanisms.

Role of antibody

Many biological systems are controlled by the product inhibiting the reaction once a certain level has been reached. This type of negative feedback is seen with antibody, which may act by blocking the epitopes on the antigen so that it can no longer stimulate the cell through its receptor.

As antibody levels rise there is competition between free antibody and the B cell receptor. Consequently, only those B cells that have a receptor with a high affinity for antigen will be stimulated and therefore produce high-affinity antibody. For this reason antibody feedback is thought to be an important driving force in affinity maturation.

Regulatory T cells

T_H cells control the generation of effector cells by producing helper factors. However, the factors that stimulate the expansion of B and T cell numbers do not work indefinitely. Maturation factors are also produced that control terminal differentiation into effector cells. Under the influence of these latter lymphokines, the action of the proliferation factors is inhibited mainly by making the effector cell unresponsive to their effects.



Other T lymphocytes have been described that provide negative signals to the immune system. Certain regulatory T cells limit the development of antibody-producing cells and effector T cells. The activity of these cells can involve both the production of soluble factors and direct cell–cell interactions.

TOLERANCE

Two forms of tolerance can be identified: *natural* and *acquired* tolerance. The non-response to self molecules is due to natural tolerance. If this tolerance breaks down and the body responds to self molecules, an autoimmune disease will develop. Natural tolerance appears during fetal development when the immune system is being formed. In experimental animals the introduction of foreign material at the time of birth leads to tolerance. Acquired tolerance arises when a potential immunogen induces a state of unresponsiveness to itself. This has consequences for host defences, as the presence of a tolerogenic epitope on a pathogen may compromise the ability of the body to resist infection.

An antigen can induce different effects on the two arms of the immune system. During an infection the host is exposed to a variety of antigenic determinants on a micro-organism. These epitopes are present at differing concentrations and possibly at different times during the infection. The epitopes can act as either immunogens or tolerogens. Therefore, it is possible that the antibody response to a particular antigen may be quite pronounced while the cell-mediated response may be lacking, or vice versa. Alternatively, both arms of the immune response may be stimulated or tolerized.

Generally, high doses of antigen tolerize B cells, whereas minute doses given repeatedly tolerize T cells. For acquired tolerance to be maintained, the tolerogen must persist or be administered repeatedly. This is probably necessary because of the continuous production of new T and B cells that must be made tolerant.

Several mechanisms play a role in the selective lack of response to specific antigens. As each lymphocyte has a receptor with a single specificity, the elimination of a specific cell will render the individual tolerant to the epitope it recognizes and leave the rest of the repertoire untouched. This mechanism relies on self molecules interacting with the receptor and causing their elimination. It is proposed that during lymphocyte development the cell goes through a phase in which contact with antigen leads to death or permanent inactivation. Immature B cells encountering antigen

for the first time are particularly susceptible to tolerization in the presence of low doses of antigen. The requirement for two signals in the stimulation of B cells and the generation of effector T cells can give rise to tolerance. Both cell types require stimulation via the antigen receptor and ‘help’ from a specific T cell. If the helper factors are not produced, the responding cells will be functionally deleted. Therefore, the elimination of self-reactive T cells in the thymus during T cell maturation is an important step in maintaining a state of tolerance. Tolerance can also be induced by active suppression. Some T cells are capable of inducing unresponsiveness by acting directly on B cells or other T cells. These regulatory T cells can be antigen-specific and probably produce signals that actively suppress cells capable of responding to a particular antigen.

It was originally thought that unresponsiveness to self was controlled by the elimination of all self-reactive cells before they matured. This cannot be true, as self-reactive B cells are found in normal adult animals. It is thought that these B cells are controlled by a lack of T cell help, that is, T_H cells have been eliminated.

IMMUNODEFICIENCY

The immunologically competent cells of the lymphoid tissues derived from, renewed by and influenced by the activities of the thymus, bone marrow and other lymphoid tissues can be the subject of disease processes. The deficiency states seen are either due to defects in one of the components of the system itself, or secondary to some other disease process affecting the normal functioning of some part of the lymphoid tissues. Deficiency of one or more of the defence mechanisms can be inherited, developmental or acquired. The types of infections and diseases seen in patients with immunodeficiencies relate to the role the affected component plays in the normal situation. An individual whose immune system has been depressed in any of these ways is said to be *immuno-compromised*. The compromised host is prone to infectious diseases that the normal individual would easily eradicate or not succumb to in the first place. Some examples of predisposing factors are given in Table 9.7.

Defective innate defence mechanisms

Defects in phagocyte function take two forms:

1. Where there is a quantitative deficiency of neutrophils that may be congenital (e.g. infantile

Table 9.7 The compromised host

| Predisposing factor | Effect on immune system | Type of infection |
|------------------------------------------------------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Immunosuppression for transplant or cancer | Diminished cell-mediated and humoral immunity | Lung infections, bacteraemia, fungal infections, urinary tract infections |
| Viral immunosuppression (e.g. measles, human immunodeficiency virus, Epstein–Barr virus) | Impaired function of infected cells | Secondary bacterial infections, opportunistic pathogens |
| Tumour of immune cells | Replacement of cells of the immune system | Bacteraemia, pneumonia, urinary tract infections |
| Malnutrition | Lymphoid hypoplasia Decreased lymphocytes and phagocyte activity | Measles, tuberculosis, respiratory infections, gastro-intestinal infections |
| Breakdown of tissue barriers (e.g. surgery, burns, catheterization) | Breach innate defence mechanisms | Bacterial infections, opportunistic pathogens |
| Inhalation of particles due to employment or smoking | Damage to cilia, destruction of alveolar macrophages | Chronic respiratory infections, hypersensitivity reactions |

agranulocytosis) or acquired as a result of replacement of bone marrow by tumour cells or the toxic effects of drugs or chemicals.

- Where there is a qualitative deficiency in the functioning of neutrophils which, while ingesting bacteria normally, fail to digest them because of an enzymatic defect.

Characteristic of these diseases is a susceptibility to bacterial and fungal, but not viral or protozoan, infections. Among the enzyme deficiency disorders are *chronic granulomatous disease* and the *Chédiak–Higashi syndrome*.

The complement system can also suffer from certain defects in function leading to increased susceptibility to infection. The most severe abnormalities of host defences occur, as would be expected, when there is a defect in the functioning of C3. Severe deficiency or absence of C3 is associated with increased susceptibility to infection, particularly septicaemia, pneumonia, meningitis, otitis and pharyngitis.

Defective acquired immune defence mechanisms

Primary immunodeficiencies

Primary deficiencies in immunological function can arise through failure of any of the developmental processes from stem cell to functional end cell. A complete lack of all leucocytes is seen in *reticular dysgenesis* due to a defect in the development of bone marrow stem cells in the fetus. A baby born with this defect usually dies within the first year of life from recurrent,

intractable infections. Defects in the development of the common lymphoid stem cell give rise to severe combined immunodeficiency. Both T and B lymphocytes fail to develop, but functional phagocytes are present.

There are several types of B cell defect that give rise to hypogammaglobulinaemias, that is, low levels of γ -globulins (antibodies) in the blood. Deficiency of immunoglobulin synthesis is almost complete in X-linked infantile hypogammaglobulinaemia (*Brunton's disease*). Male infants suffer from severe, chronic, bacterial infections once maternal antibody has disappeared. There is an absence, or deficiency, of all five classes of serum immunoglobulin. Therefore, the defect is thought to be caused by the absence of B cell precursors or their arrest at a pre-B cell stage. Cell-mediated immune mechanisms function normally and the patients seem to be able to handle viral infections relatively well.

Partial defects in immunoglobulin synthesis have been described affecting one or more of the immunoglobulin classes. In the *Wiskott–Aldrich syndrome*, which is inherited as an X-linked recessive character, there are low levels of IgM but IgA and IgE levels are raised. Patients are susceptible to pyogenic infections, along with recurrent bleeding and eczema. The bleeding is due to reduced platelet production (thrombocytopenia), and the allergy-related eczema is linked to the increased IgE levels. In patients with *dysgammaglobulinaemia* there is a deficiency in only one antibody class. Some patients have reduced levels of IgA whereas the other isotypes are normal. These patients have an increased incidence of infections in the upper



and lower respiratory tracts, where IgA is normally protective.

Individuals with T cell defects tend to have more severe and persistent infections than those with antibody deficiencies. A lack of T lymphocytes is often associated with abnormal antibody levels, as T_H cells are involved in the generation and control of humoral immunity. Patients with T cell defects suffer from viral, intracellular bacterial, fungal and protozoan infections rather than acute bacterial infections. In the *DiGeorge syndrome* (congenital thymic aplasia) the person is born with little or no thymus. Individuals who survive develop recurrent and chronic infections, including pneumonia, diarrhoea and yeast infections, once passive maternal immunity has waned.

Secondary immunodeficiencies

Acquired deficiencies can occur secondarily to a number of disease states or after exposure to drugs and chemicals.

Deficiency of immunoglobulins can be brought about by excessive loss of protein through diseased kidneys or via the intestine in protein-losing enteropathy. Mal nutrition and iron deficiency can lead to depressed immune responsiveness, particularly in cell-mediated immunity. Medical and surgical treatments such as irradiation, cytotoxic drugs and steroids often have undesirable effects on the immune system. Viral infections are often immunosuppressive. For example, measles, human immunodeficiency and other viruses infect cells of the immune system.

In contrast to the deficiency states just described, raised immunoglobulin levels are found in certain disorders of plasma cells due to malignant proliferation of a particular clone or group of plasma cells. In these conditions, such as chronic lymphocytic leukaemia and multiple myeloma, malignant clones each produce one particular type of antibody. There is usually a decreased synthesis of normal immunoglobulins and an associated deficiency in the immune response to acute bacterial infections. These B lymphoproliferative disorders contrast with the situation in *Hodgkin's disease*, a reticular cell neoplasm in which the patients show defective cell-mediated immunity and are susceptible to viruses and intracellular bacteria.

HYPERSENSITIVITY

Immunity was first recognized as a resistant state that followed infection. However, some forms of immune reaction, rather than providing exemption or safety, can produce severe and occasionally fatal results.

These are known as *hypersensitivity reactions* and result from an excessive or inappropriate response to an antigenic stimulus. The mechanisms underlying these deleterious reactions are those that normally eradicate foreign material, but for various reasons the response leads to a disease state. When considering each of the four hypersensitivity states it is important to remember this fact and consider the underlying defence mechanism and how it has given rise to the observed immunopathology.

Various classifications of hypersensitivity reactions have been proposed; probably the most widely accepted is that of Coombs and Gell. This recognizes four types of hypersensitivity that are considered in turn.

Type I: anaphylactic

If a guinea-pig is injected with a small dose of an antigen such as egg albumin, no adverse effects are noted. If a second injection of the same antigen is given intravenously after an interval of about 2 weeks, a condition known as *anaphylactic shock* is likely to develop. The animal becomes restless, starts chewing and rubbing its nose, begins to wheeze, and may develop convulsions and die. The initial injection of antigen is termed the sensitizing dose, whereas the second injection causes anaphylactic shock. Such a reaction is seen in human beings after a bee-sting or injection of penicillin in sensitized individuals. Localized reactions are seen in patients with hay fever and asthma. In all of these situations the host responds to the first injection by producing IgE, and it is the level of IgE produced to a particular antigen that determines whether an anaphylactic reaction will occur on re-exposure to the same antigen. Asthma results from a similar response in the respiratory tract.

The biologically active molecules that are responsible for the manifestations of type I hypersensitivity are stored within mast cell and basophil granules or are synthesized after cell triggering. The signal for the release or production of these molecules is the cross-linking of surface-bound IgE by antigen. The release of these molecules, vasoactive amines and chemotactic factors, is responsible for the symptoms of type I hypersensitivity. IgE has been implicated in the control of parasitic worms; the importance of this is discussed in Chapter 11.

Type II: cytotoxic

Type II reactions are initiated by the binding of an antibody to an antigenic component on a cell surface. The antibody is directed against an epitope, which can

be a self molecule or a drug or microbial product passively adsorbed on to a cell surface. The cell that is covered with antibody is then destroyed by the immune system. A variety of infectious diseases caused by salmonellae and mycobacteria are associated with haemolytic anaemia. There is evidence, particularly in studies of salmonella infection, that the haemolysis is due to an immune reaction against bacterial endotoxin that becomes coated on to the erythrocytes of the patient.

Type III: immune complex

As discussed above, when a soluble antigen combines with antibody the size and physical form of the immune complex formed depends on the relative proportions of the participating molecules and is affected by the class of antibody. Monocytes and macrophages are very efficient at binding and removing large complexes. These same cell types can also eliminate the smaller complexes made in antibody excess, but are relatively inefficient at removing those formed in antigen excess. Type III hypersensitivity reactions appear when there is a defect in the systems involving phagocytes and complement that remove immune complexes, or when the system is overloaded and the complexes are deposited in tissues. This latter situation occurs when antigens are never completely eliminated, as with persistent infection with an organism, autoimmunity and repeated contact with environmental factors.

The tissue damage that results from the deposition of immune complexes is caused by the activation of complement, platelets and phagocytes – in essence, an acute inflammatory response. In general, the degree and site of damage depend on the ratio of antigen to antibody. At equivalence or slight excess of either component, the complexes precipitate at the site of antigen injection or production and a mild, local type III hypersensitivity reaction occurs (e.g. the *Arthus reaction*). In contrast, the complexes formed in large antigen excess become soluble and circulate, causing more serious systemic reactions (e.g. *serum sickness*), or eventually deposit in organs, such as skin, kidneys and joints. The type of disease and its time course depends on the immune status of the individual.

The local release of antigens from an infectious organism can cause a type III reaction. A number of parasitic worms, although undesirable, cause little or no damage. However, if the worm is killed it can become lodged in the lymphatics, and the inflammatory response initiated by antigen–antibody complexes causes a blockage of lymph flow. This leads to the condition of elephantiasis in which enormous

swellings can occur. In some cases of tuberculosis, sarcoidosis, leprosy and streptococcal infections, vascular inflammatory lesions are seen mainly in the legs. These are variously referred to as *erythema nodosum*, *nodular vasculitis* and *erythema induratum*, and may be due to the deposition of immune complexes and the development of an Arthus reaction.

In systemic disease the clinical manifestations depend on where the immune complexes form or lodge – skin, joints, kidney and heart being particularly affected.

Drugs such as penicillin and sulphonamides can cause type III reactions. The most susceptible patients develop rashes (urticarial, morbilliform or scarlatini-form), pyrexia, arthralgia, lymphadenopathy and perhaps nephritis some 8–12 days after being given the drug. It is likely that similar events occur in many bacterial and viral infections (see Chs 12 and 10, respectively).

Type IV: cell-mediated or delayed

This form of hypersensitivity can be defined as a specifically provoked, slowly evolving (24–48 h), mixed cellular reaction involving lymphocytes and macrophages. The reaction is not brought about by circulating antibody but by sensitized lymphoid cells. This type of response is seen in a number of allergic reactions to bacteria, viruses and fungi, in contact dermatitis and in graft rejection. The classical example of this type of reaction is the tuberculin response that is seen following an intradermal injection of a purified protein derivative (see p. ••) from tubercle bacilli in immune individuals. An indurated inflammatory reaction in the skin appears about 24 h later and persists for a few weeks. In humans the injection site is infiltrated with large numbers of mononuclear cells, mainly lymphocytes, with about 10–20% macrophages. Most of these cells are in or around small blood vessels. The type IV hypersensitivity state arises when an inappropriate or exaggerated cell-mediated response occurs.

Cell-mediated hypersensitivity reactions are seen in a number of chronic infectious diseases caused by myco-bacteria, protozoa and fungi. Because the host is unable to eliminate the micro-organism, the antigens persist and give rise to a chronic antigenic stimulus. Thus, continual release of lymphokines from sensitized T cells results in the accumulation of large numbers of activated macrophages that can become epithelioid cells. These cells can fuse together to form giant cells. Macrophages express antigen fragments on their surface in association with MHC class I and II molecules, and are therefore the targets



of T_C cells and stimulate more lymphokine production. This whole process leads to tissue damage with the formation of a *chronic granuloma* and resultant cell death.

Penicillin sensitization is a common clinical complication following topical application of the antibiotic in ointments or creams. This and other substances that cause contact sensitivity are not themselves antigenic and become so only in combination with proteins in the skin. The Langerhans cells of the epidermis are efficient antigen-presenting cells favouring the development of a T cell response. These cells pick up the newly formed antigen in the skin and transport it to the draining lymph node where a T cell response is stimulated. Here, the specific T cells are stimulated to mature, and then return to the site of entry of the offending material and release their lymphokines. In a normal situation these would help to eliminate a pathogen, but in this case the continual or subsequent exposure to the foreign material leads to an inappropriate response. The reaction site is characterized by a mononuclear cell infiltrate peaking at 48 h. The clinical symptoms in these contact dermatitis lesions include redness, swelling, vesicles, scaling and exudation of fluid (i.e. eczema).

AUTOIMMUNITY

A fundamental characteristic of the immune system of an animal is that it does not, under normal circumstances, react against its own body constituents. Mechanisms, as we have seen, exist that allow the immune system to tolerate self and destroy non-self. Occasionally these mechanisms break down and autoantibodies are produced.

Genetic factors appear to play a role in the development of autoimmune diseases and there is a strong association between several autoimmune diseases and particular HLA (human leucocyte group A)

specificities, suggesting that immune response gene effects may be involved.

There are a number of examples where potential auto-antigenic determinants are present in exogenous material. These preparations may provide a new carrier, a T cell-stimulating determinant that provokes autoantibody formation. The encephalitis sometimes seen after rabies vaccination with the older vaccines is thought to result from a response directed against the brain that is stimulated by heterologous brain tissues present in the vaccine.

Micro-organisms are a source of cross-reacting antigens, sharing antigenic determinants with tissue components. This may be an important way of inducing autoimmunity. The group A streptococcus, which is closely associated with rheumatic fever, shares an antigen with the human heart. Heart lesions are a common finding in rheumatic fever, and anti-heart antibody is found in just over 50% of patients with this condition. Nephritogenic strains of type 12 group A streptococci carry surface antigens similar to those found in human glomeruli, and infection with these organisms has been associated with the development of acute nephritis. Some of the immunopathology seen in Chagas' disease has been attributed to a cross-reaction between *Trypanosoma cruzi* and cardiac muscle.

Autoimmunity can be induced by bypassing T cells. Self-reactive cells can be stimulated by polyclonal activators that directly activate B cells. A number of micro-organisms or their products are potent polyclonal activators; however, the response that is generated tends to be IgM and to wane when the pathogen is eliminated. Bacterial endotoxin, the lipopolysaccharide of Gram-negative bacteria, provides a non-specific inductive signal to B cells, bypassing the need for T cell help. A variety of antibodies are present in infectious mononucleosis, including autoantibodies, as a result of the polyclonal activation of B cells by Epstein-Barr virus.

RECOMMENDED READING

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