Chapter 9

Inflammation and fever

9.1 Inflammation

9.1.1 Principles of inflammation

A human or animal must defend itself against multitude of different pathogens including viruses, bacteria, fungi, and protozoan and metazoan parasites as well as tumours and a number of various harmful agents which are capable to derange its homeostasis. For this, a plenty of effector mechanisms capable of defending the body against such antigens and agents have developed and these can be mediated by soluble molecules or by cells. If infection occur as a consequence of the tissue damage, the innate and, later, the adaptive immune systems are triggered to destroy the infectious agent.

Inflammation is a complex stereotypical reaction of the body expressing the response to damage of its cells and vascularized tissues. In avascular tissues, e.g. in normal cornea, the true inflammation does not occure.

The discovery of the detailed processes of inflammation has revealed a close relationship between inflammation and the immune response.

The five basic symptoms of inflammation - redness (rubor), swelling (tumour), heat (calor), pain (dolor) and deranged function (functio laesa) have been known since the ancient Greek and Roman era. These signs are due to extravasation of plasma and infiltration of leukocytes into the site of inflammation. Early investigators considered inflammation a primary host defence system. From this point of view inflammation is the key reaction of the innate immune response but in fact, inflammation is more than this, since it can lead to death, as in anaphylactic shock, or debilitating diseases, as in arthritis and gout.

According to different criteria, inflammatory responses can be divided into several categories. The criteria include:

1. time - hyperacute (peracute), acute, subacute, and chronic inflammation;
2. the main inflammatory manifestation - alteration, exudation, proliferation;
3. the degree of tissue damage - superficial, profound (bordered, not bordered);
4. characteristic picture - nonspecific, specific;
5. immunopathological mechanisms
   • allergic (reaginic) inflammation,
   • inflammation mediated by cytotoxic antibodies,
   • inflammation mediated by immune complexes,
   • delayed-type hypersensitivity reactions.

9.1.1.1 The response to injury and infection

Inflammation is the body’s reaction to invasion by an infectious agent, antigen challenge or even just physical, chemical or traumatic damage.

The mechanism for triggering the response the body to injury is extremely sensitive. Responses are to tissue damage that might not normally be thought of as injury, for example when the skin is stroked quite firmly or if some pressure is applied to a tissue.
In addition, the body has the capacity to respond to both minor injuries such as bruising, scratching, cuts, and abrasions, as well as to major injuries such as severe burns and amputation of limbs.

Depending on the severity of the tissue damage resulting from an injury, the integrity of the skin or internal surfaces may be breached and damage to the underlying connective tissue and muscle, as well as blood vessels can occur. In this situation infection can, and frequently does result because the normal barrier to the entry of harmful organisms has been broken. It is obviously most important that the body can respond to injury by healing and repairing the damaged tissue, as well as by eliminating the infectious agents that may have entered the wound and their toxins. It is also important that the appropriate response to the tissue damage and infection can be made: it is no use bringing all of the body’s defences into action to repair a minor scratch, just as one would not expect a single mechanism to be able to deal with the sudden loss of a limb or a major infection.

The inflammatory reaction is phylogenetically and ontogenetically the oldest defence mechanism. The cells of the immune system are widely distributed throughout the body, but if an infection or tissue damage occurs it is necessary to concentrate them and their products at the site of damage. Three major events occur during this response:

1. An increased blood supply to the tissue "in danger". It is performed by vasodilation. The inflamed tissue looks like containing greater number of vessels.

2. Increased capillary permeability caused by retraction of the endothelial cells. This permit larger molecules than usual to escape from the capillaries, and thus allows the soluble mediators of immunity to reach the site of inflammation.

3. Leukocytes migrate out of the capillaries into the surrounding tissues. In the earliest stages of inflammation, neutrophils are particularly prevalent, but later monocytes and lymphocytes also migrate towards the site of infection.

For the possibility of surrounding tissue damage, inflammatory responses must be well ordered and controlled. The body must be able to act quickly in some situations, for example to reduce or stop the lost of blood, whereas tissue repair and reconstruction can begin a little later. Therefore, a wide variety of interconnected cellular and humoral (soluble) mechanisms are activated when tissue damage and infection occur. On the other hand if the injury is negligible, the body must have mechanisms which are able to stop the tissue damage when the injury agent was removed.

The development of inflammatory reactions is controlled by cytokines, by products of the plasma enzyme systems (complement, the coagulation clotting, kinin and fibrinolytic pathways), by lipid mediators (prostaglandins and leukotrienes) released from different cells, and by vasoactive mediators released from mast cells, basophils and platelets. These inflammatory mediators controlling different types of inflammatory reaction differ. Fast-acting mediators, such as vasoactive amines and the products of the kinin system, modulate the immediate response. Later, newly synthesized mediators such as leukotrienes are involved in the accumulation and activation of other cells. Once leukocytes have arrived at a site of inflammation, they release mediators which control the later accumulation and activation of other cells.

However, in inflammatory reactions initiated by the immune system, the ultimate control is exerted by the antigen itself, in the same way as it controls the immune response itself. For this reason, the cellular accumulation at the site of chronic infection, or in autoimmune reactions (where the antigen cannot ultimately be eradicated), is quite different from that at sites where the antigenic stimulus is rapidly cleared.

The nervous system can also participate in the control of inflammation, especially axon reflexes, but inflammation may be realized in denervated tissues as well.

Inflammation can become chronic. In certain settings the acute process, characterized by neutrophil infiltration and edema, gives way to a predominance of mononuclear phagocytes and lymphocytes. This probably occurs to some degree with the normal healing process but becomes exaggerated and chronic when there is ineffective elimination of foreign materials as in certain infections (e.g. tuberculosis) or following introduction of foreign bodies (e.g. asbestos) or deposition of crystals (e.g. urate...
9.1. Inflammation

crystals). Chronic inflammation is often associated with fusion of mononuclear cells to form multinucleated giant cells, which eventually become granuloma. Chronic inflammation is seen under conditions of delayed hypersensitivity.

Main humoral and cellular components involved in the amplification and propagation of both acute and chronic inflammation are showed in Table 9.1.

9.1.1.2 Factors involved in cell damage

There are two categories of factors capable to induce the damage of cells and tissues - endogenous and exogenous. Endogenous damaging factors include immunopathological reactions, and some neurological and genetical disorders. Exogenous factors can be divided into:

- mechanical (traumatic injury),
- physical (extremely low or high temperature, ionising irradiation, microwaves),
- chemical (caustic agents, poisons, venoms, genotoxic and proteotoxic compounds),
- nutritive (deficiency of oxygen, vitamins and basic nutrients),
- biological (viruses, microorganisms, protozoan and metazoan parasites).

Immunopathological reactions may be also triggered by exogenous antigens. Genetically caused alterations leading to inflammation are manifested by destruction of membrane structures, by derangement of transport mechanisms, or by defective activity of some enzymes and mediators. Cell damage also occurs during ageing. It is a very complicated process in which genetic, metabolic, immunologic, neurological and other factors are involved. In ageing cells, probably metabolic intermediates such as different free radicals, aldehydes, ketones, and their reaction products, or on the contrary non-degradable compounds are accumulated. This results in a serious defect in the integrity and physiological homeostasis of cells and tissue.

It seems that aging cells are losing their multiplication capacity at a particular generation. For instance, cultivated fibroblasts lose their ability to multiply between 40 and 60 generations. The cell aging may result as a tissue atrophy. In other cases, hypertrophy or hypoplasia is the compensatory mechanism for this situation. The altered cellular activities may lead to metaplasia, dysplasia, or neoplasia because aging cells are more susceptible to destruction of their DNA, RNA and vital proteins.

Extremely low temperature is able to form crystals inside the cell. Mild decrease in temperature causes paralysis of vasomotors and an increase in permeability of vessels. Blood viscosity rises proportionally with the lowering temperature and cells are destroyed by hypoxia. Low temperature acting for a longer time provokes the destruction of myelin in exposed area. Microthrombi are produced in vessels and they are the cause of gangrene.

High temperature increases the permeability of cell membranes. Very high temperature is responsible for the coagulation of vessels and denaturation of vital biopolymers, especially proteins.

According to the dose and the way of exposition ionizing irradiation may primarily damage haematopoietic, gastrointestinal or neural tissues. Whole-body irradiation produces nonspecific immunosupression which is the cause of increased sensitivity to infection. The infection is developed mainly due to leukopenia and the loss of physical integrity of mucosal membranes especially in the gastrointestinal tract. Whole-body irradiation eliminates most of the mature lymphocytes of the immune system while preserving the more radiation-resistant elements such as the thymic epithelium. Ionizing radiation is also used for the treatment of patient with cancer and sometimes in the form of local graft irradiation. An alternative form of radiation therapy is total lymphoid irradiation e.g. for the treatment of Hodgkin’s disease. Lethally irradiated persons can be given immature bone marrow cells to reconstitute the immune systems.

On the cell level, irradiation destroy important biopolymers (DNA, proteins) and biological membranes. At first, the degenerative changes of nucleus and chromosomal aberrations can be seen. The increased membrane permeability and activation of hydrolytic lysosomal enzymes disrupt cell structures and compartments. Irreversible damage of irradiated cells causes their complete destruction, necrosis.

Some chemicals, namely caustic agents and mineral acids are able to damage tissues directly, other such as heavy metals, poisons and venoms mainly
derange important enzymatic reactions. Metabolic homeostasis of cells and tissues is also disturbed by the action of genotoxic and proteotoxic agents. To the often observed defects belong: destruction of cell membranes, decrease of intracellular pH, release of lysosomal enzymes and changes similar as in hypoxia (decrease of oxidative phosphorylation). Lysosomal enzymes and free radicals derived from oxygen (reactive oxygen intermediates - ROI) or from nitrogen (reactive nitrogen intermediates - RNI) have an essential role in the damage of cell structures especially during the injuring inflammation. These substances may be also activated by the action of many amphiphilic detergents that are components of different cleaning and washing preparations and tooth pastes. They are dangerous if they reach inside the body in the inappropriate amount or in the inappropriate way.

The oxygen deficiency is manifested in 3–5 minutes. In mitochondria, oxidative phosphorylation is very quickly impaired and insufficient production of ATP appears. Deficiency of ATP activates anaerobic metabolism in which ATP is formed from glycogen. But the reserves of glycogen are again quickly depleted. Because of persistent ATP insufficiency the sodium-potassium pump loses its operating capacity. This leads to the intracellular accumulation of sodium and the leakage of potassium from cells. Accumulation of sodium induces the transfer of ions and water into cell. It is the reason of endoplasmic reticulum dilatation. The dilatation provides complete damage to ribosomes and blocks proteosynthesis.

If the hypoxia continues, the whole cell is overfilled with water, sodium, and chlorides. This state is still reversible, after the renewing of oxygen transport, the cell should recover. In the others cases, vacuoles in the cytoplasm and the damage of mitochondrial membrane appear. Now, it is the irreversible process. Because of the membrane damage, the extracellular calcium may enter the cell and accumulate in mitochondria. The production of ATP is completely terminated that is thought to be the real death of cell. The cell or tissue death is performed as necrosis.

Cell damage may be also caused by different gasses, especially by nitrogen oxides, sulphur dioxide, carbon monoxide, formaldehyde, chlorine, etc. Carbon monoxide is bound by hemoglobin with 300times higher affinity than oxygen. Therefore the exposure
to CO develops the secondary oxygen deficiency due to the termination of oxygen transport to cells.

Infections are often involved in cell damage. Virulence of microorganisms and the induction of inflammation depend on their ability to replicate in human or animal body and to destroy cellular structures. During growth and multiplication, microorganisms can produce and release different exotoxins which are potent injuring agents. Other microorganisms, after destruction or lysis, release from phospholipid and lipopolysaccharide envelops toxins known as endotoxins. The term "endotoxin" is generally used to refer to the thermostable polysaccharide toxin, firmly bound to the bacterial cell, in contrast to the thermolabile protein "exotoxin", secreted into the external environment. Endotoxin (lipopolysaccharide, LPS) is responsible for many pathophysiological symptoms observed during gram-negative bacterial infections. They include pyrogenicity (the ability to cause an increase in body temperature), changes in the number of circulating leukocytes (leukocytopenia, leukocytosis), complement activation, activation of macrophages, aggregation of platelets, increase of capillary permeability and others. In addition, LPS induces an immune response. Administration or release of a higher dose of endotoxin may produce lethal shock. All these biological activities are mediated through the endogenous mediator – tumor necrosis factor-α (TNF-α).

Viruses do not produce exotoxins or endotoxins. They are typical intracellular parasites and use cells for their own replication. During this, damage of cell structures leading to the death of cell is observed. In addition viruses may be responsible for the tumorous transformation of cells.

During the immune responses, the cells may be damaged by effector cells and molecules participating in immune mechanisms. From this point of view they are thought to be the immunopathological responses. They include:

1. **Immediated allergic anaphylactic reactions** mediated by IgE antibodies (reagines).

2. **Cytotoxic reactions** during which complement is activated by IgG or IgM antibodies reacting with antigens of self cells and structures (autoantigens) which immediately damage the target cells and surrounding tissues.

3. **Reactions of the immune complex type.** The complement system is also activated by the immune complexes. During the activation, chemotactic factors are formed which attract granulocytes to the inflammation area. Neutrophils destroy target cells by released lysosomal enzymes, especially by proteinases, and free radicals of oxygen.

4. **Reactions of delayed or cell-mediated hypersensitivity.** Specific subpopulation of T lymphocytes and several cytokines are involved in these processes.

5. **Cytotoxic reactions influencing the function of cell receptors.** They are also mediated by autoantibodies that may have a function of agonists or antagonists. Hence, the autoantibodies can pathologically stimulate and/or block the transfer of specific signal through the receptor.

It follows that cell damage following the inflammatory reaction may be useful or harmful. The useful activities include:

1. destruction of injuring and infectious agents and their elimination from the inflammatory site;
2. limitation of spreading of injuring factors;
3. stimulation of the specific immune response;
4. help in the healing process.

To the harmful inflammatory reactions belong autoimmune and other immunopathological processes.

### 9.1.1.3 The phases of inflammation

The main purpose of inflammation, this immensely complex response seems to be to bring fluid, proteins, and cells from the blood into the damaged tissues. It should be remembered that the tissues are normally bathed in a watery fluid (extracellular lymph) that lacks most of the proteins and cells that are present in blood, since the majority of proteins are too large to cross the blood vessel endothelium. Thus there have to be mechanisms that allow cells and proteins to gain access to extravascular sites where and when they are needed if damage and infection has occurred.

The main features of the inflammatory response are, therefore: **vasodilation**, i.e. widening of the blood vessels to increase the blood flow to the infected area; **increased vascular permeability**, which
allows diffusible components to enter the site; cellular infiltration by chemotaxis, or the directed movement of inflammatory cells through the walls of blood vessels into the site of injury; changes in biosynthetic, metabolic, and catabolic profiles of many organs; and activation of cells of the immune system as well as of complex enzymatic systems of blood plasma. Of course, the degree to which these occur is normally proportional to the severity of the injury and the extent of infection.

Inflammation can be divided into several phases. The earliest, gross event of an inflammatory response is temporary vasoconstriction, i.e. narrowing of blood vessels caused by contraction of smooth muscle in the vessel walls, which can be seen as blanching (whitening) of the skin. This is followed by several phases that occur over minutes, hours and days later, outlined below.

1. The acute vascular response follows within seconds of the tissue injury and last for some minutes. This results from vasodilation and increased capillary permeability due to alterations in the vascular endothelium, which leads to increased blood flow (hyperaemia) that causes redness (erythema) and the entry of fluid into the tissues (oedema). This phase of the inflammatory response can be demonstrated by scratching the skin with a finger-nail. The "wheal and flare reaction" that occurs is composed of (a) initial blanching of the skin due to vasoconstriction, (b) the subsequent rapid appearance of a thin red line when the capillaries dilate; (c) a flush in the immediate area, generally within a minute, as the arterioles dilate; and (d) a wheal, or swollen area that appears within a few minutes as fluid leaks from the capillaries. It is usually terminates after several tens minutes.

2. If there has been sufficient damage to the tissues, or if infection has occurred, the acute cellular response takes place over the next few hours. The hallmark of this phase is the appearance of granulocytes, particularly neutrophils, in the tissues. These cells first attach themselves to the endothelial cells within the blood vessels (margination) and then cross into the surrounding tissue (diapedesis). During this phase erythrocytes may also leak into the tissues and a haemorrhage can occur (e.g. a blood blister). If the vessel is damage, fibrinogen and fibronectin are deposited at the site of injury, platelets aggregate and become activated, and the red cells stack together in what are called "rouleau" to help stop bleeding and aid clot formation. The dead and dying cells contribute to pus formation.

3. If the damage is sufficiently severe, a chronic cellular response may follow over the next few days. A characteristic of this phase of inflammation is the appearance of a mononuclear cell infiltrate composed of macrophages and lymphocytes. The macrophages are involved in microbial killing, in clearing up cellular and tissue debris, and they also seem to be very important in remodelling the tissues.

4. Over the next few weeks, resolution may occur, meaning that the normal tissue architecture is restored. Blood clots are removed by fibrinolysis, and if it is not possible to return the tissue to its original form, scarring results from in-filling with fibroblasts, collagen, and new endothelial cells. Generally, by this time, any infection will have been overcome. However, if it has not been possible to destroy the infectious agents or to remove all of the products that have accumulated at the site completely, they are walled off from the surrounding tissue in granulomatous tissue. A granuloma is formed when macrophages and lymphocytes accumulate around material that has not been eliminated, together with epitheloid cells and gigant cells (perhaps derived from macrophages) that appear later, to form a ball of cell.

Inflammation is often considered in terms of acute inflammation that includes all the events of the acute vascular and acute cellular response (1 and 2 above), and chronic inflammation that includes the events during the chronic cellular response and resolution or scarring (3 and 4).

In addition, a large number of more distant effects occur during inflammation. These include: the production of acute phase proteins, including complement components, by the liver; fever, caused by pyrogens acting on the hypothalamus in the brain; and systemic immunity, resulting in part from lymphocyte activation in peripheral lymphoid tissues.
9.1.2 Exudation and swelling

9.1.2.1 Fluid exudate

In acute inflammation, the pressure in postcapillary venules may overcome the osmotic pressure of plasma proteins. Therefore fluid and low molecular substances have the tendency to penetrate into the surrounding area. The vascular permeability for proteins and some smaller molecules differs from tissue to tissue. For example, the brain and thymus vessels are less permeable. The sinusoids in liver and sinuses in spleen are highly open vessels even at normal conditions.

The increased capillary permeability for plasma proteins is the key factor for the production of inflammatory exudate. In the interstitial area, high-molecular proteins may be split into smaller fragments that participate in the raising of osmotic pressure of interstitial fluid. In addition, the alteration of general matrix is observed. It becomes more fluid which helps to make easier the diffusion of exudate. On the other hand, a sudden increase of pressure in tissue is thus prevented.

There are two phases of inflammatory infiltration. The immediate temporary phase with a peak between 8 and 10 min and duration about 30 min. It is developed by the release of fluid from venules mediated by histamine. This is followed by immediate prolonged phase which is similar, only the time of duration is greater – a few days. The second delayed phase needs a few hours for its development. The damage to capillaries and venules is observed. In the fluid exudate, all components of plasma, including fibrinogen, kinins, complement, immunoglobulins etc., are present. Fibrinogen is important for clot formation and the prevention of further loss of blood. Fibrin, which is originated from fibrinogen, acts as the beginning of a scaffold on which tissues may subsequently be repaired and on which new capillaries can be constructed, a process known as angiogenesis. Although the rapid response of the coagulation pathway is essential, the extent of blood clotting must be limited so that it does not progress to undamaged vessels. In addition, the clots must ultimately be removed from the area of damage. This is controlled by fibrinolysis (fibrin breakdown) due to the enzyme plasmin.

The kinins are important mediators of inflammatory responses. For kinin generation to proceed efficiently, activated Hageman factor activates prekallikrein via a series of prekallikrein activators, resulting in the production of kallikrein. The generation of kallikrein triggers kinin production, including the formation of bradykinin, which is responsible for induction pain, increasing vascular permeability, and causing vasodilation. Kallikrein also activates the fibrinolytic pathway, leading to the removal of blood clots.

The complement cascade, as a part of the innate immune response, may be activated via the alternative and/or collectin (lectin) pathway to destroy some invading microorganisms. In addition, during activation of complement, important opsonins (C3b), chemotactic factors for neutrophils and mononuclear phagocytes (C5a), and anaphylatoxins (C5a, C3a) are formed. They all participate in inflammation during phagocytosis or immediate allergic reactions. Immunoglobulins may act as specific or nonspecific opsonins facilitating thus the process of phagocytosis, or may participate in antibody-dependent cell-mediated cytotoxicity (ADCC) by which target cells are destroyed by killer cells.

In the fluid infiltrate, all components of plasma, including administered drugs, are present. Therefore it is important to administer effective antibiotic or other chemotherapy as soon as possible in order to reach the inflammatory area in the concentration similar to that in plasma.

Exudative infiltrate contributes to the general signs of inflammation. It is responsible for edema (swelling, tumour). The increased pressure in tissue may participate in the production of pain (dolor). Actually, the pain is observed before the occurrence of greater edema, since also other factors such as the acidic pH of exudate, the accumulation of potassium ions and the presence of bradykinin, serotonin or other mediators take part in this process.

9.1.2.2 Cellular exudate

Cellular exudate is formed during the second and the third phase of inflammation – acute and chronic cellular response. During the former, neutrophils are prevalent, whereas mononuclear cells (macrophages and lymphocytes) overcome later. Cell composition of exudate differ not only depending on the phase of inflammation but also on the type of inflamed tissue and factors triggering inflammatory process. Central effector and regulatory functions in acute in-
flammation posses neutrophils. They are also dominant when a pyogenic bacterial infection or local deposition of immune complexes containing IgG are the cause of inflammation. **Mononuclear phagocytes** represent the main infiltrating cells in subacute and chronic phase of the majority of inflammatory reactions, and in the case of infection with intracellularly parasitizing microorganisms as well. **Eosinophils** and **basophils** are predominant when inflammation has been initiated by immediate allergic reactions or by parasites.

So, a number of different cell types are recruited into the area where damage has occurred, and these are responsible for inactivation and removing of the invading infectious agents, for removing the damaged tissues, for inducing the formation of new tissue, and reconstructing the damaged cell matrix, including basement membranes and connective tissue. A new blood supply to the area is also established during the repair process.

**Professional phagocytes** (neutrophils, eosinophils, monocytes and tissue macrophages) are essential performing phagocytosis, **lymphocytes** are involved in the specific immune responses, **endothelial cell** in the regulation of leukocyte emigration from the blood into inflamed tissue and **platelets** with mast cells in the production of early phase mediators.

The accumulation of leukocytes in inflamed tissue results from adhesive interactions between leukocytes and endothelial cells within the microcirculation. These adhesive interactions and the excessive filtration of fluid and protein that accompanies an inflammatory response are largely confined to one region of the microvasculature – postcapillary venules. The nature and magnitude of the leukocyte-endothelial cell adhesive interactions that take place within postcapillary venules are determined by a variety of factors, including expression of adhesion molecules on leukocytes and/or endothelial cells, products of leukocyte (superoxide and other ROI) and endothelial cell (nitric oxide) activation, and the physical forces generated by the movement of blood along the vessel wall. The contribution of different adhesion molecules to leukocyte rolling, adhesion, and emigration in venules will be discussed later.

This process is similar for granulocytes, monocytes, and lymphocytes only different chemotactic factors and cytokines may be involved in its initiation and control. The white blood cells leave the postcapillary venule by extending pseudopodia between opposing endothelial cells and pulling themselves into the subendothelial space and the adjacent interstitial compartment. This complex event, which is often termed leukocyte extravasation, emigration, or diapedesis, is dependent not only on an array of cellular processes including adhesion molecule expression and activation, but also on cytoskeletal reorganization, and alteration in membrane fluidity.

### 9.1.3 Cells participating in inflammation

#### 9.1.3.1 Mast cells and basophils

Mast cells and basophils play a central role in inflammatory and immediate allergic reactions. They are able to release potent inflammatory mediators, such as histamine, proteases, chemotactic factors, cytokines and metabolites of arachidonic acid that act on the vasculature, smooth muscle, connective tissue, mucous glands and inflammatory cells.

**Mast cells** settle in connective tissues and usually do not circulate in the blood stream.

**Basophils** are the smallest circulating granulocytes with relatively the least known function. They arise in the bone marrow, and following maturation and differentiation, are released into the blood circulation. If they are adequately stimulated they may settle in the tissues.

Both mast cells and basophils contain special **cytoplasmic granules** which store mediators of inflammation. The extracellular release of the mediators is known as **degranulation** and may be induced by:

(a) physical destruction, such as high temperature, mechanical trauma, ionising irradiation, etc.;

(b) chemical substances, such as toxins, venoms, proteases;

(c) endogenous mediators, including tissue proteases, cationic proteins derived from eosinophils and neutrophils;

(d) immune mechanisms which may be IgE-dependent or IgE-independent. The former is elicited by aggregation of IgE bound to high-affinity receptors (FcεRI) on the surface of these cells. Specific antigen (allergen) is responsible for the IgE aggregation. In the IgE-independent
way, the anafylatoxins C5a, C3a and C4a are formed during activation of complement. Then, the degranulation is triggered through C5a-receptors on the surface of mast cells and basophils.

There are two categories of inflammatory (anaphylactic) mediators in mast cells and basophils. **Preformed mediators**, stored in secretory granules and secreted upon cell activation, include a biogenic amine, typically histamine, proteoglycans, either heparin, over-sulphate chondroitin sulphates or both, and a spectrum of neutral proteases. Released histamine acts at H1, H2 and H3 receptors on cells and tissues, and is rapidly metabolized extracellularly. The proteoglycan, which imparts the metachromatic staining characteristic of mast cells when exposed to certain basic dyes such as toluidine blue, has two functions: it may package histamine and basic proteins into secretory granules, and in human mast cells it appears to regulate the stability of the protease called tryptase. Neutral proteases, which account for the vast majority of the granule protein, serve as markers of mast cells and of different types of mast cells.

**Newly generated mediators**, often absent in the resting mast cells, are typically produced during IgE-mediated activation, and consist of arachidonic acid metabolites, principally leukotriene C4 (LTC4) and prostaglandin D2 (PGD2) and cytokines. Of particular interest in humans is the production of tumour necrosis factor (TNF-α), IL-4, IL-5 and IL-6. In the cytoplasm of both mastocytes and macrophages are special organelles – lipid bodies – where metabolism of arachidonic acid occur and where their products, including leukotrienes, may be stored.

Mast cells are heterogeneous – two types of them, mucosal and connective tissue, were reported in rodent tissue back in the 1960’s on the basis of histochemical and fixation characteristics that reflect, in part, whether heparin proteoglycan was present in secretory granules. **Neutral proteases** better reflect the heterogeneity or plasticity of mast cells in vivo and in vitro, particularly in humans where histochemical heterogeneity is less apparent (Table 9.2).

In murine mast cells, five **chymases** (mouse mast cell protease – MMCP-1,-2,-3,-4 and-5), one mast cell carboxypeptidase and two **tryptases** (MMCP-6 and -7) have been reported.

In human mast cells, genes encoding two chymotryptic enzymes (chymase and cathepsin G-like protease) and one mast cell carboxypeptidase enzyme, and at least two genes encoding tryptase peptides have been detected. The gene encoding chymase resides on chromosome 14, closely linked to the gene encoding cathepsin G, an enzyme apparently expressed in mast cells and various myelomonocytic cells, and to the genes encoding granzymes, which are expressed in cytotoxic T lymphocytes and natural killer cells. Two types of mast cells have been found by immunohistochemical analyses. The **MC\textsubscript{TC}** type contains tryptase, chymase, cathepsin G like protease and mast-cell carboxypeptidase, and predominates in normal skin and intestinal submucosa, whereas the **MC\textsubscript{T}** type contain only tryptase, and predominates in normal intestinal mucosa and lung alveolar wall. Nearly equivalent concentrations of each type are found in nasal mucosa. In MC\textsubscript{TC} cells, tryptase, chymase and mast-cell carboxypeptidase reside in macromolecular complexes with proteoglycan, but interestingly, tryptase reside in a separate complex from that in which chymase and mast-cell carboxypeptidase are found.

The biological function of mast cell neutral proteases, like mast cells themselves, remain to be fully clarified. In serum, elevated levels of tryptase are detected in systemic mast-cell disorders, such as anaphylaxis and mastocytosis. Ongoing mast-cell activation in asthma appear to be a characteristic of this chronic inflammatory disease. It is detected by elevated levels of tryptase and PGD\textsubscript{2} in bronchoalveolar lavage fluid, higher spontaneous release of histamine by mast cell obtained from the bronchoalveolar lavage fluid, than non asthmatics, and ultrastructural analysis of mast cell in pulmonary tissue.

The number of basophils and mast cells increase at sites of inflammation. To reach these areas, basophils must migrate from the blood into tissue sites. A crucial step in this process is the adherence of cells to the endothelium. **Cell adherence** is mediated by several families of adhesion molecules and adhesion receptors in the surface of basophils and mast cells that can mediate binding to other cell and to the extracellular matrix (ECM) glycoproteins. Upon stimulation, basophils and mast cells release cytokines, including TNF-α and IL-4, that can modulate adhesion molecules on endothelial cells. Activated endothelial cells express the intercellular adhe-
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<table>
<thead>
<tr>
<th>Mast cell type</th>
<th>Biogenic amine</th>
<th>Neutral protease</th>
<th>Proteoglycan</th>
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</thead>
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<tr>
<td><strong>Mouse:</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Mucosal</td>
<td>H</td>
<td>MMCP-1,-2</td>
<td>Chondroitin sulphate E</td>
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<td>Connective tissue</td>
<td>H + 5-HT</td>
<td>MMCP-3,-4,-5,-6, Carboxypeptidase</td>
<td>Heparin</td>
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<tr>
<td><strong>Human:</strong></td>
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<tr>
<td>MC&lt;sub&gt;T&lt;/sub&gt;cells</td>
<td>H</td>
<td>Tryptase</td>
<td>Heparin, chondroitin sulphate</td>
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<td>Tryptase, chymase, cathepsin G-like protease, carboxypeptidase</td>
<td>Heparin, chondroitin sulphate</td>
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H: histamine; 5-HT: serotonin; MMCP: mouse mast cell protease

### Table 9.2: Predominant granule mediators of mast cells

Adhesion molecule (ICAM-1), endothelial-leukocyte adhesion molecule (ELAM-1) and vascular cell adhesion molecule (VCAM-1) on their cell surface. Human basophils express integrins as receptors for these molecules.

Until recently, the effects of adherence on cell function were believed to result only from changes in cell shape and cytoskeletal organization. However, in addition to cell spreading, aggregated adhesion receptors transduce a variety of intracellular signals that regulate cell function. These signals include protein tyrosine phosphorylation, phosphoinositide hydrolysis, changes in intracellular pH or calcium concentration and the expression of several genes. The adhesion properties of basophils and mast cells regulate their migration, localization, proliferation and phenotype.

Different mechanisms could contribute to the increase in the number of mast cells at sites of tissue injury: mast cells or their progenitors could migrate to these sites; or resident mast-cell precursors could proliferate. Adhesion receptors and their ligands also play a role in the localization and migration of mast cells in normal tissues. ECM proteins that are the ligands for adhesion receptors are chemotactics for mast cells. Adherence of mast cells to fibroblasts, other cells or to ECM proteins can transduce signals that affect cell growth and differentiation.

The increase in the number of mast cells and basophils, and the enhanced secretion at sites of inflammation, can accelerate the elimination of the cause of tissue injury or, paradoxically, may lead to a chronic inflammatory response. Thus, manipulating mast-cell and basophil adhesion may be an important strategy for controlling the outcome of allergic and inflammatory responses.

#### 9.1.3.2 Eosinophils

The eosinophil is a terminally differentiated, end-stage leukocyte that resides predominantly in submucosal tissue and is recruited to sites of specific immune reactions, including allergic diseases. The mean generation time for eosinophils in the bone marrow is approximately 2-6 days. They mainly settle in the tissue where their number is about one hundred times higher than in the blood. Like other granulocytes, they possess a polymorphous nucleus, although with only two lobes and no nucleolus. The eosinophil cytoplasm contains large ellipsoid granules with an electron-dense crystalline nucleus and par-
tially permeable matrix. In addition to these large primary crystalloid granules, there is another granule type that is smaller and lacks the crystalline nucleus.

These large specific granules are the principal indentifying feature of eosinophils. They contain four distinct cationic proteins which exert a range of biological effects on host cells and microbial targets: major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN), and eosinophil peroxidase (EPO). Basophils contain about one fourth as much MBP as did eosinophils and detectable amounts of EDN, ECP and EPO. Small amounts of EDN and ECP were also found in neutrophils.

These proteins have major effects not only on the potential role of eosinophils in host defence against helminthic parasites, but also in contributing to tissue dysfunction and damage in eosinophil related inflammatory and allergic diseases. As MBP lack enzymatic activity, one mechanism whereby this highly cationic polypeptide may exert its toxic activities is by interactions with lipid membranes leading to their derangement. Both MBP and EPO have been shown to act as selective allosteric inhibitors of agonist binding to M2 muscarinic receptors. Thus, these proteins may contribute to M2 receptor dysfunction and enhance vagally mediated bronchoconstriction in asthma. EDN specifically damage the myelin coat of neurons.

In addition, histaminase and a variety of hydrolytic lysosomal enzymes are also present in the large specific granules.

Among the typical small granule enzymes are aryl sulphatase, acid phosphatase and a 92 kDa metalloproteinase, a gelatinase.

Only recently has it been recognized that eosinophils are capable of elaborating cytokines which include those with potential autocrine growth-factor activities for eosinophils and those with potential roles in acute and chronic inflammatory responses. Three cytokines have growth-factor activities for eosinophils: granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3 and IL-5. Other cytokines produced by human eosinophils that may have activities in acute and chronic inflammatory responses include IL-1α, IL-6, IL-8, TNF-α and both transforming growth factors, TGF-α and TGF-β.

Eosinophils also participate in hypersensitivity reactions, especially through two lipid inflammatory mediators, leukotriene C4 (LTC4) and platelet activating factor (PAF). Both mediators contract airway smooth muscle, promote the secretion of mucus, alter vascular permeability and elicit eosinophil and neutrophil infiltration. In addition to the direct activities of these eosinophil-derived mediators, MBP by a non-cytotoxic mechanism can stimulate the release of histamine from basophils and mast cells, and EPO from mast cells. Thus, once stimulated, eosinophils can serve as a local source of specific lipid mediators as well as induce the release of mediators from mast cells and basophils.

The processes that lead to the accumulation of eosinophils within tissue sites of specific inflammation, as for other leukocytes, involve numerous sequential interactions that enable eosinophils to adhere to and then transmigrate through the endothelium and to respond to local chemotactants. The adhesion of eosinophils to endothelium include CD18-dependent pathways, interaction between E-selectin and P-selectin and adherence to VCAM by means of very late antigen 4 (VLA-4) expressed on the eosinophil.

The eosinophil granule content is released following similar stimuli to neutrophil granules (e.g. during phagocytosis of opsonized particles and by chemotactic factors). However, whereas the neutrophil lysosomal enzymes act primarily on material engulfed in phagolysosomes, the eosinophil granule content act mainly on extracellular target structure such as parasites and inflammatory mediators.

The eosinophil functional activity, like the immune response in general, may be beneficial or harmful for the organism. Compared to neutrophils, eosinophils have limited phagocytic activity which is mainly aimed at killing multicellular parasites. Another beneficial activity is the inactivation of mediators of anaphylaxis. Thus, for example, acyl-sulphatase B may inactivate the slow-reacting substance of anaphylaxis (SRS-A, a mixture of LTC4, LTD4 and LTE4), phospholipase D destroys the platelet lytic factor, histaminase degrades histamine and lysophospholipase (phospholipase B) may inactivate the membrane-active lysophosphatides.

In addition to the acute release of protein, cytokine and lipid mediators of inflammation, eosinophils likely contribute to chronic inflammation, including the development of fibrosis. Eosinophils are the major source of the fibrosis-promoting cytokine...
Chapter 9. Inflammation and fever (I. Hulín, M. Ferenčík, V. Štvrtnová, J. Jakubovský)

TGF-β in nodular sclerosing Hodgkin’s disease. Additional roles for the eosinophil in modulating extracellular matrix deposition and remodeling are suggested by studies of normal wound healing. During dermal wound healing eosinophils infiltrate into the wound sites and sequentially express TGF-α early, and TGF-β later, during wound healing.

9.1.3.3 Neutrophils, central cells in acute inflammation

Neutrophils, which are also known as polymorphonuclear leukocytes (PMN), represent 50 to 60% of the total circulating leukocytes and constitute the "first line of defence" against infectious agents or "non-self" substances that penetrate the body’s physical barriers. Once an inflammatory response is initiated, neutrophils are the first cells to be recruited to sites of infection or injury. Their targets include bacteria, fungi, protozoa, viruses, virally infected cells and tumour cells. Their development in the bone marrow takes about two weeks; during this period, they undergo proliferation and differentiation. During maturation, they pass through six morphological stages: myeloblast, promyeloblast, myelocyte, metamyelocyte, non-segmented (band) neutrophil, segmented neutrophil. The segmented neutrophil is a fully functional active cell. It contains cytoplasmic granules (primary or azurophil and secondary or specific) and a lobulated chromatin-dense nucleus with no nucleolus. The bone marrow of a normal healthy adult produces more than 10¹¹ neutrophils per day and more than 10¹² per day in settings of acute inflammation. Upon release from the bone marrow to the circulation the cells are in a nonactivated state and have a half-life of only 4 to 10 h before marginating and entering tissue pools, where they survive for 1 to 2 days. Cells of the circulating and marginated pools can exchange with each other. Senescent neutrophils are thought undergo apoptosis (programmed cell death) prior to removal by macrophages. The viability is significantly shorter in individuals suffering from infectious or acute inflammatory diseases when the tissue requirement for newly recruited neutrophils increases considerably.

Subpopulations of neutrophils have been identified by various criteria. These cells exist not only in dormant (resting) or activated states but also in various intermediate stages. For, example, priming is a mechanism whereby dormant neutrophils acquire a state of preactivation that enable a more powerful response to be generated once microbial activity is initiated.

9.1.3.3.1 Neutrophil granules The neutrophil granules are of major importance for neutrophils function. When referring to phagocytes or leukocytes in general, the term granule is used more often than lysosome. The terms are not fully equivalent; the term granules was originally derived from morphological observations whereas the term lysosomes is based on functional and biochemical characteristics of these cell organelles. Not all organelles that look like granules are necessarily typical lysosomes. The granules of neutrophils are generated during cell differentiation; they are produced for storage rather than continuously. On the basis of function and enzyme content, human neutrophil granules can be divided into three main types - azurophil, specific and small storage granules. Their function is not just to provide enzymes for hydrolytic substrate degradation - as in classical lysosomes – but also to kill ingested bacteria and, finally, to secrete their contents to regulate various physiological and pathological processes, including inflammation. Individual granule populations can be characterized morphologically (e.g. azurophil granules are larger and contain more electron-dense material than specific granules), or biochemically using enzyme markers or other substances (Table 9.3).

Neutrophil granules contain antimicrobial or cytotoxic substances, neutral proteinases, acid hydrolases and a pool of cytoplasmic membrane receptors. Among azurophil granule constituents myeloperoxidase (MPO) is a critical enzyme in the conversion of hydrogen peroxide to hypochlorous acid. Together with hydrogen peroxide and a halide cofactor it forms the most effective microbialicid and cytotoxic mechanism of leukocytes - the myeloperoxidase system. MPO is responsible for the characteristic green color of pus.

Defensins, which constitute 30 to 50% of azurophilic granule protein, are small (molecule weight < 4000) potent antimicrobial peptides that are cytotoxic to a broad range of bacteria, fungi and some viruses. Their toxicity may be due to membrane permeabilization of the target cell which is similar to other channel-forming proteins (perforin).

Bacterial permeability-increasing (BPI) protein is
### Table 9.3: Enzymes and other constituents of human neutrophil granules

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Granules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Azurophil</td>
</tr>
<tr>
<td><strong>Antimicrobial</strong></td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td></td>
<td>Lysozyme</td>
</tr>
<tr>
<td></td>
<td>Defensins</td>
</tr>
<tr>
<td></td>
<td>BPI</td>
</tr>
<tr>
<td><strong>Neutral proteinases</strong></td>
<td>Elastase</td>
</tr>
<tr>
<td></td>
<td>Cathepsin G</td>
</tr>
<tr>
<td></td>
<td>Proteinase 3</td>
</tr>
<tr>
<td><strong>Acid hydrolases</strong></td>
<td>Cathepsin B</td>
</tr>
<tr>
<td></td>
<td>Cathepsin D</td>
</tr>
<tr>
<td></td>
<td>β-D-Glucuronidase</td>
</tr>
<tr>
<td></td>
<td>α-Mannosidase</td>
</tr>
<tr>
<td></td>
<td>Phospholipase A₂</td>
</tr>
<tr>
<td><strong>Cytoplasmic membrane receptors</strong></td>
<td>CR3, CR4</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>Chondroitin -4-sulphate</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

BPI: bactericidal permeability-increasing protein  
FMLP: N-formylmethionyl-leucyl-phenylalanine  

Serine proteases such as elastase and cathepsin G hydrolyze proteins in bacterial cell envelopes. Substrates of granulocyte elastase include collagen cross-linkages and proteoglycans, as well as elastin components of blood vessels, ligaments, and cartilage. Cathepsin D cleaves cartilage proteoglycans, whereas granulocyte collagenases are active in cleaving type I and, to a lesser degree, type III collagen from bone, cartilage, and tendon. Collagen breakdown prod-

Also a member of perforins. It is highly toxic to gram-negative bacteria but not to gram-positive bacteria or fungi and can also neutralize endotoxin, the toxic lipopolysaccharide component of gram-negative bacterial cell envelope.

Lactoferrin sequesters free iron, thereby preventing the growth of ingested microorganisms that survive the killing process and increases bacterial permeability to lysozyme.
ucts have chemotactic activity for neutrophils, monocytes, and fibroblasts.

Regulation of tissue destructive potential of lysosomal proteases is mediated by protease inhibitors such as α2-macroglobulin and α1-antiprotease. These antiproteases are present in serum and synovial fluids. They are thought to function by binding to and covering the active sites of proteases. Protease-antiprotease imbalance is probably important in the pathogenesis of emphysema.

Azurophil granules function predominantly in the intracellular milieu (in the phagolysosomal vacuole), where they are involved in the killing and degradation of microorganisms. On the other hand, neutrophil specific granules are particularly susceptible to release their contents extracellularly and appear to have an important role in initiating inflammation. Specific granules represent an intracellular reservoir of various plasma membrane components including cytochrome b558 (component of NADPH oxidase, enzyme responsible for the production of superoxide), receptors for complement fragment iC3b (CR3, CR4) for laminin, and formylmethionyl-peptide chemotactants. In addition, there is also histaminase capable for the degradation of histamine, vitamin B12 binding protein, plasminogen activator (responsible for plasin formation and cleavage of C5a from C5) and others.

The importance of neutrophil granules in inflammation is apparent from studies of several patient with congenital abnormalities of the granules. Patients with Chédiak-Higashi syndrome have a profound abnormality in the rate of establishment of an inflammatory response and have abnormally large lysosomal granules. The congenital syndrome of specific granule deficiency is an exceedingly rare disorder characterized by diminished inflammatory responses and severe bacterial infections of skin and deep tissues.

9.1.3.3.2 Neutrophils in host defence The major role of neutrophils is to phagocyte and destroy infectious agents but they also limit the growth of some microbes, thereby buying time for adaptive (specific) immunological responses. With many microbes, however, neutrophil defences are ineffective in the absence of opsonins and various agents that amplify the cytotoxic response.

Opsonization is a process, in which opsonins adsorb to the surface of bacteria or other particles and facilitate their adherence to the phagocyte cytoplasmic membrane through opsonin receptors. Specific binding between the particle and phagocyte which occurs during immune phagocytosis is mediated by immunoadherent receptors. There are two types of immunoadherent receptors: Fc-receptors mainly for IgG antibodies (FcR) and complement receptors (CR1, CR3). It means that function of opsonins in the first case is realized by antibodies and in the second case by iC3b. Specific binding between the particle and phagocyte may be also performed by lectins and lectin receptors (lectinophagocytosis).

To the phagocytosis itself chemotaxis of phagocytes precede into the site where phagocytosable material occurs. This is regulated by chemotactic factors generated by infectious agents themselves, as well as those release as a result of their initial contact with phagocytes and other components of the immune system.

Phagocytosis is a complex process composed of several morphological and biochemical steps. After recognition and particle binding to the phagocyte surface, ingestion (engulfment), phagosome origination, phagolysosome formation (fusion of phagosome with lysosomes), killing and degradation of ingested cells or other material proceed. Simultaneously with the recognition and particle binding a dramatic increase in oxygen consumption (the respiratory burst) is observed. It is responsible for the production of superoxide and other oxygen radicals, and also for the secretion of a variety of enzymes and biologically active substances controlling inflammatory and cytotoxic reactions.

During phagocytosis, cytosolic granules (lysosomes) fuse with the invaginating plasma membrane (around the engulfing microorganism) to form a phagolysosome into which they release their contents, thereby creating a highly toxic microenvironment. This step is of the first importance because during it two categories of cytotoxic substances, present in the preformed state in azurophil and specific granules and synthesized de novo during the respiratory burst, arrive at the same cell compartment. This degranulation normally prevents release of the toxic components into the extracellular milieu. However, some target may be too large to be fully phagocytosed or they avoid engulfment, resulting in
frustrated phagocytosis in which no phagosome is formed. These may be killed extracellularly. However, tissue damage occurs when neutrophil microbicidal products are released extracellularly to such an extent that host defences (antioxidant and antiprotease screens) in the immediate vicinity are overwhelmed.

The importance of neutrophils in fighting bacterial and fungal infections is well recognized. Recently, it has been shown that neutrophils are in abundance also in virally induced lesions. Neutrophils bind to opsonized viruses and virally infected cells via antibody (Fc) and complement (iC3b) receptors. Viruses such as influenza can be inactivated by neutrophils through damage to viral proteins (e.g. hemagglutinin and neuraminidase) mediated by the myeloperoxidase released during degranulation. In contrast to these acute diseases, chronic influenza infections can diminish or exhaust the microbicidal potency of neutrophils.

9.1.3.3.3 Neutrophils and host tissue damage

Although neutrophils are essential to host defence, they have also been implicated in the pathology of many chronic inflammatory conditions and ischemia-reperfusion injury. Hydrolytic enzymes of neutrophil origin and oxidatively inactivated protease inhibitors can be detected in fluid isolated from inflammatory sites. Under normal conditions, neutrophils can migrate to sites of infection without damage host tissues. This damage may occur through several independent mechanisms. These include premature activation during migration, extracellular release of toxic products during the killing of some microbes, removal of infected or damaged host cells and debris as a first step in tissue remodeling, or failure to terminate acute inflammatory responses.

Ischemia-reperfusion injury is associated with an influx of neutrophils into the affected tissue and subsequent activation. This may be triggered by substances released from damaged host cells or as a consequence of superoxide generation through xantine oxidase.

Under normal conditions, blood may contain a mixture of normal, primed, activated and spent neutrophils. In the inflammatory site, mainly activated and spent neutrophils are present. Activated neutrophils have enhanced production of reactive oxygen intermediates (ROI). A subpopulation of neutrophils with the enhanced respiratory burst has been detected in the blood of people with an acute bacterial infection and patients with the adult respiratory distress syndrome (ARDS). This is a good example of the neutrophil paradox. Neutrophils have been implicated in the pathology of this condition because of the large influx of these cells into the lung and the associated tissue damage caused by oxidants and hydrolytic enzymes released from activated neutrophils. The impairment of neutrophil microbicidal activity that occurs as the ARDS worsens may be a protective response on the part of the host, which is induced locally by inflammatory products. This "down-regulation" of neutrophil function may explain why many of these patients eventually die from overwhelming pulmonary infections.

The acute phase of thermal injury is also associated with neutrophil activation, and this is followed by a general impairment in various neutrophil functions. Activation of neutrophils by immune complexes in synovial fluid contributes to the pathology of rheumatoid arthritis. Chronic activation of neutrophils may also initiate tumour development because some ROI generated by neutrophils damage DNA and proteases promote tumour cell migration.

In patient suffering from severe burns, a strong correlation has been established between the onset of bacteremic infection and reduction in the proportion and absolute numbers of neutrophils positive for antibody and complement receptors.

Oxidants of neutrophil origin have also been shown to oxidize low-density lipoproteins (LDL) which are then more effectively bound to the plasma membrane of macrophages through specific scavenger receptors. Uptake of these oxidized LDL by macrophages is thought to initiate atherosclerosis.

In addition, primed neutrophils have been found in people with essential hypertension, Hodgkin’s disease, inflammatory bowel disease, psoriasis, sarcoidosis, and septicaemia, where priming correlates with high concentrations of circulating TNF-α (cachectin).

Hydrolytic damage to host tissue and therefore chronic inflammatory conditions may occur only when antioxidant and antiprotease screens are overwhelmed. Antiprotease deficiency is thought to be responsible for the pathology of emphysema. Many antiproteases are members of the serine protease inhibitor (SERPIN) family. Although the circulation is
rich in antiproteases, these large proteins may be selectively excluded at sites of inflammation because neutrophils adhere highly to their targets. Oxidative stress may initiate tissue damage by reducing the concentration of extracellular antiproteases to below the level required to inhibit released proteases. Chlorinated oxidants and $H_2O_2$ can inactivate antiproteases such as α1-protease inhibitor and α2-macroglobulin (which are endogenous inhibitors of elastase) but, surprisingly, simultaneously activate latent metalloproteases such as collagenases and gelatinase, which contribute to the further activation of antiproteases.

Cytoplasmic constituents of neutrophils may also be a cause of formation of specific anti-neutrophil cytoplasmic antibodies (ANCA) which are closely related to the development of systemic vasculitis and glomerulonephritis. ANCA are antibodies directed against enzymes that are found mainly within the azurophil or primary granules of neutrophils. There are three types of ANCA that can be distinguished by the patterns they produce by indirect immunofluorescence when tested on normal ethanol-fixed neutrophils. Diffuse fine granular cytoplasmic fluorescence (cANCA) is typically found in Wegener’s granulomatosis, in some cases of microscopic polyarteritis and Churg Strauss syndrome, and in some cases of crescentic and segmental necrotising glomerulonephritis, but is rare in other conditions. The target antigen is usually proteinase 3. Perinuclear fluorescence (pANCA) is found in many cases of microscopic polyarteritis and glomerulonephritis. These antibodies are often directed against myeloperoxidase but other targets include elastase, cathepsin G, lactoferrin, lysozyme and β-D-glucuronidase. The third group designated "atypical" ANCA includes neutrophil nuclear fluorescence and some unusual cytoplasmic patterns and while a few of the target antigens are shared with pANCA, the others have not been identified yet.

pANCA are also found in a third of patients with Crohn’s disease. The reported incidence of ANCA in rheumatoid arthritis and SLE varies considerably but the patterns are predominantly pANCA and atypical ANCA.

9.1.3.3.4 Free radicals produced by neutrophils Two types of free radicals are produced by neutrophils, macrophages, endothelial and other cells. The first type is represented by reactive oxygen intermediates which are formed in neutrophils by the activity of NADPH oxidase, the enzyme of the respiratory burst. The second type includes reactive nitrogen intermediates, the first member of them, nitric oxide being produced by nitric oxide synthase.

**Reactive oxygen intermediates (ROI)**

Upon activation neutrophils and mononuclear phagocytes have increased oxygen consumption, a process known as the respiratory burst. During this, oxygen is univalently reduced by NADPH oxidase to superoxide anion or its protonated form, perhydroxyl radical, which then is catalytically converted by action of superoxide dismutase to hydrogen peroxide:

$$O_2 + e^- + H^+ \rightarrow HO_2 = ^\cdot O_2^- + H^+$$

$$^\cdot O_2^- + ^\cdot O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

**NADPH oxidase** is an electron transport chain found in the wall of the endocytic vacuole of professional phagocytes and in B and T lymphocytes. It is so called because NADPH is used as an electron donor to reduce oxygen to superoxide and hydrogen peroxide. NADPH oxidase is a complex enzyme composed at least of five members. Two of them are p21phox and gp91phox subunits of a very unusual flavocytochrome b558 in the cytoplasmic membrane. Two cytosolic proteins (p47phox, p67phox), a RIM, and a Rac-related GTP-binding protein are thought to be the other functional components of this electron transport system. (phox means "phagocyte oxidase", p – protein, and gp – glycoprotein). The NADPH oxidase system is dissociated and thus inactive in dormant neutrophils. While some components are membrane bound, others are stored in the cytosol. Upon activation, the cytosolic components translocate to the plasma membrane to assemble the active oxidase. The absence of, or an abnormality in, any one of these components result in chronic granulomatous disease (CGD) characterized by the absence of respiratory burst from neutrophils and monocytes of these patients. The children suffer from repeated infections that respond poorly to conventional therapy and almost invariably lead to early death.

**Superoxide anion** ($^\cdot O_2^-)$ is both a one-electron reductant and a one-electron oxidant that can pass through cell membrane via anion channels. It appears
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that superoxide does not have direct toxic effects on targets but, rather exerts its toxicity by penetration to important sites where it subsequently is converted to other ROI. Hydrogen peroxide (H₂O₂), hydroxyl radical (·OH) and singlet oxygen are of the first importance of them.

**Hydrogen peroxide** interacts with myeloperoxidase (MPO), contained in neutrophil azurophil granules to produce hypochlorous acid, which is metabolized to hypochlorite (bleach) and chlorine:

\[
H_2O_2 + Cl^- + H^+ \rightarrow H_2O + HOCl
\]

\[
HOCl \rightleftharpoons H^+ + OCl^-
\]

\[
HOCl + Cl^- \rightarrow Cl_2 + OH^-
\]

**Hydroxyl radical** (·OH) is formed by several ways from which decomposition of H₂O₂ catalyzed by Fe²⁺ is the most important:

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH
\]

This reaction is supposed to be involved, for instance, in asbestosis because asbestos contains high concentrations of iron. The toxicity of ·OH is believed to result from the ability of ·OH to serve as a powerful one-electron oxidant capable of abstracting electrons from a large variety of compounds with the formation of a new radical, which can oxidize other substances:

\[
\cdot OH + R \rightarrow OH^- + R'
\]

Hydroxyl radical and hypochlorite are the most powerful substances involved in microbial and cytotoxic reactions. HOCl is 100 to 1000 times more effective than H₂O₂. Furthermore, HOCl-induced cell death occurs very rapidly in comparison to that mediated by H₂O₂.

**Singlet oxygen** (¹O₂) is an oxygen form whose electrons are excited at a higher energy level compared to the normal (ground) triplet oxygen. When returning to the ground state they emit light (chemiluminescence) which may have antimicrobial and cytotoxic effects.

These oxidants also promote the margination of neutrophils by triggering the expression of adhesion molecules on endothelial cells.

ROI are involved in a variety of pathological conditions. For example pulmonary diseases in which oxygen radicals are thought to be involved include ARDS, hyperoxia, asbestosis, silicosis, paraquat toxicity, bleomycin toxicity, cigarette smoking, ionizing radiation and others.

ROI are highly toxic also for producing cells. Therefore neutrophils have to contain large reserves of endogenous antioxidants such as glutathione and ascorbate. Their ability to maintain these antioxidants in the reduced state during phagocytosis may prevent death from oxidative suicide.

**Reactive nitrogen intermediates** (RNI)

They are sometimes also called reactive oxynitrogen intermediates (RONI). The pathway by which they are originated is an oxidative process in which short-lived nitric oxide (NO’ ) is derived from the guanidino nitrogen in the conversion of L-arginine to L-citrulline. This reaction is catalysed by NO’ synthase and, like the respiratory burst, it involves oxygen uptake.

Three distinct isofrom of nitric oxide synthase (NOS) representing three distinct gene products have been isolated and purified. The three isoforms vary considerably in subcellular location, structure, kinetics, regulation, and hence functional roles (Table 9.4).

Two of the enzymes are constantly present and termed constitutive NOS (cNOS). The endothelial cNOS is mostly membrane bound and formed only in endothelial cells. The neuronal cNOS was identified in the cytosol of central and peripheral neurons. NO’ derived from the cNOS isof orm act as a physiologic regulator by relaxing vascular smooth muscle or by functioning as a neurotransmitter. These isoforms produce small amounts of NO’ for short periods in a calcium/calmodulin dependent manner upon stimulation. Endothelial cNOS with the endothelial cell acting as a signal transducer, releases NO’ continuously in varying amounts to regulate blood vessel tone and thus also the blood flow and pressure. Large amounts of NO’ produced in a prolonged time may cause vasodilation and hypotension, whereas insufficient NO’ formation may be involved in hypertension. It seems that NO’ plays a fundamental role in the regulation of the cardiovascular system. The organic nitrates used as vasodilatation drugs for many years spontaneously release or are biotransformed to the active form which is NO’ Within the CNS, NO’
Table 9.4: Isoforms of human NO\textsuperscript{·} synthase and their characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Endothelial cNOS</th>
<th>Neuronal cNOS</th>
<th>Inducible NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depency on</td>
<td>(\text{Ca}^{2+}), calmodulin</td>
<td>(\text{Ca}^{2+}), calmodulin</td>
<td>Independent</td>
</tr>
<tr>
<td>Molecular weight x10\textsuperscript{3}</td>
<td>150–160</td>
<td>150–160</td>
<td>132</td>
</tr>
<tr>
<td>Chromosomal location</td>
<td>7q35–36</td>
<td>12q24.2</td>
<td>17q11–12</td>
</tr>
<tr>
<td>Producing cells</td>
<td>Endothelial</td>
<td>Neurons</td>
<td>Macrophages, monocytes, Kupffer’s cells neutrophils, hepatocytes, myocytes, chondrocytes, smooth muscle cells</td>
</tr>
<tr>
<td>Inductors of biosynthesis</td>
<td>No</td>
<td>No</td>
<td>LPS, TNF–(\alpha), IL–1, IFN–(\gamma), GM–CSF</td>
</tr>
<tr>
<td>Inhibition by L–arginine analogs</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Inhibition by glucocorticoids</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The third isoform of NOS is not present in resting cells but instead the cells must be induced to express the enzyme, thus the name **inducible NOS** (iNOS). Stimuli typically include cytokines and/or lipopolysaccharide (LPS), and once expressed the enzyme generates large amounts of NO. A number of cytokines is involved in the production of iNOS. Among them IFN–\(\gamma\), IL-1, IL-6, THF–\(\alpha\), GM-CSF (granulocyte-macrophage colony stimulatory factor) and PAF (platelet activating factor) exert the stimulatory effect whereas the suppression has been observed in the case of IL-4, IL-8, IL-10, TGF–\(\beta\) (transforming growth factor), PDGF (platelet-derived growth factor) and MDF (macrophage deactivating factor).

NO\textsuperscript{·} may react with superoxide to form highly toxic peroxynitrite anion:

\[
\text{NO}^\cdot + \cdot \text{O}_2^- \rightarrow \text{ONOO}^- 
\]

which may be transformed in an acid milieu to peroxynitrite acid and then to hydroxyl radical:

\[
\text{ONOO}^- + \text{H}^+ \rightarrow \text{ONO}_2 \text{H} \quad \text{ONO}_2 \text{H} \rightarrow \cdot \text{OH} + \text{NO}_2^- \rightarrow \text{NO}_3^- + \text{H}^+ 
\]

Independent pathways are involved in the synthesis of ROI and RNI. Dormant neutrophils produced
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NO\(^{-}\) continuously but activation arrest this pathway in favor of the oxidative burst. Thus, although the ROI and RNI pathways are independent, they may compete for common substrates such as NADPH and \(O_2\) and exert other modulating effects on each other. The steady-state production of these species may dictate the anti/proinflammatory balance. Microbial killing appears to ROI dependent in normal neutrophils but RNI may play a role in cells with deficiencies in the NADPH oxidase/MPO pathways. Nitric oxide may also contribute to the microbicidal activity of neutrophils by reacting with ROI to form secondary cytotoxic species such as peroxynitrite.

The main role of neutrophil-derived NO\(^{-}\) may be to facilitate the migration of neutrophils from blood vessels to surrounding tissue by causing vasodilatation. NO\(^{-}\) facilitates relaxation of vascular smooth muscle, and ROI initiate vasoconstriction through the production of superoxide, which removes NO. In addition NO\(^{-}\) inhibits neutrophil adhesion to vascular endothelium and this may prevent inflammatory and ischemia-reperfusion injuries.

The basis of the functional activity of NO\(^{-}\) is its dual actions on some enzymes of target cells. The small amount of NO\(^{-}\) released by cNOS isoforms is adequate to activate the known NO-sensitive enzymes (guanylate cyclase and ADP-ribosyl-transferase) and participate in NO signaling pathways. The larger amounts of NO\(^{-}\) generated by iNOS may also activate the NO-sensitive enzymes, but in many cell types the high output of NO\(^{-}\) also exceed the necessary concentration threshold to inhibit the action of certain iron-containing enzymes, namely aconitase, NADPH-ubiquinone oxidoreductase, succinate-ubiquinone oxidoreductase, ribonucleotide reductase, NADPH oxidase and glyceraldehyde-3-phosphate dehydrogenase.

Activation of soluble guanylate cyclase by NO\(^{-}\) leads to the synthesis of cGMP, which leads to relaxation of vascular smooth muscle cells, inhibition of platelet adherence, aggregation, inhibition of neutrophil chemotaxis, and signal transduction in the central and peripheral nervous system.

NO\(^{-}\) causes autoribosylation of glyceraldehyde-3-phosphate dehydrogenase, which inactivates this glycolytic enzyme. NO\(^{-}\) also inhibits three mitochondrial enzymes: aconitase of the Krebs cycle and NADPH ubiquinone oxidoreductase and succinate-ubiquinone oxidoreductase of the electron transport chain.

Induced NO\(^{-}\) synthesis was reported in inflammatory responses initiated by microbial products or autoimmune reactions and also in the systemic inflammatory response, also referred to as sepsis. NO\(^{-}\) likely participates in the inflammatory reaction and subsequent joint destruction in some types of arthritis. For instance synovial fluid from patients with osteoarthritis exhibits elevated nitrate concentrations (nitrate are end products of the L-arginine-NO synthase pathway). There is also evidence for chronic expression of iNOS in the smooth muscle in atherosclerotic aortic aneurysms, a disease in which there is progressive dilatation and destruction of the aortic wall leading often to fatal rupture.

9.1.3.3.5 Regulation of neutrophil function

Under normal conditions, neutrophils roll along microvascular walls via low affinity interaction of selectins with specific endothelial carbohydrate ligands. During the inflammatory response, chemotactic factors of different origin and proinflammatory cytokines signal the recruitment of neutrophils to sites of infection and/or injury. This leads to the activation of neutrophil \(\beta_2\)-integrins and subsequent high-affinity binding to intercellular adhesion molecules on the surface of activated endothelial cells in postcapillary venules. Under the influence of a chemotactic gradient, generated locally and by diffusion of chemoattractants from the infection site, neutrophil penetrate the endothelial layer and migrate through connective tissue to sites of infection (diapedesis), where they finally congregate and adhere to extra-cellular matrix components such as laminin and fibronectin. A wide variety of adhesion molecules have been characterized on the surface of phagocytic cells and will be shown later.

Cytokines are basic regulators of all neutrophil functions. Many of them including hematopoietic growth factors and pyrogens have shown to be potent neutrophil priming agents. Neutrophils also synthesize and secrete small amounts of some cytokines including IL-1, IL-6, IL-8, TNF-\(\alpha\), and GM-CSF; they may act in an autocrine or paracrine manner. The pyrogenic cytokines, IL-1, TNF-\(\alpha\), and IL-6 all prime various pathways that contribute to the activation of NADPH oxidase. Pro-inflammatory cytokine IL-8, which is also known as neutrophil-activating factor, is also a potent chemoattractant; it synergizes with IFN-\(\gamma\), TNF-\(\alpha\), GM-CSF, and G-CSF to amplify various neutrophil cytotoxic functions. Cytokines
also increase the microbiostatic and killing capacities of neutrophils against bacteria, protozoa and fungi. IFN-γ and GM-CSF independently amplify neutrophil antibody-dependent cytotoxicity. Anti-inflammatory cytokines, IL-4 and IL-10 inhibit the production of IL-8 and the release of TNF-α and IL-1 which reflects in the blockade of neutrophil activation.

Furthermore, some cytokines prolong neutrophil survival. The acute inflammatory response may be terminated by the secretion of macrophage inflammatory protein-1α (MIP-1α) from neutrophils; this protein may signal mononuclear cell recruitment and clear neutrophils from the affected tissue site. All these cytokines are produced by neutrophils themselves and/or by lymphocytes, monocytes/macrophages or endothelial cells.

Along to cytokines other mediators, including bioactive lipids, neuroendocrine hormones, histamine, and adenosine, are also involved in the regulation of neutrophil activation.

Bioactive lipids originate mainly from arachidonic acid which is an abundant constituent of neutrophil membranes. Arachidonic acid is metabolized to prostaglandins, leukotrienes and lipoxins. LTB₄ is a strong neutrophil chemoattractant that may play a role in the priming process. Vasoactive leukotrienes LTC₄, LTD₄ and LTE₄ increase microvascular permeability and may contribute to ischemia-reperfusion injury. In contrast to leukotrienes, prostaglandins suppress most neutrophil functions, possibly through their ability to elevate intracellular cAMP. Lipoxins LXA₄ and LXB₄ are potent inhibitors of neutrophil microbiocidal activity.

In many inflammatory conditions, the level of platelet-activating factor (PAF) rise in the affected tissues, but injury can be attenuated by PAF antagonists. PAF directly primes superoxide generation and elastase release.

The major "stress hormones" are involved in the regulation of inflammation at both the systemic and, perhaps, local levels. The bidirectional interactions of cytokines and neurotransmitters with nervous and immune cells, respectively, provide a means of indirect chemical communication between the neuroendocrine and immune systems. From the neuroendocrine hormones mainly growth hormone, prolactin, β-endorphin, glucocorticoids and catecholamines are involved in the neutrophil regulation. Growth hormone primes the oxidative burst of human neutrophils. This is initiated by growth hormone to the prolactin (and not the growth hormone) receptor on neutrophils in a zinc-dependent process. The growth-promoting effects of growth hormone are mediated through insulin-like growth factor 1, which is also a strong neutrophil-priming agent. Prolactin, which shares considerable functional and structural similarities with growth hormone, is also a strong immunopotentiating agent. Prolactin primes the oxidative burst of neutrophils and macrophages to the same intensity as that induced by growth hormone.

Although glucocorticoids and opioids may enhance some immune responses at very low concentrations, they are generally considered to be immunosuppressive. These contrasting responses may be controlled by the presence of multiple receptors for the same mediator that are coupled to stimulatory and inhibitory pathways. In fact, containment of the stress response may be the principal role of glucocorticoids. Glucocorticoids severely impair the phagocytic and cytotoxic activities of neutrophils and macrophages, their capacity to produce ROI and to induce iNOS, and secrete lysosomal enzymes in response to activation. Oxidative burst of professional phagocytes is also inhibited with epinephrine and β-endorphin which activity is mediated via nonopioid receptors.

Histamine is a potent inhibitor of neutrophil microbiocidal activity. Adenosine provides an interesting example of how a single mediators may play dual roles. Adenosine, a vasodilator, is a potent anti-inflammatory agent released from damaged host cells. Neutrophil chemotaxis is activated by adenosine occupancy of A₁ receptors and inhibition of the respiratory burst triggered through A2 receptors. Adenosine suppresses the respiratory burst only if it is added before the triggering agent, but it has no effect on the initiation or progress of degranulation.

The interactions between platelets and neutrophils are essential for both cell types. Activated platelets can bind to neutrophils and stimulate the oxidative burst while themselves synthesize vasoconstrictive leukotrienes. Like prostaglandins, many immunosuppressive mediators use cAMP as a second messenger. Increased intracellular cAMP in neutrophils is associated with decreases in a number of microbiocidal functions. Phagocyte priming and activation may, in fact, be controlled by shifts in the intracellular ratio
of cGMP to cAMP, since cGMP is stimulatory.

9.1.3.4 Macrophages and monocytes

Originally, monocytes and macrophages were classified as cells of the reticulo-endothelial system - RES (Aschoff, 1924). Van Furth et al. (1972) proposed the mononuclear phagocyte system – MPS, and monocytes and macrophages became basic cell types of this system. Their development takes in the bone marrow and passes through the following steps: stem cell - committed stem cell - monoblast - promonocyte - monocyte (bone marrow) - monocyte (peripheral blood) - macrophage (tissues). Monocyte differentiation in the bone marrow proceeds rapidly (1.5 to 3 days). During differentiation, granules are formed in monocyte cytoplasm and these can be divided as in neutrophils into at least two types. However, they are fewer and smaller than their neutrophil counterparts (azurophil and specific granules). Their enzyme content is similar.

The process of haematopoiesis is controlled by a group of at least 11 growth factors. Three of these glycoproteins initiate the differentiation of macrophages from uni- and bipotential progenitor cells in the bone marrow. The progression from pluripotential stem cell to myeloid-restricted progenitor is controlled by IL-3, which generates differentiated progeny of all myeloid lineages. As IL-3-responsive progenitors differentiate, they became responsive to GM-CSF and M-CSF, the two growth factors giving rise to monocyte/macrophage-restricted progeny. After lineage commitment, cells are completely dependent on these growth factors for continued proliferation and viability. More recently, TNF-α has also been implicated in growth regulation for macrophage precursors.

The blood monocytes are young cells that already possess migratory, chemotactic, pinocytic and phagocytic activities, as well as receptors for IgG Fc-domains (FcγR) and iC3b complement. Under migration into tissues, monocytes undergo further differentiation (at least one day) to become multifunctional tissue macrophages. Monocytes are generally, therefore, considered to be immature macrophages. However, it can be argued that monocytes represent the circulating macrophage population and should be considered fully functional for their location, changing phenotype in response to factors encountered in specific tissue after migration.

Macrophages can be divided into normal and inflammatory macrophages. Normal macrophages include macrophages in connective tissue (histiocytes), liver (Kupffer’s cells), lung (alveolar macrophages), lymph nodes (free and fixed macrophages), spleen (free and fixed macrophages), bone marrow (fixed macrophages), serous fluids (pleural and peritoneal macrophages), skin (histiocytes, Langerhans’ cell) and in other tissues.

The macrophage population in a particular tissue may be maintained by three mechanisms: influx of monocytes from the circulating blood, local proliferation and biological turnover. Under normal steady-state conditions, the renewal of tissue macrophages occurs through local proliferation of progenitor cells and not via monocyte influx. Originally, it was thought that tissue macrophages were long-living cells. More recently, however, it has been shown that depending on the type of tissue, their viability ranges between 6 and 16 days.

Inflammatory macrophages are present in various exudates. They may be characterized by various specific markers, e.g. peroxidase activity, and since they are derived exclusively from monocytes they share similar properties. The term exudate macrophages designates the developmental stage and not the functional state.

Macrophages are generally a population of ubiquitously distributed mononuclear phagocytes responsible for numerous homeostatic, immunological, and inflammatory processes. Their wide tissue distribution makes these cells well suited to provide an immediate defence against foreign elements prior to leukocyte immigration. Because macrophages participate in both specific immunity via antigen presentation and IL-1 production and nonspecific immunity against bacterial, viral, fungal, and neoplastic pathogens, it is not surprising that macrophages display a range of functional and morphological phenotypes.

9.1.3.4.1 Heterogeneity and activation of macrophages

Macrophage heterogeneity is a well-documented phenomenon, perhaps first observed by Metchnikoff, who described a progression of infiltrating cell types in inflammatory exudates. It has also long been recognized that macrophages isolated from different anatomical sites display a diversity of phenotypes and capabilities. Because
macrophage function is dependent in part on signals received from the immediate microenvironment, it is suggested that macrophage heterogeneity may arise from unique conditions within specific tissues. Obviously, the sterile, anaerobic environment of the spleen or peritoneum will impart different constraints on resident macrophages than does the aerobic environment of the alveolar macrophage, which contains numerous external factors. Antibodies directed against specific membrane antigens have been used to compare macrophage from different tissues. For instance, human breast milk macrophages express an antigen not observed on monocytes, alveolar macrophages, or peritoneal cells. Furthermore, human alveolar macrophage express high levels of MHC class II antigen, whereas the opposite is found for peritoneal macrophages.

It has been just as quickly recognized that macrophages isolated from a given tissue display heterogeneous function. For example, only a portion of peritoneal macrophages express low levels of 5'-nucleotidase, and immune elicitation of peritoneal macrophages results in predominantly macrophages with low 5'-nucleotidase activity, presumably because of an influx of monocytes. Thus, functional heterogeneity results from the spectrum of maturational states in a given isolate because of the influx of monocytes and/or local proliferation.

Because macrophages are responsible for numerous inflammatory processes, it becomes important to distinguish between normal or steady-state haematopoiesis and induced haematopoiesis associated with immunological challenge. Production of the macrophage lineage from bone marrow progenitors is normally controlled by M-CSF, which is constitutively produced by many cell types. In response to invasive stimuli and inflammation, monocyte numbers increase dramatically, as do serum levels of M-CSF. In addition, GM-CSF appears in the serum. Although there appear to be a large overlap of macrophage progenitors able to respond to M-CSF or GM-CSF, the very different structures and signal transduction mechanisms of the receptors for M-CSF and GM-CSF suggest that the differentiation pathways they initiate, would be dissimilar.

M-CSF-derived macrophages are larger, have a higher phagocytic capacity, and are highly resistant to infection by vesicular stomatitis virus compared to GM-CSF-derived macrophages. Conversely, GM-CSF-derived macrophages are more cytotoxic against TNF-α-resistant tumour targets, express more MHC class II antigen, more efficiently kill Listeria monocytogenes, and constitutively secrete more PGE2.

The production of functionally distinct macrophage populations gives the nonspecific immune system added flexibility to respond to immunological or inflammatory stimuli. It is probable that the nature of an immune response is dictated in large part by the functional phenotype(s) of the macrophages present within the lesion. The existence of distinct subsets of helper T lymphocytes (TH cells) also suggest that the predominance of TH1 (IFN-γ and IL-2 producing) or TH2 (IL-4 and IL-10 producing) cells may, in turn, favor the production or activation of a particular macrophage subset.

In addition, the orchestration and regulation of cytokine production during inflammatory responses constitute a key determinant of both the resolution of challenge and the limitation of host tissue damage. Hence, the sequential appearance within inflammatory lesions may allow the most appropriate response at a given stage of an immune response. Analysis of temporal production of cytokines during immune responses suggests that different macrophage populations participate at various stages, or that the changing conditions within the lesion differentially affect the functions of distinct macrophage populations.

Several studies correlate the presence of certain macrophage populations with disease states. Human macrophages expressing an undefined antigen detected by the 27E10 monoclonal antibody are observed in acute inflammatory exudates in cases of contact dermatitis, gingivitis, and psoriasis, but are absent in chronic inflammation arising from osteoarthritis, tuberculoid leprosy, and rheumatoid arthritis. In human liver, heart, and kidney grafts, strong infiltration of 27E10-positive macrophages is associated with acute rejection, whereas the RM3/1-positive phenotype is associated with an uncomplicated clinical course. Accumulation of 25F9-positive macrophages correlates with tumour progression and poor prognosis. These studies suggest that phenotypic analysis of macrophage subsets might be of use diagnostically, even if the specific role of these macrophage populations is unclear.

It follows that the term macrophage refers to a heterogeneous population of cells that differ in their origin, development stage (differentiation), local adap-
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Inflammation and thus also in their function and purpose. The nomenclature of individual macrophage types (particularly inflammatory) is rather confusing and terms such as stimulated, activated, induced, elicited etc. are often used interchangeably. Basically, two main macrophage groups can be distinguished: resident (normal) and inflammatory (exudate) macrophages.

The term activated macrophages is reserved for macrophages possessing specifically increased functional activity. The process of differentiation is not to be confused with activation, the process through which differentiated macrophages acquire an increase ability to perform specific functions. Characteristically, resident tissue macrophages are relatively quiescent immunologically, having low oxygen consumption, low levels of major histocompatibility complex (MHC) class II gene expression, and little or no cytokine secretion. Resident macrophages are, however, phagocytic and chemotactic and retain some proliferative capacity.

There are two stages of macrophage activation, the first being a primed stage in which macrophages exhibit enhanced MHC class II expression, antigen presentation, and oxygen consumption, but reduced proliferative capacity. The agent that primes macrophages for activation is IFN-γ, a product of stimulated T\(_{H1}\) and T\(_{H0}\) cells. But many other factors, including IFN-α, IFN-β, IL-3, M-CSF, GM-CSF and TNF-α, can also prime macrophages for selected functions.

Primed macrophages respond to secondary stimuli to become fully activated, a stage defined by their inability to proliferate, high oxygen consumption (through NADPH oxidase), killing of facultative and intracellular parasites, tumour cell lysis, and maximal secretion of mediators of inflammation, including TNF-α, PGE\(_2\), IL-1, IL-6, reactive oxygen species, and nitric oxide produced by iNOS. Agents capable of providing secondary signals are diverse and include LPS, heat-killed gram-positive bacteria, yeast glucans, GM-CSF and phorbol esters. The distinction between primed and fully activated macrophages is usually arbitrary, depending in large part on the stimulus used and the functional assessed.

Macrophages stimulated for tumouricidal activity show decreased MHC class II gene transcription and are generally poor presenters of antigen, despite their secretion of IL-1. Moreover, the process of tumour cell lysis is a multistep event, determined by the sensitivity of the target cell itself. Thus, activation to kill one target does not necessarily include the ability to kill all targets.

When human (but not murine) macrophages are exposed to IFN-γ they express a 1-hydroxylase. This enables them to convert inactive circulating 25-hydroxycholecalciferol into the active metabolite, 1,25-dihydroxycholecalciferol (also known as vitamin D\(_3\) or calcitriol). Macrophages have receptors for this derivative, and it exerts additional activating effects on these cells, and perhaps some negative feedback on lymphocytes. This pathways is of some importance in man, since production of calcitriol can be so great that it leaks from the site of macrophage activation into the peripheral circulation, where it can exert its better known effects on calcium and phosphate balance. Detectable hypercalcaemia can result.

There is also evidence that activated macrophages can be deactivated. Prostaglandin E may have this effect, and some effector mechanisms (but not all) are steroid sensitive. Recently a macrophage deactivating factor (MDF) has been purified from a tumour cell supernatant. This cytokine block activation by IFN-γ of increased capacity for production of ROI and, to some extent, of nitric oxide. So too do IL-4, calcitonin gene related peptide (CGRP), and TGF-β.

9.1.3.4.2 Biological functions of macrophages

Macrophages are involved at all stages of the immune response. First, as already outlined, they act as rapid protective mechanism which can respond before T cell-mediated amplification has taken place. Activated macrophages play a key role in host defence against intracellular parasitic bacteria, pathogenic protozoa, fungi and helminths as well as against tumours, especially metastasising tumours. After phagocytosis, macrophages prevent intracellularly parasitic organisms from replication at least by three ways:

1. Intracellular environment is unsuitable for microbial reproduction due to low pH and lack of nutrients in a phagolysosome.
2. The toxic reaction may be activated to against dividing organisms. This include ROI, hypochlorite, NO’, myeloperoxidase, neutral proteases and lysosomal hydrolases.
3. Macrophages may also produce microbiostatic effector molecules at a steady-state and thus maintain intracellular microorganisms in the non-replicating state. This latent infection is generally observed only in such individuals whose macrophages cannot be sufficiently activated. Generally, macrophages represent the second line of defence against different agents including pathogenic microorganisms.

In addition, macrophages are important killer cells (K cells); by means of antibody-dependent cell-mediated cytotoxicity (ADCC) they are able to kill or damage extracellular targets. They also take part in the initiation of T cell activation by processing and presenting antigen. Finally they are central effector and regulatory cells of the inflammatory response. To fulfill these functions, macrophages in their activated state are able to produce more than one hundred of different substances (Table 9.5).

Monocytes lose their myeloperoxidase activity during conversion to tissue macrophages, therefore microbicidal and cytotoxic activity of macrophages is performed mainly through ROI, NO \( \cdot \) and other substances which are similar to those in neutrophils with the exemption of thymidine, arginase and TNF-\( \alpha \). However, macrophages may acquire MPO from their environment by pinocytosis or from ingested neutrophils. In this way, especially macrophages in inflammatory site with the intensive cell destruction, can gain myeloperoxidase (or other peroxidase). Such peroxidase then participates in cytotoxic mechanisms of macrophages.

Macrophages are important producers of arachidonic acid and its metabolites. Upon phagocytosis macrophages release up to 50% of their arachidonic acid from membranous esterified glycerol phospholipid. It is immediately metabolized into different types of prostanoids. From them prostaglandins, especially PGE\(_2\), and prostacyclin (PGI\(_2\)) are characterized as pro-inflammatory agents: they induce vasodilatation, act synergetically with complement component C5a and LTB\(_4\), mediate fever and myalgia response to IL-1, in the combination with bradykinin and histamine they contribute to erythema, oedema, and pain induction. Tromboxan TXA\(_2\) is considered as an inflammatory mediator; it facilitates platelet aggregation and triggers vasoconstriction. LTB\(_4\) is the efficient chemoattractant substance. A mixture of LTC\(_4\), LTD\(_4\) and LTE\(_4\) became known as slow-reacting substance of anaphylaxis (SRS-A). These leukotrienes are important mediators of bronchial asthma, since they provoke long-term contractions of bronchial smooth muscles. More detailed data about bioactive lipids, complement, clotting factors and cytokines will be in next chapters.

Macrophages secrete not only cytotoxic and inflammation controlling mediators but also substances participating in tissue reorganization. They include enzymes, as hyaluronidase, elastase, and collagenase, inhibitors of some of them (antiproteases), regulatory growth factors and others. Hyaluronidase, by destroying hyaluronic acid, an important component of connective tissue, reduces viscosity and thus permits greater spreading of material in tissue spaces. Hyaluronidase is therefore sometimes designated the "spreading factor". Elastase and collagenase are enzymes capable to split collagen and elastin, the basic members of connective proteins.

9.1.3.4.3 The role of macrophages in angiogenesis
An important component of inflammatory reactions and subsequent repair and remodelling processes is angiogenesis or neovascularization – the formation of new capillaries from preexisting blood vessels. It is also commonly observed during physiological growth processes and during embryogenesis, where the formation of new blood vessels from angioblasts is referred to as vasculogenesis. Some diseases, such as arthritis and diabetic retinopathy, are maintained by persistent neovascularization. Interest in neovascularization was originally evoked by the phenomenon of tumour angiogenesis: Tumours do not grow beyond 2-3 mm\(^3\) and cannot metastasize unless vascularized. From the many cells and cell products as inducers or modulators of angiogenesis, macrophages have emerged as a major protagonist. The angiogenic activity of macrophages is associated with their secretory activity and needs specific activation.

Among the potential activators of macrophages, LPS is known to induce angiogenic activity but does not appear to be a general stimulus of angiogenesis. More specific activation signals could be provided by the particular metabolic conditions found in wounds. Macrophages became angiogenic when exposed to low oxygen tensions or to woundlike concentrations
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<table>
<thead>
<tr>
<th>Group of substances</th>
<th>Individual products</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbicidal and cytotoxic:</strong></td>
<td>Superoxide, hydrogen peroxide, hydroxyl radical, hypohalite, chloramines</td>
</tr>
<tr>
<td>Reactive oxygen intermediates</td>
<td></td>
</tr>
<tr>
<td>Reactive nitrogen intermediates</td>
<td>Nitric oxide, nitrates, nitrates</td>
</tr>
<tr>
<td>Oxygen independent</td>
<td>Neutral proteases, acid hydrolases, lysozyme, defensins</td>
</tr>
<tr>
<td><strong>Tumouricidal:</strong></td>
<td>H₂O₂, NO’, TNF-α, C3a, proteases, arginase, thymidine</td>
</tr>
<tr>
<td><strong>Tissue damaging:</strong></td>
<td>H₂O₂, TNF-α, NO’, neutral proteases</td>
</tr>
<tr>
<td><strong>Fever inducing:</strong></td>
<td>IL-1, TNF-α, IL-6</td>
</tr>
<tr>
<td><strong>Inflammation regulators:</strong></td>
<td></td>
</tr>
<tr>
<td>Bioactive lipids</td>
<td>Prostaglandins (PGE₂, PGF₂α), prostacyclin (PGI₂), tromboxans, leukotrienes (LTB₄, LTC₄, LTD₄, LTE₄)</td>
</tr>
<tr>
<td>Bioactive oligopeptides</td>
<td>Glutathione</td>
</tr>
<tr>
<td>Complement components</td>
<td>C1, C4, C2, C3, C5, factors B, D, P, I, H</td>
</tr>
<tr>
<td>Clothing factors</td>
<td>V, VII, IX, X, prothrombin, plasminogen activator, plasminogen activator inhibitors</td>
</tr>
<tr>
<td>Cytokines</td>
<td>IL-1, IL-6, IL-8, TNF-α, INF-γ. Macrophage inflammatory proteins (MIP-1, MIP-2, MIP-3). Regulatory growth factors (M-CSF, GM-CSF, G-CSF, PDGF).</td>
</tr>
<tr>
<td>Neutral proteinases</td>
<td>Elastase, collagenase, angiotensin convertase, stromelysin</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>α₂-Macroglobulin, α-1-proteinase inhibitor, plasmin and collagenase inhibitors, plasminogen activator inhibitors</td>
</tr>
<tr>
<td><strong>Acid hydrolases</strong></td>
<td>Acid proteases (cathepsin D and L), peptidases, lipases, lysozyme and other glycosidases, ribonucleases, phosphatases, sulphatases</td>
</tr>
<tr>
<td>Stress proteins</td>
<td>Heat shock proteins (HSP)</td>
</tr>
<tr>
<td>Participating in tissue reorganization:</td>
<td>Elastase, collagenase, hyaluronidase, regulatory growth factors, fibroblast growth factor (FGF), transforming growth factors (TGF-α, TGF-β), angiogenesis factors</td>
</tr>
<tr>
<td>Other:</td>
<td>Apolipoprotein E, IL-1 inhibitors, purine and pyrimidine derivates (thymidine, uracil, neopterin)</td>
</tr>
</tbody>
</table>

Table 9.5: Effector and regulatory products of macrophages

of lactate, pyruvate, or hydrogen ions. They can also be activated by cytokines such as IFN-γ, GM-CSF, PAF, or MCP (**monocyte chemoattractant protein**). Certain mature or immature macrophage-like cell lines obtained from mice or humans have different angiogenic activities. Differences in angiogenic acti-
vity also depend on the developmental and largely on the activation stage.

The formation of new capillaries proceeds through a series of steps. The sequence of events for endothelial cells begins with destruction of the basement membrane and local degradation of the extracellular matrix (ECM). This allows endothelial cells to migrate by extending cytoplasmic buds in the direction of chemotactic factors. Simultaneously, inflamed and neoplastic tissues present leakage of capillaries with exudation of fibrin, which also serves as a migratory matrix for endothelial and other cells. For elongation of new capillaries, migrating endothelial cells must be replaced by newly divided endothelial cells. Eventually migration and mitosis have to be stopped while capillary sprouts differentiate into mature capillaries with a new basement membrane. Macrophages are able to promote all phases of the angiogenic process by virtue of their secretory products.

Local degradation of the ECM appears to entail more than opening up a way for migrating cells. The ECM has been suggested to have a controlling role in a variety of physiological and biochemical processes including angiogenesis. Some ECM molecules become angiogenic after hydrolytic degradation. For instance, fragments of hyaluronic acid, as opposed to the complete molecule, have been shown to induce neovascularization. Similarly, fibrin, after digestion by plasmin, can provoke an angiogenic response. Macrophages release several proteases, among them plasminogen activator, whose actions could yield angiogenic fragments from ECM molecules.

Several soluble growth factors (basic fibroblast growth factor – bFGF, TGF-β3, GM-CSF) are stored in the ECM by being bound to, for example, heparin-like glycosaminoglycans. Their enzymatic release would make them available for endothelial cells. Macrophages are able to degrade heparan sulphate and to release ECM-bound bFGF by expression of urokinase-type tissue plasminogen activator.

The composition of the ECM determine the shape of endothelial cells, thereby dictating their responsiveness to individual growth factors. Changes in shape of endothelial cells with subsequently modified responsiveness towards growth factors were achieved by mere mechanical forces. Macrophages secrete several enzymes and cytokines that cause changes of the molecular or mechanical structure of the ECM. They are a rich source of metalloproteases (e.g. collagenases) and serine proteases (e.g. elastase and plasminogen activator). As mentioned above, these enzymes can degrade ECM molecules, modulate mechanical structures, and liberate ECM-bound growth factors. Plasminogen activator has been found to be indispensable for tube formation of microvascular cells in vitro. Its inhibition subsequently leads to suppression of angiogenesis. As proteolytic enzymes are capable to degrading almost all components of the ECM, control mechanisms are necessary, such as activation-dependent release of proteinases. Macrophages themselves also synthesize tissue inhibitors of metallo- and serine proteases.

Macrophages release monokines that influence changes in the ECM other than inducing protease release by endothelial cells. Some of these cytokines also have direct angiogenic effects. TGF-β3, which both stimulates and inhibits angiogenesis in vitro, modulates the expression of fibronectin or collagen type I and their incorporation into the ECM. Other macrophage-derived cytokines with angiogenic potential and modulating effects on the ECM are angiotropin, platelet-derived growth factor (PDGF), and IL-6.

In summary, macrophage can change the composition of the extracellular matrix:

1. by releasing degrading enzymes,
2. by synthesizing ECM molecules such as fibronectin or proteoglycans, and
3. by releasing monokines with modulating effects on the ECM.

Macrophages produce several factors, other than proteases, that induce migration of endothelial cells. Most of them also support other stages of the angiogenic process such as mitosis or differentiation of endothelial cells. However, two factors appear to have predominantly chemotactic effects: human angiogenic factor (HAF) and angiotropin. Their migratory effects are sufficient for initial neovascularization as migrating endothelial cells can form sprouts without proliferating. Angiogenin, another well-characterized nonmitogenic angiogenic factor does not appear to be released by macrophages.

Macrophages secrete or produce several factors that induce mitoses of capillary endothelium. Whereas the mitogenic effects of bFGF, TGF-α, GM-CSF, M-CSF, vascular endothelial growth
factor/vascular permeability factor (VEGF/VPF), IL-8, and substance P are well characterized, the proliferative actions of insulin-like growth factor I (IGF-I, somatomedin C) and PDGF on endothelial cells may need further confirmation.

The heparin-binding (fibroblast) growth factors have shown prominent angiogenic activity in almost any bioassay for angiogenesis. Basic FGF stimulates directed migration and proliferation of cultured endothelial cells and promotes formation of differentiated capillary tubes in vitro. These effects are associated with selective up-regulation of integrins on endothelial cells and induction of protease such as plasminogen activator.

Activated macrophages are the only blood cells besides platelets producing platelet-derived growth factor (PDGF). PDGF is a family of dimeric proteins consisting of A chain and/or a B chain. Activated monocytes and macrophages, but not resting monocytes, express the B-chain gene of PDGF. Direct angiogenic effects of PDGF are not unequivocal. Controversial effects of PDGF on endothelial cells may derive from the heterogeneity of endothelial cells. Capillaries that respond to PDGF have PDGF-B-type receptors, whereas endothelial cells of large vessels do not respond to PDGF. Apart from its direct effects on endothelium, PDGF has effects on other cells involved in wound healing. It is a potent chemoattractant and mitogen for mesenchymal cells. It also activates macrophages and stimulates synthesis of ECM components.

Vascular endothelial growth factor (VEGF), also called vascular permeability factor (VPF) or vasculotropin, is a PDGF-related protein whose mitogenic activity is apparently specific for endothelial cells, the only cells that normally express VEGF/VPF receptors. Besides stimulating proliferation of endothelial cells, it markedly increases vascular permeability and induces serine and metalloproteinases in endothelial cells.

Macrophages release several factors that do not directly induce angiogenesis in corresponding assays but act indirectly by attracting or activating angiogenic cells. This applies for each phase of the angiogenic process. Since angiogenesis is a component of inflammatory processes, all macrophage-derived mediators of inflammation have to be considered at least as indirect angiogenesis factors. Another macrophage-derived factors are angiogenic, but it is not known whether they are chemotactic, mitogenic, or lead to change in the ECM. As an example may serve angiotensin-converting enzyme (ACE). ACE converts angiotensin I to angiotensin II. The latter caused marked neovascularization after being implanted into rat cornea or in the chorioallantoic membrane assay. The nature of this angiogenic effect is unknown and may be indirect by causing an inflammatory infiltrate, as ACE promotes neither migration nor proliferation of endothelial cells. Most macrophages exhibit only low activity of angiotensin-converting enzyme, but macrophages of sarcoidosis granulomas present higher activities. This may be important view of a marked angiogenic effect of macrophages in sarcoidosis.

Inhibition of neovascularization is necessary to restrict the extent of the new vascular network and to facilitate differentiation of capillary sprouts into functionally mature capillaries. Macrophages release several factors that inhibit migration or mitosis of endothelial cells: monocyte-derived endothelial cell inhibitory factor (MECIF), macrophage-derived endothelial cell inhibitor (MD-ECI), thrombospondin I, IFN-α, and IFN-γ. These cytokines could thereby promote the differentiating effects of otherwise chemotactic and mitogenic factors such as angiotropin, bFGF, GM-CSF, or M-CSF.

Thrombospondin 1 (TSP 1) is one of several ECM proteins that are produced by macrophages. Its expression has been linked to a cancer suppressor gene in several cells that is down-regulated during tumourigenesis. It inhibits migration, proliferation, and capillary tube formation of endothelial cells in vitro, where it is associated with quiescent but not proliferating endothelial cells. TSP 1 also suppress neovascularization in vivo. Since TSP 1 performs several other functions in wound (serving as migratory matrix, enhancing neutrophil chemotaxis, inhibiting proteases) its role in angiogenesis is likely to depend on regulatory effects of the other mediators present during inflammation.

Histological examination of tissue sections with ongoing angiogenesis has shown that the presence of granulocytes and macrophages is a prerequisite for the neovascularization. Neutralization or depletion of either type suppressed the formation of new blood vessels. The angiogenic activity of granulocytes, however, appeared to be inferior to that of macrophages and apparently was not associated with
the release of diffusible factors. Also the half-life of extracellular granulocytes is extremely short. Mast cells or T lymphocytes, on the other hand, do not appear to be relevant for outgrowth of vascular sprouts in inflammatory angiogenesis. Instead, mast cells may have an important role in tumor angiogenesis, and the angiogenic activity of T lymphocytes seems to be relevant in graft-versus-host reaction.

9.1.4 Mediators of inflammation

Once leukocytes have arrived at a site of infection or inflammation, they release mediators which control the later accumulation and activation of other cells. However, in inflammatory reactions initiated by the immune system, the ultimate control is exerted by the antigen itself, in the same way as it controls the immune response itself. For this reason, the cellular accumulation at the site of chronic infection, or in autoimmune reactions (where the antigen cannot ultimately be eradicated), is quite different from that at sites where the antigenic stimulus is rapidly cleared.

There are four major plasma enzyme systems which have an important role in haemostasis and control of inflammation. These are the complement system, the clotting system, the fibrinolytic (plasmin) system and the kinin system.

Inflammatory mediators are soluble, diffusible molecules that act locally at the site of tissue damage and infection, and at more distant sites. They can be divided into exogenous and endogenous mediators. Bacterial products and toxins can act as exogenous mediators of inflammation. Notable among these is endotoxin, or LPS of Gram-negative bacteria. The immune system of higher organisms has probably evolved in a veritable sea of endotoxin, so it is perhaps not surprising that this substance avokes powerful responses. For example, endotoxin can trigger complement activation, resulting in the formation of anaphylatoxins C3a and C5a which cause vasodilation and increase vascular permeability. Endotoxin also activates the Hageman factor, leading to activation of both the coagulation and fibrinolytic pathways as well as the kinin system. In addition, endotoxin elicit T cell proliferation, and have been described as superantigen for T cells.

Endogenous mediators of inflammation are produced from within the (innate and adaptive) immune system itself, as well as other systems. For example, they can be derived from molecules that are normally present in the plasma in an inactive form, such as peptide fragments of some components of complement, coagulation, and kinin systems. Mediators of inflammatory responses are also released at the site of injury by a number of cell types that either contain them as preformed molecules within storage granules, e.g. histamine, or which can rapidly switch on the machinery required to synthesize the mediators when they are required, for example to produce metabolites of arachidonic acid.

Mononuclear phagocytes (monocytes and macrophages) are central to inflammation, as they produce many components which participate in or regulate the different plasma enzyme systems, and hence the mediators of the inflammatory response. They are also actively phagocytic and are involved in microbial killing, as are neutrophils. While the latter can be thought of as short-lived kamikaze cells that need to be continually replaced from the bone marrow, mononuclear phagocytes are long-lived and some can proliferate in situ. Other cells such as mast cells and basophils are much less phagocytic, but together with platelets, these cells are particularly important for secretion of vasoactive mediators. The function of these cell types is at least partially under the control of cytokines. All inflammatory cells have receptors for Fc domains of immunoglobulins and for complement components, and they possess specialized granules containing an immense variety of products that are released perhaps by common mechanisms. Cytotoxic T lymphocytes and NK cells, in general, also possess granules which are important for their cytotoxic function. In general, lymphocytes are involved in the adaptive response to inflammation, and the early events of inflammation are mediated in part by molecules produced by cells of the innate arm of the immune system.

Early phase mediators are produced by mast cells and platelets. They are especially important in acute inflammation and include mainly histamine, serotonin and other vasoactive substances. Platelets may contribute to inflammatory responses resulting as a consequence of tissue injury, through a variety of mechanisms including:

1. the release of vasoactive amines and other permeability factors,
2. the release of lysosomal enzymes,
3. the release of coagulation factors which lead to localized and generalized fibrin deposition, and
4. The formation of platelet aggregates or trombi which result in the blocking of vessels and capillaries.

To the early phase mediators also belong chemoattractants (e.g. C5a) and cytokines such as IL-1, IL-6, and TNF-\(\alpha\).

Late phase mediators are responsible for the regulation of vascular events occurring later – from about 6–12 hours after initiation of inflammation. The later vascular events are mediated, at least in part, by products of arachidonic acid.

The chemical mediators of inflammation are summarized in Table 9.6. There is considerable functional redundancy of the mediators by inflammation. This explains why certain patients may have complete absence of a humoral component (e.g., complement component C3), yet minimal problems with increased susceptibility to infection.

Edema formation can be separated from phagocyte recruitment. Vasodilation in response to histamine, bradykinin, PGE\(_2\) and PGI\(_2\), and complement fragments C3a and C5a results from a direct action of these substances on endothelial cells and smooth muscle vasculature with resulting leakage of plasma. This is accompanied by release of mediators, such as C5a, LTB\(_4\), and PAF, that act directly on the phagocytic cells. In addition N-formyl peptides are released from bacteria and mitochondria of damaged tissues. These mediators are potent chemoattractants that mobilize neutrophils, monocytes, and eosinophils, cause release of lysosomal contents, and activate the respiratory burst of the phagocytes with resulting production of toxic oxygen products.

Following intravenous endotoxin, a characteristic change in body temperature and white blood count is observed. The body temperature begins to increase after about one hour and reaches a maximum at about four hours. The leukocyte count shows a characteristic decrease at about 30 min, due to neutrophil and monocyte adherence to endothelial cells in the lung and spleen. This is followed by release of mediators, such as C5a, LTB\(_4\), and PAF, that act directly on the phagocytic cells. In addition N-formyl peptides are released from bacteria and mitochondria of damaged tissues. These mediators are potent chemoattractants that mobilize neutrophils, monocytes, and eosinophils, cause release of lysosomal contents, and activate the respiratory burst of the phagocytes with resulting production of toxic oxygen products.

9.1.4.1 Histamine and serotonin

The most important vasoactive mediators that are stored in mast cell and basophil granules are histamine in man, as well as serotonin or 5-hydroxytryptamine in rodents. They both are also present in human platelets. Histamine is stored in mast cells and basophils largely complexed to mucopolysaccharide (glycosaminoglycans) such as heparin. Histamin has diverse functions including primary, local dilatation of small vessels; widespread arteriole dilatation; local increased vascular permeability by contracting endothelial cells; the contraction of nonvascular smooth muscle; chemotaxis for eosinophils; and blocking T lymphocyte function. A number of different cells of the body have receptors for histamine. These can be of three types – H1, H2, and H3. The H1 receptors mediate acute vascular effects together with smooth muscle constriction in the bronchi (histamine act as a "spasmogen") and the stimulation of eosinophil chemotaxis. In contrast, the H2 receptors mediate a number of anti-inflammatory effects, including the inhibition of eosinophil chemotaxis, but cause the vasodilatation. The H3 receptor is mainly involved in the control of histamine release by different producing cells.

Serotonin is also capable of increasing vascular permeability, dilating capillaries and producing contraction of nonvascular smooth muscle. Most sero-
<table>
<thead>
<tr>
<th>Function</th>
<th>Mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased vascular permeability of small blood vessels</td>
<td>Histamine, serotonin, bradykinin, C3a, C5a, PGE₂, LTC₄, LTD₄, prostacyclins, activated Hageman factor, high-molecular-weight kininogen fragments, fibrinopeptides</td>
</tr>
<tr>
<td>Vasoconstriction</td>
<td>TXA₂, LTD₄, LTC₄, LTD₄, C5a, N-formyl peptides</td>
</tr>
<tr>
<td>Smooth muscle contraction</td>
<td>C3a, C5a, histamine, LTB₄, LTC₄, LTD₄, TXA₂, serotonin, PAF, bradykinin</td>
</tr>
<tr>
<td>Increased endothelial cell stickiness</td>
<td>IL-1, TNF-α, MCP, endotoxin, LTB₄</td>
</tr>
<tr>
<td>Mast cell degranulation</td>
<td>C5a, C3a</td>
</tr>
<tr>
<td><strong>Phagocytes</strong></td>
<td></td>
</tr>
<tr>
<td>Stem cell proliferation</td>
<td>IL-3, G-CSF, GM-CSF, M-CSF</td>
</tr>
<tr>
<td>Recruitment from bone narrow</td>
<td>CSFs, IL-1</td>
</tr>
<tr>
<td>Adherence/aggregation</td>
<td>iC3b, IgG, fibronectin, lectins</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>C5a, LTBR, IL-8 and other chemokines, PAF, histamine (for eosinophils), laminin, N-formyl peptides, collagen fragments, lymphocyte-derived chemotactic factor, fibrinopeptides</td>
</tr>
<tr>
<td>Lysosomal granule release</td>
<td>C5a, IL-8, PAF, most chemoattractants, phagocytosis</td>
</tr>
<tr>
<td>Production of reactive oxygen intermediates</td>
<td>C5a, TNF-α, PAF, IL-8, phagocytic particles; IFN-γ enhances</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>C3b, iC3b, IgG (Fc portion), fibronectin; IFN-γ increases Fc receptor expression</td>
</tr>
<tr>
<td>Granuloma formation</td>
<td>IFN-γ, TNF-α, IL-1</td>
</tr>
<tr>
<td>Pyrogens</td>
<td>IL-1, TNF-α, PGE₂, IL-6</td>
</tr>
<tr>
<td>Pain</td>
<td>PGE₂, bradykinin</td>
</tr>
</tbody>
</table>

Table 9.6: Mediators of inflammation

Histamin is stored in the gastrointestinal tract and central nervous system but a large amount is also stored in the dense granules of platelets.

**9.1.4.2 Lipid mediators**

The major constituent of cell membranes are phospholipids. Cellular phospholipases, especially phospholipase A₂ and C, are activated during inflammation and degrade phospholipids to arachidonic acid. Arachidonic acid has a short half-life and can be metabolized by two major routes, the cyclo-oxygenase and lipoxygenase pathways. The cyclo-oxygenase pathway produces prostaglandins, prostacyclin, and thromboxanes; the lipoxygenase pathway produces in one branch leukotrienes and in the second branch lipoxins (Figure 9.1).
The prostaglandins (PG) are a family of lipid-soluble hormone-like molecules produced by different cell types in the body. For example, macrophages and monocytes are large producers of both PGE$_2$ and PGF$_2$, neutrophils produce moderate amounts of PGE$_2$, mast cells produce PGD$_2$. It is important to note that, unlike histamine, prostaglandins do not exist free in tissues, but have to be synthesized and released in response to an appropriate stimulus. PGE$_2$ enhances vascular permeability, is pyrogenic, increases sensitivity to pain, and stimulates leukocyte cAMP, which can have an important suppressive effects on release of mediators by mast cells, lymphocytes, and phagocytes.

Thromboxane A$_2$ (TXA$_2$) is produced by monocytes and macrophages, as well as by platelets. It causes platelets to aggregate and constrict blood vessels and airways. These effects are somewhat opposed by the action of prostacyclin (PGI$_2$) which is a potent vasodilator.

Leukotrienes. LTB$_4$ and 5-hydroxyeicosatetraenoate (5-HETE), causes the chemotaxis (directed locomotion) and/or chemokinesis (general cell movement) of a number of cell types including neutrophils.
The synthesis of LTB₄ is inhibited by colchicine, an anti-inflammatory agent used for treatment of gout. The mixture of LTC₄, LTD₄ and LTF₄ originally called slow reacting substance of anaphylaxis (SRS-A), is produced by a wide variety of cells, including monocytes and macrophages. They are spasmogenic and cause contraction of smooth muscle, mainly in the bronchus, and they have effects on mucous secretion.

Lipoxins LXA₄ and LXB₄ stimulate changes in microcirculation. For example, LXA₄ induces rapid arteriolar dilation and can also antagonize LTD₄-induced vasoconstriction. It suggest that LXA₄ may regulate the action of vasoconstrictor leukotrienes. LXA₄ can block neutrophil chemotaxis induced by both LTB₄ and N-formyl-oligopeptides. Both LXA₄ and LXB₄ inhibit cytotoxicity of natural killer cells. Platelets produce a group of acetyl-alkylglycerol ether analogs of phosphatidylcholine called platelet-activating factors (PAFs). PAFs cause platelet aggregation and are potent phagocyte chemoattractants and stimuli of lysosomal enzyme release and reactive oxygen product formation by neutrophils, eosinophils, and macrophages. In addition, PAFs increase the stickiness of endothelial cells for leukocytes.

The basic activities of bioactive lipids are listed in Table 9.7.

9.1.4.3 Products of the complement system

Complement is a complex system containing more than 30 various glycoproteins present in serum in the form of components, factors, or other regulators and/or on the surface of different cells in the form of receptors. These are present in the blood serum in an inactive state and are activated by immune complexes (the classical pathway), by carbohydrates (the lectin pathway), or by other substances, mainly of bacterial origin (the alternative pathway) – Figure 9.2.

The components of the classical pathway are numbered 1 to 9 and prefixed by the letter C, e.g. C1, C2...C9. C1 is composed of three subcomponents C1q, C1r, and C1s. The early components of the alternative pathway are known as factors, and each molecule is named by a letter, for example factor B, D, P. The lectin pathway is the same as the classical pathway, only C1q is omitted. All these pathways use in the later stages of activation the same terminal components C5-C9 that form membrane attack complex (MAC) – C5b678(9)n. C3 also participates in all pathways.

Activation of each of the components results from a proteolytic cleavage event in a cascade mechanism which fragments the native molecule into two fragments. The fragment which participates further in the complement cascade is designated the b fragment (e.g. C3b) and is usually larger than the another a fragment (e.g. C5a) which possesses other biological activities.

The complement system influence the activity of numerous cells, tissues and physiological mechanism of the body. These effects may involve either the whole complement, or only individual components or fragments. Activation of the complement cascade, with the formation of the effector MAC unit, results in cytotoxic and cytolitic reactions. Target cells for MAC action may be heterologous erythrocytes, nucleated cells (autologous or foreign), bacteria (Gram-negative, susceptible to serum), microscopic fungi, viruses with a surface envelope and virus-infected cells.

The result of cytotoxic complement reaction may be beneficial for the body (elimination of the infectious agent or damaged cells) or harmful (damage to autologous normal cells by immunopathological reactions).

Different fragments, released from individual components during complement activation, operate by a non-cytolytic mechanism through specific receptors present on various cell types. The direction and intensity of the biological response depend on the state of the receptors (affinity and density) and on the function of cells bearing receptors. From the functional standpoint, complement receptors can be divided into two types: the adherent type and the other receptors. Adherent receptors mediate adherence of cells and other particles with bound C3b or C4b fragments and are known as CR1 to CR5. Adherence reaction mediated through the CR receptors on phagocytes lead to stimulation of phagocytosis, activation of metabolism and secretory function and movement of phagocytes into the inflammatory site. These receptors, present on the other cells of the immune system, are involved in a variety of immunoregulatory reactions. CR1 on erythrocytes may bind circulating immune complexes (that had activated complement) and transport them to the liver where
9.1. Inflammation

<table>
<thead>
<tr>
<th>Activity</th>
<th>Lipid mediators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrogenicity</td>
<td>PGE₂</td>
</tr>
<tr>
<td>Vasoconstriction</td>
<td>TXA₂</td>
</tr>
<tr>
<td>Vasodilatation, increase of vascular</td>
<td>PGI₂, PGE₂, PGD₂, PGF₂</td>
</tr>
<tr>
<td>permeability</td>
<td></td>
</tr>
<tr>
<td>Contraction of smooth muscle,</td>
<td>LTC₄, LTD₄, LTE₄</td>
</tr>
<tr>
<td>increase of vascular permeability</td>
<td></td>
</tr>
<tr>
<td>Chemotaxis of phagocytes</td>
<td>LTB₄, 5-HETE, PAF</td>
</tr>
<tr>
<td>Platelet aggregation</td>
<td>TXA₂, PAF</td>
</tr>
</tbody>
</table>

Table 9.7: Lipid mediators and their basic activities

The immune complexes are partially degraded and thus become more soluble.

The second group of receptors reacts with small complement fragments (C₄a, C₃a, C₅a) as well as with C₁q, Bₐ, Bₐ and factor H. Stimulation of these receptors results in various biological effects (chemotaxis, secretion of vasoactive amines, mediators of the inflammatory and anaphylactic reaction etc.).

The main functions of the complement cascade and its role in the acute inflammatory reaction are summarized in Table 9.8.

The complemet system is a potent mechanism for initiating and amplifying inflammation. This is mediated through fragments of complement components. To the most well-defined fragments belong anaphylatoxins. Anaphylatoxins are proteolytic products of the serine proteases of the complement system: C₃a, C₄a and C₅a. They are polypeptides containing approximately 75 amino acid residues and meet all the criteria which characterize local hormones. The C-terminal arginine in the molecule of C₃a is of fundamental importance for its biological activity. As soon as arginine is removed, the biological activity disappears completely. In the case of C₅a, the removal of C-terminal arginine (C₅adesArg) only decreases its biological activity.

The production of anaphylatoxins follows not only from complement activation, but also from activation of other enzyme system which may directly cleave C₃, C₄ and C₅. Such enzymes include plasmin, kallikrein, tissue and leukocyte lysosomal enzymes, and bacterial proteases.

The anaphylatoxins have powerful effects on blood vessel walls, causing contraction of smooth muscle and an increase in vascular permeability. These effects show specific tachyphylaxis (i.e. repeated stimulation induces diminishing responses) and can be blocked by antihistamines; they are probably mediated indirectly via release of histamine from mast cells and basophils. C₅a is the most powerful, approximately 100 times more effective than C₃a, and 1000 times more effective than C₄a. The smooth muscle contraction in the lungs is primarily mediated by LTC₄ and LTD₄. This activity decrease in the following order:

C₅a > histamine > acetylcholine > C₃a >> C₄a

C₅a is extremely potent at stimulating neutrophil chemotaxis, adherence, respiratory burst generation and degranulation. C₅a also stimulates neutrophils and endothelial cells to express more adhe-
sion molecules. Ligation of the neutrophil C5a receptor is followed by mobilization of membrane arachidonic acid which is metabolized to prostaglandins and leukotrienes including LTB$_4$, another potent chemoattractant for neutrophils and monocytes. Following ligation of monocyte C5a receptors, IL-1 is released. Thus the local synthesis of C5a at sites of inflammation has powerful pro-inflammatory properties.

At the same time, C3b and C4b fragments act as opsonins enhancing phagocytosis. In addition to inducing phagocytosis, ligation of complement receptors on neutrophils, monocytes and macrophages may also stimulate exocytosis of granules containing powerful proteolytic enzymes, and free radical production through the respiratory burst.

Recently it has been shown that mannan-binding protein (MBP) is the main opsonin in the human blood serum. This was confirmed by observations on infants with recurrent infections due to opsonin deficiency. All such children were found either to lack MBP, or to have very low concentrations of the lectin. MBP has been found to initiate complement-mediated lysis of mannan-coated erythrocytes and this lysis requires the presence of the classical pathway complement component C4, but not C1q. This new lectin pathway of complement activation is important not only for the killing of microorganisms through the interaction of carbohydrates on their surfaces and MBP or other collectins (humoral lectins found in humans and other mammals) but also for the opsonizing activity.

The complement cascade also interacts with other triggered-enzyme cascade: coagulation, kinin generation and fibrinolysis. There is another connection between these systems: the regulatory protein, C1 inhibitor, inhibits not only C1r and C1s but also Factor XIIa of the coagulation system, kallikrein of the kinin system and plasmin of the fibrinolytic cascade.

Under some circumstances the consequences of
9.1. Inflammation

<table>
<thead>
<tr>
<th>Activity</th>
<th>Components and fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase of vascular permeability</td>
<td>C5a, C3a, C5a_{desArg}, C4a</td>
</tr>
<tr>
<td>Smooth muscle contraction</td>
<td></td>
</tr>
<tr>
<td>Degranulation of mast cells and basophils</td>
<td></td>
</tr>
<tr>
<td>Neutrophil activation and chemotaxis</td>
<td>C5a, C5a_{desArg}</td>
</tr>
<tr>
<td>Stimulation of prostaglandin and leukotriene production</td>
<td></td>
</tr>
<tr>
<td>Opsonization of bacteria and immune complexes leading to phagocytosis</td>
<td>C3b, C4b</td>
</tr>
<tr>
<td>Stimulation of the respiratory burst of professional phagocytes</td>
<td>C3b, C5a, C5a_{desArg}, C1q</td>
</tr>
<tr>
<td>Lysis of bacteria and foreign cells</td>
<td>C5b678(9)\textsubscript{n}</td>
</tr>
<tr>
<td>Solubilization of circulating immune complexes</td>
<td>C3b, CR1</td>
</tr>
</tbody>
</table>

Table 9.8: The biological functions of complement and its role in the acute inflammatory reactions

complement activation in vivo may be deleterious rather than beneficial. The state of shock that may follow bacteraemia with Gram-negative organisms may, in part, be mediated by complement, which is extensively activated by endotoxin. The large quantities of C3a and C5a which result from this cause activation and degranulation of neutrophils, basophils and mast cells. These anaphylatoxins may stimulate intravascular neutrophil aggregation leading to clothing and deposition of emboli in the pulmonary microvasculature. At this site neutrophil products, including elastase and free radicals, may cause the condition of shock lung. This condition is characterized by interstitial pulmonary oedema due to damage to small blood vessels, exudation of neutrophils into alveoli, and arterial hypoxaemia. Extracorporeal blood circulation, for example through heart-lung bypass machines, or over cuprophane dialysis membranes, may similarly cause activation of complement, accompanied by transient leukopenia, thought to be caused by aggregation of neutrophils in the lungs.

Tissue injury following ischemic infarction may also cause complement activation. Abundant deposition of membrane attack complex may be readily seen in tissue following ischaemic injury. A possible pathophysiological role for complement activation following tissue ischaemia was demonstrated in experimental models of myocardial infarction: complement depletion reduced the size of tissue injury and infusion of soluble CR1 has recently been shown to have a similar effect.

The activation of complement by immune complexes is normally beneficial. Immune complexes bearing C3b are efficiently removed from tissues and from the circulation by monocytes and other phagocytes. However there are circumstances in which immune complex production continues at a high level; complement activation by immune complexes may then prove deleterious. Such complexes may form in tissues, for example in glomeruli of patients with autoantibodies to glomerular basement membrane (Goodpasture’s syndrome) or at motor end-plates in patients with autoantibodies to acetylcholine receptors (myasthenia gravis). Alternatively, immune complexes may become trapped in blood vessel walls.
having travelled trough the circulation. This occurs, for example in systemic lupus erythematosus, and in bacterial endocarditis in which an infected heart valve provides the source of immune complexes which deposit in the kidney and other microvascular beds.

Complement mediates inflammation in these diseases by two major pathways:

1. by activated leukocytes, which are attracted to sites of immune complex deposition by locally-produced anaphylatoxins C5a and C5a_{desArg} and which bind to C3b and C4b fixed to the immune complexes;

2. by the membrane attack complex (MAC), which cause cell lysis and thus stimulates prostaglandin synthesis from arachidonic acid, mobilized from perturbed cell membranes.

These two mechanisms of damage are well exemplified by considering two types of glomerular disease. Autoantibodies to glomerular basement membrane cause inflammation which can be inhibited by either complement depletion or by neutrophil depletion. In contrast, membranous nephritis, (which may be induced experimentally by antibodies to subepithelial antigens), is unaffected by neutrophil depletion, but almost totally abrogated in animals deficient in C5. In this disease the basement membrane is presumed to act as a physical barrier to neutrophil exudation, so that the heavy proteinuria is caused by deposition of membrane attack complex.

9.1.4.4 The coagulation mechanism

The blood clothing system or coagulation pathway, like the complement system, is a proteolytic cascade. Each enzyme of the pathway is present in the plasma as a zymogen, in other words in an inactive form, which on activation undergoes proteolytic cleavage to release the active factor from the precursor molecule. The coagulation pathway functions as a series of positive and negative feedback loops which control the activation process. The ultimate goal of the pathway is to produce thrombin, which can then convert soluble fibrinogen into fibrin, which forms a clot. The generation of thrombin can be divided into three phases, the intrinsic and extrinsic pathways that provide alternative routes for the generation of factor X, and the final common pathway which results in thrombin formation (Figure 9.3).

The intrinsic pathway is activated when blood comes into contact with sub-endothelial connective tissues or with negatively charged surface that are exposed as a result of tissue damage. Quantitatively it is the most important of the two pathways, but is slower to cleave fibrin than the extrinsic pathway. The Hageman factor (factor XII), factor XI, prekallikrein, and high molecular weight kininogen (HMWK) are involved in this pathway of activation. Thus this pathway provides a further of the interrelationship between the various enzyme cascade systems in plasma. The first step is the binding of Hageman factor to a sub-endothelial surface exposed by an injury. A complex of prekallikrein and HMWK also interacts with the exposed surface in close proximity to the bound factor XII, which becomes activated. During activation, the single chain protein of the native Hageman factor is cleaved into two chains (50 and 28 kDa), that remain linked by a disulphide bond. The light chain (28kDa) contains the active site and the molecule is referred to as activated Hageman factor (factor XIa). There is evidence that the Hageman factor can autoactivate, thus the pathway is self-amplifying once triggered (compare with the alternative pathway of complement).

Activated Hageman factor in turn activates prekallikrein. The kallikrein produced can then also cleave factor XII, and a further amplification mechanism is triggered. The activated factor XII remains in close contact with the activating surface, such that it can activate factor XI, the next step in the intrinsic pathway which, to proceed efficiently, requires Ca^{2+}. Also involved at this stage is HMWK, which binds to factor XI and facilitates the activation process. Activated factors XIa, XIIa, and kallikrein are all serine proteases, like many of the enzymes of the complement system.

Eventually the intrinsic pathway activates factor X, a process that can also be brought about by the extrinsic pathway. Factor X is the first molecule of the common pathway and is activated by a complex of molecules containing activated factor IX, factor VIII, calcium, and phospholipid which is provided by the platelet surface, where this reaction usually takes place. The precise role of factor VIII in this reaction is not clearly understood. Its presence in the complex is obviously essential, as evidenced by the serious consequences of factor VIII deficiency experienced by haemophiliacs. Factor VIII is mod-
Figure 9.3: The intrinsic, extrinsic, and common pathways of the coagulation (clotting) cascade
ified by thrombin, a reaction that results in greatly enhanced factor VIII activity, promoting the activation of factor X.

The extrinsic pathway is an alternative route for the activation of the clotting cascade. It provides a very rapid response to tissue injury, generating activated factor X almost instantaneously, compared to the seconds or even minutes required for the intrinsic pathway to activate factor X. The main function of the extrinsic pathway is to augment the activity of the intrinsic pathway.

There are two components unique to the extrinsic pathway, tissue factor or factor III, and factor VII. Tissue factor is present in most human cells bound to the cell membrane. The activation process for tissue factor is not entirely clear. Once activated, tissue factor binds rapidly to factor VII which is then activated to form a complex of tissue factor, activated factor VII, calcium, and a phospholipid, and this complex then rapidly activates factor X.

The intrinsic and extrinsic systems converge at factor X to a single common pathway which is ultimately responsible for the production of thrombin (factor IIa).

Clot formation. The end result of the clotting pathway is the production of thrombin for the conversion of fibrinogen to fibrin. Fibrinogen is a dimer soluble in plasma. Exposure of fibrinogen to thrombin results in rapid proteolysis of fibrinogen and the release of fibrinopeptide A. The loss of small peptide A is not sufficient to render the resulting fibrin molecule insoluble, a process that is required for clot formation, but it tends to form complexes with adjacent fibrin and fibrinogen molecules. A second peptide, fibrinopeptide B, is then cleaved by thrombin, and the fibrin monomers formed by this second proteolytic cleavage polymerize spontaneously to form an insoluble gel. The polymerized fibrin, held together by noncovalent and electrostatic forces, is stabilized by the transamidating enzyme factor XIIIa, produced by the action of thrombin on factor XIII. These insoluble fibrin aggregates (clots), together with aggregated platelets (thrombi), block the damaged blood vessel and prevent further bleeding.

There is an interrelationships between the coagulation pathway and other plasma enzyme systems. Contact activation of the coagulation pathway, in addition to promoting blood clotting, results in the generation of plasminogen activator activity, which is involved in fibrinolysis or clot removal. Activated Hageman factor and its peptides can also initiate the formation of kallikrein from plasma prekallikrein, and this triggers the release of bradykinin from kininogens in the plasma. Kinins are responsible for dilating small blood vessels, inducing a fall in blood pressure, triggering smooth muscle contraction, and increasing the permeability of vessel walls. In addition, activation of the coagulation pathway produces a vascular permeability factor, as well as chemotactic peptides for professional phagocytes.

9.1.4.5 Fibrinolysis

Once haemostasis is restored and the tissue is repaired, the clot or thrombus must be removed from the injured tissue. This is achieved by fibrinolytic pathway. The end product of this pathway is the enzyme plasmin, a potent proteolytic enzyme with a broad spectrum of activity. Plasmin is formed by activation of the proenzyme, plasminogen by either plasma or tissue activators. Tissue plasminogen activators are found in most tissues, except the liver and the placenta, where they are synthesized by endothelial cells and are found concentrated in the walls of blood vessels. The two best characterized are vascular activator (commonly known as tissue plasminogen activator – tPA) and urokinase. There is great interest in using tPA as a therapeutic agent for dissolving blood clots: the gene for tPA has now been cloned and the expressed gene product is available for clinical trials. Plasminogen activator is also a product of macrophages. The level of tissue activator in the plasma is normally low, but can be increased by exercise and stress.

Two forms of plasminogen are present in the plasma: one has a glutamic acid at the N-terminal of the polypeptide chain, and is called native or gluplasminogen, and the other a lysine. The latter form arise as a result of partial degradation of the parent molecule by autolysis.

Triggering of fibrinolysis occur when the plasminogen activator, plasminogen, and fibrin are all in close proximity. Both plasminogen and its activator bind avidly to fibrin as the clot forms. This close association prevents inhibition of plasmin activity by inhibitor, and allows proteolysis of the fibrin to proceed after the production of lys-plasminogen. Plasmin inhibitors (antiplasmins) which can control plasmin activity include: α1-antitrypsin, α2-antiplasmin, C1 inhibitor, antithrombin III.
Plasmin attacks fibrin at a number of different sites, at least 50, reducing its size such that it no longer has haemostatic activity. Many fragments are formed during this process, and some retain the capacity to polymerize, thus some of the early degradation products can compete with fibrinogen for thrombin and act as inhibitors of clot formation. This may prevent the clot being removed before the tissue is repaired.

9.1.4.6 The kinin-forming system

The kinins, bradykinin and lysylbradykinin, are important mediators of inflammatory responses. They are liberated from precursor molecules, kininogens, by the action of various proteases, collectively known as kininogenases. Three types of kininogen have been identified: high- and low-molecular weight kininogen (HMWK and LMWK respectively), and T-kininogen. These molecules are synthesized by hepatocytes and are released into the plasma, where in addition to releasing kinins, they function as (i) cofactors in the coagulation pathway; (ii) inhibitors of cysteine protease enzymes; and (iii) part of the acute phase response. The kinins are potent vasoactive basic peptides and their properties are wide ranging, including the ability to increase vascular permeability, cause vasodilation, pain, and the contraction of smooth muscle, and to stimulate arachidonic acid metabolism.

Three different pathways may lead to kinin formation during inflammation: (i) the generation of bradykinin as a result of activation of the Hageman factor and the production of plasma kallikrein; (ii) the production of lysylbradykinin by tissue kallikreins; and (iii) the action of cellular proteases in kinin formation.

The mechanism of bradykinin formation in plasma and in tissues is summarized in Figure 9.4.

In brief, HMWK and prekallikrein circulate in plasma as a 1:1 stoichiometric complex. This complex, together with the Hageman factor, binds to negatively charged surface or collagen. Once they are exposed by tissue damage, the Hageman factor is activated, prekallikrein is converted to kallikrein, and HMWK itself is digested to release bradykinin, a nine amino acid peptide.

As bradykinin is such a potent vasoactive peptide, its activity and its formation must be carefully controlled. Activation of the pathway is controlled internally by the presence of inhibitors for each of the active components. C1 inhibitor controls the activity of the activated Hageman factor, while α2-macroglobulin and C1 inhibitor act as kallikrein inhibitors. There are a variety of enzymes in plasma that control bradykinin activity, including carboxypeptidase N, which removes the C-terminal arginine residue, thus inactivating the peptide.

Kalikrein also act directly on the complement pathway with direct cleavage of the chemotactically active peptide C5a from the complement component C5. Cleavage of fibrinogen by plasmin results in a number of products including fibrinopeptide B, which potentiates the action of bradykinin and has also chemotactic activity for phagocytic cells.

9.1.4.7 Cytokines mediating inflammatory and effector functions

Cytokines are soluble (glyco)proteins, nonimmunoglobulin in nature, released by living cells of the host, which act nonenzymatically in picomolar to nanomolar concentrations through specific receptors to regulate host cell function. Cytokines make up the fourth major class of soluble intercellular signaling molecules, alongside neurotransmitters, endocrine hormones, and autacoids. They possess typical hormonal activities:

1. they are secreted by a single cell type, react specifically with other cell types (target cells) and regulate specific vital functions that are controlled by feedback mechanisms;

2. they generally act at short range in a paracrine or autocrine (rather than endocrine) manner;

3. they interact first with high-affinity cell surface receptors (distinct for each type or even subtype) and then regulate the transcription of a number of cellular genes by little understood second signals. This altered transcription (which can be an enhancement or inhibition) result in changes in cell behaviour.

Target cells, on which cytokines transform their information signal, may be localized in any body compartment (sometimes a long distance from the site of secretion). Other type of these molecules act mostly on neighbouring cells in the microenvironment where they have been released. These are characterized as
local hormones and their secretion is brought about by autocrine (only the cell or organ of secretion is affected) or paracrine mechanisms. During the paracrine secretion some cytokines may escape cell binding and may spill over into general circulation via lymph or plasma. This is important, especially for the products of lymphoid cells, which are mobile after having picked up the message in the microenvironment throughout the body and therefore their immunoregulatory products, (lymphokines, monokines, interleukins and other cytokines), despite being of local hormone character, may act in fact systemically.

Cytokines are synthesized, stored and transported by various cell types not only inside of the immune system (lymphokines, interleukins, monokines, tumour necrosis factors, interferons) but also by other cells which are mainly studied in haematology (colony-stimulating factors), oncology (transforming

Figure 9.4: The kinin pathway forming bradykinin
growth factors), and cell biology (peptide growth factors, heat shock and other stress proteins). The main types of cytokines are listed in Table 9.9.

**Lymphokines** are cytokines secreted mainly by activated T H lymphocytes and the term **monokines** refers to analogous immunoregulators produced by activated macrophages and monocytes. In order to unify the terminology of these factors, the term **interleukin** was accepted. Besides the term expressing their origin, cytokines may be also named according to their function, as are **interferons**, growth and differentiation factors, colony-stimulating factors, etc.

The central role of cytokines is to control the direction, amplitude, and duration of immune responses and to control the (re)modeling of tissues, be it developmentally programmed, constitutive, or unscheduled. Unscheduled remodeling is that which accompanies inflammation, infection, wounding, and repair. Individual cytokines can have pleiotropic (multiple), overlapping and sometimes contradictory functions depending on their concentration, the cell type they are acting on, and the presence of other cytokines and mediators. Thus the information which an individual cytokine conveys depend on the pattern of regulators to which a cell is exposed, and not on one single cytokine. It is supposed that all cytokines form the specific system or network of communication signals between cells of the immune system, and between the immune system and other organs. In this inter-cell signalling network, the signal is usually transferred by means of a special set of cytokines.

Because of the potent and profound biological effects of cytokines, it is not surprising that their activities are tightly regulated, most notably at the levels of secretion and receptor expression. Additional regulatory mechanisms are provided by the concomitant action of different cytokines and the presence in biological fluids of specific inhibitory proteins, soluble cytokine-binding factors and specific autoantibodies.

The cytokine system is a very potent force in homeostasis when activation of the network is local and cytokines act vicinally in surface-bound or diffusible form, but when cytokine production is sustained and/or systemic, there is no doubt that cytokines contribute to the signs, symptoms, and pathology of inflammatory, infectious, autoimmune, and malignant diseases. **TNF-α** is an excellent example of such dual action. Locally it has important regulatory and antitumour activities but when TNF-α circulates in higher concentrations beyond the organ of origin, it may be involved in the pathogenesis of endotoxic shock, cachexia and other serious diseases.

From the point of inflammation view there are two main groups of cytokines: proinflammatory and anti-inflammatory (Table 9.10). **Proinflammatory cytokines** are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions. **Anti-inflammatory cytokines** belong to the T cell-derived cytokines and are involved in the down-regulation of inflammatory reactions.

The central role in inflammatory responses have **IL-1** and **TNF-α**, because the administration of their antagonists, such as IL-1ra (IL-1 receptor antagonist), soluble fragment of IL-1 receptor, or monoclonal antibodies to TNF-α and soluble TNF receptor, all block various acute and chronic responses in animal models of inflammatory diseases. Some of these antagonists are beginning to utilize as anti-inflammatory agents in diseases such as sepsis and rheumatoid arthritis. IL-1 and TNF-α together with IL-6 serve as **endogenous pyrogens**. The up-regulation of inflammatory reaction is also performed by IL-11, IFN-α, IFN-β, and especially by the members of chemokine superfamily. On the other hand, anti-inflammatory cytokines (IL-4, IL-10, IL-13) are responsible for the down-regulation of inflammatory responses. They are able to suppress the production of proinflammatory cytokines. Their strong anti-inflammatory activity suggest possible utilization in management of many inflammatory diseases, including sepsis, rheumatoid arthritis, inflammatory bowel disease, psoriasis, T cell-mediated autoimmune diseases such as type I diabetes, as well as in acute graft-versus-host disease. IL-10 is capable of effectively protecting mice from endotoxin-induced shock, a lethal inflammatory reaction mediated by TNF-α and IL-1. The production of most lymphokines and monokines such as IL-1, IL-6 and TNF-α is also inhibited by **transforming growth factor β** (TGF-β). But, on the other hand, TGF-β has a number of proinflammatory activities including chemoattractant effects on neutrophils, T lymphocytes, and unactivated monocytes. TGF-β has been demonstrated to have in vivo immunosuppressive and anti-inflammatory effects as well as proinflammatory and selected immunoenhancing activities.
<table>
<thead>
<tr>
<th>Cytokine types</th>
<th>Individual cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphokines</td>
<td>MAF (macrophage inhibition factor), MMIF (macrophage migration inhibition factor), MCF (macrophage chemotactic factor), LMIF (leukocyte migration inhibition factor), HRFs (histamine releasing factors), TF (transfer factor)</td>
</tr>
<tr>
<td>Interleukins</td>
<td>IL-1, IL-2, ..., IL-15</td>
</tr>
<tr>
<td>Tumour necrosis factors</td>
<td>TNF-α (cachectin), TNF-β (lymphotoxin)</td>
</tr>
<tr>
<td>Interferons</td>
<td>IFN-α, IFN-β, IFN-γ, IFN-ω, IF-τ</td>
</tr>
<tr>
<td>Colony stimulating factors</td>
<td>G-CSF (granulocyte colony stimulating factor), GM-CSF (granulocyte-macrophage CSF), M-CSF (macrophage CSF), multi-CSF (IL-3)</td>
</tr>
<tr>
<td>Polypeptide growth factors</td>
<td>aFGF (acidic fibroblast growth factor), bFGF (basic fibroblast growth factor), EGF (epidermal growth factor), NGF (nerve growth factor), PDGF (platelet-derived growth factor), VEGF (vascular endothelial growth factor)</td>
</tr>
<tr>
<td>Transforming growth factors</td>
<td>TGF-α, TGF-β</td>
</tr>
<tr>
<td>α-Chemokines</td>
<td>IL-8, NAP-2 (neutrophil-activating protein 2), PF-4 (platelet factor 4), βTG (β-thromboglobulin)</td>
</tr>
<tr>
<td>β-Chemokines</td>
<td>MCP-1 (monocyte chemoattractant protein 1), MCP-3, MIP-1α, MIP-1β (macrophage inflammatory protein 1/β), RANTES</td>
</tr>
<tr>
<td>Stress proteins</td>
<td>HSPs (heat shock proteins), GRPs (glucose-regulated proteins), ubiquitin, superoxide dismutase (Mn)</td>
</tr>
</tbody>
</table>

RANTES: Regulated upon Activation Normal T Expressed and presumably Secreted chemokine

Table 9.9: Main types of cytokines

When administered systemically, TGF-β acts as an inhibitor, but if given locally can promote inflammation. Generally, TGF-β stimulates neovascularization and the proliferation and activities of connective tissue cells and is a pivotal factor in scar formation and wound healing. But TGF-β has antiproliferative effects on most other cell types including epithelial cells, endothelial cells, smooth muscle cells, fetal hepatocytes, and myeloid, erythroid, and lymphoid cells. TGF-β is a potent immunosuppressive cytokine that suppresses cell-mediated as well as humoral immunity (including tumour immunity).
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<table>
<thead>
<tr>
<th>Group</th>
<th>Individual cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proinflammatory cytokines</strong></td>
<td></td>
</tr>
<tr>
<td>Endogenous pyrogens</td>
<td>IL-1, TNF-α, IL-6</td>
</tr>
<tr>
<td>Up-regulation</td>
<td>IL-1, TNF-α, IL-6, IFN-α, IFN-β, chemokines</td>
</tr>
<tr>
<td>Stimulation of the production of acute phase reactants</td>
<td>IL-1, IL-6, IL-11, TNF-α, INF-γ, TGF-β, LIF, OSM, CNTF</td>
</tr>
<tr>
<td><strong>Chemoattractant cytokines</strong></td>
<td></td>
</tr>
<tr>
<td>CXC chemokines</td>
<td>IL-8, PF-4, PBP, NAP-2, β-TG</td>
</tr>
<tr>
<td>CC chemokines</td>
<td>MIP-1α, MIP-1β, MCP-1, MCP-2, MCP-3, RANTES</td>
</tr>
<tr>
<td>C chemokines</td>
<td>Lymphotactin</td>
</tr>
<tr>
<td>Stimulation of proinflammatory cytokines</td>
<td>IL-12</td>
</tr>
<tr>
<td><strong>Anti-inflammatory cytokines</strong></td>
<td></td>
</tr>
<tr>
<td>Inhibition of production of proinflammatory cytokines</td>
<td>IL-4, IL-10, IL-13</td>
</tr>
</tbody>
</table>

TGF-β: transforming growth factor; LIF: leukemia inhibitory factor; OSM: oncostatin M; CNTF: ciliary neurotrophic factor; PF-4: platelet factor 4; PBP: platelet basic protein; NAP-2: neutrophil activating protein 2; β-TG: β-thromboglobulin; MIP: macrophage inflammatory protein; MCP: monocyte chemoattractant protein; RANTES: Regulated upon Activation Normal T Expressed and presumably Secreted chemokine

Table 9.10: Cytokines involved in inflammatory reactions

9.1.4.7.1 Chemokines Chemokines (a shortening of chemoattractant cytokines) represent a superfamily of about 30 chemotactic cytokines acting as vital initiators and promulgators of inflammatory reactions. They range from 8 to 11 kD in molecular weight, are active over a 1 to 100 ng/ml concentration range, and are produced by a wide variety of cell types. The production of chemokines is induced by exogenous irritants and endogenous mediators such as IL-1, TNF-α, PDGF, and IFN-γ. The chemokines bind to specific cell surface receptors and can be considered second-order cytokines that appear to be less pleiotropic than first-order proinflammatory cytokines because they are not potent inducers of other cytokines and exhibit more specialized functions in inflammation and repair.

The chemokine molecules share structural similarities, including four conserved cysteine residues which form disulphide bonds in the tertiary structure of the proteins. Traditionally, the chemokine superfamily has been divided into two subgroups: C-X-C (where X is any amino acid) and C-C, according to whether an intervening residue spaces the first two cysteines. This structural distinction has been shown to delineate a general, though not absolute, distinction in the biological properties of these molecules: most C-X-C chemokines are chemoattractants for neutrophils (and to some extent lymphocytes) but
not monocytes, whereas **C-C chemokines** appear to attract monocytes, basophils, eosinophils, and lymphocytes (including NK cells) but not neutrophils. Recently, the third “C” branch of these molecules has been discovered. **Lymphotactin**, the representative of these **C chemokines**, is clearly chemotactic for lymphocytes and NK cells, but it does not attract either monocytes or neutrophils. The list of main chemokines is in the Table 9.10 and 9.11.

Not all of the known properties of the chemokines involve leukocyte migration. For example chemokines have been reported to have roles in haematopoietic precursor cell cycling regulation and differentiation. On the other hand, their involvement in such processes as leukocyte trafficking and inflammatory processes further suggest that the chemokines are important in a number of disease states. It is now clear that certain C-C chemokines, namely RANTES, MCP-1, MCP-3 and MIP-1 exhibit potent promigratory and activating potentials for eosinophils, basophils and T cells, the cells most often associated with respiratory pathologies and allergic disorders, including asthma and nasal polyposis. These observations are now being coupled with an emerging body of evidence showing that these mediators can be localized to affected tissues during these pathologies.

**IL-8** and MCP-1 are more widely produced than other chemokines and there is a suggestion that they represent the first line of defence.

**Interleukin-8** is a polypeptide consisting of 72 amino acids in its mature form. The biological profile of activity of IL-8 is very similar to that of the classical chemotactic peptides C5a and FMLP (N-formylmethionyl-leucyl-phenylalanine). It is able to induce the full pattern of responses observed in chemotactically stimulated neutrophils, i.e. activation of the motile apparatus and directional migration, expression of surface adhesion molecules, release of lysosomal enzymes, and production of reactive oxygen intermediates. IL-8 is not species-specific and is a potent angiogenic factor.

Recently, elevated urinary IL-8 levels were observed in patients with several types of glomerulonephritis including IgA nephropathy, acute glomerulonephritis, purpura nephritis, membranous proliferative glomerulonephritis, and lupus nephritis, but not in patients with focal glomerulosclerosis and membranous nephropathy. The former groups are characterized pathologically by the infiltration of neutrophils and/or mononuclear cells and proliferation of mesangial cells, whereas the latter lacks such findings. Immunohistochemical analyses demonstrated the detection of IL-8 protein in inflammatory cells infiltrated into glomeruli in IgA nephropathy, suggesting that IL-8, produced in glomeruli, promotes the infiltration of neutrophils into glomeruli, thereby inducing renal injury.

**Monocyte chemoattractant protein** – 1 (MCP-1) is a chemoattractant for human monocytes with the optimal agonist concentration of $10^{-9}$ mol/L and may play a role in the accumulation of monocytes over a period of 24-48 hours after interaction of antigen and sensitized lymphocytes. MCP-1 is nearly as effective as C5a, and much more potent than IL-8, in the degranulation of basophils, resulting in histamine release. This may play an important role in the pathogenesis of the late phase of allergic disorders such as atopic food allergies, asthma, and chronic urticaria. Histamine release also occurs after stimulation with two other C-C chemokines, RANTES and MIP-1α.

### 9.1.4.8 Chemotactic factors

The term chemotaxis refers to the movement of leukocytes (or cells in general), induced by a chemotactic stimulus. Besides chemotaxis (stimulated, directed migration), leukocytes also possess two other types of movement: random migration (undirected, spontaneous migration) and chemokinesis (stimulated, undirected migration). A chemotactic stimulus is provided by substances that can either attract or repulse the cells. Thus, chemotactic cell movement can be either positive or negative, i.e. the cells may move towards the source of chemotactic substances (towards an increasing concentration gradient) or in the opposite direction. The positive movement is typical for leukocytes. Substances possessing chemotactic activity are called **chemotactic factors** (chemotaxins, chemoattractants).

Leukocyte chemotaxis (leukotaxis) is mainly responsible for their mobilization at the inflammatory site. Both exogenous and endogenous chemotactic factors are involved (Table 9.11). **Exogenous chemotaxins** include bacterial oligopeptides of the FMLP type, lectins, denatured proteins, some lipids and lipopolysaccharides. **Endogenous chemotaxins** are produced by the host organism and are of **humoral** (complement fragment C5a, C5(desArg and
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<table>
<thead>
<tr>
<th>Chemoattractant</th>
<th>Ne</th>
<th>Mo/Ma</th>
<th>Eo</th>
<th>Ba</th>
<th>Ly</th>
<th>NK</th>
</tr>
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<tbody>
<tr>
<td><strong>Exogenous</strong></td>
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<td></td>
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<tr>
<td>FMLP</td>
<td>+</td>
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<td>–</td>
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<tr>
<td><strong>Endogenous</strong></td>
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<tr>
<td>C5a</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LTB₄, PAF</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PDGF</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
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<td>+</td>
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</tr>
<tr>
<td>TGF-β</td>
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<td>±</td>
<td>–</td>
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<tr>
<td><strong>Chemokines CXC</strong></td>
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<tr>
<td>IL-8, NAP-2</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>±</td>
<td>–</td>
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<tr>
<td>MCP-1</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>MCP-3, MIP-1, RANTES</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>Chemokines C</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lymphotactin</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

Ne: neutrophils; Mo: monocytes; Ma: macrophages; Eo: eosinophils; Ba: basophils; Ly: lymphocytes (T cells); NK: NK cells; FMLP: N-formylmethionyl-leucyl-phenylalanine

Table 9.11: The main chemotactic factors for leukocytes

Ba, fibrinopeptides, kallikrein and plasminogen activator) or cellular type (from different cells – LTB₄, PAF, chemotactic cytokines etc.)

Interaction between the chemotactic factor and its corresponding receptor triggers a series of coordinated biochemical events which include changes in the cell transmembrane potential, altered cyclic nucleotide levels and ion flow across the cytoplasmic membrane and increased glucose utilization and oxygen consumption. The composition of membrane phospholipids is altered and arachidonic acid, released by phospholipases, is metabolized into a number of biologically active intermediates and products. Within a few minutes, the leukocyte changes from a round to a triangular shape that is oriented along the direction of chemotactic gradient. Reorganization of cytoskeletal contractile elements, particularly actin microfilaments and microtubular structures, contributes to this shape change. Activation of the contractile cell system not only results in migration but also in other form of movement such as enhanced adherence, spreading, endocytosis and secretion of lysosomal enzymes.

9.1.4.9 The acute phase reactants

9.1.4.9 The acute phase reactants

Within the spectrum of systemic reaction to inflammation two physiological responses in particular are
regarded as being associated with **acute inflammation**. The first involves the alteration of the temperature set-point in the hypothalamus and the generation of the febrile response. The second involves alterations in metabolism and gene regulation in the liver. Three cytokines that are released from the site of tissue injury – IL-1, TNF-α and IL-6 are considered to regulate the febrile response, possibly as a protective mechanism. These cytokines mediate fever through the induction of PGE$_2$. At the same time, IL-1 and IL-6 can act on the adrenal pituitary axis to generate adrenocorticotropic hormone (ACTH) and, subsequently, induce the production of cortisol. This provides a negative feedback loop, since corticosteroids inhibit cytokine gene-expression.

It is important to consider the acute phase response (and inflammation) as a dynamic homeostatic process that involves all of the major systems of the body, in addition to the immune, cardiovascular and central nervous system. Normally, the acute phase response lasts only a few days; however, in cases of chronic or recurring inflammation, an aberrant continuation of some aspects of the acute phase response may contribute to the underlying tissue damage that accompanies the disease, and may also lead to further complications, for example cardiovascular diseases or protein deposition diseases such as reactive amyloidosis.

The second important aspect of the acute phase response is the radically altered biosynthetic profile of the liver. Under normal circumstances, the liver synthesizes a characteristic range of plasma proteins at steady state concentrations. Many of these proteins have important functions and higher plasma levels of these **acute phase reactants** (APRs) or **acute phase proteins** (APPs) are required during the acute phase response following an inflammatory stimulus. Although most APRs are synthesized by hepatocytes, some are produced by other cell types, including monocytes, endothelial cells, fibroblasts and adipocytes. Most APRs are induced between 50% and several-fold over normal levels. In contrast, the **major APRs** can increase to 1000-fold over normal levels. This group includes serum amyloid A (SAA) and either C-reactive protein (CRP) or serum amyloid P component (SAP) depending on the species. Ironically, of all the APRs, the activities of these three are among the least well-known. Nevertheless, their interactions with other well-defined defence systems and the magnitude and rapidity of their induction following an acute phase stimulus, together with their short half-lives, suggest a particularly critical requirement for these proteins very early in the establishment of host defence. Significantly, individuals unable to synthesize these proteins have not been described; these major APRs are therefore likely to be of considerable clinical importance.

**CRP** and **SAP** are pentraxins, proteins with a characteristic pentameric organization of identical subunits arranged as single and double annular pentagonal discs, respectively. Generally, only one of these proteins is an APR in a given mammalian species: in humans, normal plasma SAP levels are approximately 30 mg.L$^{-1}$ and remain constant during inflammation but CRP levels can increase up to 1000-fold from approximately 1 mg.L$^{-1}$, depending on the disease and its severity. CRP was originally
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<table>
<thead>
<tr>
<th>Group</th>
<th>Individual proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive APRs</td>
<td>Serum amyloid A, C-reactive protein, serum amyloid P component</td>
</tr>
<tr>
<td>Major APRs</td>
<td>C2, C3, C4, C5, C9, B, C1 inhibitor, C4 binding protein</td>
</tr>
<tr>
<td>Complement proteins</td>
<td>Fibrinogen, von Willebrand factor</td>
</tr>
<tr>
<td>Coagulation proteins</td>
<td>α1-Antitrypsin, α1-antichymotrypsin, α2-antiplasmin, heparin cofactor II, plasminogen activator inhibitor I</td>
</tr>
<tr>
<td>Proteinase inhibitors</td>
<td>Haptoglobin, haemopexin, ceruloplasmin, manganese superoxide dismutase</td>
</tr>
<tr>
<td>Metal-binding proteins</td>
<td>α1-Acid glycoprotein, haeme oxygenase, mannose-binding protein, leucocyte protein I, lipoprotein (a), lipopolysaccharide-binding protein</td>
</tr>
<tr>
<td>Other proteins</td>
<td>Albumin, pre-albumin, transferin, apoAI, apoAII, α2-HS glycoprotein, inter-α-trypsin inhibitor, histidine-rich glycoprotein</td>
</tr>
</tbody>
</table>

Table 9.12: Acute phase reactants

SAA is the collective name given to a family of polymorphic proteins encoded by multiple genes in a number of mammalian species. Functionally, SAAs are small apolipoproteins that associate rapidly during the acute phase response with the third fraction of high-density lipoprotein (HDL3), on which they become the predominant apolipoprotein. SAA enhances the binding of HDL3 to macrophages during inflammation, concomitant with a decrease in the binding capacity of HDL3 to hepatocytes. It suggest that SAA may remodel HDL3 and act as a signal to redirect it from hepatocytes to macrophages, which can then engulf cholesterol and lipid debris at sites of necrosis. Excess cholesterol could thus be redistributed for use in tissue repair or excreted. Other putative protective roles for SAA are the inhibition of thrombin-induced platelet activation, as well as inhibition of the oxidative burst in neutrophils, which would help prevent oxidative tissue destruction.

The exquisite responsiveness of CRP to acute phase stimuli, along with its wide concentration range and ease of measurement, have led to plasma CRP levels being used to monitor accurately the severity of inflammation and the efficacy of disease management during an infection. Conversely, some diseases (e.g. systemic lupus erythematosus) are associated with relatively low plasma levels of CRP.

SAA and SAP are archetypal examples of plasma proteins that are likely to be beneficial during the transient acute phase response but which have detrimental effects in chronic inflammation. These major APRs have been implicated in a number of clinical conditions. Secondary, or reactive amyloidosis is the occasional consequence of a variety of chronic and recurrent inflammatory diseases, for example leprosy, tuberculosis, systemic lupus erythematosus and rheumatoid arthritis. It is characterized by the ultimately fatal deposition of insoluble fibrils in a number of tissues, including spleen, liver and kidney. Secondary amyloid deposits are composed mainly of amyloid A derived (probably by proteolysis) from the...
precursor SAA. Amyloid P component (AP), derived from SAP, is associated with secondary AA plaques and all other forms of amyloid deposits, including those present in Alzheimer’s disease. AP has also been shown to act as an elastase inhibitor, which suggests a role for SAP on amyloid deposit in protecting the fibrils from degradation by proteolytic enzymes.

APR synthesis is under control performed by inflammatory mediators from which several cytokines and hormones specifically regulate the transcription of human APRs (Figure 9.5). These include TNF-α, IL-1, IL-6, IL-11, IFN-γ, LIF, OSM, CNTF, TGF-β, and glucocorticoids. In addition, insulin and akadai acid have recently been shown to act as inhibitors of the cytokine-driven induction of some APRs. There is considerable heterogeneity in the response of individual APR genes to the listed cytokines. An important feature of the acute phase response is that IL-1 and TNF-α stimulate, via the CNS, the synthesis of glucocorticoids by the adrenal glands, which results in co-operative enhancement of the IL-1 and TNF-α-mediated induction of APR synthesis in the liver. This effect is coincident with the glucocorticoid-mediated downregulation of IL-1 synthesis by macrophages, thereby creating a negative-feedback loop between the immune and CNS systems to reduce de novo cytokine synthesis. Most of the increase (or decrease, in the case of negative APRs) in APR biosynthesis is due to increased (or decreased) gene transcription.

9.1.5 Molecular mechanisms of the acute cell-mediated inflammatory reaction

The accumulation of leukocytes in inflamed tissue results from adhesive interactions between leukocytes and endothelial cells within the microcirculation. These adhesive interactions and the excessive filtration of fluid and protein that accompanies an inflammation play a role in SAP on amyloid deposit in protecting the fibrils from degradation by proteolytic enzymes.

9.1.5.1 Adhesion molecules

During an inflammatory response adhesion molecules serve to enhance pairing between many less avid receptors and their ligands and transmit signals that direct specific effector functions. At least four superfamilies of adhesion molecules participate in these events: the selectins, the integrins, certain members of the immunoglobuline superfamily and cadherins.

The selectin family is composed of three members named according to the cells in which they were originally discovered. L-selectin (CD62L) is constitutively expressed on leukocytes and its target cells are activated endothelial cells. E-selectin (CD62E) is produced exclusively by endothelial cells after cytokine activation and its counter-receptors are on neutrophils, monocytes, eosinophils, lymphocyte subsets and some tumour cells. P-selectin (CD62P) is preformed and stored for rapid release in the granules of platelets or the Weibel-Palade bodies of endothelial cells but in the latter P-selectin is expressed only after cytokine activation. Its target cells are the same as those for E-selectin. Each selectin receptor molecule contains a lectin-like N-terminal domain, followed by an epidermal growth factor–like motif, a series of consensus repeats similar to those in complement-regulatory proteins, a transmembrane domain, and a cytoplasmic tail. The lectin domain is directly involved in mediating cell-cell contact through Ca$^{2+}$-dependent interactions with cell-surface carbohydrates, particularly the sialylated Lewis X antigen (sLe$^x$).

The integrins are a large family of heterodimeric glycoproteins which can be subdivided according to the particular β subunit they possess, which is shared by all members of the group. On this basis, this family can be subdivided into the β1, β2, and β3
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Abbreviations: + stimulation of activity; – inhibition of activity; E: enhancement of activity; LIF: leukemia inhibitory factor; OSM: oncostatin M; CNTF: ciliary neurotrophic factor.

Figure 9.5: Inflammatory mediators that modulate hepatic APR synthesis in humans

integrins. The β2 integrins are expressed particularly by leukocytes, giving rise to their alternative name, the leukocyte integrins, whereas the others, in general, are more widely distributed. The β2 leukocyte integrins are represented by three heterodimeric molecules: LFA-1 (CD11a/CD18), CR3 (DC11b/CD18), and CR4 (CD11c/CD18). Each of them contains the same 95 kDa β2 integrin subunit, also designated CD18 and different α chains designated CD11a (180 kDa), CD11b (165 kDa), and CD11c (150 kDa). The intact CD11a/CD18 molecule is the lymphocyte function-associated antigen-1, LFA-1 and is expressed by lymphocytes (including T cells), myeloid cells (monocytes, macrophages, and granulocytes), and a variety of other cell type. CD11b/CD18 is the complement receptor type 3, CR3, and CD11c/CD18 is CR4 (also called p150,95). Both CR3 and CR4 are expressed by myeloid cells.

There are two or more ligands for LFA-1, those defined to date being ICAM-1 (CD54) and ICAM-2 (CD102), which are members of the immunoglobulin superfamily. CR3 can bind fragments of complement components, particularly iC3b, and it mediates phagocytosis of complement-coated particles by professional phagocytes.

Alongside with intercellular adhesion molecules ICAM-1 and ICAM-2 there is additional member of the diverse immunoglobulin superfamily - platelet-endothelial cell adhesion molecule, PECAM-1 (CD31). Both ICAM-1 and ICAM-2
are expressed by endothelial cells. PECAM-1 has been identified on neutrophils, monocytes, platelets and is present in large amounts on endothelial cells (about $10^6$ copies per cell), where it is concentrated at cell-cell junction.

The main adhesion molecules participating in the interactions between neutrophils and endothelial cells of postcapillary venules are shown in Table 9.13).

### 9.1.5.2 Leukocyte mobility and chemotaxis

The emigration of circulating leukocytes from the blood into inflamed tissues have been refined into a "three step" process comprising: (a) rolling of leukocytes along the vasculature (mediated through transient interactions between selectin proteins and their carbohydrate ligands), followed by (b) activation of both neutrophils and endothelial cells and a high affinity interaction between $\beta_2$ integrins and glyco-proteins of immunoglobulin superfamily, leading ultimately to (c) extravasation (crawling along the endothelium, diapedesis, and migration into tissue) in response to a chemoattractant gradient (Figure 9.6).

Rolling leukocytes are generally defined as white cells that move through microvessels at a rate that is lower than that of red blood cells. In $30 \mu m$ diameter postcapillary venules, the red blood cell velocity is usually 1-3 mm/s, whereas leukocytes roll at velocities ranging between 5 and 300 $\mu m/s$, with the most frequently observed rolling velocities lying between 20 and 60 $\mu m/s$. Rolling leukocytes are not always committed to either firmly adhering to the vessel wall or rolling along the entire vessel length; rolling leukocytes frequently detach and return to the mainstream of flowing blood. Leukocyte rolling is likely to occur also under normal physiological conditions in all tissues (gastrointestinal mucosa, skin, lung) that are continually exposed to external inflammatory stimuli that are physical and/or chemical in nature.

In inflamed tissue, leukocyte rolling frequently (but not always) leads to a stationary state in which the leukocyte remains firmly attacked to the endothelial cell surface, without rotation motion. This strong (high-affinity) adhesive interaction is often referred to as leukocyte sticking, firm adhesion, or adherence, terms that denote the absence of movement of the leukocyte along the length of the venuile.

In the initial phase of an acute inflammatory response, circulating leukocytes are activated by exposure to inflammatory mediators including complement fragments (C5a), cytokines such as IL-1, IL-8 and TNF-α, and lipopolysaccharide or classical chemoattractants such as formylated methionine – leucine - phenylalanine (FMLP) leading to their microvascular sequestration due in part to decreased deformability (i.e. increased cell stiffness) and in part to increased adhesiveness of the circulating leukocytes. Endothelial cells are similiarly activated, leading to enhanced expression of several adhesion molecules. Platelet activating factor (PAF) produced by endothelial cells may act on nearby neutrophils to potentiate their adhesion to the endothelium.

Transmigration of neutrophils across the endothelial barrier involves interaction between leukocyte integrins and endothelial ICAM, and between glycosylated aminoglycans on the neutrophil plasma membrane and PECAM-1, which is localized in the intercellular junctions of endothelial cells. In extravascular locations, interaction between extracellular matrix proteins and adhesion molecules, possibly by activation of cytosolic tyrosine kinases, facilitate the release of large quantities of toxic oxygen radicals and proteolytic enzymes.

### Chemokines

Chemokines due to their selective chemoattractant activities for different types of leukocytes (Table 9.11) play an important role in the process of transmigration. The model of chemokine involvement in leukocyte trafficking might be summarized as follows:

(a) a chemokine, sequestered in solid phase on the endothelial cell surface, is presented as a signal to trap a specific type of leukocyte as the cell is undergoing selectin-mediated rolling along the endothelium;

(b) the leukocyte is selectively activated by the chemokine so that the cell stops rolling and become firmly adhered;

(c) the adhered leukocyte rather "crawls" than swims along the chemotactic gradient formed by the chemokines on the endothelium;

(d) the leukocyte undergoes diapedesis and migrates into the tissue space, while still responding to a chemotactic gradient.

In general, cell mobility represents the integration of many processes including adhesion (integrin-
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<table>
<thead>
<tr>
<th>Selectin interactions</th>
<th>Adhesion receptor on neutrophils</th>
<th>Counter-receptor on endothelial cells</th>
<th>Implicated in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p150sLe^x(CD15)</td>
<td>P-selectin(CD62P)</td>
<td>Leukocyte rolling, binding to high endothelial venules</td>
</tr>
<tr>
<td></td>
<td>sLe^x(CD15s), L-selectin(CD62L)</td>
<td>E-selectin(CD62E), GlyCAM-1 and others neutrophil addressins</td>
<td></td>
</tr>
</tbody>
</table>

| Integrin–immunoglobulin superfamily interactions | LFA-1, (CD11a/CD18), VLA-4, (CD49d/CD29) | ICAM-1(CD54), ICAM-2(CD102), VCAM-1(CD106) | Secondary adhesion (sticking), spreading, homing to inflamed tissue |

| Immunoglobulin superfamily interactions | PECAM-1(CD31), HCAM(CD44) | PECAM-1(CD31) | Potentiating adhesion, transendothelial migration Receptor binding hyaluronate and other molecules of connective tissue |

GlyCAM: Glycosaminoglycan–cell adhesion molecule; HCAM: homing cell adhesion molecule; ICAM-1, ICAM-2: intercellular adhesion molecule 1,2; LFA-1: leukocyte function associated antigen 1; PECAM: platelet endothelial adhesion molecule; sLex: sialylated Lewis antigen X; VCAM-1: vascular cell adhesion molecule; VLA-4: very late antigen 4

Table 9.13: Adhesion molecules involved in leukocyte binding to endothelium

dependent), lamellar protrusion (actin-dependent), deadhesion (integrin-dependent), and contraction (actin and possibly myosin-dependent). Moving neutrophils assume a polarized morphology with an anterior lamellipodium extended in the direction of movement, a cell body that is elongated parallel to the axis of lamellar protrusion, and a knob-like tail or "uropod".

The importance of leukocyte adhesion molecules may be documented by the existence of leukocyte adhesion deficiency (LAD), a congenital disorder manifest as LAD-1 and LAD-2 syndromes. Leukocytes of patients with **LAD-1 syndrome** lack β2 integrin expression. It occurs in two main forms: one with severe and the other with moderate clinical manifestations. In severe form, both α- and β-chains in the molecule of the LFA-1 subfamily are completely lacking. The severe deficiency affecting both boys and girls, is manifested by severe, life-threatening infections with high mortality (patients seldom survive beyond two years of age). The moderate deficiency is accompanied by partially β2 integrin expression; patients express 2.5 to 6.0% of normal LFA-1, CR3 and CR4 levels and usually have only recurrent skin infections. Leukocytes from patients with **LAD-2 syndrome** failed to express sLe^x(CD15s) and therefore they are not able to bind to E-selectin and P-selectin. Consistent with the proposed role of selectins, there was a marked reduction in the rolling of leukocytes from these patients. This clearly indicates a requirement for the carbohydrate ligands recognized by the selectins. The LAD-2 patients, are suffering from recurrent bacterial infections but they can survive into childhood, with short stature and mental retardation due to disorder of fucose metabolism since fucose is an important component of sLe^x.
9.1.6 Categories of inflammation mediated by the immune system

The immune processes are probably ongoing and, in most cases, lead to the elimination of antigens without producing clinically detectable inflammation. The development of clinically apparent inflammation indicates that the immune system has encountered either an unusually large amount of antigen, antigen in an unusual location, or antigen that is difficult to digest. In some diseases, such as rheumatoid arthritis, the initiating agent is unknown or may be normal host tissue components. In others (e.g. systemic lupus erythematous), inherent or acquired immunoregulatory abnormalities may contribute to the sustained nature of the inflammatory process.

Coombs and Gell divided inflammatory responses mediated by the immune system into four categories, called I, II, III, and IV, which represent four distinct immune mechanisms that result in tissue injury. These same four processes represent mechanisms of immune protection from infectious agents:

I. Immediate hypersensitivity (allergic, or reaginic acute inflammation).

II. Cytotoxic (inflammation mediated by cytotoxic antibodies).
III. Immune complex (inflammation mediated by immune complex).

IV. Delayed hypersensitivity (chronic inflammation mediated by lymphocytes and macrophages).

9.1.6.1 Allergic (reaginic) acute inflammation

Type I hypersensitivity is characterized by an allergic reaction that occurs immediately following contact with antigen, which is referred to as the allergen. The term allergy means "changed reactivity" of the host when meeting an "agent" on a second or subsequent occasion. In some individuals, certain allergens have a propensity to stimulate production of IgE antibodies. IgE antibodies bind nonspecifically, via their high affinity Fc receptors, to mast cells and basophils. Subsequent attachment of antigen to the Fab portion of cell-bound IgE antibodies results in release of contents of cytoplasmic granules from mast cells and basophils (e.g., histamine), as well as in synthesis and secretion of biologically active products of arachidonic acid (e.g., leukotrienes). Mast cell products increase vascular permeability and constrict bronchial smooth muscle. A wheal and flare reaction occurs within seconds to minutes. Neutrophils and eosinophils characteristically predominate and mononuclear cells can also be seen.

Reaginic reactions are responsible for such allergic phenomena as urticaria, seasonal rhinitis, asthma, and in settings where large amounts of antigens (allergens) enter the host circulation, systemic anaphylaxis. These occur when an IgE response is directed against innocuous environmental antigens, such as pollen, house-dust mites or animal dander. The resulting release of pharmacological mediators by IgE-sensitized mast cell produces an acute inflammatory reaction with symptoms such as asthma or rhinitis. The importance of type I reactions in protection from infectious organisms is uncertain, although the increased vascular permeability mediated by these reactions probably facilitates the capacity of antibody and inflammatory cells to arrive at the infected site. In addition, homocytotropic IgE antibodies and cells containing inflammatory mediators probably participate in the defense against large, non-phagocytobable organisms, most notably the multicellular helminthic parasites.

There is an important question why one individual express atopic diseases and another does not. At least two reasons exist – environmental exposure and genetics. A third reason – an external event that alters IgE regulation – may be important in certain clinical situation but may represent a rare cause of atopic diseases.

The atopic diseases, allergic rhinitis, asthma, and atopic dermatitis have a genetic component. Some or all of these clinical syndromes can be present in a single member or in several member of the same family. The natural history of atopic diseases is not known, but it appears that atopic individuals appear to have a relatively high frequency of food allergy before the age of two years; food allergy then becomes rarer but the patients develop IgE antibodies to inhalant allergens and manifest allergic rhinitis and/or asthma.

In general, atopy is a hereditary feature manifested by abnormal immediate – type hypersensitivity to a certain allergen or a group of allergens.

Anaphylaxis denotes an acute systemic immediate reaction to allergen, typically mediated by IgE antibodies. The mildest form of anaphylaxis, involving only the skin, is termed urticaria or "hives". More severe reactions involve the mucous membranes and the gastrointestinal, pulmonary, and cardiovascular organs. Anaphylaxis may be life-threatening. The manifestations range from urticaria to angioedema (swelling of mucous membranes, for example, of the lips, tongue, palate, and larynx), nausea and vomiting (edema and smooth muscle contraction of gastrointestinal tract), asthma (bronchial smooth muscle contraction), and hypotension (increased vascular permeability resulting in a loss of blood volume into tissue and thus a fall in blood pressure; reducing contractility of the heart also contributes to hypotension). Life-threatening reactions involve laryngeal edema, severe asthma, or severe hypotension and circulatory collapse. Agents that induce IgE-mediated anaphylaxis include penicillin, insect venoms, foods, and occasionally immunotherapy (i.e. injection of allergen to which a person is allergic, in order to treat allergic diseases.

Identical symptoms, which are not immune mediated, are sometimes termed anaphylactoid. Anaphylactoid reactions may be caused by radiocontrast dye (used for x-ray studies) and exercise.

Although antigen-IgE antibody interaction is the major cause of anaphylaxis, other immune mecha-
nisms may occasionally induce the syndrome. Thus immune complexes may mediate anaphylaxis in some patients who are IgA-deficient and receive infusions of IgA, which interacts with preformed anti-IgA antibody. Anaphylactoid reactions may also occur after repeated intravenous administration of normal human immunoglobulin preparation that contain more than 5% of IgG aggregates in agammaglobulinaemic or hypogammaglobulinaemic patients. These aggregates activate complement to produce C5a and C3a anaphylatoxins which stimulate mediator release from basophils and perhaps some subsets of mast cells.

9.1.6.2 Acute inflammation mediated by cytotoxic antibodies

Type II, or antibody-dependent cytotoxic hypersensitivity occurs when antibody binds to either self antigen or foreign antigen on cells, and leads to phagocytosis, killer cell activity or complement-mediated lysis.

Both type II and type III hypersensitivity are caused by IgG and IgM antibodies. The main distinction is that type II reactions involved antibodies directed to antigens on the surface of specific cells or tissues, whereas type III reactions involve antibodies against widely distributed soluble antigens in the serum. Thus, damage caused by type II reactions is localized to a particular tissue or cell type, whereas damage caused by type III reactions affects those organs where antigen-antibody complexes are deposited.

These hypersensitivity reactions are related to normal immune responses seen against microorganisms and larger multicellular parasites. Indeed, in mounting a reaction to a pathogen, exaggerated immune reactions may sometimes be as damaging to the host as the effects of the pathogen itself. In such cases the borderline between a normal, useful immune response and hypersensitivity is blurred. Hypersensitivity reactions may also occur in many other conditions involving immune reactions, particularly autoimmunity and transplantation.

In type II hypersensitivity, antibody directed against cell surface or tissue antigens forms immune complex which interacts with complement and a variety of effector cells to bring about damage to the target cells. Antibodies can link the target cells to effectors cells, such as macrophages, neutrophils, eosinophils and generally, K cells, by means of Fc receptors on these effector cells. This is so-called antibody-dependent cell-mediated cytotoxicity (ADCC). Alternatively, the antibodies after binding to tissue antigens can interact with complement by activating C1 of the classical pathway. This results in the deposition of the C5b678(9) membrane attack complex and following lysis of antibody-sensitized cells.

Both the complement fragments and IgG can act as opsonins bound to host tissues or to microorganisms, and phagocytes take up the opsonized particles. By enhancing the lysosomal activity of phagocytes, and potentiating their capacity to produce reactive oxygen intermediates, the opsonins increase the phagocytes’ capacity to destroy pathogen, but also increase their ability to produce immunopathological damage in hypersensitivity reactions. For example, neutrophils from the synovial fluid of patients with rheumatoid arthritis produce more superoxide when stimulated than neutrophils from the blood. This is thought to be related to their activation, in the rheumatoid joint, by mediators which include immune complexes and complement fragments.

The accumulation of inflammatory cells (neutrophils), with release of neutrophil lysosomal enzymes and generation of toxic oxygen intermediates, together with complement-mediated tissue lysis, leads to destruction of tissues as in the glomerular and pulmonary basement membrane damage in Goodpasture’s syndrome or in the autoimmune haemolytic anemia and immune-mediated thrombocytopenia of systemic lupus erythematosus.

There are three main subtypes of cytotoxic hypersensitivity:

1. Type II reactions between members of the same species. They are caused by isoinmunization and include transfusion reactions after transfusion of blood incompatible in the AB0 system, haemolytic disease of the newborn due to rhesus incompatibility and/or transplantation reaction provoked by antibodies in the recipient directed against surface transplantation antigens on the graft.

2. Autoimmune type II hypersensitivity reactions are evoked by antibodies in the host directed against his own cell or tissue antigens (autoantibodies). As an example may serve autoimmune haemolytic anaemia caused by autoantibodies
to the patient’s own red cells; Hashimoto’s thyroiditis with autoantibodies against thyroid peroxidase surface antigen; idiopathic thrombocytopenic purpura manifest by platelet destruction evoked by anti-platelet antibodies; Goodpasture’s syndrome in which complement-mediated damage to basement membrane due to specific autoantibodies is observed.

Many diseases are caused by autoantibodies against hormone receptors. Recently, they are also known as type V hypersensitivity reactions. Autoantibodies directed against receptors can have the function of agonist resulting in stimulatory hypersensitivity and/or of antagonist leading to the blockade of signal transmitted through the receptor occupied by such an autoantibody. The example of stimulatory hypersensitivity is thyrotoxicosis where pathological stimulation of TSH receptor occurs, whereas to the blocking hypersensitivity belong primary myxoedema (blockade of TSH receptor) or myasthenia gravis (blockade of acetylcholine receptor).

3. Type II drug reactions are very complicated. Drugs may become coupled to body components and thereby undergo conversion from a hapten to a full antigen which may sensitive certain individuals. If, during this response, IgE antibodies are produced, anaphylactic reactions can result. In some circumstances, cell-mediated hypersensitivity may be induced. In other cases where coupling to serum proteins occurs, the possibility of type III immune complex-mediated reactions may arise. Finally, the drug antigenic complex with a molecule on the surface of host cells may evoke the production of antibodies which are cytotoxic for the cell-drug complex. Examples of this mechanism have been seen in the haemolytic anaemia sometimes associated with continued administration of chlorpromazine or phenacetin, in the agranulocytosis associated with the taking of amiodopyrine or of quinidine, and now classic situation of thrombocytopenic purpura which may be produced by a sedative edormid. When the drug is withdrawn, the hypersensitivity is no longer evident.

9.1.6.3 Acute inflammation mediated by immune complexes

Type III hypersensitivity develops when immune complexes are formed in large quantities, or cannot be cleared adequately by the reticulo-endothelial system, leading to serum-sickness type reactions.

Repeated cutaneous injection of antigen was shown by Arthus in 1903 to initiate, within hours, acute local inflammation. This form of inflammation, called the ”Arthus reaction”, was ultimately shown to require immune complexes. Deposition of immune complexes in local tissues with resultant inflammation is common in rheumatic diseases. The combination of IgM or IgG antibodies with antigen activates the complement cascade, generating active peptides such as C5a, which, in addition to dilating capillaries and increasing vascular permeability, contracts smooth muscle and mobilizes phagocytic cells. Binding of immune complexes to neutrophils and macrophages also activates the respiratory burst with generation of toxic oxygen products such as hydrogen peroxide, hydroxyl radical, hypochlorous acid, and chloramines. Lyssonal proteolytic enzymes, together with toxic oxygen products, produce a potent system that can damage protein and lead to blood vessel damage with haemorrhagic necrosis and local tissue destruction.

When large amounts of antigen enter the circulation (as following administration of heterologous serum), a serum sickness reaction may ensue. As antibody is produced, antigen-antibody complexes are formed. Such complexes may localize to small vessels, resulting in local inflammation and vasculitis. Phagocytosis of immune complexes by macrophages can result in release of cytokines, such as IL-1 and TNF-α, which initiate fever. Deposition of immune complexes in the glomerular basement membrane can lead to glomerulitis. By similar mechanisms arthritis may result. Rheumatoid arthritis has many characteristics of a local immune complex reaction, whereas systemic lupus erythematosus has many clinical features of serum sickness.

Diseases resulting from immune complex formation can be placed broadly into three groups:

1. The combined effects of a low-grade persistent infection (such as occur with α-haemolytic Streptococcus viridans or staphylococcal infective endocarditis, or with a parasite such as Plasmodium vivax, or in viral hepatitis), to-
Chapter 9. Inflammation and fever (J. Hulín, M. Ferenčík, V. Štvrtinová, J. Jakubovský)

1. Together with a weak antibody response, leads to chronic immune complex formation with the eventual deposition of complexes in the tissues.

2. Immune complex disease is a frequent complication of autoimmune disease where the continued production of antibody to a self-antigen leads to prolonged immune complex formation. The mononuclear phagocyte, erythrocyte, and complement systems (which are responsible for the removal of complexes) become overloaded and the complexes are deposited in the tissues, as occurs in systemic lupus erythematosus.

3. Immune complexes may be formed at body surfaces, notably in the lungs following repeated inhalation of antigenic material from moulds, plants or animals. This is exemplified in Farmer's lung and Pigeon fancier's lung, where there are circulating antibodies to the actinomycete fungi found in mouldy hay, or to pigeon antigens. Both diseases are forms of extrinsic allergic alveolitis, and they only occur after repeated exposure to the antigen.

The main diseases in which immune complexes are important are summarized in Table 9.14. The sites of immune-complex deposition are partly determined by localization of the antigen in the tissue, and partly by how circulating immune complexes become deposits.

Experimental models are available for each of the three main types of immune complex disease described above: serum sickness induced by injections of foreign antigen, to represent the presence of a persistent infection; the NZB/NZW mouse, for autoimmunity; and the Arthus reaction, for local damage by extrinsic antigen. Care must be taken when interpreting animal experiments as the erythrocytes of rodents and rabbits lack the receptor for iC3b (known as CR1) which readily binds immune complexes that have fixed complement. This is present on primate erythrocytes.

9.1.6.4 Chronic inflammation (delayed-type of hypersensitivity reaction)

Type IV or delayed type hypersensitivity (DTH), is most seriously manifested when antigens (for example those of tubercle bacilli) are trapped in a macrophage and cannot be cleared. T cells are then stimulated to elaborate lymphokines which mediate a range of inflammatory responses. Other aspects of DTH reactions are seen in graft rejection and allergic contact dermatitis. DTH is used as a general category to describe all those hypersensitivity reactions which take more than 12 hours to develop, and which involve cell-mediated immune reactions rather than humoral immune reactions. Whereas allergic reactions occur within seconds and minutes and immune complex reactions occur within several hours to one day, DTH reactions peak at 2 to 3 days.

Unlike other forms of hypersensitivity, type IV hypersensitivity cannot be transferred from one animal to another by serum, but can be transferred by T cells (Th1 cells in mice). In humans, transfer from a sensitized to a non-sensitized individual can be also achieved only by T lymphocytes and, interestingly, by a low molecular weight material extracted from them (transfer factor). Delayed type hypersensitivity is obviously associated with T cell protective immunity but does not necessarily run parallel with it – there is not always a complete correlation between delayed hypersensitivity and protective immunity. The T cells necessary for producing the delayed response are cells which have become specifically sensitized to the particular antigen by a previous encounter, and they act by recruiting other cell types to the site of the reaction.

Three types of delayed hypersensitivity reaction are recognized: Contact hypersensitivity and tuberculin-type hypersensitivity both occur within 72 hours of antigen challenge, whereas granulomatous reactions develop over a period of weeks. The granulomas are formed by the aggregation and proliferation of macrophages, and may persist for weeks. This reaction is, in terms of its clinical consequences, by far the most serious type of delayed type hypersensitivity response. The position is complicated because these different types of reaction may overlap, or occur sequentially following a single antigenic challenge.

The delayed type hypersensitivity reactions are probably important for host defence against intracellular parasites such as tuberculosis and certain viruses and are prevalent in certain disease such as sarcoidosis, Wegener’s granulomatosis, and polymyositis. In some diseases, such as chronic granulomatous disease of childhood, granuloma formation can lead to obstruction of vital structures such
### Table 9.14: Some of the main diseases in which immune complexes are implicated

<table>
<thead>
<tr>
<th>Site of deposition</th>
<th>CIC</th>
<th>VS</th>
<th>Kidneys</th>
<th>Joints</th>
<th>Skin</th>
<th>Others</th>
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<td><strong>Autoimmune diseases</strong></td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>Heart</td>
</tr>
<tr>
<td>Leprosy</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Eyes</td>
</tr>
<tr>
<td>Malaria</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Trypanosomiasis (African)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>Heart, brain</td>
</tr>
<tr>
<td>Dengue haemorrhagic fever</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
</tbody>
</table>

CIC: circulating immune complexes; VS: the vascular system

as the esophagus or ureters. The contact dermatitis is caused by sensitization to certain simple chemicals.

Perhaps the best known example of cell-mediated hypersensitivity is the *Mantoux reaction* obtained by injection of tuberculin into the skin of an individual in whom previous infection with the mycobacterium had induced a state of cell-mediated immunity. The reaction is characterized by erythema and induration which appears only after several hours and reach a maximum at 24–48 hours, thereafter subsiding. Histologically the earliest phase of the reaction is seen as a perivascular cuffing with mononuclear cells followed by a more extensive exudation of mononuclear and polymorphonuclear cells. The latter soon migrate out of the lesion leaving behind a predominantly mononuclear cell infiltrate consisting of lymphocytes and cells of the monocyte - macrophage series. This contrasts with the essentially "polymorph" character of the Arthus reaction.