# Innate immunity



## INTRODUCTION

We live in a potentially hostile world filled with a bewildering array of infectious agents (figure 1.1) of diverse shape, size, composition and subversive character which would very happily use us as rich sanctuaries for propagating their 'selfish genes' had we not also developed a series of defense mechanisms at least their equal in effectiveness and ingenuity (except in the case of many parasitic infections where the situation is best described as an uneasy and often unsatisfactory truce). It is these defense mechanisms which can establish a state of immunity against infection (Latin *immunitas*, freedom from) and whose operation provides the basis for the delightful subject called 'Immunology'.

Aside from ill-understood constitutional factors which make one species innately susceptible and another resistant to certain infections, a number of relatively nonspecific antimicrobial systems (e.g. phagocytosis) have been recognized which are **innate** in the sense that they are not intrinsically affected by prior contact with the infectious agent. We shall discuss these systems and examine how, in the state of **specific acquired immunity**, their effectiveness can be greatly increased.

## EXTERNAL BARRIERS AGAINST INFECTION

The simplest way to avoid infection is to prevent the microorganisms from gaining access to the body (figure 1.2). The major line of defense is of course the skin which, when intact, is impermeable to most infectious agents; when there is skin loss, as for example in burns, infection becomes a major problem. Additionally, most bacteria fail to survive for long on the skin because of the direct inhibitory effects of lactic acid and fatty acids in sweat and sebaceous secretions and the low pH which they generate. An exception is *Staphylococcus aureus* which often infects the relatively vulnerable hair follicles and glands.

Mucus, secreted by the membranes lining the inner surfaces of the body, acts as a protective barrier to block the adherence of bacteria to epithelial cells. Microbial and other foreign particles trapped within the adhesive mucus are removed by mechanical stratagems such as ciliary movement, coughing and sneezing. Among other mechanical factors which help protect the epithelial surfaces, one should also include the washing action of tears, saliva and urine. Many of the secreted body fluids contain bactericidal components, such as acid in gastric juice, spermine and zinc in semen, lactoperoxidase in milk and lysozyme in tears, nasal secretions and saliva.

A totally different mechanism is that of microbial

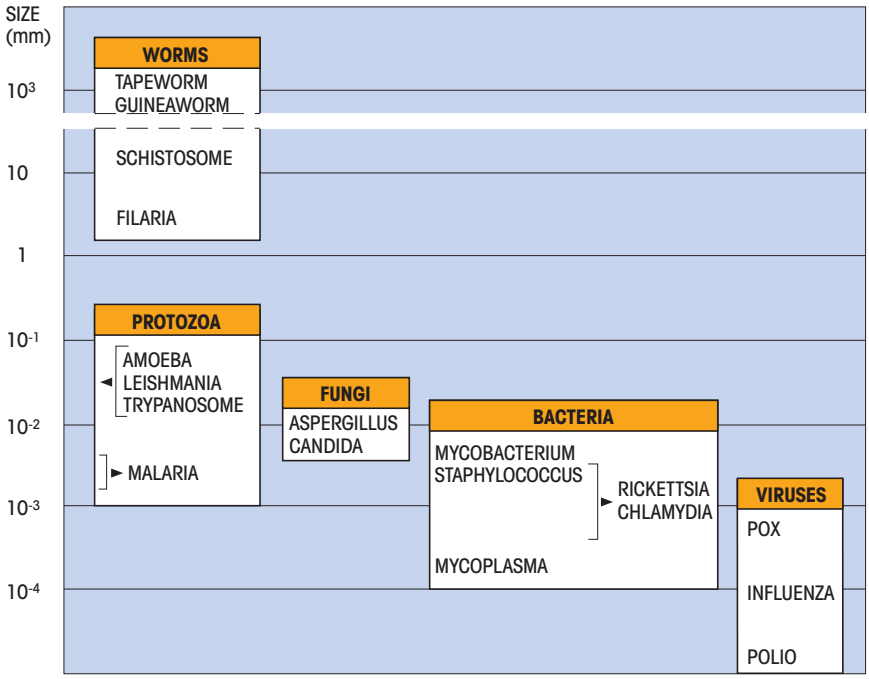



**Figure 1.1. The formidable range of infectious agents which confronts the immune system.** Although not normally classified as such because of their lack of a cell wall, the mycoplasmas are included under bacteria for convenience. Fungi adopt many forms and approximate values for some of the smallest forms are given. ]►, range of sizes observed for the organism(s) indicated by the arrow; ◄[, the organisms listed have the size denoted by the arrow.

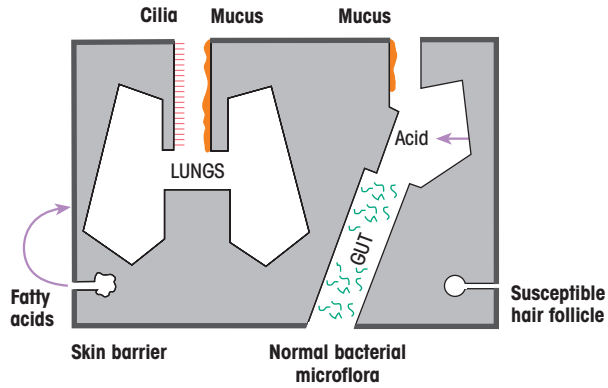



**Figure 1.2. The first lines of defense against infection:** protection at the external body surfaces.

antagonism associated with the normal bacterial flora of the body. This suppresses the growth of many potentially pathogenic bacteria and fungi at superficial sites by competition for essential nutrients or by production of inhibitory substances. To give one example, pathogen invasion is limited by lactic acid produced by particular species of commensal bacteria which metabolize glycogen secreted by the vaginal epithelium. When protective commensals are disturbed by antibiotics, susceptibility to opportunistic infections by *Candida* and *Clostridium difficile* is increased. Gut commensals may also produce colicins, a class of bactericidins which bind to the negatively charged surface of susceptible bacteria and insert a hydrophobic helical hairpin into the membrane; the molecule then undergoes a 'Jekyll and Hyde' transformation to become completely hydrophobic and forms a voltage-dependent channel in the membrane which kills by destroying the cell's energy potential. Even at this level, survival is a tough game.

If microorganisms do penetrate the body, two main defensive operations come into play, the destructive effect of soluble chemical factors such as bactericidal enzymes and the mechanism of **phagocytosis**—literally 'eating' by the cell (Milestone 1.1).

## PHAGOCYTIC CELLS KILL MICROORGANISMS

### Neutrophils and macrophages are dedicated 'professional' phagocytes

The engulfment and digestion of microorganisms are assigned to two major cell types recognized by Metchnikoff at the turn of the last century as microphages and macrophages.

#### *The polymorphonuclear neutrophil*

This cell, the smaller of the two, shares a common hematopoietic stem cell precursor with the other formed elements of the blood and is the dominant white cell in the bloodstream. It is a nondividing short-lived cell with a multilobed nucleus and an array of granules which are virtually unstained by histologic dyes such as hematoxylin and eosin, unlike those

# Milestone 1.1 — Phagocytosis

The perceptive Russian zoologist, Elie Metchnikoff (1845–1916), recognized that certain specialized cells mediate defense against microbial infections, so fathering the whole concept of cellular immunity. He was intrigued by the motile cells of transparent starfish larvae and made the critical observation that, a few hours after the introduction of a rose thorn into these larvae, they became surrounded by these motile cells. A year later, in 1883, he observed that fungal spores can be attacked by the blood cells of *Daphnia*, a tiny metazoan which, also being transparent, can be studied directly under the microscope. He went on to extend his investigations to mammalian leukocytes, showing their ability to engulf microorganisms, a process which he termed **phagocytosis**.

Because he found this process to be even more effective in animals recovering from infection, he came to a somewhat polarized view that phagocytosis provided the main, if not the only, defense against infection. He went on to define the existence of two types of circulating phagocytes: the polymorphonuclear leukocyte, which he termed a 'microphage', and the larger 'macrophage'.

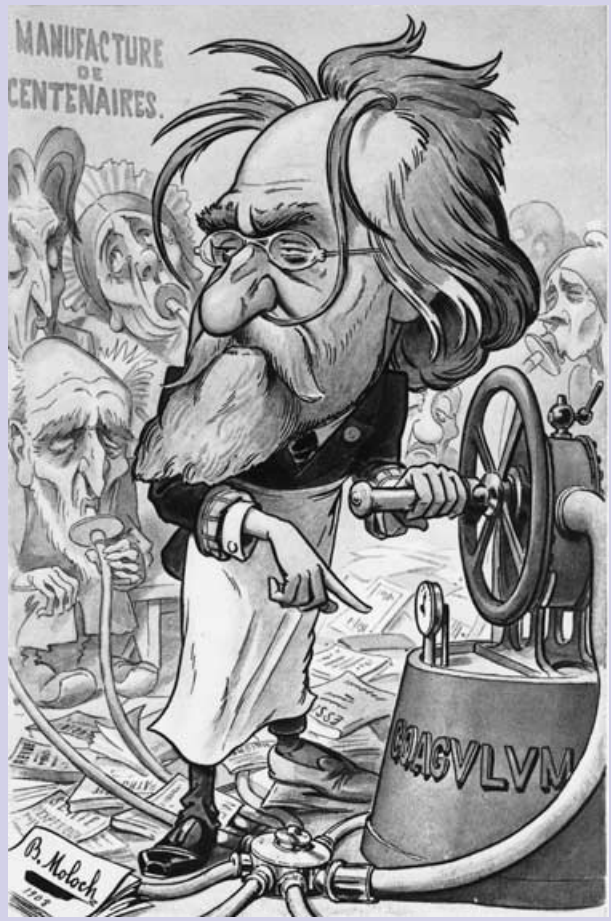



**Figure M1.1.1.** Caricature of Professor Metchnikoff from *Chanteclair*, 1908, No. 4, p. 7. (Reproduction kindly provided by The Wellcome Institute Library, London.)

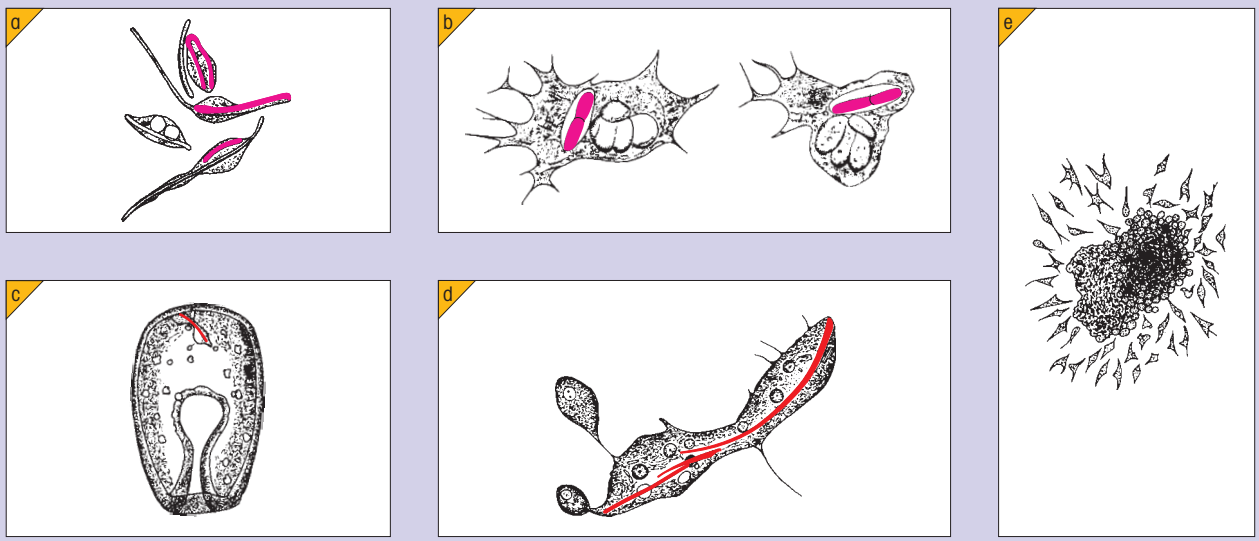
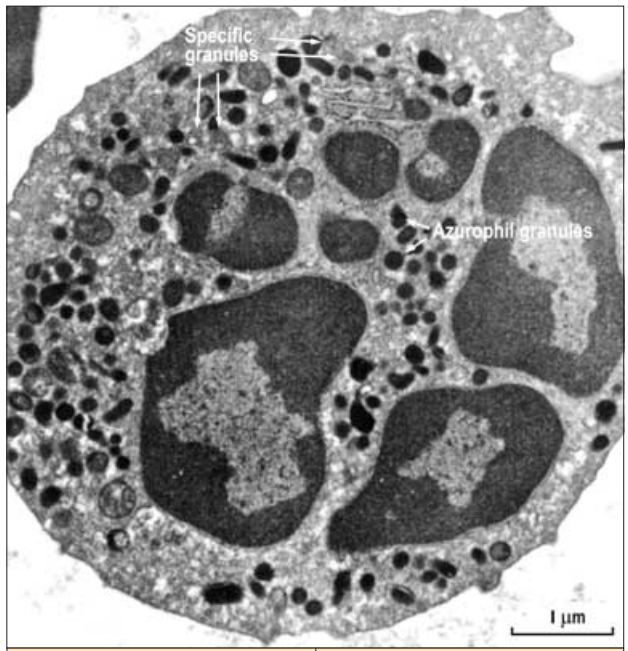



**Figure M1.1.2.** Reproductions of some of the illustrations in Metchnikoff's book, *Comparative Pathology of Inflammation* (1893). (a) Four leukocytes from the frog, enclosing anthrax bacilli; some are alive and unstained, others which have been killed have taken up the vesuvine dye and have been colored; (b) drawing of an anthrax bacillus, stained by vesuvine, in a leukocyte of the frog; the two figures represent two phases of movement of the same frog leukocyte which contains stained anthrax bacilli within its phagocytic vacuole; (c and d) a foreign body (colored) in a starfish larva surrounded by phagocytes which have fused to form a multinucleate plasmodium shown at higher power in (d); (e) this gives a feel for the dynamic attraction of the mobile mesenchymal phagocytes to a foreign intruder within a starfish larva.




| AZUROPHIL GRANULES | SPECIFIC GRANULES |
|---|---|
| 0.5μm<br>1500/cell | 0.2μm<br>3000/cell |
| Lysozyme<br>Myeloperoxidase<br>Elastase<br>Cathepsin G<br>$H^+$ hydrolases | Lysozyme<br>Cytochrome $b_{558}$<br>$OH^-$ phosphatase |
| Defensins<br>BPI | Lactoferrin<br>Vit $B_{12}$ binding protein |

**Figure 1.3. Ultrastructure of neutrophil.** The multilobed nucleus and two main types of cytoplasmic granules are well displayed. (Courtesy of Dr D. McLaren.)

structures in the closely related eosinophil and basophil (figures 1.3 and 1.4). These neutrophil granules are of two main types: (i) the **primary azurophil granule** which develops early (figure 1.4e), has the typical lysosomal morphology and contains myeloperoxidase together with most of the nonoxidative antimicrobial effectors including defensins, bactericidal permeability increasing (BPI) protein and cathepsin G (figure 1.3), and (ii) the peroxidase-negative **secondary specific granules** containing lactoferrin, much of the lysozyme, alkaline phosphatase (figure 1.4d) and membrane-bound cytochrome $b_{558}$ (figure 1.3). The abundant glycogen stores can be utilized by glycolysis enabling the cells to function under anerobic conditions.

### *The macrophage*

These cells derive from bone marrow promonocytes which, after differentiation to blood monocytes, finally settle in the tissues as mature macrophages where they constitute the **mononuclear phagocyte system** (figure 1.5). They are present throughout the connective tissue and around the basement membrane of small blood vessels and are particularly concentrated in the lung (figure 1.4h; alveolar macrophages), liver (Kupffer cells) and lining of spleen sinusoids and lymph node medullary sinuses where they are strategically placed to filter off foreign material. Other examples are mesangial cells in the kidney glomerulus, brain microglia and osteoclasts in bone. Unlike the polymorphs, they are long-lived cells with significant rough-surfaced endoplasmic reticulum and mitochondria (figure 1.8b) and, whereas the polymorphs provide the major defense against pyogenic (pus-forming) bacteria, as a rough generalization it may be said that macrophages are at their best in combating those bacteria (figure 1.4g), viruses and protozoa which are capable of living within the cells of the host.

## Pattern recognition receptors (PRRs) on phagocytic cells recognize and are activated by pathogen-associated molecular patterns (PAMPs)

It hardly needs to be said but the body provides a very complicated internal environment and the phagocytes continuously encounter an extraordinary variety of different cells and soluble molecules. They must have mechanisms to enable them to distinguish these friendly self components from unfriendly and potentially dangerous microbial agents—as Charlie Janeway so aptly put it, they should be able to discriminate between 'noninfectious self and infectious nonself'. Not only must the infection be recognized, but it must also generate a signal which betokens 'danger' (Polly Matzinger).

In the interests of survival, phagocytic cells have evolved a system of receptors capable of recognizing molecular patterns expressed on the surface of the pathogens (PAMPs) which are conserved (i.e. unlikely to mutate), shared by a large group of infectious agents (sparing the need for too many receptors) and clearly distinguishable from self patterns. By and large these pattern recognition receptors (PRRs) are lectin-like and bind multivalently with considerable specificity to exposed microbial surface sugars with their characteristic rigid three-dimensional configurations (PAMPs). They do not bind appreciably to the galatose or sialic acid groups which are commonly the penultimate and ultimate sugars of mammalian surface polysaccharides. PAMPs linked to extracellular infections include Gram-negative lipopolysaccharide (LPS), Gram-positive lipoteichoic acid, yeast cell wall mannans (cf. figure 1.8) and mycobacterial glycolipids. Unmethy-

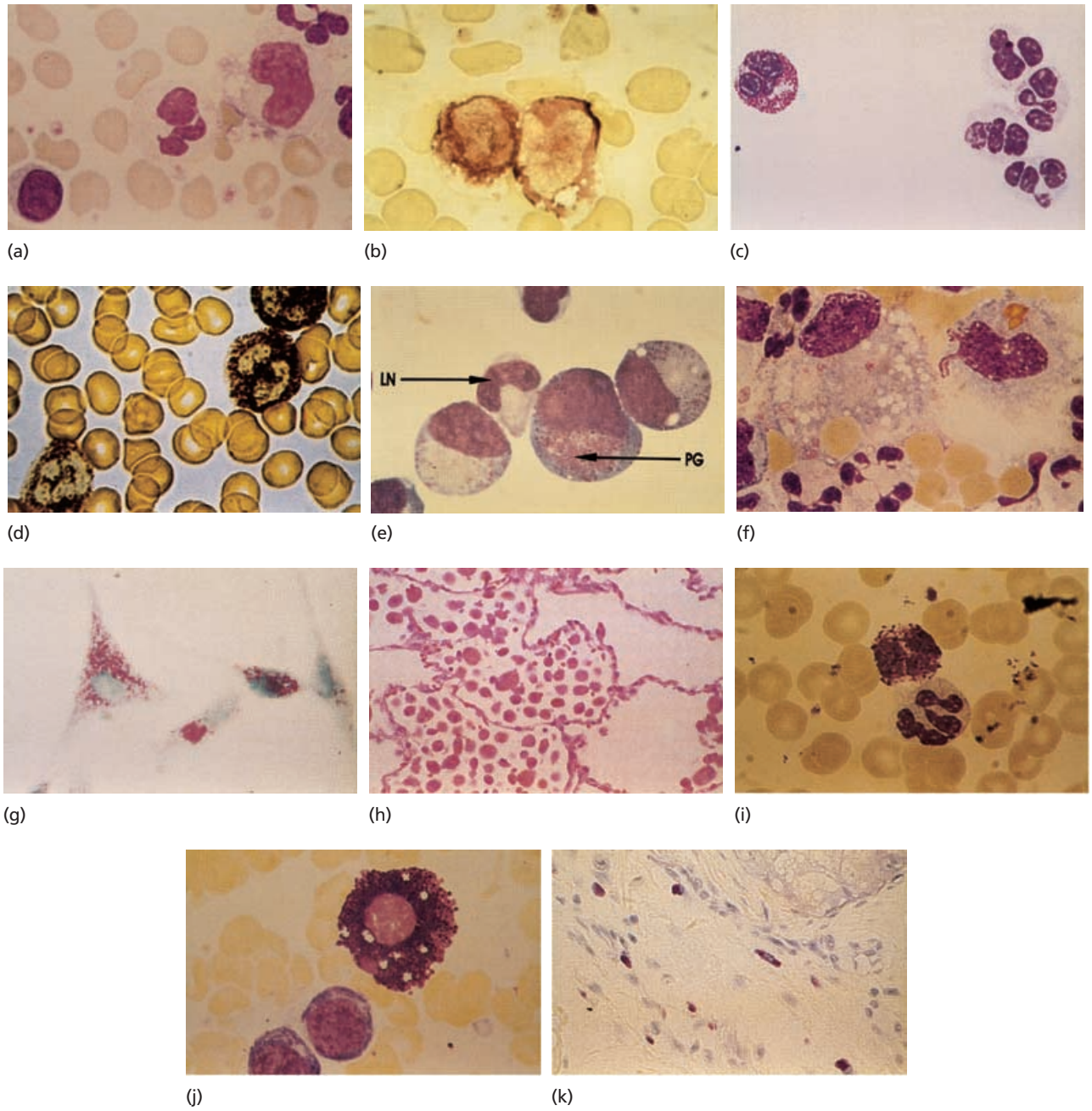



**Figure 1.4. Cells involved in innate immunity.** (a) Monocyte, showing 'horseshoe-shaped' nucleus and moderately abundant pale cytoplasm. Note the three multilobed polymorphonuclear neutrophils and the small lymphocyte (bottom left). Romanowsky stain. (b) Two monocytes stained for nonspecific esterase with α-naphthyl acetate. Note the vacuolated cytoplasm. The small cell with focal staining at the top is a T-lymphocyte. (c) Four polymorphonuclear leukocytes (neutrophils) and one eosinophil. The multilobed nuclei and the cytoplasmic granules are clearly shown, those of the eosinophil being heavily stained. (d) Polymorphonuclear neutrophil showing cytoplasmic granules stained for alkaline phosphatase. (e) Early neutrophils in bone marrow. The primary azurophilic granules (PG), originally clustered near the nucleus, move towards the periphery where the neutrophil-specific granules are generated by the Golgi apparatus as the cell matures. The nucleus gradually becomes lobular (LN). Giemsa. (f) Inflammatory cells from the site of a brain hemorrhage showing the large active macrophage in the center with phagocytosed red cells and prominent vacuoles. To the right is a monocyte with horseshoe-shaped nucleus and cytoplasmic bilirubin crystals (hematoidin). Several multilobed neutrophils are clearly delineated. Giemsa. (g) Macrophages in monolayer cultures after phagocytosis of mycobacteria (stained red). Carbol-Fuchsin counterstained with Malachite Green. (h) Numerous plump alveolar macrophages within air spaces in the lung. (i) Basophil with heavily staining granules compared with a neutrophil (below). (j) Mast cell from bone marrow. Round central nucleus surrounded by large darkly staining granules. Two small red cell precursors are shown at the bottom. Romanowsky stain. (k) Tissue mast cells in skin stained with Toluidine Blue. The intracellular granules are metachromatic and stain reddish purple. Note the clustering in relation to dermal capillaries. (The slides from which illustrations (a), (b), (d), (e), (f), (i) and (j) were reproduced were very kindly provided by Mr M. Watts of the Department of Haematology, Middlesex Hospital Medical School; (c) was kindly supplied by Professor J.J. Owen; (g) by Professors P. Lydyard and G. Rook; (h) by Dr Meryl Griffiths; and (k) by Professor N. Woolf.)

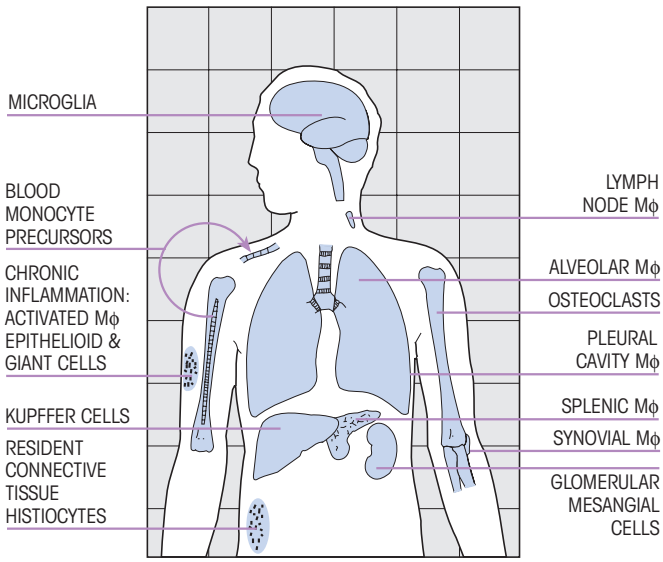



**Figure 1.5. The mononuclear phagocyte system.** Promonocyte precursors in the bone marrow develop into circulating blood monocytes which eventually become distributed throughout the body as mature macrophages (Mϕ) as shown. The other major phagocytic cell, the polymorphonuclear neutrophil, is largely confined to the bloodstream except when recruited into sites of acute inflammation.

lated CpG (guanosine–cytosine) sequences in bacterial DNA and the double-stranded RNA from RNA viruses are examples of PAMPs linked to intracellular infections.

Engagement of the pattern recognition receptor generates a signal through an NFκB transcription factor pathway which alerts the cell to danger and initiates the phagocytic process. It may be worthwhile looking more closely at the handling of Gram-negative LPS (endotoxin) since failure to do so can result in septic shock. The biologically reactive lipid A moiety of LPS is recognized by a plasma LPS-binding protein and the complex captured by the CD14 scavenger molecule on the phagocytic cell. This then activates a Toll-like receptor which in turn unleashes a series of events culminating in the release of NFκB from its inhibitor; the free NFκB translocates to the nucleus and triggers phagocytosis with the release of proinflammatory mediators (figure 1.6).

Programed cell death (apoptosis; see below) is an essential component of embryonic development and the maintenance of the normal physiologic state. The dead cells need to be removed by phagocytosis but since they do not herald any 'danger' this must be done silently without setting off the alarm bells. Accordingly, recognition of apoptotic cells by macrophages directly through the CD14 receptor and indirectly through the binding of C1q to surface nucleosome blebs (see p. 425) proceeds without provoking the release of proinflammatory mediators. In sharp contrast, cells which are injured by infection and become necrotic release endogenous heat-shock protein 60 which acts as a danger signal to the phagocytic cells and establishes a protective inflammatory response.

## Microbes are engulfed by activated phagocytic cells

After adherence of the microbe to the surface of the neutrophil or macrophage through recognition of a PAMP (figure 1.7.2), the resulting signal (figure 1.7.3) initiates the ingestion phase by activating an actin–myosin contractile system which extends pseudopods around the particle (figures 1.7.4 and 1.8); as adjacent receptors sequentially attach to the surface of the microbe, the plasma membrane is pulled around the particle just like a 'zipper' until it is completely enclosed in a vacuole (phagosome; figures 1.7.5 and 1.9). Events are now moving smartly and, within 1 minute, the cytoplasmic granules fuse with the phagosome and discharge their contents around the imprisoned microorganism (figure 1.7.7 and 1.9) which is subject to a formidable battery of microbicidal mechanisms.

## There is an array of killing mechanisms

### *Killing by reactive oxygen intermediates*

Trouble starts for the invader from the moment phagocytosis is initiated. There is a dramatic increase in activity of the hexose monophosphate shunt generating reduced nicotinamide-adenine-dinucleotide phosphate (NADPH). Electrons pass from the NADPH to a flavine adenine dinucleotide (FAD)-containing membrane flavoprotein and thence to a unique plasma membrane **cytochrome (cyt $b_{558}$)**. This has the very low midpoint redox potential of −245 mV which allows it to reduce molecular oxygen directly to superoxide anion (figure 1.10a). Thus the key reaction catalysed by this NADPH oxidase, which initiates the formation of reactive oxygen intermediates (ROI), is:

$$NADPH + O_2 \xrightarrow{\text{oxidase}} NADP^+ + \cdot O_2^- \quad \text{(superoxide anion)}$$

The superoxide anion undergoes conversion to hydrogen peroxide under the influence of superoxide dismutase, and subsequently to hydroxyl radicals (·OH). Each of these products has remarkable chemical reactivity with a wide range of molecular targets, making them formidable microbicidal agents; ·OH in particular is one of the most reactive free radicals known. Furthermore, the combination of peroxide, myeloper-

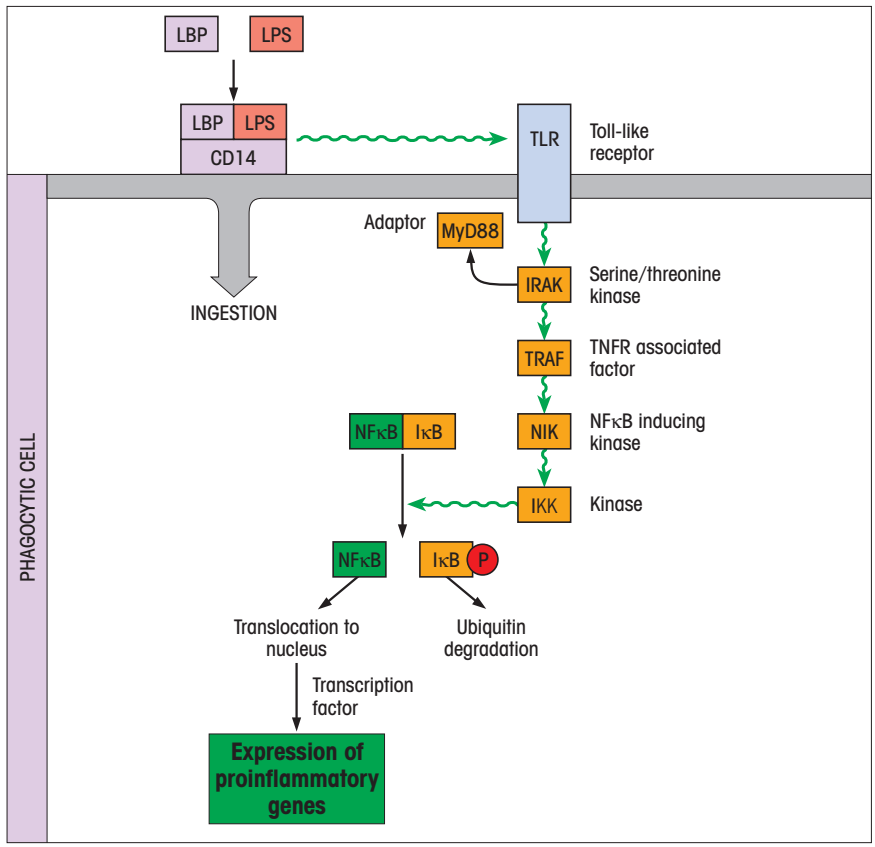



**Figure 1.6. Activation of a phagocytic cell by a Gram-negative LPS (endotoxin) danger signal.** Circulating LPS is complexed by LPS-binding protein (LBP) and captured by the CD14 (see p. 147 for CD definitions) surface scavenging receptor. This signals internalization of the complex and activates the Toll-like receptor (TLR), which then initiates a phosphorylation cascade mediated by different kinase enzymes, as a result of which the transcription factor NFκB is released from its inhibitor IκB and translocates to the nucleus, where it upregulates genes encoding defensive factors such as tumor necrosis factor (TNF), antibiotic peptides and the NADPH oxidase which generates reactive oxygen intermediates (see below). The Toll-like receptor is a leucine-rich molecule homologous with the Toll component which signals early embryonic differentiation events in *Drosophila*. The TLR is not itself a PRR and does not provide a signal for internalization, as shown by the ability of a double mutant of the MyD88 adaptor to internalize microorganisms attached to a PRR without producing inflammatory mediators such as TNF. The TLR appears to control the type of defensive response to different microbes. Thus TLR4 engineers the response to Gram-negative bacteria and LPS while TLR2 plays a key role in yeast and Gram-positive infections.

oxidase and halide ions constitutes a potent halogenating system capable of killing both bacteria and viruses (figure 1.10a). Although $H_2O_2$ and the halogenated compounds are not as active as the free radicals, they are more stable and therefore diffuse further, making them toxic to microorganisms in the extracellular vicinity.

### *Killing by reactive nitrogen intermediates*

Nitric oxide surfaced prominently as a physiologic mediator when it was shown to be identical with endothelium-derived relaxing factor. This has proved to be just one of its many roles (including the mediation of penile erection, would you believe it!), but of major interest in the present context is its formation by an inducible NO· synthase (iNOS) within most cells, but particularly macrophages and human neutrophils, thereby generating a powerful antimicrobial system (figure 1.10b). Whereas the NADPH oxidase is dedicated to the killing of extracellular organisms taken up by phagocytosis and cornered within the phagocytic vacuole, the NO· mechanism can operate against microbes which invade the cytosol; so, it is not surprising that the majority of nonphagocytic cells which may be infected by viruses and other parasites are endowed with an iNOS capability. The mechanism of action may be through degradation of the Fe–S prosthetic groups of certain electron transport enzymes, depletion of iron and production of toxic ·ONOO radicals. The *N-ramp* gene linked with resistance to microbes such as bacille Calmette–Guérin (BCG), *Salmonella* and *Leishmania*, which can live within an intracellular habitat, is now known to express a protein forming a

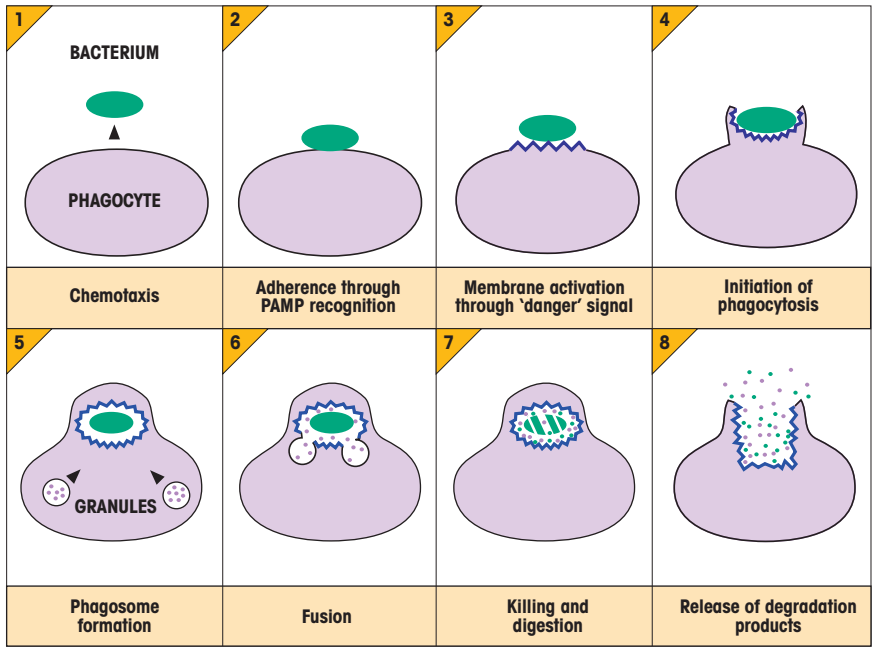



**Figure 1.7. Phagocytosis and killing of a bacterium.** Stage 3/4, respiratory burst and activation of NADPH oxidase; stage 5, damage by reactive oxygen intermediates; stage 6/7, damage by peroxidase, cationic proteins, antibiotic peptide defensins, lysozyme and lactoferrin.

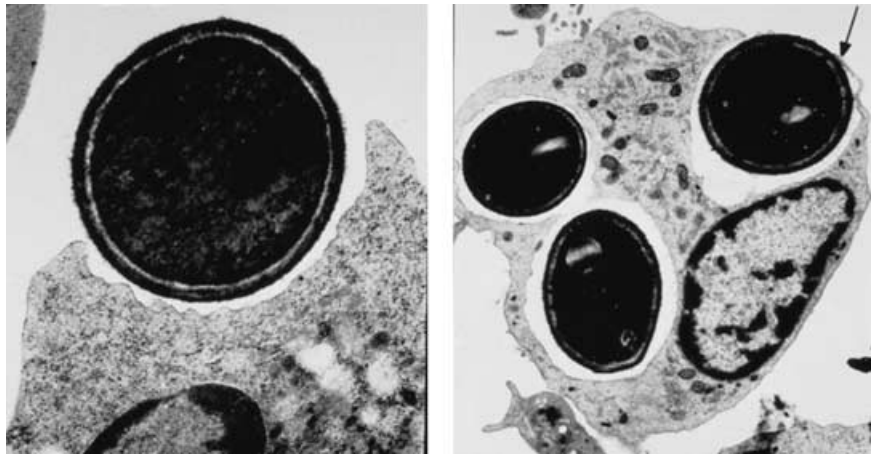



**Figure 1.8. Adherence and phagocytosis.** (a) Phagocytosis of *Candida albicans* by a polymorphonuclear leukocyte (neutrophil). Adherence to the yeast wall surface mannan initiates enclosure of the fungal particle within arms of cytoplasm. Lysosomal granules are abundant but mitochondria are rare (×15 000). (b) Phagocytosis of *C. albicans* by a monocyte showing near completion of phagosome formation (arrowed) around one organism and complete ingestion of two others (×5000). (Courtesy of Dr H. Valdimarsson.)

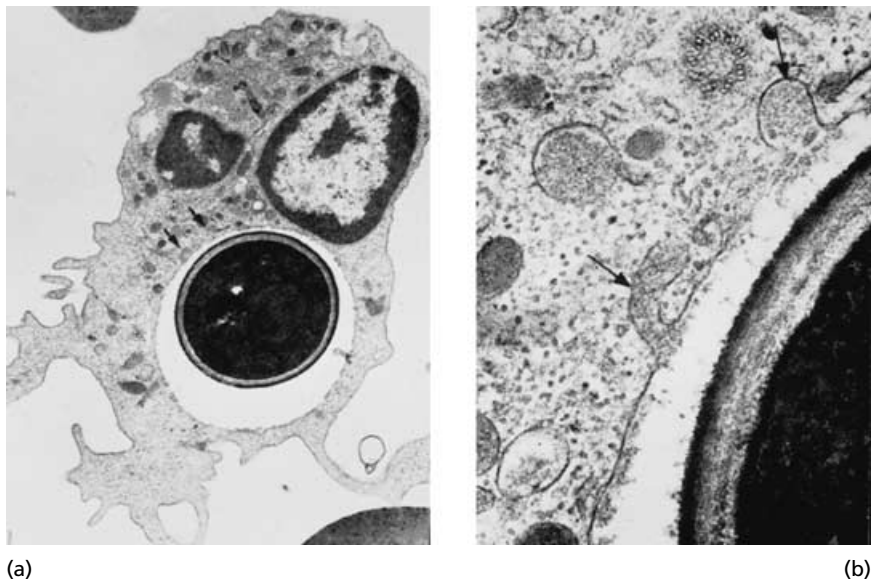



**Figure 1.9. Phagolysosome formation.** (a) Neutrophil 30 minutes after ingestion of *C. albicans*. The cytoplasm is already partly degranulated and two lysosomal granules (arrowed) are fusing with the phagocytic vacuole. Two lobes of the nucleus are evident (×5000). (b) Higher magnification of (a) showing fusing granules discharging their contents into the phagocytic vacuole (arrowed) (×33 000). (Courtesy of Dr H. Valdimarsson.)

transmembrane channel which may be involved in transporting NO· across lysosome membranes.

### *Killing by preformed antimicrobials* ( figure 1.10c)

These molecules, contained within the neutrophil granules, contact the ingested microorganism when fusion with the phagosome occurs. The dismutation of superoxide consumes hydrogen ions and raises the pH of the vacuole gently, so allowing the family of cationic proteins and peptides to function optimally. The latter, known as **α-defensins**, are approximately 3.5–4 kDa and invariably rich in arginine, and reach incredibly high concentrations within the phagosome, of the order of 20–100 mg/ml. Like the bacterial colicins described above, they have an amphipathic structure which allows them to insert into microbial membranes to form destabilizing voltage-regulated ion channels (who copied whom?). These antibiotic peptides, at concentrations of 10–100 µg/ml, act as disinfectants against a wide spectrum of Gram-positive and -negative bacteria, many fungi and a number of enveloped viruses. Many exhibit remarkable selectivity for prokaryotic and eukaryotic microbes relative to host cells, partly dependent upon differential membrane lipid composition. One must be impressed by the ability of this surprisingly simple tool to discriminate large classes of nonself cells, i.e. microbes from self.

As if this was not enough, further damage is inflicted on the bacterial membranes both by neutral proteinase (cathepsin G) action and by direct transfer to the microbial surface of BPI, which increases bacterial permeability. Low pH, lysozyme and lactoferrin constitute bactericidal or bacteriostatic factors which are oxygen independent and can function under anerobic circumstances. Finally, the killed organisms are digested by hydrolytic enzymes and the degradation products released to the exterior (figure 1.7.8).

By now, the reader may be excused a little smugness as she or he shelters behind the impressive antimicrobial potential of the phagocytic cells. But there are snags to consider; our formidable array of weaponry is useless unless the phagocyte can: (i) 'home onto' the microorganism, (ii) adhere to it, and (iii) respond by the membrane activation which initiates engulfment.

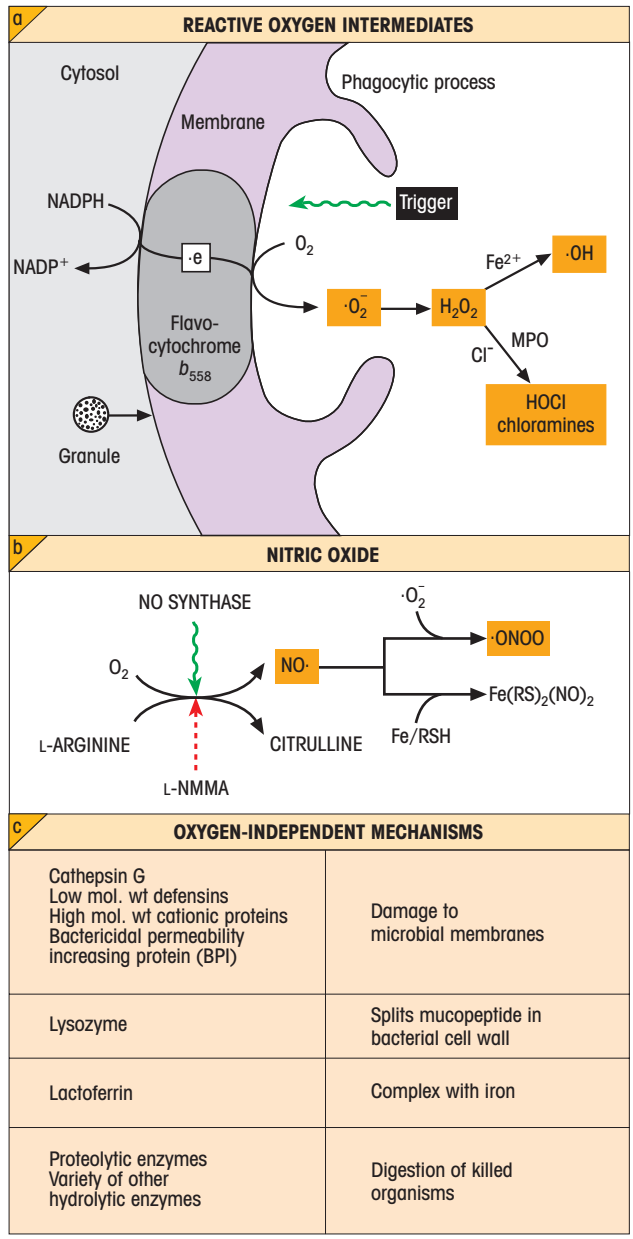



**Figure 1.10. Microbicidal mechanisms of phagocytic cells.** (a) Production of reactive oxygen intermediates. Electrons from NADPH are transferred by the flavocytochrome oxidase enzyme to molecular oxygen to form the microbicidal molecular species shown in the boxes. (*For the more studious*—The phagocytosis triggering agent binds to a classic G-protein-linked seven transmembrane domain receptor which activates an intracellular guanosine triphosphate (GTP)-binding protein. This in turn activates an array of enzymes: phosphoinositol-3 kinase concerned in the cytoskeletal reorganization underlying chemotactic responses (p. 10), phospholipase-Cγ2 mediating events leading to lysosome degranulation and phosphorylation of p47 phox through activation of protein kinase C, and the MEK and MAP kinase systems (cf. figure 9.6) which oversee the assembly of the NADPH oxidase. This is composed of the membrane cytochrome $b_{558}$, consisting of a p21 heme protein linked to gp91 with binding sites for NADPH and FAD on its intracellular aspect, to which phosphorylated p47 and p67 translocate from the cytosol on activation of the oxidase.) (b) Generation of nitric oxide. The enzyme, which structurally resembles the NADPH oxidase, can be inhibited by the arginine analog *N*-monomethyl-L-arginine (L-NMMA). The combination of NO· with superoxide anion yields the highly toxic peroxynitrite radical ·ONOO which cleaves on protonation to form reactive ·OH and $NO_2$ molecules. NO· can form mononuclear iron dithioldinitroso complexes leading to iron depletion and inhibition of several enzymes. (c) The basis of oxygen-independent antimicrobial systems.

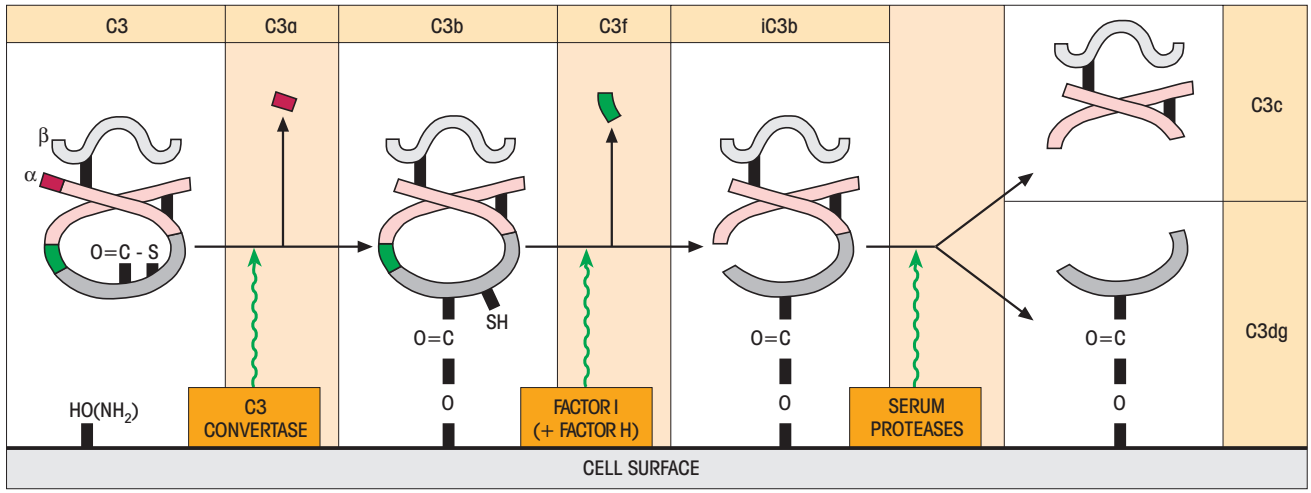



**Figure 1.11. Structural basis for the cleavage of C3 by C3 convertase** and its covalent binding to ·OH or ·$NH_2$ groups at the cell surface through exposure of the internal thiolester bonds. Further cleavage leaves the progressively smaller fragments, C3dg and C3d, attached to the membrane. (Based essentially on Law S.H.A. & Reid K.B.M. (1988) *Complement*, figure 2.4. IRL Press, Oxford.)

Some bacteria do produce chemical substances, such as the peptide formyl.Met.Leu.Phe, which directionally attract leukocytes, a process known as **chemotaxis**; many organisms do adhere to the phagocyte surface and many do spontaneously provide the appropriate membrane initiation signal. However, our teeming microbial adversaries are continually mutating to produce new species which may outwit the defenses by doing none of these. What then? The body has solved these problems with the effortless ease that comes with a few million years of evolution by developing the **complement** system.

## COMPLEMENT FACILITATES PHAGOCYTOSIS

### Complement and its activation

Complement is the name given to a complex series of some 20 proteins which, along with blood clotting, fibrinolysis and kinin formation, forms one of the triggered enzyme systems found in plasma. These systems characteristically produce a rapid, highly amplified response to a trigger stimulus mediated by a cascade phenomenon where the product of one reaction is the enzymic catalyst of the next.

Some of the complement components are designated by the letter 'C' followed by a number which is related more to the chronology of its discovery than to its position in the reaction sequence. The most abundant and the most pivotal component is C3 which has a molecular weight of 195 kDa and is present in plasma at a concentration of around 1.2 mg/ml.

#### *C3 undergoes slow spontaneous cleavage*

Under normal circumstances, an internal thiolester bond in C3 (figure 1.11) becomes activated spontaneously at a very slow rate, either through reaction with water or with trace amounts of a plasma proteolytic enzyme, to form a reactive intermediate, either the split product C3b, or a functionally similar molecule designated C3i or C3($H_2O$). In the presence of $Mg^{2+}$ this can complex with another complement component, factor B, which then undergoes cleavage by a normal plasma enzyme (factor D) to generate $C\overline{3bBb}$. Note that, conventionally, a bar over a complex denotes enzymic activity and that, on cleavage of a complement component, the larger product is generally given the suffix 'b' and the smaller 'a'.

$C\overline{3bBb}$ has an important new enzymic activity: it is a **C3 convertase** which can split C3 to give C3a and C3b. We will shortly discuss the important biologic consequences of C3 cleavage in relation to microbial defenses, but under normal conditions there must be some mechanism to restrain this process to a 'tick-over' level since it can also give rise to more $C\overline{3bBb}$, that is, we are dealing with a potentially runaway **positive-feedback loop** (figure 1.12). As with all potentially explosive triggered cascades, there are powerful regulatory mechanisms.

#### *C3b levels are normally tightly controlled*

In solution, the $C\overline{3bBb}$ convertase is unstable and factor B is readily displaced by another component, factor

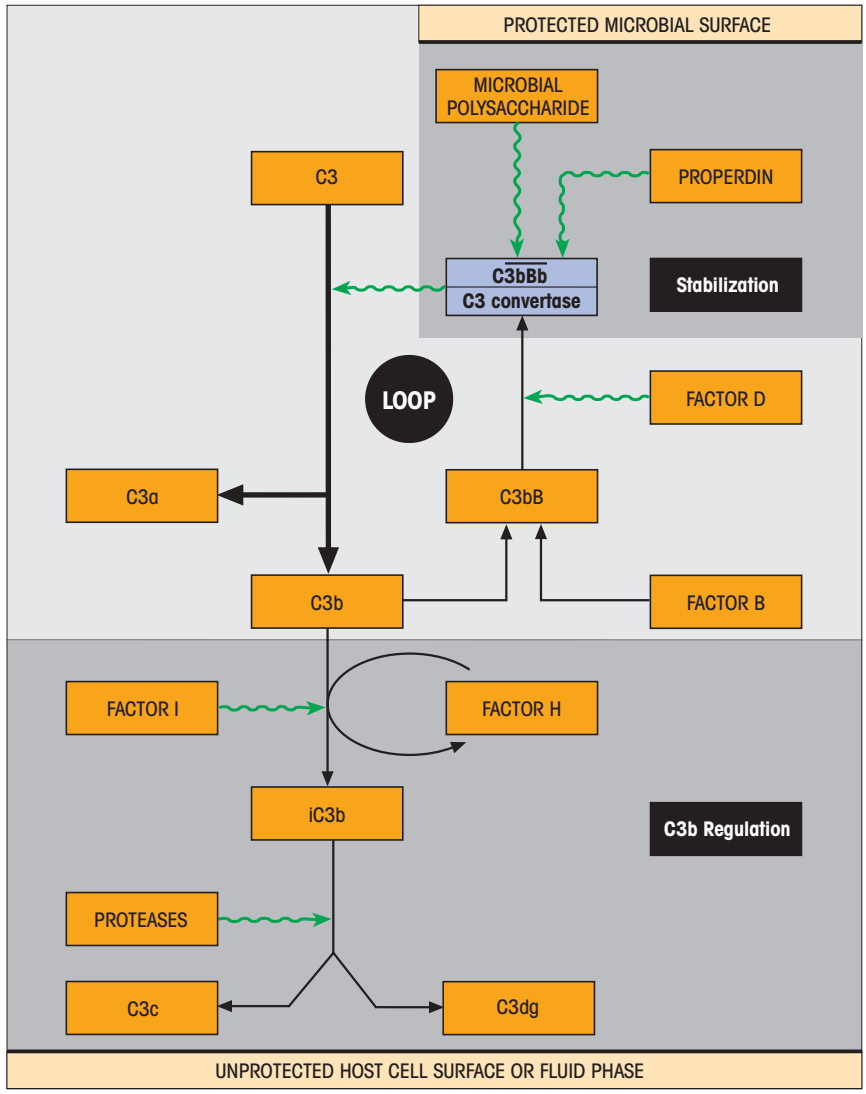



**Figure 1.12. Microbial activation of the alternative complement pathway** by stabilization of the C3 convertase ($C\overline{3bBb}$), and its control by factors H and I. When bound to the surface of a host cell or in the fluid phase, the C3b in the convertase is said to be 'unprotected' in that its affinity for factor H is much greater than for factor B and is therefore susceptible to breakdown by factors H and I. On a microbial surface, C3b binds factor B more strongly than factor H and is therefore 'protected' from or 'stabilized' against cleavage—even more so when subsequently bound by properdin. Although in phylogenetic terms this is the oldest complement pathway, it was discovered after a separate pathway to be discussed in the next chapter, and so has the confusing designation 'alternative'. ⇝ represents an activation process. The horizontal bar above a component designates its activation.

H, to form C3bH which is susceptible to attack by the C3b inactivator, factor I (figure 1.12; further discussed on p. 307). The inactivated iC3b is biologically inactive and undergoes further degradation by proteases in the body fluids. Other regulatory mechanisms are discussed at a later stage (see p. 307).

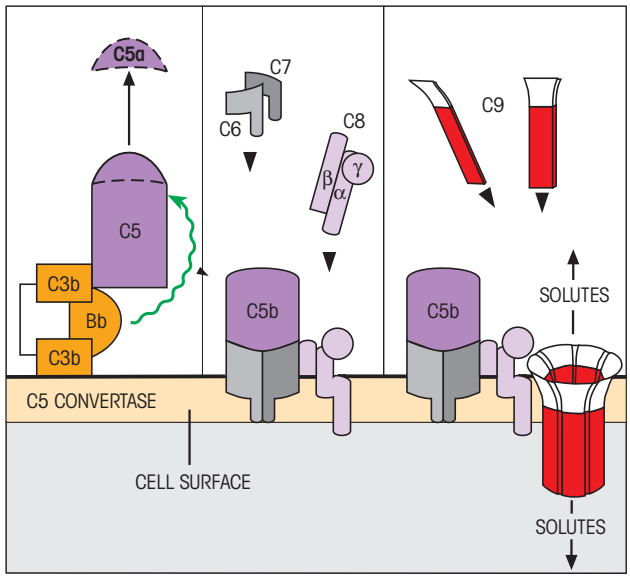
### *C3 convertase is stabilized on microbial surfaces*

A number of microorganisms can activate the $C\overline{3bBb}$ convertase to generate large amounts of C3 cleavage products by stabilizing the enzyme on their (carbohydrate) surfaces, thereby protecting the C3b from factor H. Another protein, properdin, acts subsequently on this bound convertase to stabilize it even further. As C3 is split by the surface membrane-bound enzyme to nascent C3b, it undergoes conformational change and its potentially reactive internal thiolester bond becomes exposed. Since the half-life of nascent C3b is less than 100 µsec, it can only diffuse a short distance before reacting covalently with local hydroxyl or amino groups available at the microbial cell surface (figure 1.11). Each catalytic site thereby leads to the clustering of large numbers of C3b molecules on the microorganism. This series of reactions leading to C3 breakdown provoked directly by microbes has been called **the alternative pathway** of complement activation (figure 1.12).

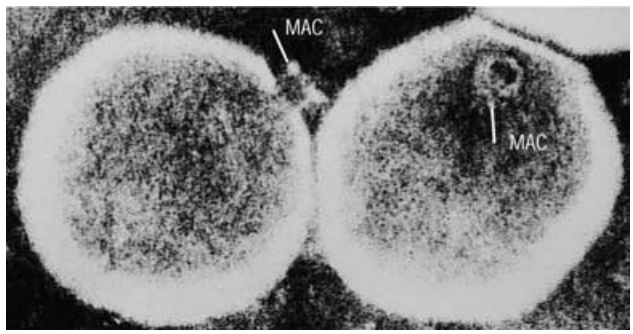
### *The post-C3 pathway generates a membrane attack complex*

Recruitment of a further C3b molecule into the $C\overline{3bBb}$ enzymic complex generates a C5 convertase which

activates C5 by proteolytic cleavage releasing a small polypeptide, C5a, and leaving the large C5b fragment loosely bound to C3b. Sequential attachment of C6 and C7 to C5b forms a complex with a transient membrane-binding site and an affinity for the β-peptide chain of C8. The C8α chain sits in the membrane and directs the conformational changes in C9 which transform it into an amphipathic molecule capable of insertion into the lipid bilayer (cf. the colicins, p. 2) and polymerization to an annular **membrane attack complex** (MAC; figures 1.13 and 2.4). This forms a transmembrane channel fully permeable to electrolytes and water, and due to the high internal colloid osmotic pressure of cells, there is a net influx of $Na^+$ and water frequently leading to lysis.




(a)




(b)

**Figure 1.13. Post-C3 pathway generating C5a and the C5b–9 membrane attack complex (MAC).** (a) Cartoon of molecular assembly. The conformational change in C9 protein structure which converts it from a hydrophilic to an amphipathic molecule (bearing both hydrophobic and hydrophilic regions) can be interrupted by an antibody raised against linear peptides derived from C9; since the antibody does not react with the soluble or membrane-bound forms of the molecule, it must be detecting an intermediate structure transiently revealed in a deep-seated structural rearrangement. (b) Electron micrograph of a membrane C5b–9 complex incorporated into liposomal membranes clearly showing the annular structure. The cylindrical complex is seen from the side inserted into the membrane of the liposome on the left, and end-on in that on the right. Although in itself a rather splendid structure, formation of the annular C9 cylinder is probably not essential for cytotoxic perturbation of the target cell membrane, since this can be achieved by insertion of amphipathic C9 molecules in numbers too few to form a clearly defined MAC. (Courtesy of Professor J. Tranum-Jensen and Dr S. Bhakdi.)

## Complement has a range of defensive biological functions

These can be grouped conveniently under three headings.

### *1 C3b adheres to complement receptors*

Phagocytic cells have receptors for C3b (CR1) and iC3b (CR3) which facilitate the adherence of C3b-coated microorganisms to the cell surface (discussed more fully on p. 258).

### *2 Biologically active fragments are released*

C3a and C5a, the small peptides split from the parent molecules during complement activation, have several important actions. Both act directly on phagocytes, especially neutrophils, to stimulate the respiratory burst associated with the production of reactive oxygen intermediates and to enhance the expression of surface receptors for C3b and iC3b. Also, both are **anaphylatoxins** in that they are capable of triggering mediator release from mast cells (figures 1.4k and 1.14) and their circulating counterpart, the basophil (figure 1.4i), a phenomenon of such relevance to our present discussion that we have presented details of the mediators and their actions in figure 1.15; note in particular the chemotactic properties of these mediators and their effects on blood vessels. In its own right, C3a is a chemoattractant for eosinophils whilst C5a is a potent neutrophil chemotactic agent and also has a striking ability to act directly on the capillary endothelium to produce vasodilatation and increased permeability, an effect which seems to be prolonged by leukotriene $B_4$ released from activated mast cells, neutrophils and macrophages.

### *3 The terminal complex can induce membrane lesions*

As described above, the insertion of the membrane attack complex into a membrane may bring about cell lysis. Providentially, complement is relatively inefficient at lysing the cell membranes of the autologous host due to the presence of control proteins (cf. p. 307).

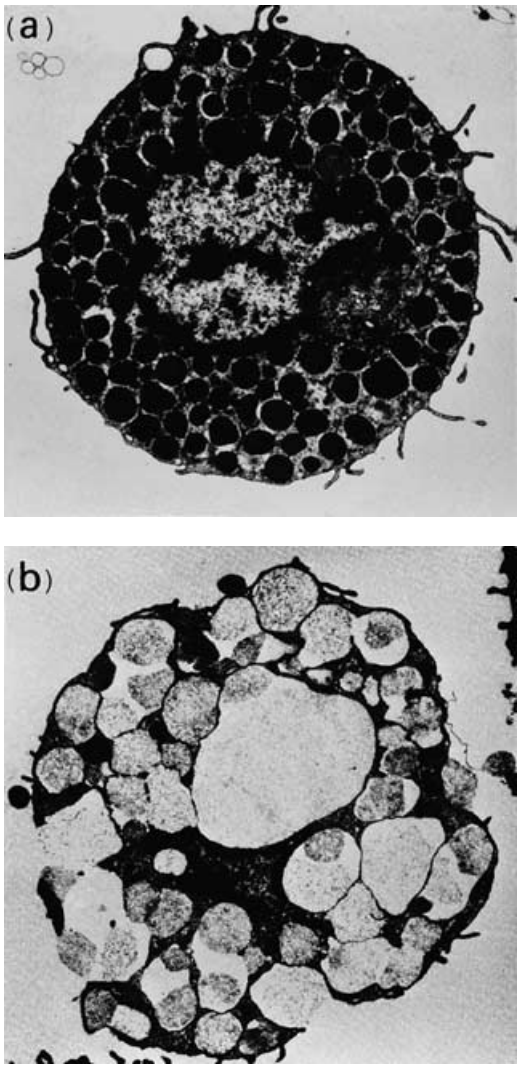



**Figure 1.14. The mast cell.** (a) A resting cell with many membrane-bound granules containing preformed mediators. (b) A triggered mast cell. Note that the granules have released their contents and are morphologically altered, being larger and less electron dense. Although most of the altered granules remain within the circumference of the cell, they are open to the extracellular space. (Electron micrographs ×5400.) (Courtesy of Drs D. Lawson, C. Fewtrell, B. Gomperts and M.C. Raff from (1975) *Journal of Experimental Medicine* **142**, 391.)

## COMPLEMENT CAN MEDIATE AN ACUTE INFLAMMATORY REACTION

We can now put together an effectively orchestrated defensive scenario initiated by activation of the alternative complement pathway (see figure 1.16).

In the first act, $C3b\overline{Bb}$ is stabilized on the surface of the microbe and cleaves large amounts of C3. The C3a fragment is released but C3b molecules bind copiously to the microbe. These activate the next step in the sequence to generate C5a and the membrane attack complex (although many organisms will be resistant to its action).

### The mast cell plays a central role

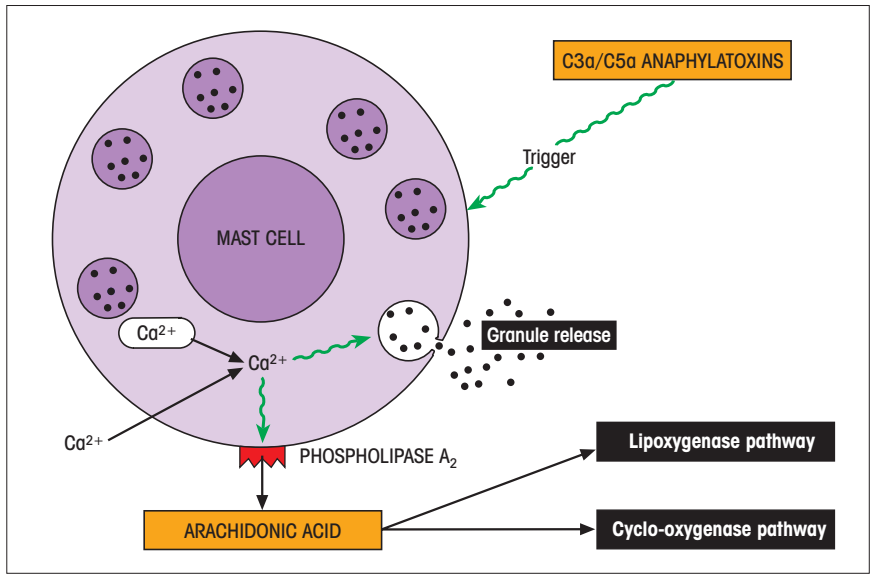
The next act sees C3a and C5a, together with the mediators they trigger from the mast cell, acting to recruit polymorphonuclear phagocytes and further plasma complement components to the site of microbial invasion. The relaxation induced in arteriolar walls causes increased blood flow and dilatation of the small vessels, while contraction of capillary endothelial cells allows exudation of plasma proteins. Under the influence of the chemotaxins, neutrophils slow down and the surface adhesion molecules they are stimulated to express cause them to marginate to the walls of the capillaries where they pass through gaps between the endothelial cells (diapedesis) and move up the concentration gradient of chemotactic factors until they come face to face with the C3b-coated microbe. Adherence to the neutrophil C3b receptors then takes place, C3a and C5a at relatively high concentrations in the chemotactic gradient activate the respiratory burst and, hey presto, the slaughter of the last act can begin!

The processes of capillary dilatation (redness), exudation of plasma proteins and also of fluid (edema) due to hydrostatic and osmotic pressure changes, and accumulation of neutrophils are collectively termed the **acute inflammatory response**.

### Macrophages can also do it

Although not yet established with the same confidence that surrounds the role of the mast cell in acute inflammation, the concept seems to be emerging that the tissue macrophage may mediate a parallel series of events with the same final end result. Nonspecific phagocytic events and certain bacterial toxins such as the lipopolysaccharides (LPSs) can activate macrophages, but the phagocytosis of C3b-opsonized microbes and the direct action of C5a generated through complement activation are guaranteed to goad the cell into copious secretion of soluble mediators of the acute inflammatory response (figure 1.17).

These upregulate the expression of adhesion molecules for neutrophils on the surface of endothelial cells, increase capillary permeability and promote the chemotaxis and activation of the polymorphonuclear neutrophils themselves. Thus, under the stimulus of complement activation, the macrophage provides a pattern of cellular events which reinforces the




**Granule release**

| PRE-FORMED | EFFECT |
|---|---|
| HISTAMINE | Vasodilatation, incr. capillary permeability chemokinesis, bronchoconstriction |
| PROTEOGLYCAN | Binds granule proteases |
| NEUTRAL PROTEASES<br>β-GLUCOSAMINIDASE | Activates C3<br>Splits off glucosamine |
| ECF<br>NCF | Eosinophil chemotaxis<br>Neutrophil chemotaxis |
| PLATELET ACTIVATING FACTOR | Mediator release |
| INTERLEUKINS 3,4, 5 & 6<br>GM-CSF, TNF | Multiple, including macrophage activation, trigger acute phase proteins, etc. (cf. Chapter 10) |

| NEWLY SYNTHESIZED | EFFECT |
|---|---|
| **Lipoxygenase pathway** → LEUKOTRIENES $C_4$, $D_4$ (SRS-A), $B_4$ | Vasoactive, bronchoconstriction, chemotaxis |
| **Cyclo-oxygenase pathway** → PROSTAGLANDINS<br>THROMBOXANES | Affect bronchial muscle, platelet aggregation and vasodilatation |

**Figure 1.15. Mast cell triggering leading to release of mediators by two major pathways:** (i) release of preformed mediators present in the granules, and (ii) the metabolism of arachidonic acid produced through activation of a phospholipase. Intracellular $Ca^{2+}$ and cyclic AMP are central to the initiation of these events but details are still unclear. Mast cell triggering may occur through C3a, C5a and even by some microorganisms which can act directly on cell surface receptors. Mast cell heterogeneity is discussed on p. 323. ECF, eosinophil chemotactic factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; NCF, neutrophil chemotactic factor. Chemotaxis refers to directed migration of granulocytes up the pathway concentration gradient of the mediator.

mast cell-mediated pathway leading to acute inflammation—yet another of the body's fail-safe redundancy systems (often known as the 'belt and braces' principle).

## HUMORAL MECHANISMS PROVIDE A SECOND DEFENSIVE STRATEGY

### Microbicidal factors in secretions

Turning now to those defense systems which are mediated entirely by soluble factors, we recollect that many microbes activate the complement system and may be lysed by the insertion of the membrane attack complex. The spread of infection may be limited by enzymes released through tissue injury which activate the clotting system. Of the soluble bactericidal substances elaborated by the body, perhaps the most abundant and widespread is the enzyme lysozyme, a muramidase which splits the exposed peptidoglycan wall of susceptible bacteria (cf. figure 13.5).

Like the α-defensins of the neutrophil granules, the human β-defensins are peptides derived by proteolytic cleavage from larger precursors; they have β-sheet structures, 29–40 amino acids and three intramolecular disulfide bonds, although they differ from

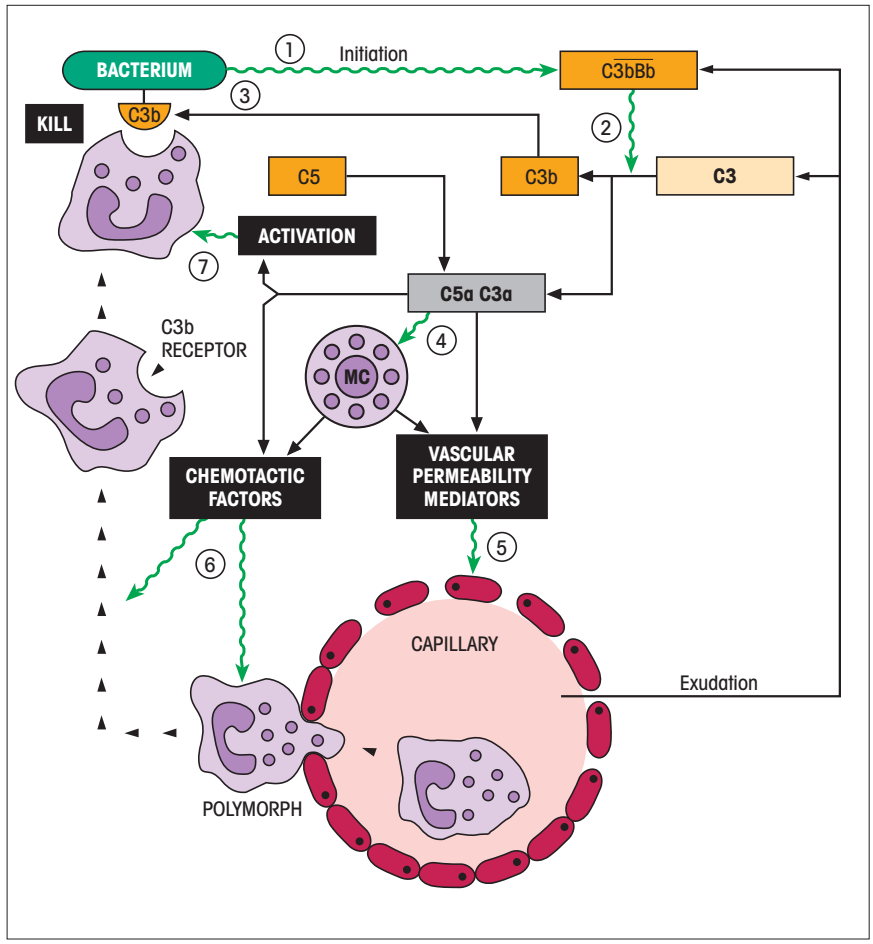



**Figure 1.16. The defensive strategy of the acute inflammatory reaction initiated by bacterial activation of the alternative C pathway.** Directions: ① start with the activation of the C3bBb C3 convertase by the bacterium, ② notice the generation of C3b (③ which binds to the bacterium), C3a and C5a, ④ which recruit mast cell mediators; ⑤ follow their effect on capillary dilatation and exudation of plasma proteins and ⑥ their chemotactic attraction of neutrophils to the C3b-coated bacterium and triumph in ⑦ the adherence and final activation of neutrophils for the kill.

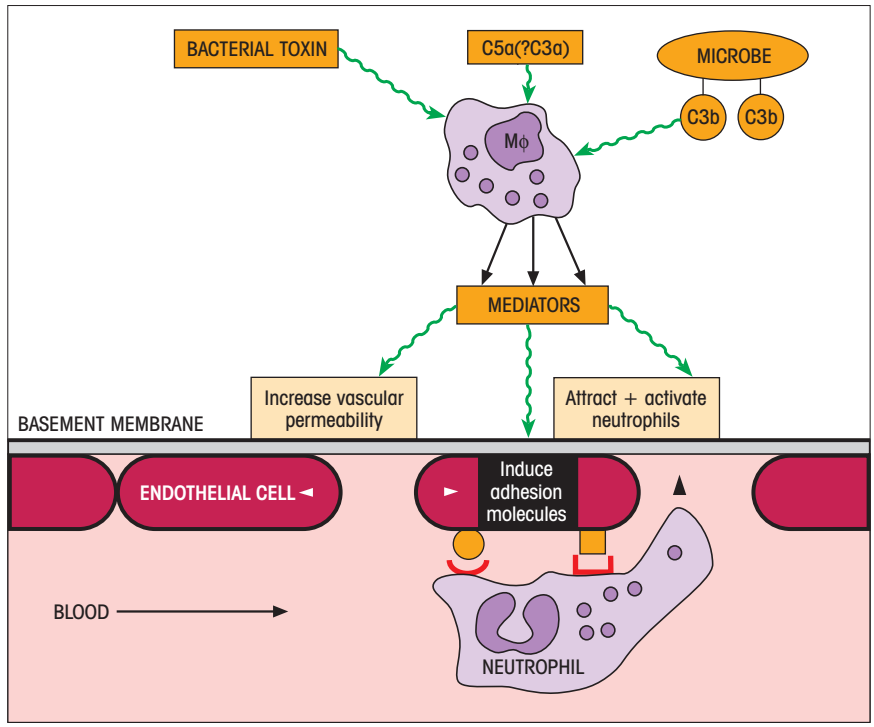



**Figure 1.17. Stimulation by complement components and bacterial toxins such as LPS induces macrophage secretion of mediators of an acute inflammatory response.** Blood neutrophils stick to the adhesion molecules on the endothelial cell and use this to provide traction as they force their way between the cells, through the basement membrane (with the help of secreted elastase) and up the chemotactic gradient.

the α-defensins in the placement of their six cysteines. The main human β-defensin, hDB-1, is produced abundantly in the kidney, the female reproductive tract, the oral gingiva and especially the lung airways. Since the word has it that we are all infected every day by tens of thousands of airborne bacteria, this must be an important defense mechanism. This being so, inhibition of hDB-1 and of a second pulmonary defensin, hDB-2, by high ionic strength could account for the susceptibility of cystic fibrosis patients to infection since they have an ion channel mutation which results in an elevated chloride concentration in airway surface fluids. Another airway antimicrobial active against Gram-negative and -positive bacteria is LL-37, a 37-residue α-helical peptide released by proteolysis of a cathelicidin (cathepsin L-inhibitor) precursor.

This theme surfaces again in the stomach where a peptide split from lactoferrin by pepsin could provide the gastric and intestinal secretions with some antimicrobial policing. A rather longer two-domain peptide with 108 residues, termed secretory leukoprotease inhibitor (SLPI), is found in many human secretions. The C-terminal domain is anti-protease but the N-terminal domain is distinctly unpleasant to metabolically active fungal cells and to various skin-associated microorganisms, which makes its production by human keratinocytes particularly appropriate. In passing, it is worth pointing out that many D-amino acid analogs of peptide antibiotics form left-handed helices which retain the ability to induce membrane ion channels and hence their antimicrobial powers and, given their resistance to catabolism within the body, should be attractive candidates for a new breed of synthetic antibiotics. Lastly, we may mention the two lung surfactant proteins SP-A and SP-D which, in conjunction with various lipids, lower the surface tension of the epithelial lining cells of the lung to keep the airways patent. They belong to a totally different structural group of molecules termed collectins (see below) which contribute to innate immunity through binding of their lectin-like domains to carbohydrates on microbes, and their collagenous stem to cognate receptors on phagocytic cells—thereby facilitating the ingestion and killing of the infectious agents.

## Acute phase proteins increase in response to infection

A number of plasma proteins collectively termed acute phase proteins show a dramatic increase in concentration in response to early 'alarm' mediators such as macrophage-derived interleukin-1 (IL-1) released as a result of infection or tissue injury. These include C-reactive protein (CRP), mannose-binding protein (MBP) and serum amyloid P component (table 1.1). Other acute phase proteins showing a more modest rise in concentration include $\alpha_1$-antichymotrypsin, fibrinogen, ceruloplasmin, C9 and factor B. Overall, it seems likely that the acute phase response achieves a beneficial effect through enhancing host resistance, minimizing tissue injury and promoting the resolution and repair of the inflammatory lesion.

**Table 1.1. Acute phase proteins.**

| Acute phase reactant | Role |
|---|---|
| Dramatic increases in concentration: | |
| C-reactive protein | Fixes complement, opsonizes |
| Mannose binding protein | Fixes complement, opsonizes |
| $\alpha_1$-acid glycoprotein | Transport protein |
| Serum amyloid P component | Amyloid component precursor |
| Moderate increases in concentration: | |
| $\alpha_1$-proteinase inhibitors | Inhibit bacterial proteases |
| $\alpha_1$-antichymotrypsin | Inhibit bacterial proteases |
| C3, C9, factor B | Increase complement function |
| Ceruloplasmin | $\cdot O_2^-$ scavenger |
| Fibrinogen | Coagulation |
| Angiotensin | Blood pressure |
| Haptoglobin | Bind hemoglobin |
| Fibronectin | Cell attachment |

To take an example, during an infection, microbial products such as endotoxins stimulate the release of IL-1, which is an endogenous pyrogen (incidentally capable of improving our general defenses by raising the body temperature), and IL-6. These in turn act on the liver to increase the synthesis and secretion of CRP to such an extent that its plasma concentration may rise 1000-fold.

Human CRP is composed of five identical polypeptide units noncovalently arranged as a cyclic pentamer around a Ca-binding cavity. These protein **pentraxins** have been around in the animal kingdom for some time, since a closely related homolog, limulin, is present in the hemolymph of the horseshoe crab, not exactly a close relative of *Homo sapiens*. A major property of CRP is its ability to bind in a Ca-dependent fashion, as a pattern recognition molecule, to a number of microorganisms which contain phosphorylcholine in their membranes, the complex having the useful

property of activating complement (by the classical and not the alternative pathway with which we are at present familiar). This results in the deposition of C3b on the surface of the microbe which thus becomes **opsonized** (i.e. 'made ready for the table') for adherence to phagocytes.

Yet another member of this pentameric family is the serum amyloid P (SAP) component. This protein can complex with chondroitin sulfate, a cell matrix glycosaminoglycan, and subsequently bind lysosomal enzymes such as cathepsin B released within a focus of inflammation. The degraded SAP becomes a component of the amyloid fibrillar deposits which accompany chronic infections—it might even be a key initiator of amyloid deposition (cf. p. 385).

A most important acute phase opsonin is the Ca-dependent **mannose-binding protein (MBP)** which can react not only with mannose but several other sugars, so enabling it to bind with an exceptionally wide variety of Gram-negative and -positive bacteria, yeasts, viruses and parasites; its subsequent ability to trigger the classical C3 convertase through two novel associated serine proteases (MASP-1 and MASP-2) qualifies it as an opsonin. (Please relax, we unravel the secrets of the classical pathway in the next chapter.) MBP is a multiple of trimeric complexes, each unit of which contains a collagen-like region joined to a globular lectin-binding domain. This structure places it in the family of **collectins** (**col**lagen + **lectin**) which have the ability to recognize 'foreign' carbohydrate patterns differing from 'self' surface polysaccharides normally decorated by terminal galactose and sialic acid groups, whilst the collagen region can bind to and activate phagocytic cells through complementary receptors on their surface. The collectins, especially MBP and the alveolar surfactant molecules SP-A and SP-D mentioned earlier, have many attributes that qualify them for a first-line role in innate immunity. These include the ability to differentiate self from nonself, to bind to a variety of microbes, to generate secondary effector mechanisms, and to be widely distributed throughout the body including mucosal secretions.

Interest in the collectin conglutinin has perked up recently with the demonstration, first, that it is found in humans and not just in cows, and second, that it can bind to *N*-acetylglucosamine; being polyvalent, this implies an ability to coat bacteria with C3b by cross-linking the available sugar residue in the complement fragment with the bacterial proteoglycan. Although it is not clear whether conglutinin is a member of the acute phase protein family, we mention it here because it embellishes the general idea that the evolution of lectin-like molecules which bind to microbial rather than self polysaccharides, and which can then hitch themselves to the complement system or to phagocytic cells, has proved to be such a useful form of protection for the host (figure 1.18).

## Interferons inhibit viral replication

These are a family of broad-spectrum antiviral agents present in birds, reptiles and fishes as well as the higher animals, and first recognized by the phenomenon of viral interference in which an animal infected with one virus resists superinfection by a second unrelated virus. Different molecular forms of interferon have been identified, all of which have been gene cloned. There are at least 14 different α-interferons (IFNα) produced by leukocytes, while fibroblasts, and probably all cell types, synthesize IFNβ. We will keep a third type (IFNγ), which is not directly induced by viruses, up our sleeves for the moment.

Cells synthesize interferon when infected by a virus and secrete it into the extracellular fluid where it binds to specific receptors on uninfected neighboring cells. The bound interferon now exerts its antiviral effect in the following way. At least two genes are thought to be derepressed in the interferon-treated cell allowing the synthesis of two new enzymes. The first, a protein kinase, catalyses the phosphorylation of a ribosomal protein and an initiation factor necessary for protein synthesis, so greatly reducing mRNA translation. The other catalyses the formation of a short polymer of adenylic acid which activates a latent endonuclease; this in turn degrades both viral and host mRNA.

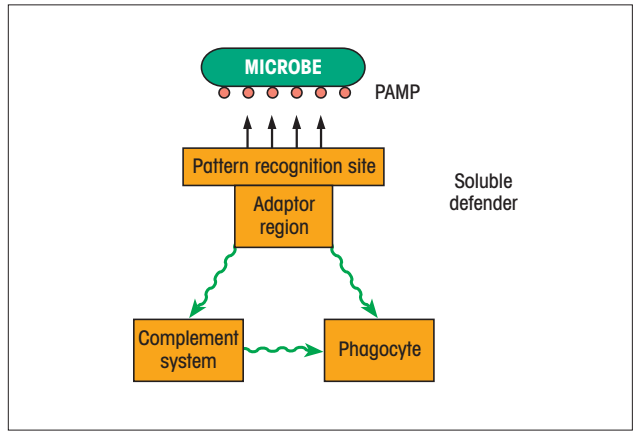



**Figure 1.18. A major defensive strategy by soluble factors.** The recognition elements link the microorganism to a microbicidal system through the adaptor region. PAMP, pathogen-associated molecular pattern.

Whatever the precise mechanism of action ultimately proves to be, the net result is to establish a cordon of uninfectable cells around the site of virus infection so restraining its spread. The effectiveness of interferon *in vivo* may be inferred from experiments in which mice injected with an antiserum to murine interferons could be killed by several hundred times less virus than was needed to kill the controls. However, it must be presumed that interferon plays a significant role in the recovery from, as distinct from the prevention of, viral infections.

As a group, the interferons may prove to have a wider biologic role than the control of viral infection. It will be clear, for example, that the induced enzymes described above would act to inhibit host cell division just as effectively as viral replication. The interferons may also modulate the activity of other cells, such as the natural killer cells, to be discussed in the following section.

## EXTRACELLULAR KILLING

### Natural killer (NK) cells

Viruses lack the apparatus for self renewal and so it is essential for them to penetrate the cells of the infected host in order to take over its replicative machinery. It is clearly in the interest of the host to find a way to kill such infected cells before the virus has had a chance to reproduce. NK cells appear to do just that when studied *in vitro*.

They are large granular lymphocytes (figure 2.6a) with a characteristic morphology (figure 2.7b). Killer and target are brought into close opposition (figure 1.19a) through recognition by lectin-like (i.e. carbohydrate-binding) and other receptors on the NK cell (cf. p. 69) of structures on high molecular weight glycoproteins on the surface of virally infected cells. Activation of the NK cell ensues and leads to polarization of granules between nucleus and target within minutes and extracellular release of their contents into the space between the two cells followed by target cell death.

One of the most important of the granule components is a **perforin** or cytolysin bearing some structural homology to C9; like that protein, but without any help other than from $Ca^{2+}$, it can insert itself into the membrane of the target, apparently by binding to phosphorylcholine through its central amphipathic domain. It then polymerizes to form a transmembrane pore with an annular structure, comparable to the complement membrane attack complex (figure 1.19a).

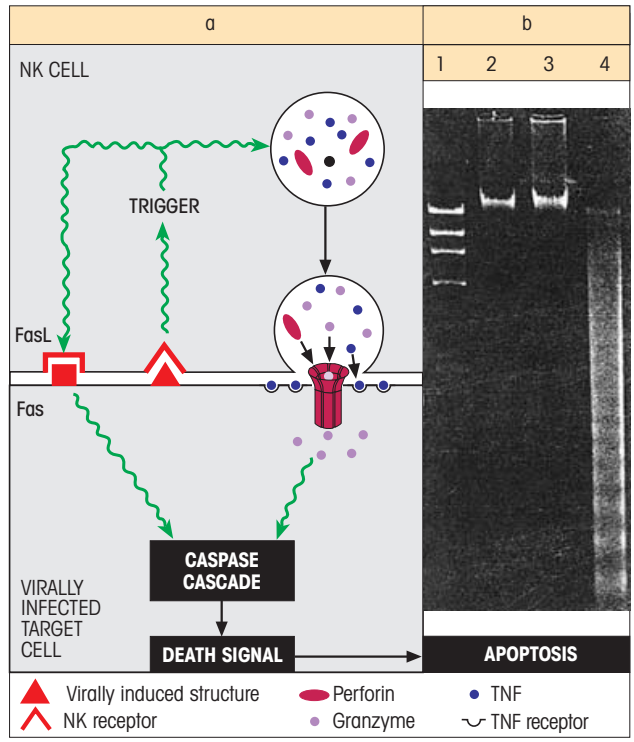



**Figure 1.19. Extracellular killing of virally infected cell by natural killer (NK) cell.** (a) Binding of the NK receptors to the surface of the virally infected cell triggers the extracellular release of perforin molecules from the granules; these polymerize to form transmembrane channels which may facilitate lysis of the target by permitting entry of granzymes which induce apoptotic cell death through activation of the caspase protease cascade and ultimate fragmentation of nuclear DNA. (Model resembling that proposed by Hudig D., Ewoldt G.R. & Woodward S.L. (1993) *Current Opinion in Immunology* **5**, 90.). Another granule component, TNF, activates caspase-dependent apoptosis through the 'death domains' of the surface TNF receptors on the target cell. Engagement of the NK receptor also activates a parallel killing mechanism mediated through the binding of the Fas-ligand (FasL) on the effector to the target cell Fas receptor whose cytoplasmic death domains activate procaspase-8. (b) Fragmentation of nucleosome DNA into 200 kb 'ladder' fragments following programed cell death (kindly provided by Professor S. Martin). Lane 1: standards obtained by digestion of λ DNA by *Hin*dIII; lanes 2 and 3: undegraded DNA from normal control cells; lane 4: characteristic breakdown of DNA from apoptotic cells. Because this is such a fundamental 'default' mechanism in every cell, it is crucial for there to be heavy regulation: thus a large group of regulatory proteins, the Bcl-2 subfamily, inhibit apoptosis while the Bax and BH3 subfamilies promote it. The word 'apoptosis' in ancient Greek describes the falling of leaves from trees or of petals from flowers and aptly illustrates apoptosis in cells where they detach from their extracellular matrix support structures. (See figure 12.7 for morphologic appearance of apoptotic cells.)

## Target cells are told to commit suicide

Whereas C9-induced cell lysis is brought about through damage to outer membranes followed later by nuclear changes, NK cells kill by activating **apoptosis** (programed cell death), a mechanism present in every cell which leads to self immolation. Apoptosis is mediated by a cascade of proteolytic enzymes termed **caspases**. Like other multicomponent cascades, such as the blood clotting and complement systems, it depends upon the activation by proteolytic cleavage of a proenzyme next in the chain, and so on. The sequence terminates with very rapid nuclear fragmentation effected by a Ca-dependent endonuclease which acts on the vulnerable DNA between nucleosomes to produce the 200 kb 'nucleosome ladder' fragments (figure 1.19b); only afterwards can one detect release of $^{51}$Cr-labeled cytoplasmic proteins through defective cell surface membranes. These nuclear changes are not produced by C9. Thus, although perforin and C9 appear to produce comparable membrane 'pores', there is a dramatic difference in their killing mechanisms.

In addition to perforin, the granules contain tumor necrosis factor-α (TNFα), lymphotoxin-β and a family of serine proteases termed **granzymes**, one of which, granzyme B, can function as an NK cytotoxic factor by passing through the perforin membrane pore into the cytoplasm where it can split procaspase-8 and activate the apoptotic process. Tumor necrosis factor can induce apoptotic cell death through reaction with cell surface TNF receptors whose cytoplasmic 'death domains' can also activate procaspase-8. Chondroitin sulfate A, a protease-resistant highly negatively charged proteoglycan present in the granules, may subserve the function of protecting the NK cell from autolysis by its own lethal agents.

Killing by NK cells can still occur in perforin-deficient mice, probably through a parallel mechanism involving **Fas** receptor molecules on the target cell surface. Engagement of Fas by the so-called **Fas-ligand (FasL)** on the effector cell provides yet another pathway for the induction of an apoptotic signal in the unlucky target.

The various interferons augment NK cytotoxicity and, since interferons are produced by virally infected cells, we have a nicely integrated feedback defense system.

## Eosinophils

Large parasites such as helminths cannot physically be phagocytosed and extracellular killing by eosinophils would seem to have evolved to help cope with this situation. These polymorphonuclear 'cousins' of the neutrophil have distinctive granules which stain avidly with acid dyes (figure 1.4c) and have a characteristic appearance in the electron microscope (figure 13.22). A major basic protein is localized in the core of the granules while an eosinophilic cationic protein together with a peroxidase have been identified in the granule matrix. Other enzymes include arylsulfatase B, phospholipase D and histaminase. They have surface receptors for C3b and on activation produce a particularly impressive respiratory burst with concomitant generation of active oxygen metabolites. Not satisfied with that, nature has also armed the cell with granule proteins capable of producing a transmembrane plug in the target membrane like C9 and the NK perforin. Quite a nasty cell.

Most helminths can activate the alternative complement pathway, but although resistant to C9 attack, their coating with C3b allows adherence of eosinophils through their C3b receptors. If this contact should lead to activation, the eosinophil will launch its extracellular attack which includes the release of the major basic protein and especially the cationic protein which damages the parasite membrane.

## SUMMARY

A wide range of innate immune mechanisms operate which do not improve with repeated exposure to infection.

### Barriers against infection

- Microorganisms are kept out of the body by the skin, the secretion of mucus, ciliary action, the lavaging action of bactericidal fluids (e.g. tears), gastric acid and microbial antagonism.
- If penetration occurs, bacteria are destroyed by soluble factors such as lysozyme and by phagocytosis with intracellular digestion.

### Phagocytic cells kill microorganisms

- The main phagocytic cells are polymorphonuclear neutrophils and macrophages.
- The phagocytic cells use their pattern recognition

(*continued p. 20*)

receptors (PRRs) to recognize and adhere to pathogen-associated molecular patterns (PAMPs) on the microbe surface.

- Organisms adhering to the phagocyte surface activate the engulfment process and are taken inside the cell where they fuse with cytoplasmic granules.
- A formidable array of microbicidal mechanisms then come into play: the conversion of $O_2$ to reactive oxygen intermediates, the synthesis of nitric oxide and the release of multiple oxygen-independent factors from the granules.

### Complement facilitates phagocytosis

- The complement system, a multicomponent triggered enzyme cascade, is used to attract phagocytic cells to the microbes and engulf them.
- The most abundant component, C3, is split by a convertase enzyme formed from its own cleavage product C3b and factor B and stabilized against breakdown caused by factors H and I, through association with the microbial surface. As it is formed, C3b becomes linked covalently to the microorganism.
- The next component, C5, is activated yielding a small peptide, C5a; the residual C5b binds to the surface and assembles the terminal components C6–9 into a membrane attack complex which is freely permeable to solutes and can lead to osmotic lysis.
- C5a is a potent chemotactic agent for neutrophils and greatly increases capillary permeability.
- C3a and C5a act on mast cells causing the release of further mediators, such as histamine, leukotriene $B_4$ and tumor necrosis factor (TNF), with effects on capillary permeability and adhesiveness, and neutrophil chemotaxis; they also activate neutrophils.

### The complement-mediated acute inflammatory reaction

- Following the activation of complement with the ensuing attraction and stimulation of neutrophils, the activated phagocytes bind to the C3b-coated microbes by their surface C3b receptors and may then ingest them. The influx of polymorphs and the increase in vascular permeability constitute the potent antimicrobial **acute inflammatory response** (figure 2.18).
- Inflammation can also be initiated by tissue macrophages which subserve a similar role to the mast cell, since signaling by bacterial toxins, C5a or iC3b-coated bacteria adhering to surface complement receptors causes release of neutrophil chemotactic and activating factors.

### Humoral mechanisms provide a second defensive strategy

- In addition to lysozyme, peptide defensins and the complement system, other humoral defenses involve the acute phase proteins, such as C-reactive and mannose-binding proteins, whose synthesis is greatly augmented by infection. Mannose-binding protein is a member of the collectin family including conglutinin and surfactants SP-A and SP-D, notable for their ability to distinguish microbial from 'self' surface carbohydrate groups by their pattern recognition molecules.
- Recovery from viral infections can be effected by the interferons which block viral replication.

### Extracellular killing

- Virally infected cells can be killed by large granular lymphocytes with NK activity through a perforin/granzyme and a separate Fas-mediated pathway, leading to programed cell death (apoptosis) mediated by activation of the caspase protease cascade which fragments the nuclear DNA.
- Extracellular killing by C3b-bound eosinophils may be responsible for the failure of many large parasites to establish a foothold in potential hosts.

See the accompanying website (**www.roitt.com**) for multiple choice questions.