CHAPTER 1

Innate immunity

We live in a potentially hostile world filled with a bewildering array of infectious agents against which we have developed a series of defense mechanisms at least their equal in effectiveness and ingenuity. It is these defense mechanisms that can establish a state of immunity against infection (Latin *immunitas*, freedom from) and whose operation provides the basis for the delightful subject called "Immunology."

A number of 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 and are usually present before the onset of the infectious agent. The innate response is not enhanced by previous exposure to the foreign organism and the response time is very rapid usually occurring in minutes or hours. 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. 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 that 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 antagonism where the normal bacterial flora of the body suppresses the growth of many potentially pathogenic bacteria and fungi. This is due to competition for essential nutrients or by the production of microbicidal substances. For example, pathogen invasion of the vagina is limited by lactic acid produced by commensal organisms which metabolize glycogen secreted by

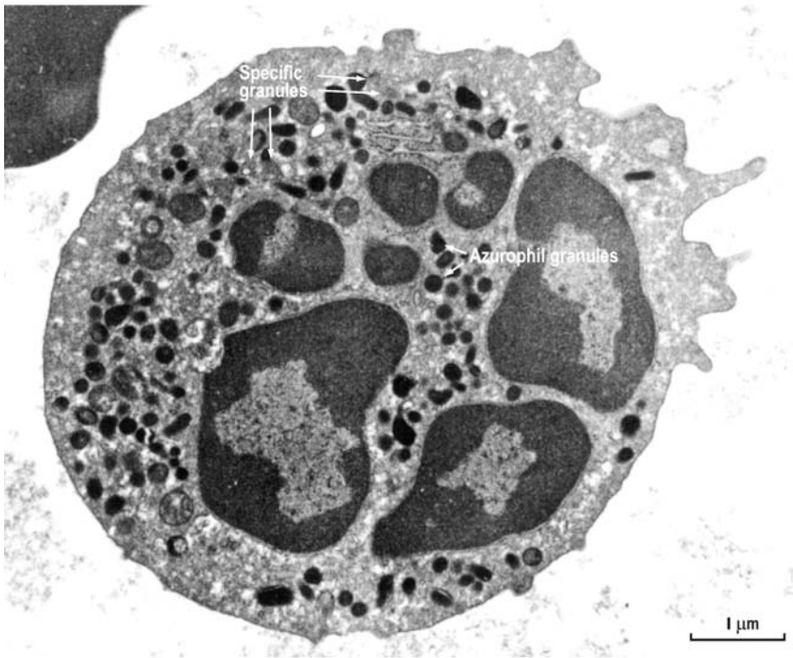


Figure 1.1 Ultrastructure of neutrophil. The multilobed nucleus and two main types of cytoplasmic granules are well displayed. (Courtesy of Dr D. McLaren.)

the vaginal epithelium. When protective commensals are disturbed by antibiotics, susceptibility to opportunistic infections such as *Candida albicans* and *Clostridium difficile* is increased.

If microorganisms do penetrate the body, two further innate 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

The polymorphonuclear neutrophil

This cell 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 (figures 1.1 & 1.2a,b) and an array of granules which are of two main types (figure 1.1): (i) the **primary azurophil granule**, which develops early and contains myeloperoxidase together with most of the nonoxidative antimicrobial effectors, including defensins, bactericidal/permeability-increasing (BPI) protein and cathepsin G, and (ii) the peroxidase-negative **secondary specific granules**, containing lactoferrin and much of the lysozyme, alkaline phosphatase (figure 1.2c) and membrane-bound cytochrome b_{558}.

The macrophage

These cells derive from bone marrow promonocytes which, after differentiation to blood monocytes (figure 1.2a), finally settle in the tissues as mature macrophages where they constitute the **mononuclear phagocyte system** (figure 1.2d). They are present throughout the connective tissue and around the basement membrane of small blood vessels and are particularly concentrated in the lung (figure 1.2f, 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 polymorphonuclear neutrophils, they are long-lived cells with significant rough-surface endoplasmic reticulum and mitochondria. Whereas the neutrophils 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 1.2e), viruses and protozoa that are capable of living within the cells of the host.

Milestone 1.1—Phagocytosis

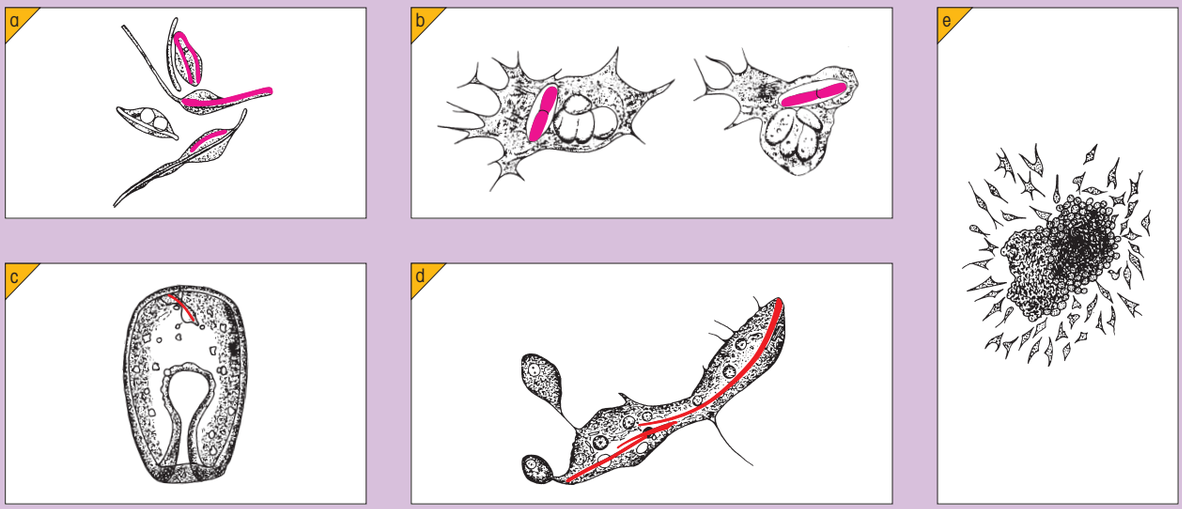


Figure M1.1.1 Reproductions of some of the illustrations in Metchnikoff's book, *Comparative Pathology of Inflammation* (1893). (a) Four leukocytes from the frog, enclosing anthrax bacilli. Some are alive and unstained; others, which have been killed, have taken up the vesuvine dye and have been colored. (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.

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 these motile cells surrounded it. A year later, in 1883, he observed that fungal spores can be attacked by the blood cells of *Daphnia*, a tiny metozoan 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."

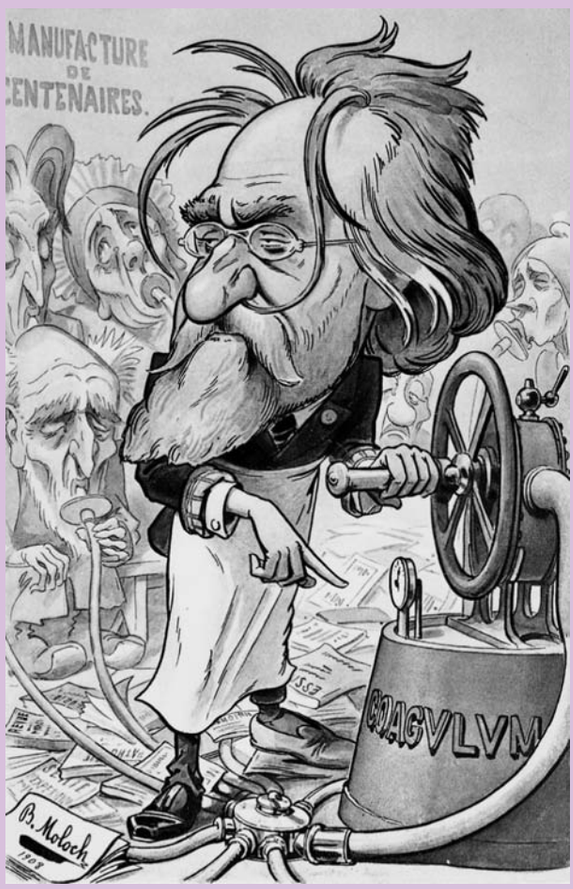


Figure M1.1.2 Caricature of Professor Metchnikoff (from *Chanteclair*, 1908, **4**, p. 7). (Reproduction kindly provided by The Wellcome Institute Library, London.)

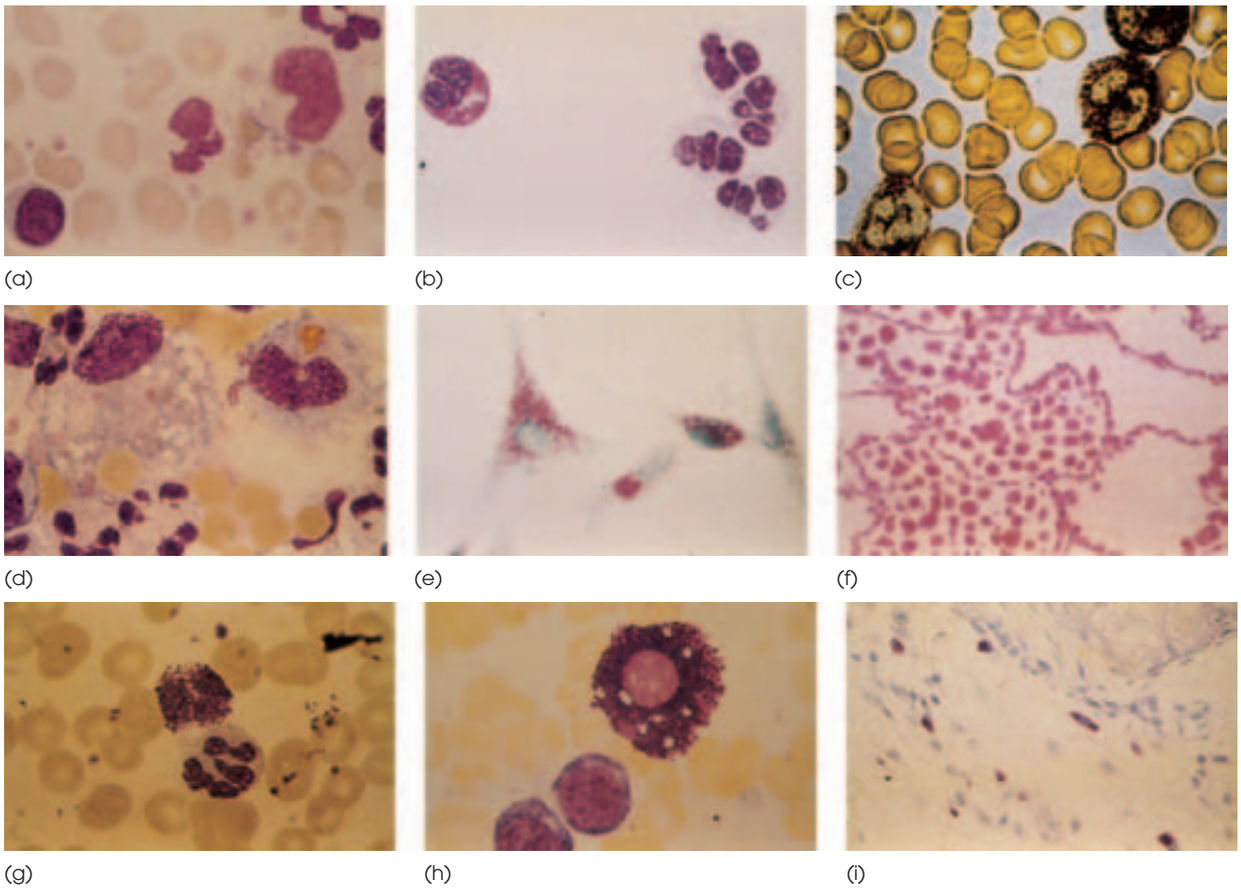


Figure 1.2 Cells involved in innate immunity. (a) Monocyte, showing "horseshoe-shaped" nucleus and moderately abundant pale cytoplasm. Note the three multilobed polymorphonuclear neutrophils and the small lymphocyte (bottom left) (Romanowsky). (b) Four polymorphonuclear neutrophils and one eosinophil. The multilobed nuclei and the cytoplasmic granules are clearly shown, those of the eosinophil being heavily stained. (c) Polymorphonuclear neutrophil showing cytoplasmic granules stained for alkaline phosphatase. (d) 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). (e) Macrophages in monolayer cultures after phagocytosis of mycobacteria (stained red) (Carbol-Fuchsin counterstained with Malachite Green.) (f) Numerous plump alveolar macrophages within air spaces in the lung. (g) Basophil with heavily staining granules compared with a neutrophil (below). (h) 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). (i) Tissue mast cells in skin stained with Toluidine Blue. The intracellular granules are metachromatic and stain reddish purple. (The slides for (a), (c), (d), (g) and (h) were very kindly provided by Mr M. Watts. (b) was kindly supplied by Professor J.J. Owen; (e) by Professors P. Lydyard and G. Rook; (f) by Dr Meryl Griffiths and (i) by Professor N. Woolf.)

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

Phagocytes must have mechanisms to enable them to distinguish friendly self-components from unfriendly and potentially dangerous microbial agents. Phagocytic cells have therefore evolved a system of receptors called **pattern recognition receptors** (PRRs) capable of recognizing PAMPs expressed on the surface of infectious agents. These PAMPs are essentially polysaccharides and polynucleotides that differ minimally from one pathogen to another but are not found in the host. By and large the PRRs are lectin-like and bind multivalently with considerable specificity to exposed microbial surface sugars. Engagement of the PRR generates a signal through a NFκB (nuclear factor-kappa B) transcription factor pathway which alerts the cell to danger and initiates the phagocytic process.

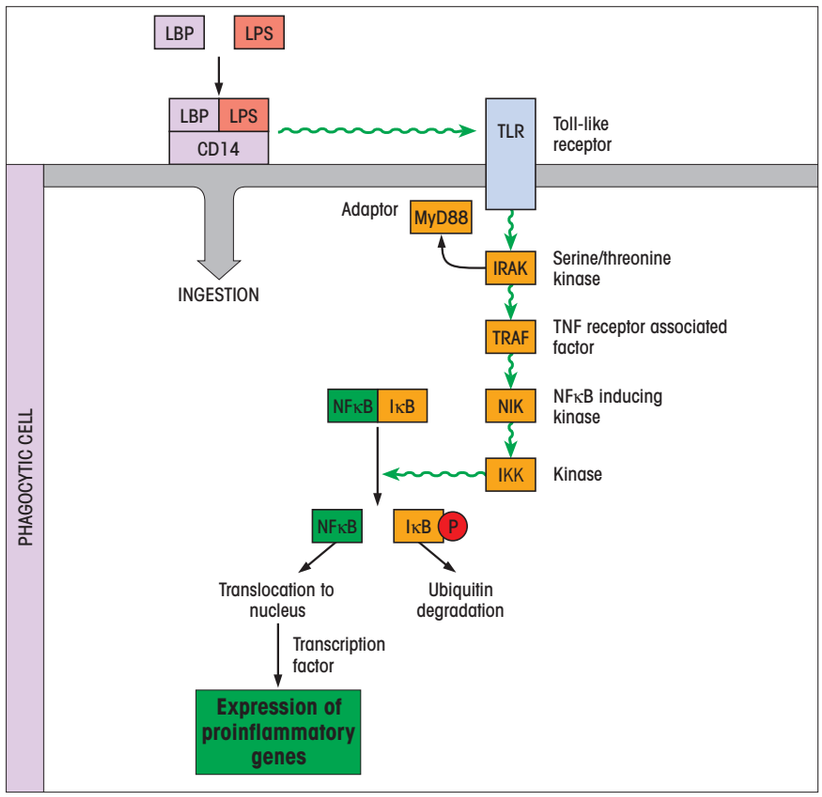


Figure 1.3 Activation of a phagocytic cell by a Gram-negative lipopolysaccharide (LPS) (endotoxin) danger signal. Circulating LPS is complexed by LPS-binding protein (LBP) and captured by the CD14 surface scavenging receptor. This signals internalization of the complex and activates the Toll-like receptor (TLR), which then initiates a phosphorylation cascade mediated by different kinase enzymes. As a result the transcription factor nuclear factor-kappa B (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 nicotinamide adenine dinucleotide phosphate (NADPH) oxidase which generates reactive oxygen intermediates (ROIs). 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.

Toll-like receptors (TLRs) recognize PAMPs and cause cytokine release

Toll-like receptors are a family of at least 10 transmembrane proteins that recognize various microbial products. For example TLR2 recognizes Gram-positive bacterial peptidoglycan, TLR4 is specialized for the recognition of Gram-negative bacterial lipopolysaccharide (LPS) (endotoxin) and TLR3 and TLR5 are important in the recognition of virus derived double-stranded RNA. When the TLRs are activated they trigger a biochemical cascade with activation of NFκB and ultimately synthesis of proinflammatory cytokines and other antimicrobial peptides that lead to the development of adaptive immunity (figure 1.3).

Microbes are engulfed by phagocytosis

Before phagocytosis can occur, the microbe must first adhere to the surface of the polymorph or macrophage through recognition of a PAMP. The resulting signal initiates the ingestion phase by activating an actin–myosin contractile system which results in pseudopods being extended around the particle (figures 1.4 & 1.5a). 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 called a phagosome (figures 1.4 & 1.5b). Within 1 min the cytoplasmic granules fuse with the phagosome and discharge their contents around the imprisoned microorganism (figure 1.5c), which is now

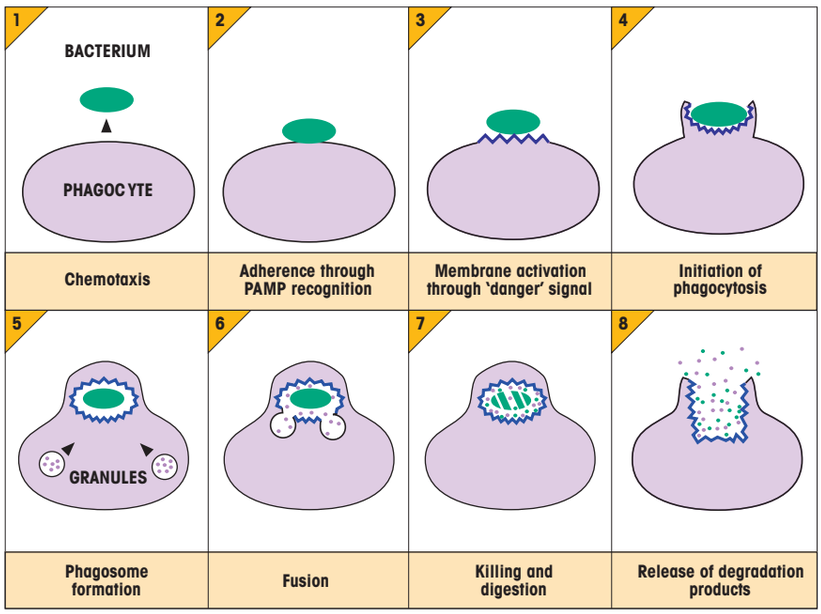
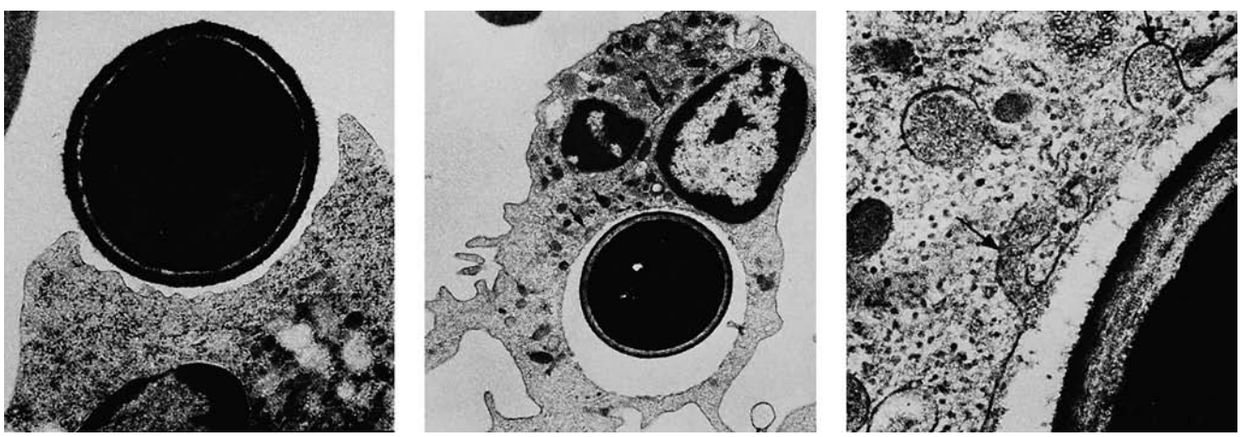


Figure 1.4 Phagocytosis and killing of a bacterium.



(a) (b) (c)

Figure 1.5 Adherence and phagocytosis. (a) Phagocytosis of *Candida albicans* by a polymorphonuclear neutrophil. Adherence to the surface initiates enclosure of the fungal particle within arms of cytoplasm (×15000). (b) Phagolysosome formation by a neutrophil 30 min 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). (c) Higher magnification of (b) showing fusing granules discharging their contents into the phagocytic vacuole (arrowed) (×33000). (Courtesy of Dr H. Valdimarsson.)

subject to a formidable battery of microbicidal mechanisms.

Killing by reactive oxygen intermediates (ROIs)

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 unique plasma membrane **cytochrome (cyt b_{558})**, which reduces molecular oxygen directly to superoxide anion (figure 1.6). Thus, the key reaction catalysed by this NADPH oxidase, which initiates the formation of ROIs, is:

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

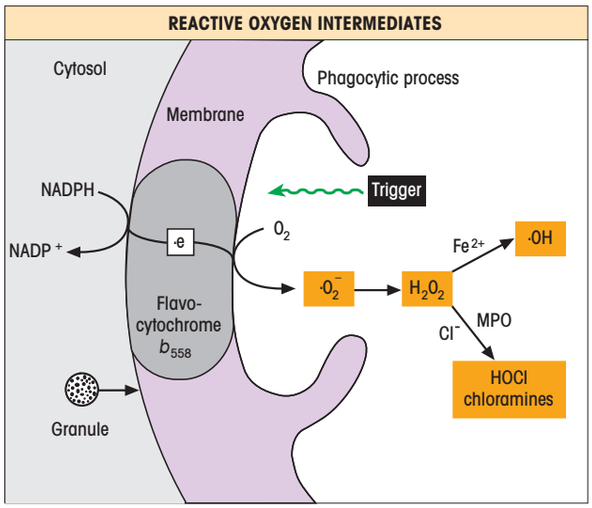


Figure 1.6 Microbicidal mechanisms of phagocytic cells. Production of reactive oxygen intermediates (ROIs). Electrons from nicotinamide adenine dinucleotide phosphate (NADPH) are transferred by the flavocytochrome oxidase enzyme to molecular oxygen to form the microbicidal molecular species shown in the boxes.

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, myeloperoxidase (MPO) and halide ions constitutes a potent halogenating system capable of killing both bacteria and viruses (figure 1.6).

Other killing mechanisms

Nitric oxide (NO) can be formed by an inducible NO synthase (iNOS) in many cells of the body. In macrophages and human neutrophils it generates a powerful antimicrobial system. Whereas NADPH oxidase is dedicated to the killing of extracellular organisms taken into phagosomes by phagocytosis, the NO mechanism can operate against microbes that invade the cytosol. It is not surprising therefore that iNOS capability is present in many nonphagocytic cells that may be infected by viruses and other parasites.

If microorganisms are not destroyed by these systems, they will be subjected to a family of peptides called defensins, which reach very high levels within the phagosome and act as disinfectants against a wide variety of bacteria, fungi and enveloped viruses. Further damage is inflicted on the bacterial membranes by neutral proteinase (cathepsin G) action and by the bactericidal or bacteriostatic factors, lysozyme and lactoferrin. Finally, the killed organisms are digested by hydrolytic enzymes and the degradation products released to the exterior (figure 1.4).

COMPLEMENT FACILITATES PHAGOCYTOSIS

Complement and its activation

Complement is the name given to a complex series of over 30 proteins found in plasma and on cell surfaces 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. The activated or the split products of the cascade have a variety of defensive functions and the complement proteins can therefore be regarded as a crucial part of the innate immune system.

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.

C3 continuously undergoes slow spontaneous cleavage

Under normal circumstances, small amounts of C3 are continuously broken down into the split product C3b, or a functionally similar molecule designated C3bi. 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 the suffix "a."

$C\overline{3bBb}$ has an important new enzymic activity: it is a C3 convertase which can now split large amounts of C3 to give C3a and C3b. We will shortly discuss the important biological 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 even more $C\overline{3bBb}$. That is, we are dealing with a potentially

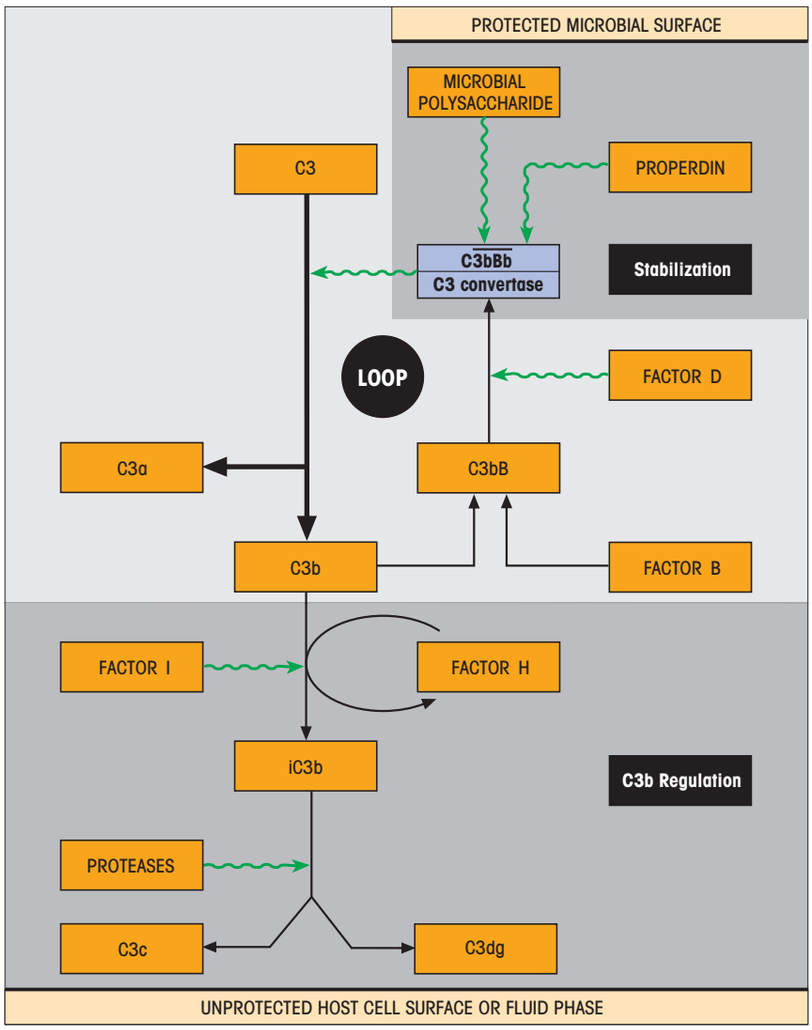


Figure 1.7 Microbial activation of the alternative complement pathway loop by stabilization of the C3 convertase ($C\overline{3b}\overline{Bb}$), and its control by factors H and I. When bound to the surface of a host cell or in the fluid phase, the C3b in the convertase is said to be "unprotected" in 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.

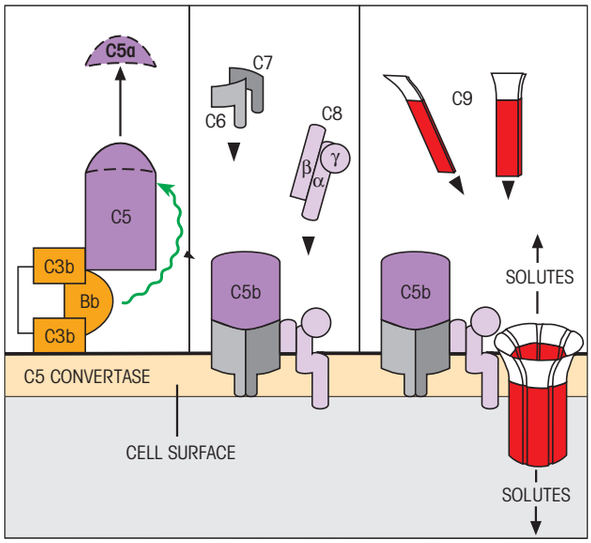
runaway **positive-feedback** or **amplification loop** (figure 1.7). As with all potentially explosive triggered cascades, there are powerful regulatory proteins in the form of factor H and factor I which control this feedback loop.

During infection C3 convertase is stabilized and the alternative complement pathway is activated

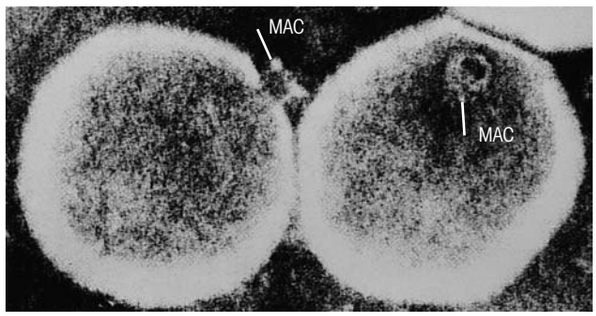
A number of microorganisms can activate the $C\overline{3b}\overline{Bb}$ convertase to generate large amounts of C3 cleavage products by stabilizing the enzyme on their (carbohydrate) surfaces. This protects the C3b from factor H and allows large quantities of $C\overline{3b}\overline{Bb}$ to build up and cleave C3. Another protein, properdin, acts subsequently on the bound convertase to stabilize it even further. This series of reactions provoked directly by microbes leads to the clustering of large numbers of C3b molecules on the microorganism and has been called the **alternative pathway** of complement activation (figure 1.7).

Complement can be activated when carbohydrates on bacterial surfaces combine with a serum protein called mannose-binding lectin (MBL)

Mannose-binding lectin is found at low levels in normal serum and binds to mannose and other carbohydrates on bacterial surfaces. This initiates a series of reactions which culminate in complement activation. Mannose-binding lectin activates complement by interacting with two serine proteases



(a)



(b)

Figure 1.8 Post-C3 pathway generating C5a and the C5b–9 membrane attack complex (MAC). (a) Cartoon of molecular assembly. (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. (Courtesy of Professor J. Tranum-Jensen and Dr S. Bhakdi.)

called MASP1 and MASP2. It is known that MASP2 cleaves and activates C4 and C2, generating a C3 convertase called $C\overline{4b2a}$ which we shall discuss in Chapter 2. Activation of C3 initiates the alternative pathway loop and the formation of the membrane-attack complex.

The post-C3 pathway generates a membrane attack complex (MAC)

Recruitment of a further C3b molecule into the $C\overline{3bBb}$ enzymic complex generates a C5 convertase. This 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 C8. The C8 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 and polymerization to an annular MAC (figures 1.8 & 2.3). This forms a transmembrane channel fully permeable to electrolytes and water. Due to the high internal colloid osmotic pressure of cells, there is a net influx of Na^+ and water, leading to cell lysis.

Complement has a range of defensive biological functions

1 C3b adheres to complement receptors

Phagocytic cells have receptors for C3b (CR1) and C3bi (CR3) which facilitate the adherence of C3b-coated microorganisms to the cell surface and their subsequent phagocytosis. This process is called opsonization and is perhaps the most important function resulting from complement activation.

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 are **anaphylatoxins** in that they are capable of triggering the release of host defence mediators such as histamine, leukotriene B4 and tumor necrosis factor (TNF) from mast cells (figures 1.2i, 1.9 & 1.10) and their circulating counterparts the basophils. C5a acts directly on neutrophils, and both C3a and C5a on eosinophils (described later in this chapter), to stimulate the respiratory burst associated with production of ROIs and to enhance the expression of surface receptors for C3b. Importantly, C5a is also a potent neutrophil chemotactic agent. Both C3a and C5a have a striking ability to act directly on the capillary endothelium to produce vasodilatation and increased permeability, an effect that seems to be prolonged by leukotriene B4 released from activated mast cells, neutrophils and macrophages.

3 The terminal complex can induce membrane lesions

As described above, the insertion of the MAC into a membrane may bring about cell lysis.

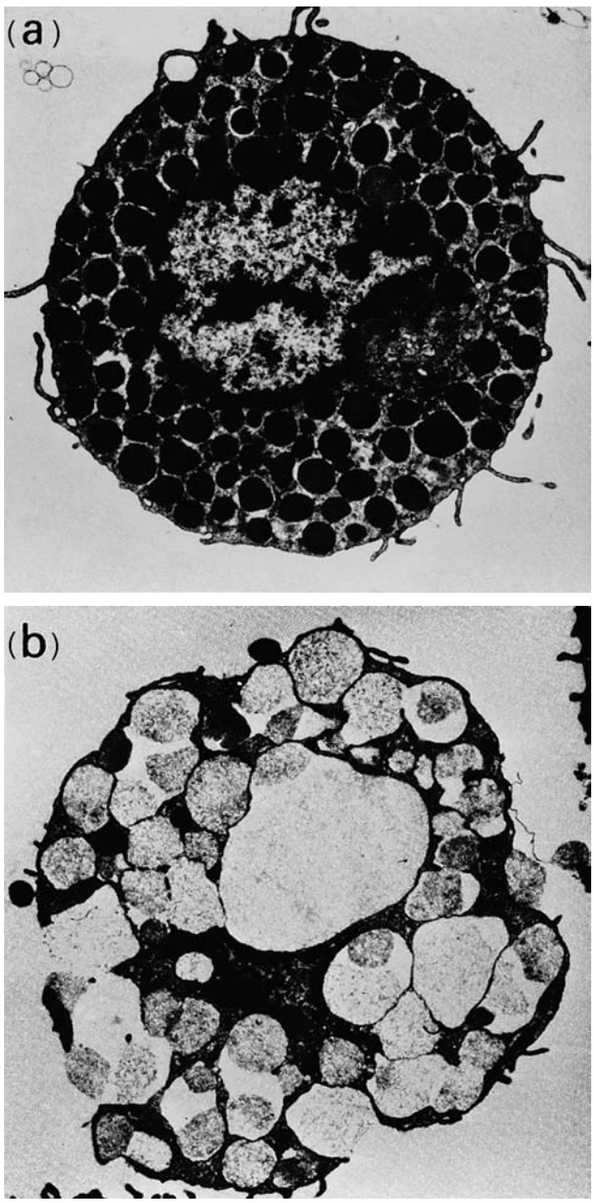


Figure 1.9 The mast cell. (a) A resting cell with many membrane-bound granules containing preformed mediators. (b) A triggered mast cell. Note that the granules have released their contents and are morphologically altered, being larger and less electron dense. Although most of the altered granules remain within the circumference of the cell, they are open to the extracellular space (electron micrographs ×5400). (Reproduced from D. Lawson, C. Fewtrell, B. Gomperts and M.C. Raff (1975) *Journal of Experimental Medicine* **142**, 391–402. Copyright permission of The Rockefeller University Press.)

4 Complement plays a role in the induction of antibody responses

As shall be described in detail later, B-cells proliferate and produce antibody when antigen binds to its surface receptors. This activation is modulated by co-receptors, including one for C3b. Therefore, when a B-cell is activated in the presence of C3b, the threshold for activation is lowered, and much less antigen is required to activate the B-cell.

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 (figure 1.10).

In the first act, $C\overline{3bBb}$ 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. $C\overline{3bBb}$ activates the next step in the sequence to generate C5a and the MAC.

The next act sees the proinflammatory peptides, C3a and C5a (anaphylatoxins), together with the mediators they trigger from the mast cell, recruiting polymorphonuclear neutrophils and further plasma complement components to the site of microbial invasion. Complement activation also causes the expression of the adhesion molecules P-selectin and ICAM-1 (intercellular adhesion molecule-1) on endothelial cells. 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. Here they first adhere to the endothelial cells, then pass through gaps between these cells (diapedesis), and then move up the concentration gradient of chemotactic factors until they come face to face with the C3b-coated microbe. C5a, which is at a relatively high concentration in the chemotactic gradient, activates the respiratory burst in the neutrophils, with subsequent generation of toxic oxygen radicals and other phagocytic bactericidal mechanisms.

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

Tissue macrophages also play a crucial role in acute inflammatory reactions. They may be activated by the

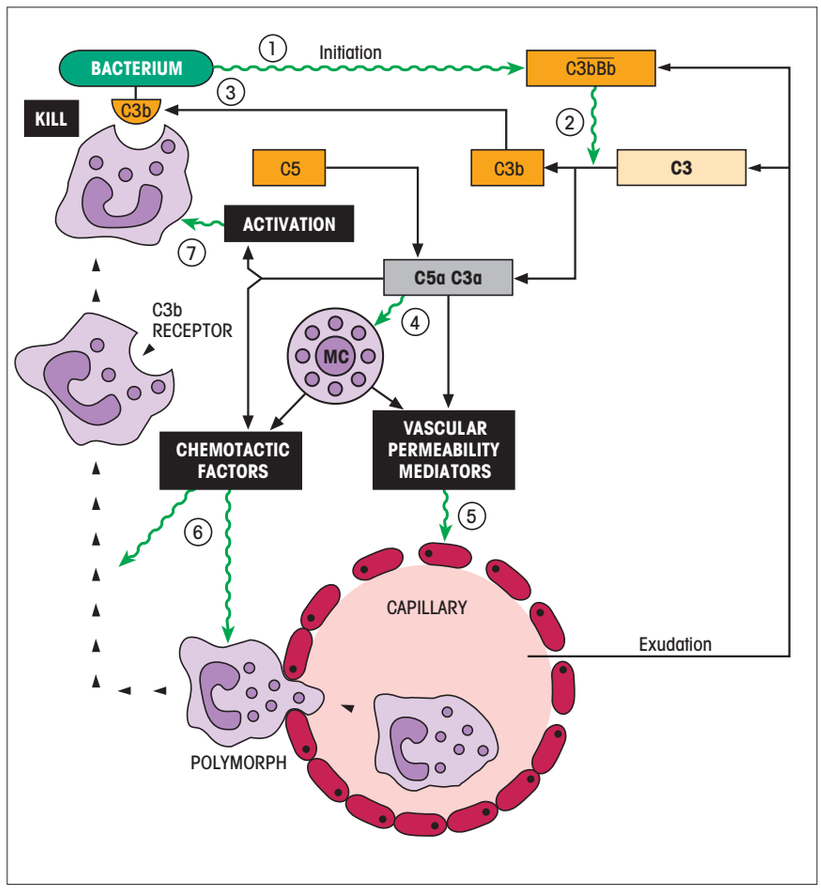


Figure 1.10 The defensive strategy of the acute inflammatory reaction initiated by bacterial activation of the alternative C pathway. Directions: ① start with the activation of the $C\overline{3bBb}$ C3 convertase by the bacterium, ② notice the generation of C3b (③ which binds to the bacterium), C3a 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.

direct action of C5a or certain bacterial toxins such as the LPSs acting on the TLRs, or by the phagocytosis of C3b-opsonized microbes. Following activation, the macrophages will secrete a variety of soluble mediators which amplify the acute inflammatory response (figure 1.11). These include cytokines such as interleukin-1 (IL-1) and TNF, which 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 acute inflammation.

HUMORAL MECHANISMS PROVIDE A SECOND DEFENSIVE STRATEGY

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 MAC. 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. Interferons are a family of broad-spectrum antiviral agents induced by viruses and act to limit proliferation and spread of the infection. Interferon α (IFNα) is produced particularly by leukocytes, and interferon β (IFNβ) especially by fibroblasts, although all nucleated cells can probably synthesize these molecules. 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, which contribute to innate immunity through binding of their lectin-like domains to carbohydrates on microbes and their

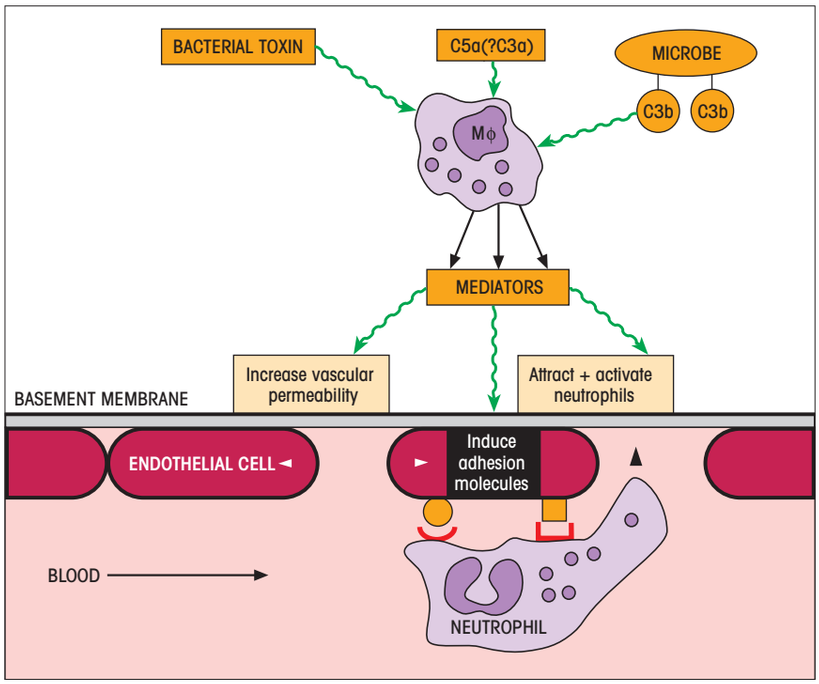


Figure 1.11 Stimulation by complement components and bacterial toxins such as lipopolysaccharide (LPS) induces macrophage secretion of mediators of an acute inflammatory response. Blood neutrophils stick to the adhesion molecules on the endothelial cell and use this to provide traction as they force their way between the cells, through the basement membrane (with the help of secreted elastase) and up the chemotactic gradient.

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

During an infection, microbial products such as endotoxins (LPS) activate macrophages and other cells to release various cytokines including IL-1, which is an endogenous pyrogen (incidentally capable of improving our general defenses by raising the body temperature), TNF and IL-6. These in turn act on the liver to increase the synthesis and secretion of a number of plasma proteins collectively termed acute phase proteins. These include C-reactive protein (CRP, the plasma concentration of which may increase 1000-fold), serum amyloid P component and MBL (Table 1.1). We have previously described the role that MBL plays in activating the complement system. Other acute phase proteins showing a more modest rise in concentration include α_1-antitrypsin, 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. For example, CRP can bind to numerous

Table 1.1 Acute phase proteins.

Acute phase reactant	Role
Dramatic increases in concentration:	
C-reactive protein	Fixes complement, opsonizes
Mannose binding lectin	Fixes complement, opsonizes
α_1-acid glycoprotein	Transport protein
Serum amyloid P component	Amyloid component precursor
Moderate increases in concentration:	
α_1-proteinase inhibitors	Inhibit bacterial proteases
α_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

microorganisms forming a complex that may activate the complement pathway (by the classical pathway, 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. Measurement of CRP is a useful laboratory test to assess the activity of inflammatory disease.

EXTRACELLULAR KILLING

Natural killer (NK) cells are part of the innate immune system

Viruses lack the apparatus for self-renewal 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. **Natural killer cells** appear to do just that.

Natural killer cells are large granular lymphocytes (figure 2.4a) with a characteristic morphology. They possess activating receptors which recognize structures on glycoproteins on the surface of virally infected cells or on tumor cells, and which bring killer and target into close apposition (figure 1.12). Many of the ligands for the activating receptors can also be present on noninfected normal cells, and therefore the NK cells also possess inhibitory receptors to prevent killing of normal cells. These inhibitory receptors, which override the signals from the activating receptors, recognise ubiquitous molecules such as the major histocompatibility complex (MHC) class I glycoprotein normally found on the surface of all nucleated cells. However, virally infected or tumor cells often lose expression of MHC class I. Thus, only in the absence of MHC class I is the killing of the target cell allowed to proceed (see figure 4.4). 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. This is followed by target cell death.

The most important of these granule contents 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 forming a transmembrane pore with an annular structure, comparable to the complement MAC (figure 1.8). In addition to perforin, the granules contain lymphotoxin α and a family of serine proteases termed granzymes, one of which, granzyme B, can

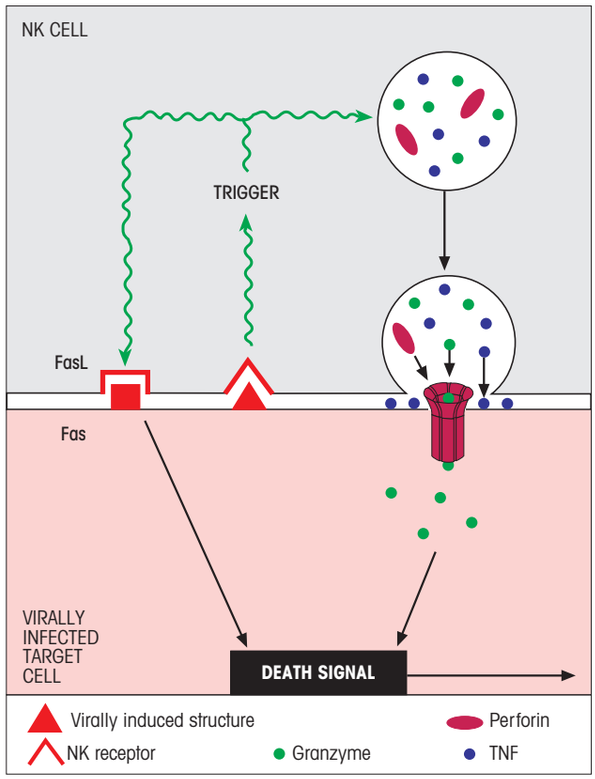


Figure 1.12 Extracellular killing of virally infected cell by natural killer (NK) cell. Binding of the NK receptors to the surface of the virally infected cell triggers the extracellular release of perforin molecules from the granules. These polymerize to form transmembrane channels which may facilitate lysis of the target by permitting entry of granzymes, tumor necrosis factor (TNF) and other potentially cytotoxic factors derived from the granules. (Model resembling that proposed by D. Hudig, G.R. Ewoldt and S.L. Woodward in (1993) *Current Opinion in Immunology* **5**, 90–6.) Engagement of the NK receptor also activates a parallel killing mechanism mediated through the binding of the FasL (Fas-ligand) on the effector to the target cell Fas receptor thereby delivering a signal for apoptosis.

function as an NK cytotoxic factor by inducing **apoptosis** (programmed cell death) in the target cell. Very rapid nuclear fragmentation effected by a Ca-dependent endonuclease that acts on the vulnerable DNA between nucleosomes can be detected.

An alternative recognition system for NK-cell-mediated killing can involve engagement of upregulated Fas receptor molecules on the target cell surface by the FasL (Fas-ligand) on the effector NK cell, a process which also induces an apoptotic signal in the unlucky target. Tumor necrosis family members, interacting with TNF receptors on target cells can also mediate cytotoxicity. One member of the family that is

expressed by activated NK cells is TRAIL (tumor necrosis factor-related apoptosis-inducing ligand).

Natural killer cells also produce cytokines which regulate inflammation and acquired immune function

Not only do NK cells have the ability to lyse virally infected and tumor cells, but they also produce a wide range of cytokines once they are activated. These include cytokines such as IL-1 and TNF, which play an important role in inflammation, and granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon γ (IFNγ) and transforming growth factor-β (TGFβ), which modulate the acquired immune response (see Chapter 2). Natural killer cells also express costimulatory molecules such as CD40L (CD40-ligand) and have been shown to regulate B-cell function when they are activated.

Eosinophils

Large parasites such as helminths (worms) cannot physically be phagocytosed and extracellular killing by eosinophils would seem to have evolved to help cope with this situation. Eosinophils, when released from the bone marrow, circulate in the peripheral blood and then traffic to peripheral tissue especially to the lung and the gut. Their prominent location in these sites suggests that they play an important role in host defence surveillance of mucosal surfaces. Eosinophils have distinctive granules which stain avidly with acid dyes (figure 1.2b). They have surface receptors for cytokines, chemokines, adhesion molecules and complement components, and on activation produce an impressive respiratory burst with concomitant generation of active oxygen metabolites and proinflammatory cytokines.

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

REVISION

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 and antibacterial action of fluids and microbial antagonism.
- If penetration occurs, bacteria are destroyed by soluble factors such as lysozyme and by phagocytosis which is followed by intracellular digestion.
- Phagocytic cells kill microorganisms.
- The main phagocytic cells are polymorphonuclear neutrophils and mononuclear macrophages. Organisms adhere via their pathogen-associated molecular patterns (PAMPs) to pattern recognition receptors (PRRs) on the phagocytic cell surface.
- Toll-like receptors (TLRs) are transmembrane proteins that recognize bacterial products. When activated they trigger the release of proinflammatory cytokines.
- Binding to PRRs activates the engulfment process and the microorganism is taken inside the cell where it fuses with cytoplasmic granules.
- A formidable array of microbicidal mechanisms then come into play including the conversion of oxygen to reactive oxygen intermediates (ROIs), the synthesis of nitric oxide and the release of multiple oxygen-independent factors from the granules.
- 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 to form C3b, which binds the adjacent microorganisms.
- Mannose-binding lectin (MBL) binds to mannose on the surface of microorganisms and initiates complement activation by binding the proteases MASP1 and MASP2.
- Once C3 is split the next component, C5, is activated yielding a small peptide, C5a. The residual C5b binds to the surface of the organism and assembles the terminal components C6–9 into a membrane attack complex

(MAC), which is freely permeable to solutes and can lead to osmotic lysis of the offending pathogen.

Complement has a range of defensive functions

- C3b coated organisms bind to C3b receptor (CR1) on phagocytic cells and are more readily phagocytosed.
- C5a is highly chemotactic for, and can activate, neutrophils. Both C3a and C5a are potent chemotactic and activating agents for eosinophils and they both greatly increase capillary permeability.
- C3a and C5a act on mast cells causing the release of further mediators such as histamine, leukotriene B4 and tumor necrosis factor (TNF) with effects on capillary permeability and adhesiveness, and neutrophil chemotaxis; they also activate neutrophils.
- Insertion of the MAC into an organism brings about cell lysis.
- C3b plays a role in facilitating antibody production by B-cells.

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**.
- Complement activation induces endothelial cells to express adhesion molecules which attach to leukocytes and cause them to move between endothelial cells into the area of the microbes.
- Phagocytic cells are activated by C5a to ingest and kill invading microbes.
- Inflammation can also be initiated by tissue macrophages, which can be activated by C5a or by bacterial products such as endotoxin acting on the TLRs. These cells secrete cytokines including interleukin-1 (IL-1) and TNF which increase the adhesiveness of endothelial cells thereby bringing more cells to the site of inflammation.

Humoral mechanisms provide a second defensive strategy

- In addition to lysozyme, defensins and the complement system, other humoral defenses involve the acute phase proteins such as C-reactive protein and mannose-binding ligand whose synthesis is greatly augmented by infection.
- Recovery from viral infections can be effected by the interferons, which block viral replication.
- Collectins bind to carbohydrates on organisms and also to receptors on phagocytic cells thereby facilitating phagocytosis.

Acute phase proteins increase during infection

- Cytokines such as IL-1 and TNF, released during acute inflammation, act on the liver that synthesises plasma proteins called acute phase proteins.
- These have a beneficial effect on host defence.
- Measurement of C-reactive protein (CRP) is useful to assess the activity of inflammatory processes.

Extracellular killing

- Natural killer (NK) cells possess killer activating receptors recognizing glycoproteins on the surface of the virally infected cell or tumor cell, and dominant inhibitory receptors recognizing major histocompatibility complex (MHC) class I on normal cells.
- Virally infected cells can be destroyed by NK cells using programmed cell death (apoptosis) through a perforin/granzyme pathway, or by FasL (Fas-ligand) on the NK cell engaging Fas on the target cell.
- 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

FURTHER READING

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