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Neutrophils and host defence: The fight against infection

The neutrophil, the subject of this book, plays a key role as part of the immune response to microbial infections. Its major function is the rapid killing of bacteria and fungi before they multiply and spread throughout the body. The neutrophil is only one arm of the immune system, which includes other leukocytes, lymphocytes and molecular components such as complement, antibodies, acute phase proteins and cytokines. These cellular and molecular components of immunity constitute a co-ordinated and sophisticated network that has evolved in order to maximise the survival of the host against the range of pathogens it encounters daily. This chapter describes the role of the neutrophil within this immune network. Other elements of the immune system (the cellular and molecular components) are also briefly described but only with emphasis on how they interact with neutrophil function; thus, the descriptions of these systems focus upon how the cellular and molecular elements assist neutrophil function during infection and how neutrophils themselves may affect and regulate other aspects of the immune response. A more complete description of the immune system may be found in texts such as Davey (1989), Roitt (1990) and Benjamini and Leskowitz (1991).

1.1 The immune system

The immune system protects humans and animals from microbial infections by such infectious agents as bacteria, yeasts and fungi, viruses and protozoa. These differ greatly not only in their size but in their structural and molecular properties, as well as in the ways in which they seek to infect our bodies. Some of these pathogens infect bodily fluids, some penetrate tissues and some even survive and multiply within individual host cells. These intracellular pathogens include viruses, some parasitic protozoa (such as *Plasmodium*, the causative agent of malaria, which infects erythrocytes) and

pathogenic bacteria such as *Listeria* and *Legionella*. Infections with these organisms are difficult to counteract because the immune system must recognise the presence of the pathogen within a host cell. On the other hand, many micro-organisms that do not cause disease in humans or animals can survive and multiply within certain parts of our body (e.g. in the gastrointestinal tract). However, if some of these organisms invade other parts of our body, then they are not well tolerated and can cause disease. Perhaps the most striking examples of such organisms are the gut bacteria. In an adult human there may be up to 10^{13} bacteria present in the alimentary canal, but if only small numbers of these enter the bloodstream or urinogenital tract, then they can cause disease.

In the developed countries, infection accounts for less than 2% of all deaths, although the newborn and the elderly are more susceptible to infections than are healthy adults. This is because the immune system is somewhat underdeveloped in newborns, and even more so in premature babies, whose susceptibility to infections is even greater. At the other end of the age spectrum, the immune system becomes defective; this, coupled with a generalised decreased function of our bodily organs and decreased capacity for repair, allows infections to become more common again. Other conditions that lead to a greater susceptibility to infections include poor diet, diseases such as diabetes, disorders of the immune systems (e.g. genetic diseases or haematological disorders) and drug therapy. In patients receiving cytotoxic therapy or radiotherapy for the treatment of cancers, for example, infections are the major causes of morbidity and mortality because the therapy used to kill the tumour also destroys the immune system. Major reasons for the relatively low death rate from infections in the developed countries include the widespread immunisation programs available, good sanitation and adequate diets. There is also an impressive array of antimicrobial agents available, especially antibiotics and antifungal agents, that can be administered to patients if a microbial pathogen evades the immune system.

In the developing countries, however, death from infection is far more widespread, and in some parts of the world 70–80% of all deaths arise from infectious disease. In part, this is geographical: many tropical/subtropical environments allow the proliferation of disease-carrying biting insects (e.g. flies, ticks and beetles) that pass infections (e.g. malaria, sleeping sickness, leishmaniasis) from host to host during blood meals. However, the majority of problems with infections in these developing countries are due to poverty. Infections are common because of poor diet, poor sanitation and contaminated water supplies, lack of effective immunisation programs and unavailability of antibiotics. It is estimated that about 20 million children under the

age of 5 years die each year in these countries from diarrhoeal disease. Improved sanitation, clean water and simple therapy would save many of these lives.

The major protection against infection is provided by the skin, which is largely impermeable to water, gases and micro-organisms. It thus serves to protect internal structures and tissues whilst being sufficiently elastic to allow movement. Infectious organisms that penetrate this physical barrier do so via cuts, scratches, burns or bites. Skin, although an effective and efficient barrier, cannot cover the entire body, however: the eyes, the alimentary canal, respiratory tract and urogenital tract are covered not by skin but by a lining of cells (a transparent layer, in the eyes) that permits absorption and secretion of liquids and gases. These parts of the body, prime routes for penetration by infectious organisms, possess specialised structures and features to protect against infection but still allow normal function. The tracts in question secrete mucus, a hydrated gel acting to waterproof and lubricate whilst still allowing the transport of gases, liquids and nutrients. They also are lined with epithelial cells that may be continuously shed and replaced, so as to replace lining cells that may have become damaged or infected; this phenomenon occurs in the gut, but may also occur at lower rates in the respiratory and urogenital tracts. Some epithelial cells are ciliated, and in the lung a major mechanism for the physical removal of micro-organisms and dust particles is the *mucociliary escalator,* in which epithelial cell cilia constantly 'brush' mucus and foreign particles upwards and out of the lungs so that they are cleared via coughing. Mucus and other bodily secretions, such as tears, saliva, nasal secretions and breast milk, also contain constituents that are antimicrobial, such as lysozyme, acid pH, bile and immunoglobulins.

Once a microbe has penetrated these physical barriers, the immune system is then responsible for destroying it before it can multiply and disperse to other parts of the body. The system faced with this task in animals and humans comprises both cellular and molecular components. The cells of the immune system include the *phagocytes* (neutrophils and macrophages) and the *lymphocytes* (primarily T and B cells); these function in close collaboration with the molecular components, which include complement, acute-phase proteins, antibodies and cytokines. Whilst complement (sometimes working alone or sometimes working with antibodies) can kill pathogens, the molecular elements of the immune system usually function to regulate the activity of the cellular components. Thus, the molecular and cellular components act co-ordinately to ensure maximal efficiency of the immune system in recognising and eliminating pathogenic organisms.

1.2 Cellular components of the immune system

1.2.1 Polymorphonuclear leukocytes

The cell types classified as *polymorphonuclear leukocytes* include neutrophils, basophils and eosinophils. These cells are identifiable in blood films because of the unusual morphology of their nucleus, which is irregularly shaped, often multilobed and hence polymorphic. As these are white blood cells, the general description 'polymorphonuclear leukocytes' describes their colour and nuclear morphology (Fig. 1.1a,b). The cytoplasm of these cells has a granular appearance because of the abundance of 'granules' that are, in fact, membrane-bound organelles. Because the distinct properties of these cells and their function in immunity are largely dictated by the constituents of these granules, polymorphic cells are often termed *granulocytes.*

These 'polymorphs' are divided into three subgroups by virtue of the staining properties of their cytoplasmic contents when treated with dye mixtures. Thus, *eosinophils* stain with acid dyes such as eosin and appear red in stained blood films, *basophils* stain with basic dyes and appear blue whilst *neutrophils* stain with both types of dye and their cytoplasm appears purple. The differences between the cytoplasmic contents in these cells types is much more fundamental than is suggested by these simple staining properties. The granules of basophils, neutrophils and eosinophils contain distinct molecular constituents that confer upon the cells their specialised functions during infection and inflammation.

1.2.1.1 Neutrophils

The full name of these cells is *neutrophilic polymorphonuclear leukocytes,* but the terms *neutrophil* and, less-commonly now, *polymorph* are generally used to describe this cell (Fig.1.1a). In fact, most preparations of 'neutrophils' contain about 95–97% neutrophils, the remainder being largely eosinophils, because the commonly-used separation techniques do not efficiently separate these cell types. Neutrophils are the most abundant white cell in the blood, accounting for 40–65% of white blood cells, and are found at concentrations usually in the range $3–5 \times 10^6$ cells/ml blood. This number can increase dramatically (up to tenfold) in cases of infection. They have a relatively short half-life in the circulation (estimated at about 8–20 h), but this may be extended to up to several days if the cells leave the circulation and enter tissues – although it is difficult to measure the lifespan of a tissue neutrophil. Because of the large numbers of neutrophils in the circulation and their relatively short lifespan, vast numbers of neutrophils enter and

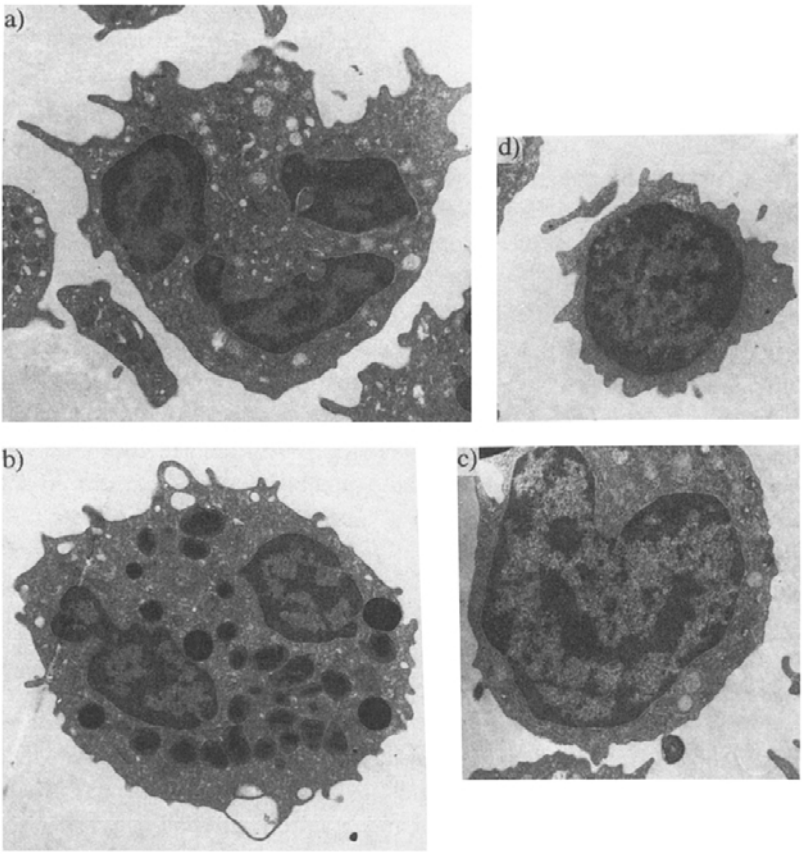

Figure 1.1. Electron micrographs of leukocytes: (a) neutrophil, showing polymorphic nucleus and numerous cytoplasmic granules; (b) eosinophil, showing distinctive granules with 'crystalline' core; (c) monocyte, with horseshoe-shaped nucleus; (d) small lymphocyte. Magnification ×7000.

leave the circulation daily. For example, in an adult human with five litres of blood in the circulation, the total neutrophil pool (not accounting for cells adhering to capillary walls or in tissues) is around 2×10^{10} cells. If these are replaced two or three times a day due to turnover, then the bone marrow, which synthesises these cells, must be able to generate about 5×10^{10} cells every day. In an individual who is infected, this number may increase by an order of magnitude. The huge number of neutrophils that must be produced daily indicates how important these cells are in host protection against infections, and provides clues as to their functional properties. Neu-

trophils are the first line of defence of the body against bacterial and fungal infections: they are the first cells to be recruited to a site of infection and must respond quickly and potently. These cells are thus highly motile (moving into tissues in response to chemical signals or chemoattractants) and contain an impressive armoury of cytotoxic mechanisms that are capable of killing a range of microbial pathogens.

Neutrophils are about 10 μm in diameter and, whilst in the circulation, are spherical with few, if any, cytoplasmic extrusions. Blood neutrophils are thus said to be in a resting or *non-activated* state. However, once they are *activated,* either by chemical stimuli or by attachment to surfaces, their morphology changes. They may become polarised so that they assume a front end and a rear end, and this polarisation is required before directional movement can occur. They then flatten to assume a classic amoeboid shape with extended pseudopodia and thus become *primed* and ready for action. Priming can be induced by many pathological or physiological stimuli and involves preparing the neutrophil for a state of readiness prior to its full-scale activation. This two-stage activation process probably guards against the non-specific activation of neutrophils: because some of the components that neutrophils use to kill bacteria can also indiscriminately attact host tissues, the possibility of non-specific activation must be minimised. Priming involves movement of some of the cytoplasmic granules, which fuse with the plasma membrane. During this process there is an increase in the number of receptors and some other important proteins on the cell surface, and with more surface receptors – the 'eyes' of the cell – the neutrophil is capable of detecting subtle changes in its microenvironment that may be caused directly or indirectly by the presence of the invading pathogen.

Neutrophils kill their target pathogens by the process of *phagocytosis* (Fig. 1.2). Efficient phagocytic killing is, however, regulated at several steps (Fig. 1.3). Firstly, the neutrophil must be able to recognise the pathogen as 'foreign'. Sometimes the surface properties of the pathogen are so unusual (compared to the surfaces of host tissues) that this recognition is achieved without the involvement of any other factors. More usually, this recognition is aided by the coating of the pathogen with *opsonins,* such as antibodies, complement fragments, acute-phase proteins and fibronectin. This *opsonisation* process is important because neutrophils possess receptors for portions of the opsonin molecules (e.g. immunoglobulin and complement receptors) so that any particle coated with opsonins is labelled as a target for neutrophil phagocytosis (Fig. 1.4).

After the binding of the pathogen to the neutrophil surface via these receptors, the neutrophil must respond by activating its bactericidal arsenal. This activation is achieved through the occupancy of receptors, which trig-

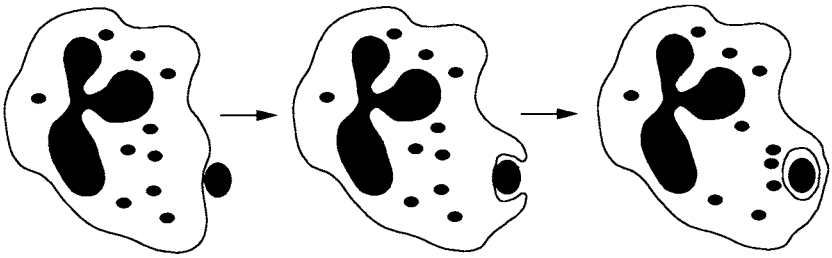

Figure 1.2. Phagocytosis by neutrophils. A bacterium is recognised by receptors of the plasma membrane of a neutrophil. This interaction triggers the formation of pseudopodia around the bacterium so that the bacterium eventually becomes fully enclosed within a phagocytic vesicle. The membrane of this vesicle is derived from the plasma membrane. Cytoplasmic granules then fuse with this vesicle to form a phagolysosome. During this process the granule membranes incorporate into the membrane of the phagolysosome, whilst the contents of the granules are discharged into the vesicle.

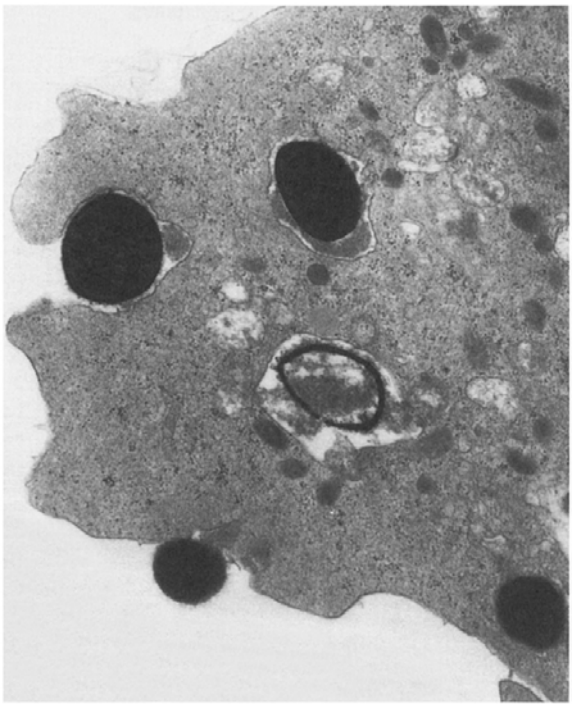

Figure 1.3. Phagocytosis of *Staphylococcus aureus* by a human neutrophil. Neutrophils were incubated with opsonised *S. aureus* and fixed after 15 min incubation. The bacterium in the centre has been lysed, and only the cell wall remains. *Source:* Experiment of Bernard Davies and John Humphreys, reproduced with permission from *Colour Atlas of Paediatric Infectious Diseases,* by Hart and Broadhead (Mosby Year Book Europe).

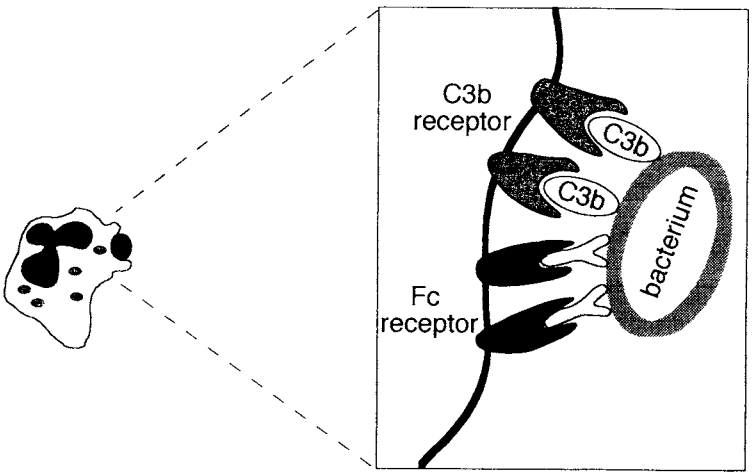

Figure 1.4. Recognition of bacteria by neutrophils. Invading bacteria are opsonised by serum proteins, such as complement fragments (e.g. C3b) and immunoglobulins. The plasma membranes of neutrophils possess receptors for these opsonins (e.g. Fc receptors and complement receptors). Thus, occupancy of these opsonin receptors triggers phagocytosis and activates events such as the respiratory burst and degranulation. Note that the receptors and opsonins are not drawn to scale.

gers the formation of second messenger molecules that directly or indirectly activate specific enzyme systems (e.g. via phosphorylation reactions). These signalling processes and *signal transduction systems* (which 'transduce' a chemical signal from the outside to the inside of the cell) in the neutrophil are complex for several reasons. One such reason for this complexity is that whilst neutrophils function to destroy microbial pathogens, this process is controlled at several stages (Fig. 1.5), including:

i. attachment of the cell to the capillary walls (*margination*) prior to leaving the circulation;
ii. squeezing through gaps between adjacent endothelial cells (*diapedesis*);
iii. migration into tissues (*chemotaxis*);
iv. recognition of the pathogen as 'foreign', initiation of phagocytosis and activation of the bactericidal mechanisms;
v. release of cytotoxic products if the pathogen is too large to be fully enclosed within a phagocytic vesicle (*frustrated phagocytosis*);
vi. release of pro-inflammatory molecules (e.g. chemoattractants) or other immune stimulants (cytokines) if more cells of the immune system (including more neutrophils) must be recruited to the site of infection.

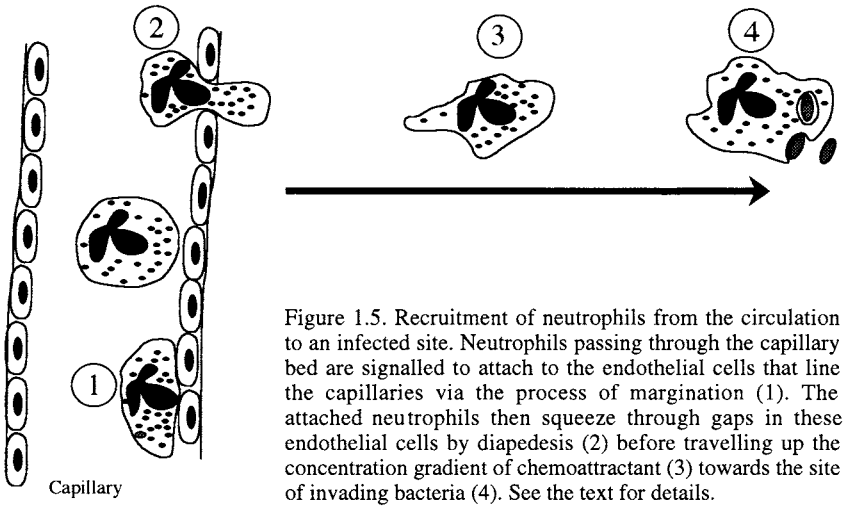

Figure 1.5. Recruitment of neutrophils from the circulation to an infected site. Neutrophils passing through the capillary bed are signalled to attach to the endothelial cells that line the capillaries via the process of margination (1). The attached neutrophils then squeeze through gaps in these endothelial cells by diapedesis (2) before travelling up the concentration gradient of chemoattractant (3) towards the site of invading bacteria (4). See the text for details.

Thus, multiple signalling systems are required in order to specifically activate these various processes. Furthermore, there is an element of overlap or redundancy in these signalling systems: if neutrophil function were controlled by a single signalling system, then a defect in that system would completely abolish all aspects of cell activation. In view of the importance of neutrophils in host protection against infection, such a defect would have devastating consequences, and the host would inevitably have an increased risk of death from infections. Possession of multiple pathways thus guards against a fatal defect that may arise due to mutation.

Another reason for complexity of cell-activation mechanisms resides in the end response of neutrophils – that is, the delivery of cytotoxic products. Whilst these products are highly lethal towards pathogens, they can also attack and destroy host tissues, and this can have deleterious effects on tissue function. Complex intracellular signalling mechanisms to activate these cytotoxic pathways also guards against non-specific activation, which could lead to host tissue damage.

Once the pathogen is enclosed within a phagocytic vesicle, these cytotoxic processes must be activated and delivered to the pathogen (see Fig. 1.2). Here, the plasma membrane and cytoplasmic granules play important roles. The plasma membrane contains an unusual enzyme that is capable of generating a series of reactive oxygen metabolites with broad antimicrobial properties. The enzyme responsible for this is an NADPH oxidase, which although it has a relatively simple task to perform (to transfer a single electron to O_2) has an extraordinarily complex structure and a complex mecha-

nism of activation. The oxidase comprises individual components that are present on the plasma membrane, on the membranes of some granules and in the cytoplasm. An active enzyme complex is assembled by the bringing together of these constituent parts during phagocytosis. Reactive oxidants are thus generated on the membrane (derived from the plasma membrane) that lines the phagocytic vesicle. Hence, the production of these oxidants is efficiently targeted, and they are delivered to the pathogen in high concentrations (Fig. 1.6). This complex structure and complicated activation mechanism also guards against the non-specific activation of the oxidase: the products of the oxidase can also cause considerable damage to host tissues.

The cytoplasmic granules also play an important function in pathogen killing because they contain a range of proteins with cytotoxic properties. These include a range of proteases, hydrolytic enzymes, a peroxidase (myeloperoxidase) and also a number of highly-specialised proteins that affect the permeability of microbial targets. Although the list of antimicrobial proteins present in these granules continues to grow as more constituents are discovered, two facts are apparent:

i. These antimicrobial proteins are 'packaged' within the granules, sometimes in a latent form, and hence are only active when they are released from these stores (Fig. 1.6): this prevents damage to host tissues or, indeed, damage to the neutrophil itself.
ii. By having this broad range of biochemically-distinct antimicrobials, the neutrophil has a degree of overkill, enabling it to attack a variety of microbial targets that may have differing susceptibilities to any one process. This in itself serves two functions: (a) should an organism develop resistance to one of the many different types of pathogens killed, that type may still be effectively killed via an alternative mechanism; (b) should a genetic defect arise that renders a particular antimicrobial system ineffective, then an alternative system will be available and hence will function to protect the host. (Defects in neutrophil function, described in Chapter 8, are usually associated with impaired defence against certain types of microbial pathogens.)

In summary, the importance of the neutrophil in protection against infection is highlighted by the presence of large numbers of these cells in the circulation and their production in vast numbers by the bone marrow. Neutrophil function is regulated via its ability to detect pathogens or signals (generated from host tissue, immune cells or the pathogens themselves) that may be generated during infections, and to leave the circulation and migrate into the infected tissues. Once at the infected site, it recognises the 'foreign'