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## Early Stages of Infection After Pathogen Entry

### OUTLINE

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### GROWTH IN EPITHELIAL CELLS

Some of the most successful microorganisms multiply in the epithelial surface at the site of entry into the body, produce a spreading infection in the epithelium and are shed directly to the exterior (Table 3.1). This is the simplest, most straightforward type of microbial parasitism. If the infection progresses rapidly and microbial progeny are shed to the exterior within a few days, the whole process may have been completed before the immune response has had a chance to influence the course of events. It takes at least a few days for antibodies or immune cells to be formed in appreciable amounts and delivered to the site of infection. However, we may underestimate the time of appearance of antibodies, as the first antibodies formed are immediately complexed with the microorganism and no free antibody appears until antibody is present in excess. With a variety of respiratory virus infections, especially those caused by rhinoviruses, coronaviruses, parainfluenza viruses and influenza viruses, epithelial cells are destroyed, and inflammatory responses induced, but there is little or no virus invasion of underlying tissues. The infection is terminated partly by the innate immune response, and partly because most locally available cells have been infected. Interferons are important resistance factors. They are low

**TABLE 3.1** Microbial Infections that are Generally Associated with Epithelial Surfaces of the Body

Microbe	Respiratory Tract and Conjunctiva	Urinogenital Tract	Skin	Intestinal Tract
Viruses	Influenza Parainfluenza 1–4 Rhinoviruses Coronaviruses	Certain papilloma viruses	Papilloma viruses (warts) Molluscum contagiosum	Rotaviruses of man, mouse, etc.
Chlamydias	Trachoma inclusion conjunctivitis	Nonspecific urethritis	–	–
Mycoplasma	<i>Mycoplasma pneumoniae</i> (atypical pneumonia)	T strains (nonspecific urethritis)	–	–
Bacteria	<i>Bordetella pertussis</i> <i>Corynebacterium diphtheriae</i> <i>Streptococci</i>	Gonococcus	Staphylococci <i>Corynebacterium minutissimum</i> <sup>a</sup>	Most Salmonellae; Shigellae <i>Campylobacter</i> sp.
Rickettsias	–	–	–	–
Fungi	<i>Candida albicans</i> (thrush)	<i>Candida albicans</i>	<i>Trichophyton</i> spp. (athlete's foot, ringworm, etc.)	–
Protozoa	–	<i>Trichomonas vaginalis</i>	–	<i>Entamoeba coli</i> <i>Giardia lamblia</i>

<sup>a</sup>This bacterium commonly infects the stratum corneum and causes erythrasma, a scaly condition of the axilla, groin and between toes.

molecular weight proteins, coded for by the cell, and formed in response to infection with nearly all viruses (see Chapter 9). The interferon formed by the infected cell is released and can act on neighbouring or distant cells, protecting them from infection. Freshly formed virus particles from the first infected epithelial cell enter the fluids bathing the epithelial surface and are borne away to initiate fresh foci of infection at more distant sites. Interferons too can reach these sites, and as more and more interferon is formed on the epithelial sheet, more and more cells are protected, so that the infectious process is slowed and finally halted. Other antiviral factors probably play a part, and the adaptive immune response itself comes into action in the final stages. Interferons are produced a few hours after infection of the first epithelial cell at a site where they are needed and without the delay characteristic of the immune response. The immune response provides resistance to subsequent re-infection, but it does not appear to be of primary importance in recovery from respiratory infections of this type.

The spread of infection is very rapid on epithelial surfaces that are covered with a layer of liquid because of the ease with which the microorganism in the fluid film encounters cells and is disseminated over the surface. This is true for the respiratory infections mentioned above, and also for infections of intestinal epithelium, such as those caused by the human diarrhoea viruses, e.g. rotavirus. The argument does not apply, however, to local infections of the skin. In this case, where the microorganism is not carried across the epithelial surface in a liquid film to establish fresh foci of infection, the whole process takes a

much longer time. Papilloma (wart) viruses, for instance, cause infection in a discrete focus of epidermal cells; indeed a wart consists of a clone of cells produced by the division of a single initially infected cell. The inevitably slow evolution of single virus-rich lesions should mean that immune responses have the opportunity to respond to and limit the infection. Papillomaviruses, however, escape the attention of the immune system. In the basal layer of the epidermis, adjacent to the antibodies and immune cells that arrive from dermal blood vessels, the virus infection is incomplete; in this layer of the epidermis, only a subset of virus genes are transcribed, no virus structural proteins are produced and no virus DNA replication occurs, therefore no virus particles are produced. Some of the proteins which are produced are involved in driving the cell into proliferation or differentiation, others act to downregulate the IFN system and MHC presentation. The infected basal cell is therefore not recognised and not a target for the immune response. As the cells move further away from these immune forces, approaching the epidermal surface and becoming keratinised, more and more virus is produced for liberation to the exterior. Neither antibodies nor immune cells are present on this dry surface to influence virus multiplication and shedding.

The respiratory viruses described above have a hit and run type of infection of epithelial cells, and are very successful parasites. A number of other viruses, including measles and chickenpox, infect inconspicuously via the respiratory tract, then spread systemically through the body and only emerge again to cause widespread respiratory infection and shedding to the exterior after a prolonged incubation period. The limitation of rhinoviruses and human coronaviruses to the surface of the upper respiratory tract is at least partly determined by their optimum growth temperature. Many of them replicate successfully at 33°C, the temperature of nasal epithelium, but not very well at the general body temperature, 37°C. Thus, they do not spread systemically nor to the lung. It is also likely that a lack of expression of the virus receptor involved in entry of the virus to the cell plays a major role in determining spread or lack thereof. Viruses of the influenza and parainfluenza groups can infect the lung as well as the nasal mucosa, but they are generally limited to the epithelial surfaces. The limitation is not absolute. Occasionally in adults and more often in infants, influenza and parainfluenza viruses spread to infect the heart, striated muscle or the central nervous system.

The spread of infection from epithelial surfaces is also controlled by the site of virus maturation from cells. Influenza and parainfluenza viruses are liberated (by budding) only from the free (external) surface of epithelial cells, as is appropriate for infection limited to surface epithelium. A similar restriction in the topography of budding is seen with rabies virus in the infected salivary gland. However, vesicular stomatitis virus is released only from the basal surface of the epithelial cell, from whence it can spread to subepithelial tissues and then through the body; topographical restriction in the site of virus release from epithelial cells reflects the polarisation of function in these cells, which in turn depends on the maintenance of tight junctions between them.

Many bacterial infections are largely confined to epithelial surfaces (Table 3.1). This is a feature, for instance, in diphtheria and streptococcal infections of the throat, gonococcal infections of the conjunctiva or urethra and most *Salmonella* infections of the intestine. To a large extent this is because host antibacterial forces, to be described at a later stage, do not permit further invasion of tissues. Under most circumstances, these bacteria are not able to overcome the host defences, but gonococci and streptococci, at least, often spread locally

through tissues and occasionally systemically through the body. For example, Group A Streptococcus occasionally causes necrotizing fasciitis or 'flesh-eating disease'. Gonococci cause a patchy infection of the columnar epithelium of the male urethra, reaching subepithelial tissues 3–4 days after infection; the yellow discharge consists of desquamated epithelial cells, inflammatory exudate, leucocytes and gonococci. Subepithelial spread probably takes the infection to other parts of the urethra and to local glands.

Most Gram-negative bacteria have only a very limited ability to invade a given host. In man, *E. coli*, *Proteus* spp. and *Pseudomonas aeruginosa* are only capable of invasion when defences are impaired or when bacteria are inadvertently introduced into a suitable site in the body (see Chapter 2). They cause systemic infection in debilitated, malnourished, or immunosuppressed patients; they produce sepsis in the uterus after abortion, and when they are introduced into the body by intravascular devices or catheters. Certain Gram-negative bacteria penetrate the intestinal epithelium but get no further, as in *Shigella* dysentery and salmonellosis. One or two highly specialised Gram-negative bacteria penetrate intestinal epithelium, enter lymphatics and spread systemically through the body to cause enteric or typhoid fever (*Salmonella typhi* and *paratyphi*).

A few bacteria show a temperature restriction similar to that described above for rhinoviruses, which prevents anything more than local spread. For instance, the lesions in leprosy (*Mycobacterium leprae*) are confined to cooler parts of the body (skin, superficial nerves, nasal mucosa, testicles, etc.). Other mycobacteria (*Mycobacterium ulcerans* and *M. marinum*) occur in water and enter human skin through superficial abrasions, especially in warm countries, and cause chronic skin ulcers. These bacteria, which also infect fish, have an optimum growth temperature of 30–33°C and remain restricted to the skin.

Fungi of the dermatophyte group (ringworm, athlete's foot<sup>1</sup>) infect skin, nails and hair, but are restricted to the dead keratinised layers of epithelium. Fungal antigens are absorbed from the site of infection and immune (including allergic) responses are generated, which at least partly account for the failure to invade deeper tissues.

## INTRACELLULAR MICROORGANISMS AND SPREAD THROUGH THE BODY

Some of the important microorganisms that regularly establish systemic infections after traversing epithelial surfaces are listed in [Table 3.2](#).

There is one important distinction between intracellular and extracellular microorganisms. If an obligate intracellular microbe is to spread systemically from the body surface, it must first enter the blood or lymph. This means gaining access to the lumen of a subepithelial lymphatic or blood vessel, either as a free microorganism, or alternatively after entering a mobile cell (leucocyte) that will carry it to other parts of the body. The microorganism cannot replicate until it reaches a susceptible cell, and the absence or shortage of such cells except at the body surface would prevent or seriously hinder its spread through

<sup>1</sup>Fungi causing this condition flourish in a moist environment, and athlete's foot is restricted to those who encase their feet in shoes. However, those who do not wear shoes (e.g. tropical Africa) are vulnerable to other fungi that enter skin at sites of injury and cause deeper lesions called mycetomas.

**TABLE 3.2** Examples of Infections in Which Microorganisms Enter Across Epithelial Surfaces and Subsequently Spread Through the Body

Microbe	Respiratory Tract and Conjunctiva	Urinogenital Tract	Skin	Intestinal Tract
Viruses	Measles	Herpes simplex 2	Arboviruses	Enteroviruses
	Rubella			Certain adenoviruses
	Varicella			
Bacteria	Psittacosis	Lympho-granuloma venereum	–	–
	<i>Mycobacterium tuberculosis</i>	<i>Treponema pallidum</i>	<i>Bacillus anthracis</i>	<i>Salmonella typhi</i>
	<i>Yersinia pestis</i>			
	Q fever	–	Typhus	Q fever?
Fungi	Cryptococcosis	–	Maduromycosis	Blastomycosis
	Histoplasmosis			
Protozoa	Toxoplasmosis	–	Malaria Trypanosomiasis	<i>Entamoeba histolytica</i>

the body. Thus, rotaviruses and rhinoviruses replicate at the epithelial surface but cannot infect leucocytes, and in any case would be unlikely to find susceptible cells elsewhere in the body if they entered blood or lymphatic vessels. Certain viruses (yellow fever, poliovirus) spread through the body to reach susceptible target organs (liver, central nervous system) after free virus particles have entered vessels below the skin or intestinal epithelium. Measles virus and tubercle bacilli infect leucocytes, which carry them through the body to organs such as the liver, spleen, skin and lung. A remarkable example of an intracellular bacterium which can manipulate host cells to assist in dissemination is *M. leprae*. *M. leprae* targets Schwann cells which are their primary niche and re-programmes them so that they return to a stem cell-like state. The properties of these cells include plasticity and migration and this facilitates the dissemination of *M. leprae* within those cells by differentiation and via macrophage release. These findings demonstrate how at least one intracellular bacteria can hijack host cell programming in order to promote bacterial spread within the host.

If, on the other hand, the microbe is able to replicate outside cells and does not have to find a susceptible cell, it can in principle multiply locally, in the blood and lymph, and in whatever part of the body it gets to. Extracellular replication itself, however, conveys a serious disadvantage, because the microorganism is exposed to all the antimicrobial forces that the body can summon up. Indeed, bacteria and other microorganisms that are capable of extracellular replication generally advertise their presence by releasing a variety of products into surrounding fluids, many of which cause inflammation and thus bring antibacterial agents such as immunoglobulins, complement and leucocytes to the site of the infection. Lymphatics are also dilated and carry the infecting organisms to lymph nodes for further exposure to antibacterial and immune forces. Intracellular microorganisms in contrast, although exposed to the infected cell's own defence mechanisms, are directly

**TABLE 3.3** Early Defences<sup>a</sup>

Acute Phase Proteins	Current Chapter
Lysozyme	See Glossary
Lactoferrin	Current chapter
Interferons and other cytokines	See Chapter 9
Complement activation (by the alternative pathway)	See Chapter 6
Phagocytosis	See Chapter 4
Natural killer (NK) cells	See Chapter 6
Apoptosis	See Glossary and Chapter 8
Collectins	See end of Chapter 6

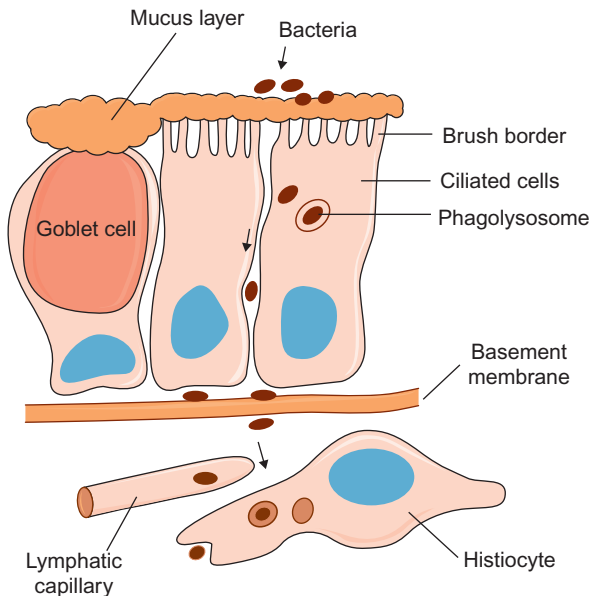
<sup>a</sup>These early or 'innate' defences operate immediately after a microbe has penetrated the body, during that critical period before immune responses have had time to come into action.

exposed to the general bodily defences only during transit from one infected cell to another. However, if the infected cell is recognised as such by the immune defences, it can be destroyed (see Chapters 6 and 9). A number of bacteria and protozoa, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Brucella abortus* or *Leishmania donovani*, carry out much of their multiplication in macrophages that have ingested them. Although they are not obligate intracellular parasites, this shifts the host–microbe battlefield into the cell. The battle is then waged in the infected macrophage, whose antimicrobial powers (see Chapter 4) and participation in immune defences (see Chapters 6 and 9) become of critical importance.

The infected host has a variety of defences that operate without delay, before the immune response comes into action (Table 3.3). These 'early' defences are referred to in this and in subsequent chapters, and they are the type of defences that mattered before the immune system had evolved. Many microbes have strategies for interfering with these defences. For example, cells infected with a virus can commit suicide before the virus has completed its growth cycle in the cell. This is called apoptosis and occurs after reovirus, HIV, and other infections. The fact that viruses (e.g. adenoviruses) have developed mechanisms for inhibiting apoptosis indicates that it plays an important part in defence. Apoptosis occurs also in bacterial infections. When uropathogenic strains of *E. coli* infect bladder epithelium, the host responds by apoptosis of the infected cells. The actual value of this response is not clear.

## SUBEPITHELIAL INVASION

After traversing the epithelial cell layer, a microorganism encounters the basement membrane. The basement membrane acts as a filter and can to some extent hold up the infection, but its functional integrity is soon broken by inflammation or epithelial cell damage. The invading microorganism has now reached the subepithelial tissues (Figure 3.1),



**FIGURE 3.1** Microbial invasion across an epithelial surface.

and here it is exposed to three important host defence systems. These are (i) the tissue fluids, (ii) the lymphatic system leading to the lymph nodes and (iii) phagocytic cells.

These three host defence mechanisms are of supreme importance and come into play whatever part of the body is infected, whether the nasal mucosa, meninges, urethra, cardiac muscle or liver lobule. Each depends for its action on the inflammatory response, because this response brings the phagocytes and serum factors to the site of infection and promotes drainage from the site by the lymphatic system. Therefore a short account of the inflammatory response will be given, and after this, each of the three antimicrobial factors will be considered separately. [Table 3.2](#) shows some of the important microorganisms that regularly spread through the body in spite of these antimicrobial factors.

## The Inflammatory Response

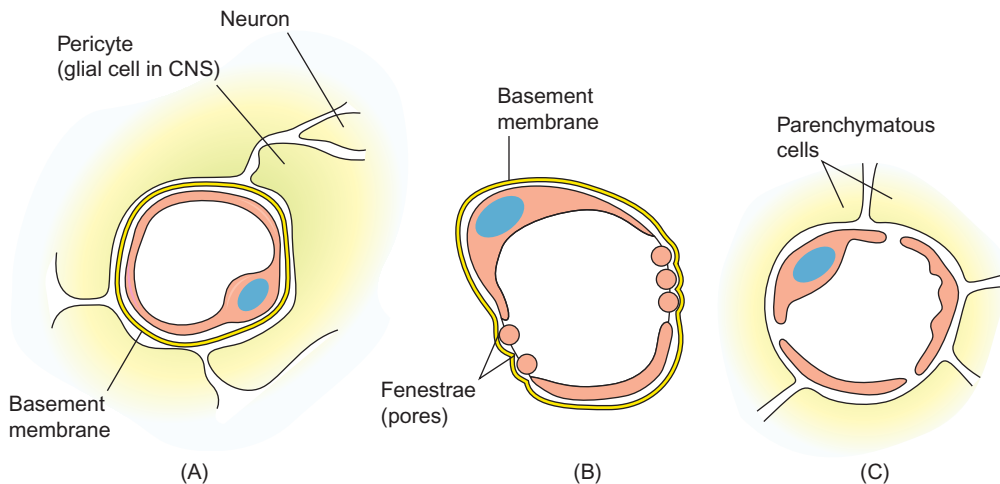
The capillary blood vessels supplying a tissue bring oxygen and low molecular weight materials to the cells, taking away carbon dioxide and metabolic or secretory products. There is also a constant passage of plasma proteins and leucocytes from capillaries into normal tissues, and these are returned to the blood via the lymphatic system after entering lymphatic capillaries. Indeed, their presence in tissues is inferred from their presence in lymphatics draining these tissues. The cells are nearly all T lymphocytes, which leave blood capillaries by actively passing through endothelial cells. After moving about and performing any necessary tasks in the tissues, the lymphocytes penetrate lymphatic capillaries and thus enter the lymph. The lymph, with its content of proteins and cells, then passes through the local lymph nodes and generally at least one more lymph node before entering the thoracic lymph duct and being discharged into the great veins in the



thorax or abdomen. Blood lymphocytes also enter lymph nodes directly and in larger numbers through post-capillary venules. The constant movement of lymphocytes from blood to tissues or lymph nodes, and back via lymphatics to the blood again, is called *lymphocyte recirculation*. Circulating lymphocytes are mostly T cells, and in the course of their continued entries into tissues and lymph nodes they have regular opportunities to encounter any microbial antigens that may be present. There is in fact a regular monitoring of tissues by T lymphocytes, and this is referred to as *immune surveillance*.

The various plasma proteins occur in the tissues in much the same proportion as in plasma, the actual concentrations depending on the structure of the capillary bed. As determined by concentrations in local lymphatics, the leaky sinusoids of the liver let through 80–90% of the plasma proteins into liver tissue, the less leaky capillaries of the intestine admit 40–60% into intestinal tissues and capillaries of skeletal muscle with their continuous lining only 10–30% (Figure 3.2). Thus, immunoglobulins, complement components, etc. occur regularly in normal tissues, but in lesser concentrations than in blood. There is some discrimination against very large molecules because the largest immunoglobulins (IgM) do not leave the blood vessels and are not detectable in afferent lymph.

There is a prompt and vigorous change in the microcirculation when tissues are damaged or infected. Capillaries and post-capillary vessels are dilated, gaps appear between endothelial cells, and the permeability of these vessels increases, allowing leakage from the blood of a protein-rich fluid. Increased amounts of immunoglobulins, complement components and other proteins are then present in tissues, and fibrinogen, for instance, may be converted into fibrin so that a diffuse network of fibrils is laid down. Circulating leucocytes (especially neutrophils and monocytes) adhere to endothelial cells, and this is followed by active passage (diapedesis) of leucocytes between endothelial cells and out



**FIGURE 3.2** Diagram to show types of blood–tissue junction in capillary, venule, or sinusoid. (A) Continuous endothelium (transport of tissue nutrients and metabolites): central nervous system, connective tissue, skeletal and cardiac muscle, skin, lung. (B) Fenestrated endothelium (transport of secreted, excreted or digested materials): renal glomerulus, intestinal villi, choroid plexus, pancreas, endocrine glands. (C) Sinusoid (reticuloendothelial system): liver, spleen, bone marrow, adrenal, parathyroid.

into tissues. The affected part now shows the four cardinal signs of inflammation, being RED and WARM (vasodilation), SWOLLEN (vasodilation, cell and fluid exudate) and often PAINFUL (distension of tissues, presence of pain mediators).

Lymphatic capillaries also become dilated, taking up the inflammatory fluids and carrying them to local lymph nodes. There is a greatly increased turnover of plasma components in the inflamed tissue. Initially, the predominant cell is the neutrophil, a reflection of the situation in the blood, but neutrophils only live for a day or two in tissues, and as the acute inflammatory state subsides mononuclear cells become more prominent, especially macrophages, which phagocytose dead neutrophils and tissue debris.

The initial stages of the inflammatory response tend to be consistent, whatever the nature of the tissue insult, and this is partly because the changes are caused by the same mediators of acute inflammation. These include histamine (released from mast cells lying close to blood vessels), kinins (polypeptides derived from precursors in plasma; see Glossary) and products of complement activation by the alternative pathway (C3a and C5a). Some of the kinins are highly active and kallidin, for instance, a decapeptide formed from kallidinogen (an  $\alpha_2$  globulin) is about 15 times more active (on a molar basis) than histamine in causing inflammation. Most bacteria form inflammatory materials during their growth in tissues, but these are not very potent compared with the activation of C3 and other molecules by carbohydrates (e.g. polysaccharides) present on bacterial surfaces (see Figure 6.6). Macrophages, when they are stimulated, release a variety of inflammatory mediators and, in addition, immune-mediated inflammation results from interaction of microbial antigen with antibody (via C3a and C5a) or reaction of antigen with IgE antibody on mast cells. The final mediators include molecules such as TNF (tumour necrosis factor), ICAM-1 (intercellular adhesion molecule-1) and ELAM-1 (endothelial cell leucocyte adhesion molecule-1). Inflammatory responses, like other powerful tissue responses, must be controlled and terminated, and the mediators of inflammation not only have a variety of inhibitors but are also inactivated locally (e.g. kinins inactivated by kininases). At a later stage, prostaglandins (a family of 20-carbon fatty acid molecules) and leukotrienes (a group of biologically active lipids) come into play.<sup>2</sup> They are produced from leucocytes, endothelial cells and platelets, and they both mediate and control the response.

If inflammation is due to infection with one of the pyogenic bacteria and the infection continues, then the continued supply of inflammatory and chemotactic products from the multiplying bacteria maintains vasodilation and the flow of neutrophils to the affected area. There is an increase in the number of circulating neutrophils, because of an increase in the rate of release from the bone marrow. The bone marrow holds a vast reserve supply with 20 times as many neutrophils as are present in the blood. If the tissue demand continues, the rate of production in the bone marrow is increased, and circulating neutrophils may remain elevated in persistent bacterial infections such as subacute bacterial endocarditis. Neutrophil production in the bone marrow is regulated by certain colony-stimulating factors, and it is a serious matter if something goes wrong and the marrow supplies are exhausted. A fall in circulating neutrophils (neutropenia) during a bacterial infection is of ominous significance.

<sup>2</sup>The terminology becomes complicated. Eicosanoids are produced by metabolism of arachidonic acid and include leukotrienes, prostaglandins, thromboxanes and lipoxins.

Viruses produce inflammatory products in tissues in the form of necrotic host cell materials or antigen–antibody complexes, but these are less potent than bacterial products, and the acute inflammatory response is of shorter duration, neutrophils being replaced by mononuclear cells. Mononuclear infiltrates are also favoured in virus infections because the infected tissues themselves are often one of the sites for the immune response, with mononuclear infiltration and cell division.

After extravasation from blood vessels, leucocytes would not automatically move to the exact site of infection. Neutrophils show random movement in tissues and also a directional movement (chemotaxis) in response to chemical gradients produced by chemotactic substances. Monocytes show little or no random movement, but they too respond to similar chemotactic substances. Chemotactic substances such as leukotrienes, C3a and C5a are formed during the inflammatory response itself. Also, many bacteria, such as *Staphylococcus aureus* or *S. typhi*, form chemotactic substances, and thus automatically betray their presence and attract phagocytic cells. It would obviously be an advantage to an infectious agent if no inflammatory or chemotactic products were formed, but for most large microorganisms (bacteria, fungi, protozoa) these products seem an almost inevitable result of microbial growth and metabolism. However, some pathogens have evolved ways of interfering with the chemotactic process by producing substances that block chemotactic receptors. The early stages of the inflammatory response in particular are known to have an important protective effect against microorganisms. In experimental staphylococcal skin infections, for instance, if the early inflammatory response is inhibited by adrenalin, and the early delivery of plasma factors and leucocytes to the site of infection thus reduced, bacteria multiply more rapidly and produce a more severe lesion. Perhaps it is not surprising that many bacterial pathogens can suppress the early inflammatory response. For example the chemotactic inhibitory protein of *S. aureus* (Chips) bind to the C5a and fmp receptors on macrophages, blocking the recognition of C5a and formylated peptides.

If inflammation becomes more severe or widespread, it is generally modulated by increased output of corticosteroid hormones, but at the same time it is backed up by a general metabolic response in the body. This is called the *acute phase response*. The liver releases about 30 different proteins, including C-reactive protein and serum amyloid protein, which undergo 1000-fold increases in concentration, as well as mannose-binding protein, haptoglobulins ( $\alpha_2$ -glycoproteins), protease inhibitors and fibrinogen. The exact function of these *acute phase proteins* is not clear, but they are protective; they fix complement, opsonize and inhibit bacterial proteases. Their presence is associated with an increased erythrocyte sedimentation rate. The patient may develop headache, muscle pains, fever and anaemia, with decreased iron and zinc and increased copper and ceruloplasmin in the serum. Proteins in muscle are broken down, partly to provide energy required during fever and fasting, and partly to provide amino acids needed by proliferating cells and for the synthesis of immunoglobulins and acute phase proteins.

Many of the features of the acute phase response appear to be due to the action of interleukin-1 (see Glossary), and also IL-6 and TNF released from macrophages and lymphocytes. It is a complex response, which on the whole would be expected to serve useful purposes, although some less obviously beneficial 'side effects' may be unavoidable.

## Tissue Fluids

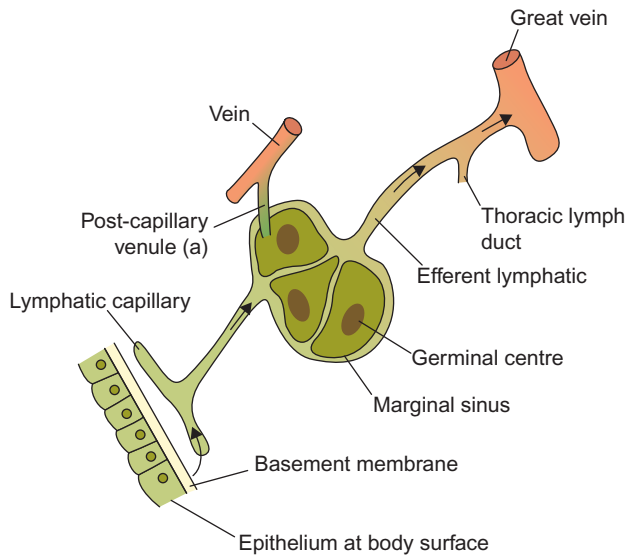
Tissue fluids normally contain variable amounts of plasma proteins, including IgG antibodies as discussed above. In the absence of specific antibodies and complement, tissue fluids make a good culture medium for most bacteria, but bacterial multiplication almost inevitably causes some inflammation. Powerful inflammatory events are set in motion when molecules on the bacterial surface (e.g. endotoxin) activate the alternative complement pathway. Larger amounts of IgG as well as activated complement components, will then be present in tissue fluids. At a later stage, secretory products from phagocytes (lysosomal enzymes, oxygen radicals, lactoferrin, etc.) will also be present, and finally tissue breakdown products and additional antimicrobial substances liberated from dead platelets, neutrophils and macrophages.

## Lymphatics and Lymph Nodes

A complex network of lymphatics lies below the epithelium at body surfaces. After reaching subepithelial tissues, foreign particles of all kinds, including microorganisms, rapidly enter lymphatic capillaries after uptake by or passage between lymphatic endothelial cells. There is a particularly rich superficial plexus of lymphatics in the skin and in the intestinal wall. Microorganisms scratched or injected into the skin inevitably enter lymphatics almost immediately. The intestinal lymphatics not only take up microorganisms that have breached the epithelial surface but also have an important role in the uptake of fat in the form of chylomicrons.

Microorganisms in peripheral lymphatics are rapidly moved (within minutes) to the local lymph nodes strategically placed to deal with the flow of lymph before it returns to the blood. The rate of flow of lymph is greatly increased during inflammation, when there is increased exudation of fluid from local blood vessels and the lymphatics are dilated. Microorganisms carried to the node in the lymph are exposed to the macrophages lining the marginal sinus (Figure 3.3), and these cells take up particles of all types from the lymph and thus filter it. The efficiency of filtration depends on the nature of the particles, on the physiological state of the macrophages, and also on the particle concentration and flow rate, the efficiency falling off at high particle concentrations or high flow rates (see Chapter 5).

All infecting microorganisms are handled in the same way and delivered via lymphatics to the local lymph node. When there has already been microbial multiplication at the site of initial infection, very large numbers may be delivered to the node. The efficiency of the node as a defence post depends on its ability to contain and destroy microorganisms rather than allow them to replicate further in the node and spread to the rest of the body. The antimicrobial forces are the macrophages of the node, the neutrophils and serum factors accumulating during inflammation, and the immune response which is initiated in the node. Under normal circumstances, as the first trickle of microorganisms reaches the node, the most important event is the encounter with macrophages in the marginal sinus. Microorganisms escaping phagocytosis by these cells enter the intermediate sinuses where they run the gauntlet of a further set of macrophages before leaving the node. If there is an inflammatory reaction in the node, a substantial migration of neutrophils



**FIGURE 3.3** Diagram of the lymphatic system, showing pathways from body surface to venous system. (a) indicates a site of lymphocyte circulation from blood to lymph node and back to blood.

into the sinuses greatly increases the phagocytic forces and thus the filtering efficiency. There is usually a further node to be traversed before the lymph is discharged into the venous system.

As well as functioning as filters, the lymph nodes, of course, are sites where the immune response comes into play. Soon after infection, as inflammatory products of microbial growth arrive in the node, there is some swelling and inflammation. The microbial antigens, some of which are already associated with antigen-presenting cells encountered at the body surface, generate an immune response, and there is further swelling of the node as cells divide and additional lymphoid cells are recruited into the node from the blood. The ability of viruses and other intracellular microorganisms to bypass the defences of the node and spread to the bloodstream is discussed in Chapter 5.

## Phagocytic Cells

Specialised phagocytic cells are divided into two main types: the macrophages, scattered through all the major compartments of the body (see Chapter 4) and the circulating neutrophils. The phagocytic cells to which microbes are exposed in the subepithelial tissues are the local macrophages (histiocytes) and also the cells arriving from the small blood vessels during inflammation. These comprise the blood monocytes which become macrophages after extravasation, and the neutrophils. From the time of the Russian zoologist, Elie Metchnikoff, who described phagocytosis in 1883, the importance of the phagocyte in defence against disease organisms has been accepted, and children have learnt of the white blood cells that act both as scavengers and policemen, removing debris, foreign particles and microorganisms. Because of the central importance of the phagocytic defence mechanisms, the subject will receive a chapter to itself.

## NUTRITIONAL REQUIREMENTS OF INVADING MICROBES

In addition to being able to resist host defence mechanisms, a pathogenic organism – be it an obligate intracellular, facultative intracellular or extracellular pathogen – must also overcome the problem of obtaining essential nutrients if it is to be successful. Two examples illustrate this point. Iron is essential for bacterial growth but most iron is sequestered in the host and the concentration of free iron in body fluids is too low (ca.  $10^{-18}$  M in serum) to support growth. In the host, iron is bound by both intracellular (ferritin, haemosiderin and haem) and extracellular (transferrin in serum and milk, and lactoferrin in milk), Fe-binding proteins with high association constants for iron. In order to overcome this problem, bacteria have evolved numerous ways of acquiring iron from the host in concentrations high enough for growth. For example, *Listeria monocytogenes* produces a soluble reductant which removes iron from transferrin. A common strategy by many bacterial pathogens is the synthesis of low molecular weight compounds called siderophores, which have an extraordinary affinity for iron. At the same time the bacteria express outer membrane proteins that act as receptors for the Fe-siderophore complexes so that Fe is taken up into the cell. *E. coli* has been extensively studied, and three classes of siderophore have been recognised; ferrichrome, the hydroxymates and aerobactin. *Salmonella* and *Shigella* spp. and *P. aeruginosa* produce more than one siderophore. *Salmonella* is also known to synthesise receptors for siderophores other than its own, which could be advantageous when present with other organisms, particularly in the competitive environment of the gut. The mycobacterial siderophores (Mycobactins) are lipid soluble and membrane associated, and exochelins are water soluble, extracellular and are the more important of the two.

Another common bacterial strategy for dealing with the Fe shortage involves the synthesis of new outer membrane proteins which interact directly with the host's own Fe-binding proteins. This is the method used by *Neisseria meningitidis* and *N. gonorrhoeae* which are able to scavenge iron directly from the host.

Perhaps the most dramatic example of Fe uptake and storage is exhibited by *Yersinia*. *Yersinia pestis* is the agent of bubonic plague and has been responsible for devastating epidemics throughout human history. This pathogen persists in wild rodent populations in many parts of the world except Australia, and is transmitted to humans by the bites of fleas. The blockage of the proventriculae of fleas by *Y. pestis* forces infected fleas to bite and subsequently regurgitate the infected blood meal into the bite wound of a new host. The ensuing bacteraemia in rodents completes the rodent–flea–rodent cycle essential for *Y. pestis* spread. However the ecology, pathogenicity and host range of *Yersinia pseudotuberculosis* (the predicted ancestor of *Y. pestis*) and *Yersinia enterocolitica* are quite different from *Y. pestis*. These are orally transmitted from contaminated food or water. As described in Chapter 2, they invade Peyer's patches and disseminate to mesenteric lymph nodes (where they multiply extracellularly) and occasionally beyond causing septicemic plague-like infections; normally these infections are self-limiting. Despite their disease-causing differences, the three *Yersinia* species do have some common pathogenic strategies, in particular the mechanism(s) for acquiring iron. *Yersinia* have two important sets of pathogenicity genes: the 70 kb virulence plasmid which has genes encoding proteins involved in



inhibition of phagocytosis to which we will return in Chapter 4, and chromosomal genes encoding virulence factors including an ‘invasin’ (involved in interaction with Peyer’s patches) and the *pgm* (pigmentation) locus. Virulent *Y. pestis* strains accumulate huge quantities of exogenous haemin to form pigmented colonies on haemin agar (hence the alternative acronym for the locus, *hms*). Contiguous with the *hms* is the *ybt* gene cluster responsible for the synthesis of the siderophore yersiniabactin. In *Y. pseudotuberculosis*, *hms* and *ybt* are not contiguous, and in *Y. enterocolitica*, *hms* is absent. Inactivation of *hms* renders *Y. pestis* avirulent and unable to develop blockages in fleas, whereas inactivation of *ybt* in *Y. enterocolitica* hugely reduces virulence for laboratory animals. In fact, the *ybt* gene cluster has been designated the ‘high pathogenicity island’ (HPI) because biotype IB strains of *Y. pestis*, *Y. tuberculosis* and *Y. enterocolitica* (New World strains) all possess *ybt* biosynthetic genes and kill mice with very low infectious doses. In contrast, biotypes 2–5 (Old World strains) are much less virulent for mice and do not possess *ybt* genes. Accordingly, the capacity to acquire high concentrations of iron for metabolism is associated with a high virulence phenotype.

Other nutrients in short supply in the mammalian host are aromatic amino acids like tryptophan. Interestingly, the expression of the *trp* operon (which comprises the genes responsible for the synthesis of tryptophan) is controlled by Fe as well as by tryptophan levels. A functional aromatic biosynthetic pathway is absolutely vital for growth *in vivo* since aromatic amino acids are generated via chorismic acid. The latter is a branch point at which the biosynthesis of p-amino benzoic acid (PABA) also begins. PABA is required as a precursor of folic acid; it is as a competitor for PABA that the sulphonamide drugs work. By introducing lesions in one or more genes in this system (*aroA* and *aroD*), it has been possible to attenuate strains of pathogenic bacteria such as *S. typhimurium*, *E. coli*, and *Aeromonas salmonicida*, such that their initial invasive properties are unaltered but their ability to grow *in vivo* is severely restricted. In some cases, such crippled constructs can be used as vaccines to protect against subsequent infection or manipulated to carry genes encoding heterologous antigens which may offer immune protection.

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