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Mechanisms of Cell and Tissue Damage

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The impact on the host of microbial damage depends very much on the tissue involved. Damage to muscle in the shoulder or stomach wall, for instance, may not be serious, but in the heart the very existence of the host depends on a strong muscle contraction continuing to occur every second or so, and here the effect of minor functional changes may be catastrophic. The central nervous system (CNS) is particularly vulnerable to slight damage. The passage of nerve impulses requires normal function in the neuronal cell membrane, and viruses especially have important effects on cell membranes. Also a degree of cellular or tissue oedema that is tolerable in most tissues may have serious consequences if it occurs in the brain, enclosed in that more or less rigid box, the skull. Therefore, encephalitis and meningitis tend to cause more severe illness than might be expected from the histological changes themselves. Oedema is a serious matter also in the lung. Oedema fluid or inflammatory cell exudates appear first in the space between the alveolar capillary and the alveolar wall, decreasing the efficiency of gaseous exchanges. Respiratory function is more drastically impaired when fluid or cells accumulate in the alveolar air space.* The effect of tissue damage is much less in the case of organs, such as the liver, pancreas or kidney, which have considerable functional reserves. More

* In the normal lung, bronchioles and alveoli have an immense capacity to absorb surplus fluid, as indicated by the observation that 21 litres of fluid can be given intratracheally to a horse over the course of 3.5 h with no ill effect. There are nearly 10^9 alveoli in man, richly supplied with lymphatics, and with a combined absorptive area of 90 m^2 .

than two-thirds of the liver must be removed before there are signs of liver dysfunction.

Cell damage has profound effects if it is the endothelial cells of small blood vessels that are involved. The resulting circulatory changes may lead to anoxia or necrosis in the tissues supplied by these vessels. Here too, the site of vascular lesions may be critical, effects on organs such as the brain or heart having a greater impact on the host, as discussed above. Rickettsiae characteristically grow in vascular endothelium and this is an important mechanism of disease production. By a combination of direct and immunopathological factors there is endothelial swelling, thrombosis, infarcts, haemorrhage and tissue anoxia. This is especially notable in the skin, and forms the basis for the striking rashes in typhus and the spotted fevers. These skin rashes, although important for the physician, are less important for the patient than similar lesions in the central nervous system or heart. It is damage to cerebral vessels that accounts for the cerebral disturbances in typhus; involvement of pulmonary vessels causes pneumonitis, and involvement of myocardial vessels causes myocardial oedema. In Q fever, rickettsiae sometimes localise in the endocardium, and this causes serious complications.

Sometimes an infectious agent damages an organ, and loss of function in this organ leads to a series of secondary disease features. The signs of liver dysfunction are an accepted result of infections of the liver, just as paralysis or coma is an accepted result of infection of the central nervous system. Diabetes may turn out to be caused by infection of the islets of Langerhans in the pancreas. Coxsackie and other virus infections of the islets of Langerhans can certainly cause diabetes in experimental animals, and coxsackie viruses have been associated with juvenile diabetes in man.

There are many diseases of unknown aetiology for which an infectious origin has been suggested. Sometimes it is fairly well established that an infectious agent can at least be one of the causes of the disease, but in most instances it is no more than a hypothesis, with little or no good evidence. For conditions as common and as serious as multiple sclerosis, cancer and rheumatoid arthritis it would be of immense importance if a microorganism were incriminated, since this would give the opportunity to prevent the disease by vaccination. Accordingly, there is a temptation to accept or publicise new reports even though the evidence is weak or the observations poorly controlled. As if to warn us about this and remind us of possibilities from environmental toxins, Parkinson's disease, a chronic neurological condition in which there is a loss of neurons in a sharply defined region of the brain (substantia nigra), can be caused by exposure to the chemical MPTP. One example which raises the possibility that subtle CNS disturbances may be caused by viruses is experimental infection with Borna disease virus. This virus was used to infect tree shrews (*Tupaia glis*) which are primitive primates. There is little overt disease, but afterwards the male is

no longer able to enact the ritual courtship behaviour, which (as students well know), is an essential preliminary to mating in all primates, and the frustrated male usually ends up bitten by the female. Thus it can be said that infection with Borna disease virus renders the male psychologically sterile. Presumably the virus in some way alters the functioning of neurons concerned in this particular pathway. All other behavioural and physiological aspects appear normal. Borna disease virus is not known to occur in man, but speculation about an analogous human situation is fuelled by the finding of Borna disease virus-specific antibodies in patients with psychiatric/behavioural disorders. In an entirely different clinical context, infection of a particular strain of rats with Borna disease virus causes immense obesity, the underlying physiological basis of which is not understood. Since the aetiology of such diseases raises interesting problems in pathogenesis, the present state of affairs is summarised in Table 8.1, which includes some of the human diseases whose infectious origin is probable, possible, conceivable, or inconceivable.

Causal connections between infection and disease states are particularly difficult to establish when the disease appears a long time after infection. It was not too difficult to prove and accept that the encephalitis that occasionally occurs during or immediately after measles was due to measles virus. But it was hard to accept that a very rare type of encephalitis (subacute sclerosing panencephalitis or SSPE), occurring up to 10 years after apparently complete recovery from measles, was also due to measles virus and this was only established after careful studies and the eventual difficult isolation of a mutant form of measles virus from brain cells. 'Slow' infections, in which the first signs of disease appear a long time after infection, are now an accepted part of our outlook. The disease kuru occurred in New Guinea and was transmitted from person to person by cannibalism. The incubation period in man appears to be 12–15 years, and the disease was caused by an unconventional infectious agent that grew in the brain. This was established when the same disease appeared in monkeys several years after the injection of material from the brain of Kuru patients (see pp. 351–353). A similar agent called scrapie (see Ch. 7) infects sheep, mice and other animals and also has an incubation period representing a large portion of the life span of the host. In both Kuru and SSPE the agent was eventually shown to be present in the brains of patients. So far this has only been demonstrated indirectly as, despite strenuous efforts, the causative agent has yet to be isolated. If in a slow infection, the microorganism that initiated the pathological process is no longer present by the time the disease becomes manifest, then the problem of establishing a causal relationship will be much greater. This may possibly turn out to be true for diseases like multiple sclerosis and rheumatoid arthritis. Liver cancer in humans and certain leukaemias in mice, cats, humans and cattle can be caused by slow-type virus infections. Cancer or leukaemia appears as a late and occa-

Table 8.1. Microorganisms as causes of human diseases of unknown aetiology

| Disease | Features | Microorganism | Pathogenic mechanism | Comments | Status of infectious aetiology |
|-------------------------|--|--|---|--|--------------------------------|
| Juvenile diabetes | Onset early in life; sensitive to insulin | Coxsackie B viruses | Infection and damage of islets of Langerhans; secondary immune phenomena | Accounts for some cases. | + |
| | | Mumps Rubella | | No direct evidence Late result congenital rubella | ± + |
| Crohn's disease | Granulomatous inflammation of intestine | Mycobacteria Viruses | Not clear; secondary immune phenomena | } No good evidence | - |
| Ulcerative colitis | Inflammation of colon | Viruses | Not clear; secondary immune phenomena | | |
| Multiple sclerosis | Demyelinating disease of central nervous system. Waxes and wanes | A variety of enveloped viruses? | Autoimmunity triggered by presentation of brain autoantigens in the envelope of a succession of different viruses | No direct evidence | - |
| Rheumatoid arthritis | Chronic inflammation and damage to joints | Mycoplasmas | ? | Cause arthritis in animals but no evidence for man | - |
| | | Viruses (Epstein-Barr, rubella, parvovirus B 19) | ? | No good evidence | - |
| Paget's disease of bone | Localised deformation of bone | Measles virus | Persistent infection of osteoclasts | | ± |

| Disease | Features | Microorganism | Pathogenic mechanism | Comments | Status of infectious aetiology |
|---------------------------|--|---|--|---|--------------------------------|
| Duodenal ulcer, gastritis | Ulceration, inflammation | <i>Helicobacter pylori</i> ^a | Bacterial cytotoxins? | Treated with antacids and antibiotics | + |
| Ankylosing spondylitis | Chronic arthritis of spine | <i>Klebsiella</i> spp. | Immune response to bacterial antigen cross-reacts with joint antigen, giving autoimmune damage | Strong association with HLA B27 genotype | + |
| Chronic fatigue syndrome | Tiredness, muscle weakness, lasting months or years | Epstein-Barr virus? HHV6, etc. | Unknown Upset of hypothalamic-adrenal axis (p. 380)? | Some cases (see p. 358) | |
| Alzheimer's disease | Presenile (<55 years) dementia | } 'Slow virus'? | } Infectious agent replicates slowly in brain, destroying cells | Some cases? | ± |
| Senile dementia | Loss of neurons; very common at 65+ years | | | No evidence | - |
| Old age | Not a disease but early death offers reliable prophylaxis. Degenerative changes | ? | ? | No evidence. Universal infection plus very long incubation period could give onset of 'disease' with ageing | - |

| | | | | | | |
|------------|--------------------|------------------------------------|--|--|--|---|
| Cancer | Carcinomas | Nasopharyngeal carcinoma | Epstein–Barr virus | Transformation of epithelial cell | Susceptibility gene in Chinese people | + |
| | | Cervical/penile carcinoma | Papillomaviruses | Transformation of epithelial cell | Associated with sexual promiscuity | + |
| | | Carcinoma of liver | Hepatitis B virus | Transformation of hepatic cell | Liver cancer especially common in those with persistent hepatitis B infection ^b | + |
| | | Skin cancer (basal cell carcinoma) | Papillomaviruses | Ultraviolet light as co-carcinogen | Evidence in animals but so far not in humans | + |
| | | Stomach cancer | <i>Helicobacter pylori</i> | Chronic inflammation? | Association (in small proportion of cases) is with chronic gastritis and ulcer (? role of host genes, diet, cofactors) | ± |
| Lymphomas | Burkitt's lymphoma | Epstein–Barr virus | Transformation of B lymphocyte plus cofactor (? malaria) | Evidence compelling but not conclusive | + | |
| | Hodgkin's disease | Epstein–Barr virus | Transformation of B lymphocyte | No direct evidence | – | |
| Leukaemias | | Retroviruses | Transformation of white cell precursor | Cause leukaemia in animals, and certain T-cell leukaemias in humans (HTLV 1 and 2) | + | |

^aAn ancient human parasite (see p. 26) detected in 2000-year-old corpses from Chile.

^bIn a study of 22 797 civil servants in Taiwan, 1.2% of hepatitis B carriers developed liver cancer compared with 0.005% of noncarriers.

sional sequel to infection. The virus, its antigens or fragments of its nucleic acid are detectable in malignant cells.

One important factor that often controls the speed of an infectious process and the type of host response, is the rate of multiplication of a microorganism.* Different infectious agents show doubling times varying from 20 min to 2 weeks, and some of these are listed in Table 8.2. Often the rate of multiplication in the infected host, in the presence of antimicrobial and other limiting factors, and when many bacteria are obliged to multiply inside phagocytic cells, is much less than the optimal rate in artificial culture. Clearly a microorganism with a doubling time of a day or two will tend to cause a more slowly evolving infection and disease than one that doubles in an hour or less.

It is uncommon for an infectious agent to cause exactly the same disease in all those infected. Its nature and severity will depend on infecting dose and route, and on the host's age, sex, nutritional status, genetic background, and so on (see Ch. 11). Many infections are asymptomatic in more than 90% of individuals, clinically characterised disease occurring in only an occasional unfortunate host, as 'the tip of the iceberg'.† Asymptomatically infected individuals are important because they are not identified, move normally in the community, and play an important part in transmission.

Table 8.2. Growth rates of microorganisms expressed as doubling times

| Microorganisms | Situation | Mean doubling time |
|---|--|--------------------|
| Most viruses | In cell | <1 h |
| <i>E. coli</i> , staphylococci, streptococci etc. | <i>In vitro</i> | 20–30 min |
| <i>Salmonella typhimurium</i> | Mouse spleen | 5–12 h |
| | <i>In vitro</i> | 30 min |
| Tubercle bacillus | <i>In vitro</i> | 24 h |
| | <i>In vivo</i> | Many days |
| Fungi | | |
| <i>Candida albicans</i> | <i>In vitro</i> (37°C) | 30 min |
| Dermatophytes | <i>In vitro</i> (28°C) | 1–24 h |
| <i>Treponema pallidum</i> | <i>In vivo</i> (rabbit) ^a | 30 h |
| Scrapie group | Mouse brain ^a | 4–7 days |
| Leprosy bacillus | <i>In vivo</i> ^a | 2 weeks |
| <i>Plasmodium falciparum</i> | <i>In vivo</i> or <i>in vitro</i> ^b | 8 h |

^a Cannot be cultivated *in vitro*.

^b Erythrocyte or hepatic cell.

* Every infection is a *race* between the spread and multiplication of the microbe and the generation of an antimicrobial response by the host. A day or two's delay in this response may let the microbe reach the critical levels of growth that give tissue damage and disease.

† The incidence of clinical disease varies from zero (*P. carinii*), through 1–2% (polio-myelitis and Epstein–Barr virus infections in small children) to virtually 100% (measles and HIV).

This chapter deals with demonstrable cell and tissue damage or dysfunction in infectious diseases. But one of the earliest indications of illness is malaise, 'not feeling very well'. This is distinct from fever or a specific complaint such as a sore throat and, although it is difficult to define and impossible to measure, we all know the feeling. It can precede the onset of more specific signs and symptoms, or accompany them. Sometimes it is the only indication that an infection is taking place. Almost nothing is known of the basis for this feeling. 'Toxins', of course, have been invoked and the earliest response to pyrogens (see pp. 329–331) before body temperature has actually risen, may play a part. Interferons may have something to do with it because pure preparations of human α or β interferons cause malaise and often headaches, and muscle aches after injection into normal individuals. Soluble mediators of immune and inflammatory responses, such as interleukin-1 (IL-1; see Glossary) or other cytokines doubtless also play a part. Several cytokines induce release of prostaglandin E₂ which, in addition to its effect on fever, reduces the pain threshold in neurons, and this could account for aches and pains. In some infectious diseases weakness and debility are prominent during convalescence. This can be especially notable following influenza and hepatitis, but its basis is as mysterious as in the case of malaise.

Infection with no Cell or Tissue Damage

The infections that matter are those causing pathological changes and disease. Before giving an account of the mechanisms by which these changes are produced, it is important to remember that many infectious agents cause little or no damage in the host. Indeed, it is of some advantage to the microorganism to cause minimal host damage, as discussed in Ch. 1. Virus infections as often as not fall into this category. Thus, although infection with rabies or measles viruses nearly always causes disease, there are many enterovirus, reovirus and myxovirus infections that are regularly asymptomatic. Even viruses that are named for their association with disease (poliomyelitis, influenza, Japanese encephalitis) often give an antibody response as the only sign of infection in the host. Tissue damage is too slight to cause detectable illness. There is also a tendency for persistent viruses to cause no more than minor or delayed cellular damage during their persistence in the body, even if the same virus has a more cytopathic effect during an acute infection, e.g. adenoviruses, herpes simplex (see Ch. 10). A few viruses are remarkable because they cause no pathological changes at all in the cell, even during a productive infection in which infectious virus particles are produced. For instance, mouse cells infected with LCM (see Glossary) or leukaemia virus show no pathological changes. A mouse congenitally infected with LCM virus shows a

high degree of immune tolerance, and all tissues in the body are infected. Throughout the life of the animal, virus and viral antigens are produced in the cerebellum, liver, retina, etc. without discernible effect on cell function. But sometimes there are important functional changes in infected cells which lead to a pathological result. For example, the virus infects growth-hormone-producing cells in the anterior pituitary. Although the cells appear perfectly healthy, the output of growth hormone is reduced, and as a result of this, suckling mice fail to gain weight normally and are runted.

When bacteria invade tissues, they almost inevitably cause some damage, and this is also true for fungi and protozoa. The extent of direct damage, however, is sometimes slight. This is true for *Treponema pallidum*, perhaps because the lipopolysaccharide-protein components that might have induced inflammatory responses, are not exposed on the surface of the bacteria. It produces no toxins, does not cause fever, and attaches to cells *in vitro* without harmful effects. Leprosy and tubercle bacilli eventually damage and kill the macrophages in which they replicate, but pathological changes are to a large extent caused by indirect mechanisms (see below). In patients with untreated lepromatous leprosy, the bacteria in the skin invade blood vessels, and large numbers of bacteria, many of them free, may be found in the blood. In spite of the continued presence of up to 10^5 bacteria ml^{-1} of blood there are no signs or symptoms of septicaemia or toxæmia. *Mycobacterium leprae* can be regarded as a very successful parasite that induces very little host response in these patients, even when the bloodstream is invaded. The resident bacteria inhabiting the skin and intestines of man and animals do not invade tissues and are normally harmless; indeed, as discussed in Ch. 1, they may benefit the host. Bacteria such as meningococci and pneumococci, whose names imply pathogenicity, spend most of their time as harmless inhabitants of the normal human nasopharynx: only occasionally do they have the opportunity to invade tissues and give rise to meningitis or pneumonia.

Direct Damage by Microorganisms

Cell and tissue damage are sometimes due to the direct local action of the microorganism. However, it is not at all clear how viruses cause the death of cells. Many virus infections result in a shutdown of RNA synthesis (transcription), protein synthesis (translation) and DNA synthesis in the host cell, but usually these are too slow to account for the death of the cell. After all, cells like neurons never synthesise DNA, and the half-life of most proteins and even RNAs is at least several hours. A possible alternative mechanism is the alteration of the differential permeability of the plasma membrane. This is important as the

cell has a high internal K^+ concentration and low Na^+ concentration, while the reverse is true of body fluids. Viruses do alter membrane permeability, but the unresolved question is whether or not this is responsible for the death of the cell or whether it is merely an after effect.

It now appears that in many virus infections (including HIV, adenoviruses, herpesviruses, influenza virus, and picornaviruses) the cells commit suicide by a mechanism called 'programmed cell death' or 'apoptosis'. This is the natural process by which the body controls cell numbers and rids itself of superfluous or redundant cells during development. A familiar example is a tadpole 'losing' its tail. Cells do not disintegrate but round up, and are then removed by phagocytes. Apoptosis in virus infections can be regarded as a host strategy for destroying infected cells.* The chromatin condenses round the edge of the nucleus and a cellular endonuclease cleaves the DNA into 180–200 base pair fragments. The cell membrane forms blebs but stays intact while the cell as a whole breaks up into smaller bodies. The suicide process is more controlled, almost more dignified, than mere disintegration and necrosis. In the latter there is early loss of membrane integrity, spillage of cell contents and random break up of DNA.

There are two more characteristic types of morphological change produced by certain viruses, and these were recognised by histologists more than 50 years ago. The first are inclusion bodies, parts of the cell with altered staining behaviour which develop during infection. They often represent either cell organelles or virus factories in which viral proteins and/or nucleic acids are being synthesised and assembled. Herpes group viruses form intranuclear inclusions, rabies and poxviruses intracytoplasmic inclusions, and measles virus both intranuclear and intracytoplasmic inclusions. The second characteristic morphological change caused by viruses is the formation of multinucleate giant cells. This occurs, for instance when human immunodeficiency virus (HIV) 'fusion' proteins (gp120–gp41) present in nascent virus particles budding from an infected cell attach to CD4 receptors in the plasma membranes of neighbouring cells; membranes then fuse and multinucleate cells are formed. It also happens in measles and certain herpes virus infections.

Before leaving the subject of direct damage by viruses, one supreme example will be given. Here the direct damage is of such a magnitude that the susceptible host dies a mere 6 h after infection. If Rift Valley Fever virus, an arthropod-borne virus infecting cattle, sheep and man in Africa, is injected in very large doses intravenously into mice, the injected virus passes straight through the Kupffer cells and endothe-

* Although viruses often prevent apoptosis while they replicate in the cell, it can be useful to make the cell disintegrate at a later stage. Adenoviruses have a 'death protein' (E3 11.6K), a nuclear envelope transmembrane protein, that acts a few days after infection, breaking up the nucleus and allowing the progeny virus to escape.

lial cells lining liver sinusoids (see Ch. 5) and infects nearly all hepatic cells. Hepatic cells show nuclear inclusions within an hour, and necrosis by 4 h. As the single cycle of growth in hepatic cells is completed, massive liver necrosis takes place, and mice die only 6 h after initial infection. The host defences in the form of local lymph nodes, local tissue phagocytes, etc. are completely overcome by the intravenous route of injection, and by the inability of Kupffer cells to prevent infection of hepatic cells. Direct damage by the replicating virus destroys hepatic cells long before immune or interferon responses have an opportunity to control the infection. This is the summit of virulence. The experimental situation is artificial, but it illustrates direct and lethal damage to host tissues after all host defence mechanisms have been overwhelmed.

Most rickettsiae and *Chlamydia* damage the cells in which they replicate, and it is possible that some of this damage is due to the action of toxic microbial products. This action, however, is confined to the infected cell and toxic microbial products are not liberated to damage other cells. Mycoplasma (see Table A.3) can grow in special cell-free media, but in the infected individual they generally multiply while attached to the surface of host cells. As studied in culture and on the respiratory epithelium, they 'burrow' down between cells, inhibit the beat of cilia and cause cell necrosis and detachment. The mechanism is not clear. If a complete lawn of mycoplasma covers the surface of the host cell, some effect on the health of the cell is to be expected, but it is possible that toxic materials are produced or are present on the surface of the mycoplasma.

Bacteria generally damage the cells in which they replicate, and these are mostly phagocytic cells (see Ch. 4). *Listeria*, *Brucella* and *Mycobacteria* are specialists at intracellular growth, and the infected phagocyte is slowly destroyed as increasing numbers of bacteria are produced in it. Bacteria such as staphylococci and streptococci grow primarily in extracellular fluids, but they are ingested by phagocytic cells, and virulent strains of bacteria in particular have the ability to destroy the phagocyte in which they find themselves, even growing in the phagocytes, as described in Ch. 4. Many bacteria cause extensive tissue damage by the liberation of toxins into extracellular fluids. Various toxins have been identified and characterised. Most act locally, but a few cause pathological changes after spreading systemically through the body.

Dental caries provides an interesting example of direct pathological action. Colonisation of the tooth surface by *Streptococcus mutans* leads to plaque formation, and the bacteria held in the plaque utilise dietary sugar and produce acid (see p. 40). Locally produced acid decalcifies the tooth to give caries. Caries, arguably the commonest infectious disease of Western man, might logically be controlled by removing plaque, withholding dietary sugar, or vaccinating against *Streptococcus mutans*. However, fluoride in the water supply or in toothpaste has

been the method of choice, and has been very successful. It acts by making teeth more resistant to acid.

Microbial Toxins

This is a huge and growing part of our subject and we need to define the term toxin, a task which is more difficult than one might think. An attempt was made by Bonventre who in 1970 defined toxins as a 'special class' of poisons which differ from, for example, cyanide or mercury by virtue of their microbial origin, protein structure, high molecular weight, and antigenicity. This view is too embracing, because it includes proteins of doubtful significance in disease, and also too restrictive, because it excludes nonprotein toxic complexes such as endotoxin. Another suggestion is that toxin must include all naturally occurring substances (of plant, animal, bacterial or whatever origin) which, when introduced into a foreign host, are adverse to the well-being or life of the victim. This, too, is unsatisfactory because some substances – potent toxins within the scope of this definition – are being used in some contexts as therapeutic agents! Perhaps it is pointless to strive for an all-embracing definition, although the obvious differences between bacterial and fungal toxins warrant the continued use of the appropriate prefix. For example, bacterial toxins are usually of high molecular weight and hence antigenic, whereas fungal toxins tend to be low molecular weight and not antigenic.

The problem of definition is compounded because there are substances (aggressins) which help to establish an infective focus as well as those whose action is uniquely or largely responsible for the disease syndrome. Also there are substances known to be produced by bacteria *in vitro*, whose properties on a priori grounds make them potential determinants of disease, but which have not been shown to play a role *in vivo*.

In the last few years a huge effort has been devoted to understanding the genetic basis of toxin acquisition, expression, assembly and secretion of toxins, the resolution of the three-dimensional structure of toxins, and their biochemical modes of action. As a result we now know a great deal about the spread of some virulence determinants in bacterial populations via bacteriophages and other transmissible genetic elements, the conditions under which toxins are expressed both *in vitro* and *in vivo*, how to disassemble complex protein toxins and form chimeric derivatives of known and potential use as therapeutic agents, and how to use some of the deadliest poisons known to man in treating certain physiological disorders. Elucidation of biochemical modes of action has resulted in toxins being used increasingly as important tools for the dissection of cell biological processes. Also, some new insights as to the role(s) of toxins in disease causation have been developed. The

latter is the result of using isogenic *tox(-)* mutants *in vivo*, using more relevant biological test systems, and concentrating more on the effects of sublethal doses of toxin and less on the effects of injecting a toxin bolus into some animal. It is beyond the scope of this book to attempt to cover all these subjects, so only an outline treatment will be given with some examples. Fortunately, for a fuller treatment one can refer the reader to the recent excellent text on bacterial protein toxins by Alouf and Freer (1999).

Protein toxins

These are either secreted by, or released upon lysis from both Gram-positive and Gram-negative bacteria, and historically referred to as exotoxins. They are proteins, some of which are enzymes. When liberated locally they can cause local cell and tissue damage. Those that damage phagocytic cells and are therefore particularly useful to the microorganism have been described in Ch. 4. Those that promote the spread of bacteria in tissues have been referred to in Ch. 5. A description of some protein toxins, or families of toxins follows.

Toxins which act extracellularly

Helicobacter pylori is a specific human pathogen affecting billions of people world-wide. It is transmitted via the oro-faecal route and colonises the seemingly inhospitable niche of the stomach. Some 20% of infected patients can develop ulcers or stomach cancer. An essential virulence factor of *H. pylori* is a potent urease which is synthesised in vast quantity by the organism, and (at least in culture) released by autolysis and efficiently absorbed on to the surface of viable organisms. As noted in Ch. 2, it is important in local neutralisation of stomach acidity thereby allowing *H. pylori* to penetrate the protective mucus layer overlying the lining of the stomach where the organism attaches to gastric epithelial cells. However, urease is now considered by some as a toxin which acts outside cells, since NH_3 , the product of urease activity, is toxic to cells.

Toxins which affect extracellular 'structural' elements

Proteases and *hyaluronidases*, which help the spread of bacteria through tissues have already been mentioned in Ch. 5. Here we consider toxins which act on extracellular substances and are responsible for many of the main features of the diseases caused by the infecting organism. *Pseudomonas aeruginosa* elastase, and one of at least six proteases of *Legionella pneumophila*, both induce fibrinopurulent exudation in the rat lung (a model for *P. aeruginosa*-induced pneumonia in human cystic fibrosis) and the guinea-pig lung (a model

for legionnaires' disease), respectively. These characteristics almost certainly arise from the release of oligopeptides from extracellular matrix components of the host which are chemotactic for leucocytes and fibroblasts. The *L. pneumophila* protease is the same major secretory protein (the zinc metalloprotease) already considered in Ch. 4 in relation to survival within macrophages.

Staphylococcal exfoliatin (epidermolysin) is important in staphylococcal 'scalded skin syndrome' (SSSS), a disease of newborn babies. The disease is characterised by a region of erythema which usually begins around the mouth and, in 1–2 days, extends over the whole body. During this period, small yellowish exudative lesions often appear. The most striking feature of the disease, however, is that the epidermis, although apparently healthy, can be displaced and wrinkled like the skin of a ripe peach by the slightest pressure. Soon large areas of epidermis become lifted by a layer of serous fluid and peel at the slightest touch. Large areas of the body rapidly become denuded in this way and the symptoms resemble those of massive scalding. The toxin causes cleavage of desmosomes (specialised cell membrane thickenings through which cells are attached to each other) in the stratum granulosum. However, despite numerous attempts to characterise the biological activity of exfoliatin, the genetically predicted serine protease and/or lipase activity has never been demonstrated.

Vibrio cholerae ZOT (zonula occludens toxin) alters the permeability of junctional complexes in rabbit gut epithelia. In human cholera patients there is evidence of widening of the zonula adherens (Fig. 8.1). Again, like staphylococcal exfoliatin, we do not know the precise mode of action of the toxin responsible for such alterations in epithelial junctional complexes.

In the two preceding examples, it has not been formally proved that these cell–cell splitting toxins act from the outside. In contrast, *Clostridium difficile* toxins A and B which also affect epithelial tight junctions are first internalised and, by virtue of their ability to inhibit Rho (an intracellular target; see Fig. 4.1), cause the collapse of tight junctions thereby increasing the ease with which inflammatory cells arrive at the site of *C. difficile* infection, a highly characteristic feature of such infections.

Toxins which act on cell membranes

Some enterotoxigenic *E. coli* elaborate families of low-molecular-weight heat-stable (ST) peptides as well as heat-labile (LT; cholera-like) toxins. STs bind to a receptor which then activates a tightly coupled membrane-bound guanylate cyclase in gut cells, resulting in the transmission of a signal to the inside of the cell, thereby elevating cGMP, or some other second message. As described later in the section on diarrhoea, this gives rise to efflux of ions, and hence water, from enterocytes.

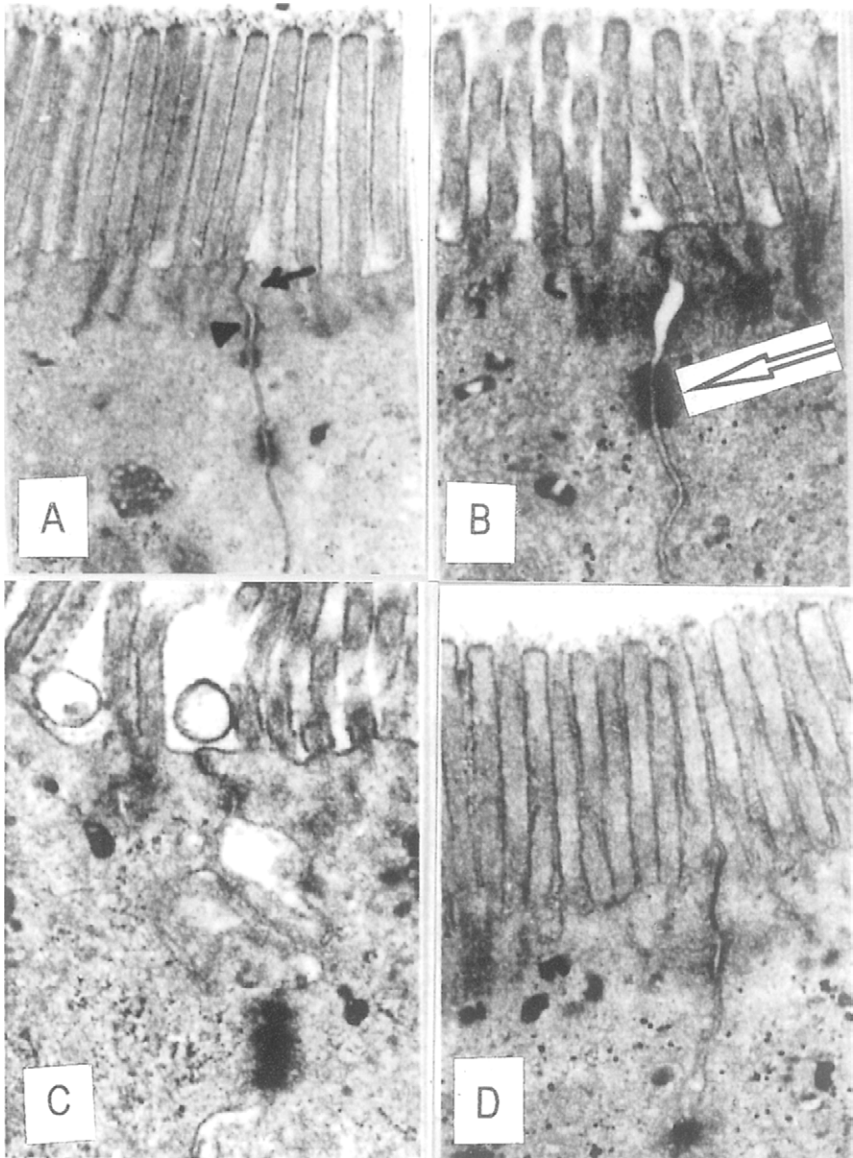


Fig. 8.1 Apical junctional complexes in duodenal biopsies from (A) a control and patients with cholera (B–D). (A) Control upper third of villus showing tightly apposed cell membranes in zonula occludens region (arrow) and widened space between cell membranes in zonula adherens (arrowhead). (B) Upper third of villus from patient with cholera. Note widening of zonula adherens and intact desmosome (arrow). (C) Saccular dilatation of zonula adherens in upper third of villus. (D) Lower third of villus from same sample as (B) showing no abnormalities. Crypt from same sample as B showed normal junctional complexes (not shown). Reproduced from *Gastroenterology* 1995, 109, 422–430, Fig. 3, with kind permission of Professor M. M. Mathan, and the publisher W.B. Saunders.

Toxins which damage membranes

Some toxins destroy membranes by virtue of their proteolytic activities, and some by their ability to degrade lipid components, while others are pore-forming or detergent-like in their mode of action.

Proteases

In addition to their action on protein components of lung connective tissue referred to above, *Pseudomonas aeruginosa* elastase and the zinc metalloprotease of *Legionella pneumophila* are believed to destroy cell membranes by their proteolytic activity. This is the probable reason for the haemorrhage associated with lung infections caused by these pathogens, i.e. effects on type I alveolar epithelial and endothelial cells.

Phospholipases

***Clostridium perfringens* α -toxin**

A large number of bacterial enzymes are phospholipases, some of which, but by no means all, are important toxins. The best example is the α -toxin of *Clostridium perfringens*, the organism most commonly associated with gas gangrene. It is strictly anaerobic and occurs as a normal inhabitant in the large intestines of man and animals; its spores are ubiquitous in soil, dust and air. *C. perfringens* does not multiply in healthy tissues, but grows rapidly when it reaches devitalised and therefore anaerobic tissues. This could be after contamination of a natural wound with soil or dust, particularly on battlefields or in automobile accidents, or after contamination of a surgical operation site with clostridia from the patient's own bowels or skin. After abortions, particularly in the old days before antibiotics, intestinal clostridia often gained access to necrotic or devitalised tissues in the uterus and set up life-threatening infections. Invasion of the blood was common and soon resulted in death, the clostridia localising and growing in internal organs such as the liver after death. *C. perfringens* has various enzymes that enable it to break down connective tissue materials, including collagen and hyaluronidase, thereby facilitating spread of the infection along tissue planes. Most of these enzymes are toxic to host cells and tissues, but α -toxin is easily the most important one. It is dermonecrotic, haemolytic (a feature seen mainly in tissues close to the focus of infection but sometimes responsible for large-scale intravascular haemolysis in infected patients), causes turbidity in lipoprotein-rich solutions and is lethal. While it is still true that these activities are all due to one molecular species, they are not (as was once thought) different expressions of the one enzymic activity.

Historically, *C. perfringens* α -toxin was the first bacterial toxin to be characterised as an enzyme: it is a zincmetallophospholipase C (PLC) which removes the head group, phosphoryl choline, from phosphatidyl choline and from sphingomyelin. It is of undoubted importance in gas

gangrene. Toxoid prepared by formalin-treated toxin will protect sheep against infection caused by *C. perfringens*. However, one might ask why all enzymes with such biochemical specificity are not equally toxic or important in determining virulence; there are several reasons which can be put forward. We know that there are at least two functional domains in *C. perfringens* α -toxin. Comparison of *C. perfringens* α -toxin with the phosphatidyl choline-preferring, nontoxic zincmetallophospholipase of *Bacillus cereus*, reveals that two-thirds of the N-terminal sequence of *C. perfringens* α -toxin shows homology with the entire sequence of *B. cereus* PLC; this portion of *C. perfringens* α -toxin retains its PLC activity, but not its haemolytic and lethal activities. The C-terminal part is not haemolytic, not enzymatically active and not cytotoxic for mouse lymphocytes, but is necessary for conferring toxicity on the N-terminal part of the protein. In fact, the C-terminus is a potent immunogen that will solidly protect mice – and hopefully man – against experimental infection with *C. perfringens*. Surprisingly, the C-terminal domain of the nontoxic *C. bifermentans* enzyme shows sequence similarity with that of its *C. perfringens* α -toxin counterpart. The relative nontoxicity of this enzyme is ascribed to its comparatively much lower turnover rate, i.e. it is a much less efficient enzyme.

While haemolysis does occur in experimental gas gangrene (evidenced by haematuria), there is little evidence of massive haemolysis in naturally occurring cases of gangrene. It is now considered more likely that the basis of toxicity is not cytolysis, but rather the consequence of the ability of the α -toxin, in sublytic doses, to cause profound metabolic changes arising from release of phospholipid derivatives. For example production of inositol triphosphate (IP₃), a potent secondary messenger, would affect many cell functions. The activation of the arachidonic acid cascade would result in the production of leukotrienes (increasing vascular permeability), prostaglandins and thromboxanes (causing inflammation, muscle contraction and platelet aggregation). This toxin also upregulates expression of endothelial leucocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1) and neutrophil chemoattractant-activator IL-8, thereby impairing delivery of phagocytes to the site of infection.

There are other pathogenic clostridia that cause gas gangrene and produce similar toxins. Infected tissues show inflammation, oedema and necrosis, not necessarily with the formation of gas, and the illness can be mild or very severe according to the extent of bacterial spread, and the nature and quantity of toxins that are formed and absorbed. Since the bacteria grow and produce their toxins only in devitalised tissues, the most important form of treatment is to remove such tissues. Clostridia are strictly anaerobic, and exposure of the patient to hyperbaric oxygen (pure oxygen at 2–3 atmospheres in a pressure chamber) has been found useful in addition to chemotherapy.

Staphylococcal β -toxin (haemolysin) is known to be produced *in vivo*. In Ch. 4, studies with isogenic mutants were described which indicate

that it is important in killing neutrophils. It probably has the narrowest substrate specificity among the phospholipases, and is a hot-cold haemolysin: lysis of erythrocytes occurs only on cooling after incubation at 37°C. The phenomenon, although of doubtful significance *in vivo*, has attracted attention and generated speculation about its mechanism. Perhaps the most likely explanation is that, when cooled below their phase-transition temperature, the remaining phospholipids undergo quasi-crystalline formation, thereby generating intramembranous stresses incompatible with structural integrity.

Pore-forming toxins

Cholesterol-binding cytolysins (CBCs)

These proteins, more commonly known as 'SH-activated cytolysins', are made by some 23 taxonomically different species of Gram-positive bacteria, not all of which are pathogens. They are lethal, cardiotoxic, antigenically related, and their lytic and lethal activities are blocked by cholesterol. Recent work requires that we abandon certain perceptions about these toxins which are enshrined in the older nomenclature. For example, *purified* toxins are not O₂-labile, and are not activated by sulfhydryl compounds, and do not depend on a cysteine residue for activity. Alouf has suggested the generic term used as our heading, since the one common feature which correctly applies to all members of this group is the ability of cholesterol to irreversibly inhibit the lytic and lethal properties of these toxins. Interaction with cholesterol is thought to be the key primary event in their interaction with susceptible membranes, which leads to the impairment of the latter; cholesterol plays no further part in the subsequent damage process. However, the role of cholesterol has been interpreted in terms of mediating the oligomerisation process (illustrated in Fig. 8.2) which leads to membrane damage. Four examples of CBCs from pathogenic species are considered briefly below. Despite the similarities which warrant their inclusion in the same toxin group, they play entirely different roles in disease causation by the organisms expressing these toxins.

Streptolysin O (SLO)

Some streptococci produce two haemolysins. The one considered here was originally thought to be oxygen-labile and designated streptolysin O (SLO), the other oxygen-stable and designated streptolysin S (SLS). In Ch. 4 (Table 4.1) the *in vitro* cytolytic properties of SLO were listed. However, the situation *in vivo* is far from clear. An injection of a bolus of SLO is lethal almost certainly due to its cardiotoxicity. More recently, SLO has been implicated (alone or in combination with other streptococcal toxins) in tissue damage. The accumulation of polymorphonuclear leucocytes (PMNs) in lung and soft tissue in cases of streptococcal toxic shock syndrome, has been attributed to SLO. SLO-induced increases in proinflammatory cytokines IL-1 β and tumour necrosis factor α (TNF- α) and several leukotrienes have also

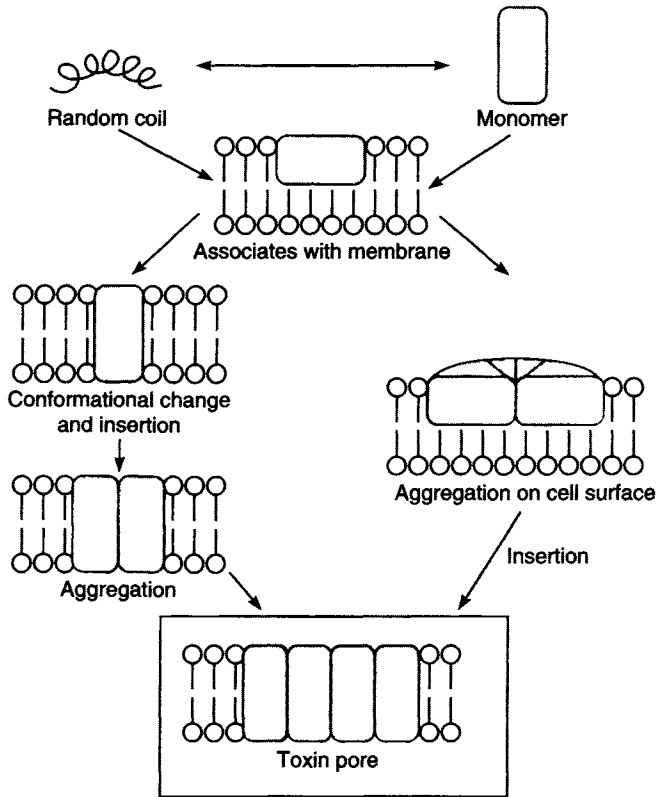


Fig. 8.2 Pore formation by pore-forming toxins. Newly synthesised proteins are soluble. On interaction with cell membranes they undergo conformational changes which allow reorganisation on and insertion into target cell membranes. Cholesterol is involved as primary receptor or mediator of aggregation for the CBC group. Others have specific receptors, but staphylococcal δ -toxin does not. (Reproduced with permission of authors (T. J. Mitchell *et al.*) and publisher (Gustav Fischer Verlag, Stuttgart, Germany) from Fig. 1, in 'Molecular studies of pneumolysin, the thiol-activated toxin of *Streptococcus pneumoniae* as an aid to vaccine design', Fifth European Workshop, Veldhoven; (B. Witholt *et al.* (eds)), *Zentralbl. Bakteriol.* 1992, Suppl. 23, p. 431.)

been demonstrated. SLO-deficient mutants of *S. pyogenes* induced less of these substances and SLO-deficient group A streptococci are less virulent in chick embryos. However, the precise role of SLO in the pathogenesis of infections caused by *S. pyogenes* and their non-suppurative sequelae (e.g. rheumatic fever) is still not clear due almost certainly to the lack of suitable animal models.

Perfringolysin O (PFO; Clostridium perfringens θ toxin)

This toxin is the first of the group for which a three-dimensional crystallographic structure has been obtained: it is a four-domain molecule. Elucidation of its structure has spawned a great deal of biochemical

and biophysical activity. However, the role of PFO in disease causation is still not entirely clear, probably due to the fact that it is only one of a very large number of known or potential virulence determinants produced by *C. perfringens*. The major features of the pathogenesis of *C. perfringens*-mediated gas gangrene is unquestionably explicable in terms of *C. perfringens* α -toxin described above. Experimental evidence suggests that PFO plays only a minor role in the pathogenesis of gas gangrene by upregulating ICAM-1 (see above for α -toxin) thereby slightly contributing to the inhibition of PMN migration to the site of infection.

Listeriolysin O (LLO; listeriolysin)

In contrast to the first two examples, LLO is the most important virulence determinant of *Listeria monocytogenes*. We have already met listeriolysin in Ch. 4: it plays an important part in mediating the escape of *L. monocytogenes* from intraphagocytic vacuoles. Exogenous addition of LLO will, like all other members of this group, rapidly kill cells by rupturing the cytoplasmic membrane. However, rupture of the phagocytic vacuole with release of organisms does not result in immediate death of the cell. This has been explained in terms of the initial acidification of the vacuole with concomitant activation of LLO; the subsequent rise in pH deactivates LLO. LLO is also a potent trigger of host cell-signalling molecules. Many of the responses elicited by LLO are believed to be the result of the activation of cytosolic NF- κ B (host-cell stress-inducible transcription factor) and its translocation into the nucleus where it acts as a transcriptional activator of different genes involved in the immune response. The pore-forming activity of LLO results in the release of antigens from the vacuole and stimulation of CD8⁺ cytotoxic T cells which are known to afford protection against *L. monocytogenes*. This helps explain why patients recovering from *L. monocytogenes* infection have high levels of anti-LLO which are themselves non-protective in experimental infections.

Pneumolysin (PLY)

This protein is produced by the pathogen *Streptococcus pneumoniae* (pneumococcus) which causes bacteraemia, pneumonia, meningitis, and otitis media in humans. PLY is different from all other members of this group in that it is not actively secreted by the pathogen, but remains in the cytoplasm until released by lysis of the pneumococcus. Recent work has shown this toxin is, like PFO, a four-domain molecule. It possesses properties which could never have been predicted or deduced from classical studies of its haemolytic activity. Comparative studies in mice with both wild-type and a pneumolysin-negative mutant (PLN-A) of serotype-2 pneumococci demonstrated that pneumolysin was important in the induction of inflammation in the lung (not cell wall components as had long been believed), conferring ability to replicate in the lung and invade into the bloodstream, and altering alveolar permeability. It inhibits ciliary beat in respiratory mucosa. In

experimental meningitis in guinea pigs, in contrast to the mouse lung model, PLY was not responsible for the potent inflammatory response, but did cause an increase in protein content of cerebrospinal fluid (CSF) presumably reflecting its ability to alter cell barriers. PLY has also been implicated in causing sensorineural deafness associated with meningitis caused by the pneumococcus (Fig. 8.3). PLY also activates the complement cascade thereby diverting complement from bacteria. From the foregoing, it is clear that PLY is an important virulence determinant of the pneumococcus.

Attempts to develop protective antipneumococcal vaccines have hitherto been based on the type-specific capsular polysaccharides. Unfortunately, there are at least 90 known types and current vaccine preparations comprise a blend of polysaccharides from some 23 types. Currently, efforts are being made to develop a broadly effective vaccine based on genetically engineered PLYs which are sufficiently non-toxic but immunogenic. Watch this space.

RTX toxins

This group of toxins has been designated RTX (repeats in toxin) toxins by virtue of a common structural feature – the presence of an array of a nine amino acid repeat (*ca.* 10–40) to which Ca^{2+} binds thereby activating the toxins which form membrane pores of varying sizes. They constitute the largest group of bacterial pore-forming toxins and are widespread among Gram-negative pathogens. In general, the role of RTXs in disease is not clear but three examples are given where RTXs are important. *E. coli* α -haemolysin, regarded as the prototype of this group, is important in extraintestinal infections caused by this organism; the toxin is active against a broad range of mammalian cells. Leukotoxin from *Pasteurella haemolytica* exhibits narrow target cell and host specificities; it specifically kills ruminant leucocytes and is important in bovine pneumonic pasteurellosis. The third example is the ‘invasive’ adenylate cyclase toxin of *Bordetella pertussis*. This toxin is unique among this group in that it is a large bifunctional toxin: it has both haemolytic and adenylate cyclase activities hence the designations AC-Hly, AC toxin, CyaA. It is one of several virulence attributes expressed by *B. pertussis* (see below) and is known to be important in the early stages of respiratory tract colonization. Strictly it is the haemolysin part of the molecule which belongs to the RTX family and its main function appears to be in translocation of the AC moiety into the cell where cAMP levels are elevated with ensuing pathophysiological sequelae.

Staphylococcal α -toxin

Staphylococci produce a range of toxins some of which we have already met. The α -toxin is easily the most studied from a biophysical point of view and is considered the main cytolysin produced by *S. aureus*. Like streptolysin-O and staphylococcal δ -toxin, it is secreted as a water-soluble protein and undergoes self-induced oligomerisation on cell membranes to form heptameric pores. In systemic staphylococcal infec-

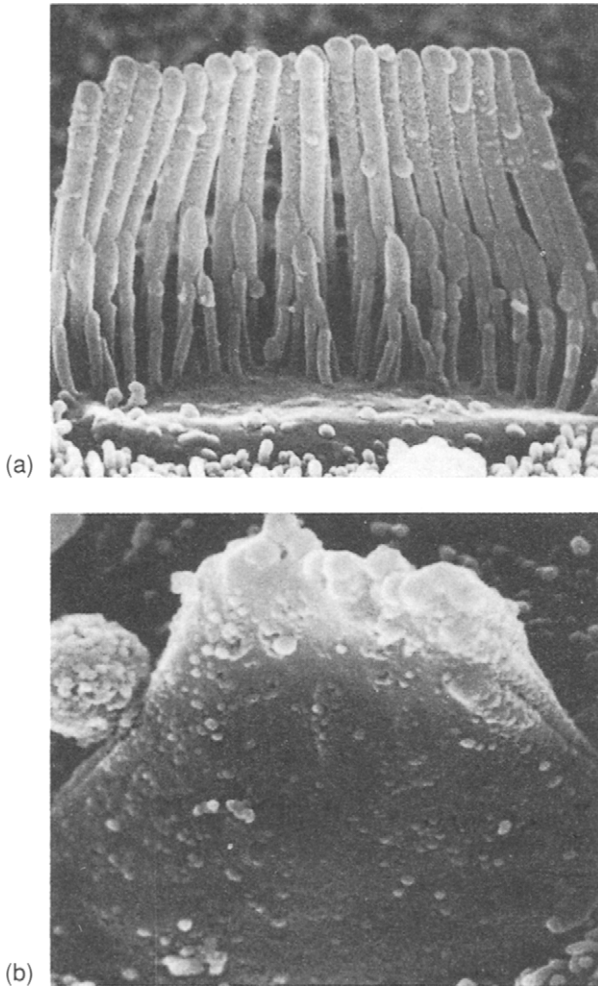


Fig. 8.3 The effect of pneumolysin on the hair cells of the inner ear of a guinea-pig. The effect of pneumolysin on the hair cells of the inner ear of a guinea-pig. (a) A scanning electron micrograph of normal hair cells. (b) Hair cells after exposure to pneumolysin; note disappearance of hairs. Hearing depends on the transmission to the hair cells of pressure waves generated in the fluid-filled chamber (scala tympani) of the cochlea. This causes lateral displacement of the hairs. Inelastic links between hairs in different rows results in membrane deformation, opening of ion channels and influx of ions. This generates an action potential in the underlying auditory nerves. (Kindly provided by Drs M. P. Osborne and S. D. Comis, Department of Physiology, The Medical School, University of Birmingham, UK.)

tions, death is most probably due to the potent α -toxin but in localised pyogenic infections – such as mastitis in cattle, goats, rabbits and mice – its role is most likely one of killing phagocytes or conferring survivability on intracellular bacteria.

Detergent-like toxins

Staphylococcal δ -toxin acts in a manner similar to that of the cholesterol-binding cytolysins, with an important difference: the binding is nonspecific with no requirement for cholesterol. It initially forms small pores and then islands of membrane or large micelles; this gives rise to its perceived detergent-like properties. There is a family of closely related δ -toxins which inhibit the growth of gonococci. It is not often that one can ascribe a positive function to a toxin which is beneficial for the organism producing it. In this case δ -toxin(s) could have important ecological significance in the mixed culture that is characteristic of the real microbial world. Of great interest is the synergy that δ -toxin displays. Sublytic amounts of δ -toxin cause release of cell constituents without lysis. However, only 0.01 haemolytic units of staphylococcal β -toxin will cause lysis of cells in the presence of 0.004 lytic units of δ -toxin. This synergistic interaction could be the way in which staphylococcal toxins, which rarely exert their lethal effects in the majority of infections, exercise important cytolytic effects. Of less obvious significance is the fact that δ -toxin is a poor antigen; for a long time its antigenicity was controversial. If δ -toxin were to prove of crucial importance as a cytolytic potentiator, then this could also partly explain why natural acquired immunity to staphylococcal infection is either non-existent or sufficiently low as to be easily overcome.

Binary toxins

These comprise two proteins, only one of which is toxic but the other is necessary at some stage for manifestation of toxicity. Two examples are given, *Serratia* and *Proteus* cytolysins and staphylococcal leukocidins.

Serratia marcescens is an opportunistic pathogen which causes a range of infections including respiratory and urinary tract infections; *Proteus mirabilis* causes acute pyelonephritis. Pathogenicity is multifactorial but the accumulated evidence is that a membrane-active cytolysin plays an important role in disease causation by both pathogens. *S. marcescens* haemolysin (Sh1A) and *P. mirabilis* haemolysin (HpmA) represent a new type of cytolytic toxin: they are described as 'cell-associated' and have a specialised means of delivery from the bacterial cell to the target host cell. Sh1A and HpmA each require a second protein, Sh1B and HpmB, respectively. The B proteins form pores in the bacterial outer membrane facilitating the secretion of the corresponding A components, their concomitant activation and insertion into eukaryotic membranes. These A proteins are also cytotoxic, causing vacuolation in cells and release of a range of inflammatory mediators.

Formerly, the second example would have been limited to a discussion of staphylococcal 'Panton Valentine' (PV) leukocidin and staphylococcal γ -toxin; the latter is one of the much studied group (α , β , γ and δ) staphylococcal haemolysins. However, the problems generated by antibiotic-resistant staphylococci has stimulated a great deal of new

research on this pathogen arising from which is the recognition that PV leukocidin and γ -toxin belong to a very large family of binary leukocidins. Each leukocidin consists of two proteins – S (so called because it elutes slowly) and F (it elutes fast) from an ion-exchange column. S binds first to as yet ill-defined cell receptors (important in defining target cell specificity), followed by F which acts synergistically with S to create functional pores in the target membrane. There are at least six class S proteins and five class F proteins which can give rise to ca. 30 biologically active combinations, a fact which could be highly significant in that some strains produce more than one binary leukocidin. Although various S-F combinations exhibit different target cell specificities, most are active against PMNs. It has been shown that PV, which is highly active against human PMNs, causes release of leukotriene B₄, IL-8, histamine and tissue degradative enzymes, which would give rise to the chemotactic invasion of more PMNs and subsequent tissue damage.

Toxins with intracellular targets

Many toxins have intracellular targets. There is intense interest in seeking to understand the mechanism(s) of uptake of the active moieties of toxins whose targets are intracellular. This is driven by the desire to understand fundamental mechanisms in cell biology and to develop selective ‘cytotoxic therapies’ in clinical medicine as well as to unravel the molecular mechanisms of disease causation. To reach an intracellular target, a protein must first be translocated across the cytoplasmic membrane. There are at least three ways in which this can be achieved: self-translocation across cytoplasmic membrane; direct injection; and receptor-mediated endocytosis.

Self-translocation

There is only one example of self-translocation across cytoplasmic membrane known to date: the invasive adenylate cyclase of *B. pertussis* described above.

Direct injection

Recognition of a direct injection mechanism has resolved a problem in *Pseudomonas aeruginosa* pathogenicity. *P. aeruginosa* possesses many potential virulence determinants three of which have definitely been implicated as being important in pathogenesis: elastase (already referred to), and two ADP-ribosylating (ADPR; see below) proteins, exotoxin A (PEA, see below), and exoenzyme S (PES). PES is non-toxic when injected into animals or added to cells on its own. This problem has now been resolved. It is a single polypeptide with no receptor-binding component or translocation domain (see below): it is ‘injected’ directly across cell membranes by a mechanism functionally similar to that already described for the translocation of Tir by *E. coli* (see Ch. 2,

Fig. 2.7). PES is activated by a cytoplasmic protein FAS, and ADP-ribosylates the small G-protein Ras resulting in the collapse of the cytoskeleton (see Ch. 4; Fig. 4.1). Also included in this category are the 'phagocyte toxins' injected into phagocytes by *Yersinia enterocolitica* (Ch. 2). It is possible that other similar bacterial enzymes, e.g. *Clostridium botulinum* C3 which ADP-ribosylates G-protein Rho, will be shown to be internalised in a similar manner.

Receptor-mediated endocytosis

There are several variations on the receptor-mediated endocytosis theme reflecting the structure of the toxins; in some cases the process involves the subversion of normal processes used by the host cell to regulate movement and organisation of cellular membranes and substituent components. Toxins first bind to their respective receptors and become internalised via coated pits, vesicles or caveolae, into endosomes from which they still must escape into the cytoplasm.

Three types of toxin with intracellular targets have been recognised reflecting their genetic origin. Some toxins consist of a single peptide, the product of a single gene, which undergoes post-translational modification into A and B fragments which are covalently linked (Fig. 8.4).

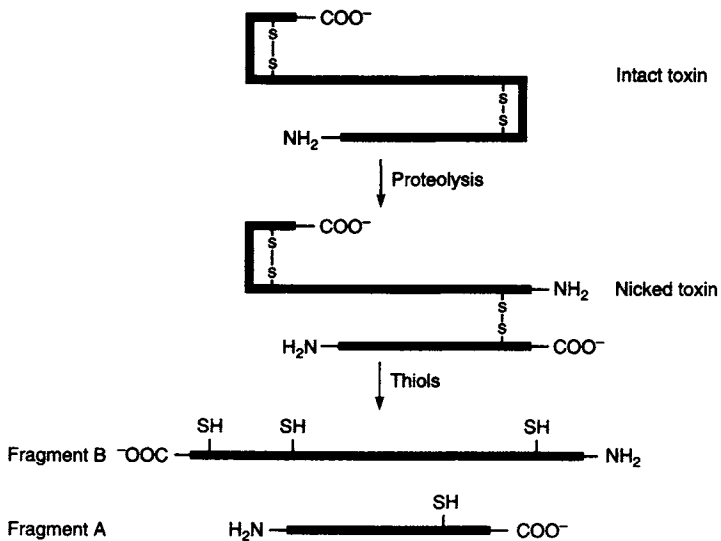


Fig. 8.4 Diphtheria toxin: post-translational modification of the single peptide into A and B fragments. The toxin is synthesised as a single polypeptide but is cleaved (nicked) by proteases into two fragments designated A and B, held together by an -S-S- bond. The latter is reduced during translocation of the A fragment into the cytosol. The B fragment consists of a receptor domain (R) which recognises the DT receptor – an EGF-like precursor which happens to be widely distributed throughout all organ systems – and a T domain which facilitates the translocation of A into the cytoplasm (see Fig. 8.5).

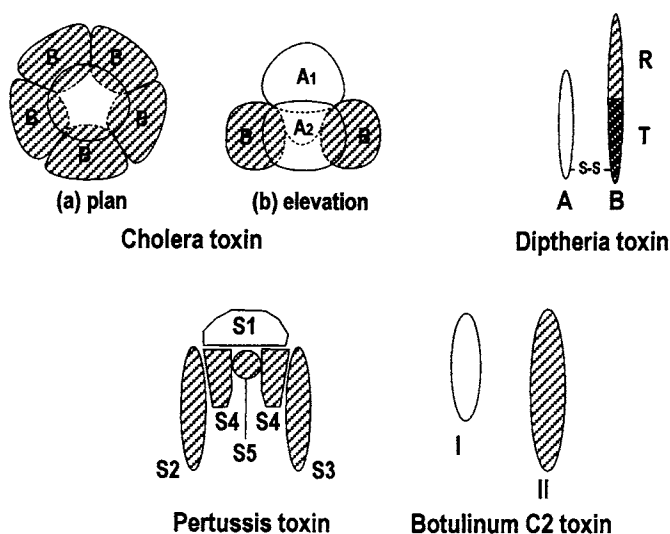


Fig. 8.5 Schematic structure of three types of A-B-type toxins. The hatched regions are the binding/translocation-facilitating parts ('B subunits'). Diphtheria toxin is synthesised as a single peptide (see Fig. 8.4). Cholera toxin (CT) is represented in plan and elevation views; *E. coli* LTs are structurally and functionally very similar to CT. CTA comprises CTA1-A2. A1 is the toxiphore which is held in association with B via A2. A2 has no known enzymic activity but plays some as yet undefined part in toxicity. Differences in CT A2 and LT A2 have been implicated as part of the reason for the lesser severity of disease caused by enterotoxigenic *E. coli*. Pertussis toxin B subunits are heterogeneous. Botulinum C2 toxin is a two-component binary toxin, in which two proteins do not form stable complexes prior to cell attachment. Not to scale. (Modified with permission of authors (I. H. Madshus and H. Stenmark) and publisher (Springer-Verlag GmbH & Co. KG, Heidelberg, Germany) from Fig. 1 in 'Entry of ADP-ribosylating toxins into cells', *Curr. Topics Microbiol. Immunol.*, 1992, **175**, 3, edited by K. Aktories.)

The A fragment is the 'active' toxiphore and the B fragment bears the receptor-binding domain and also mediates translocation of A into the cytoplasm. Examples include diphtheria toxin (DT), *Pseudomonas aeruginosa* exotoxin A (PEA) (Fig. 8.5), and the clostridial neurotoxins (BoNT and TeTx) (Fig. 8.6).

A second group of toxins are the products of separate genes giving rise to A and B subunits which noncovalently associate into stable complexes. They are also designated A-B type toxins, in which the number and nature of B subunits vary, but the connotations of A and B are as for DT. Examples include classical cholera toxin (CT), *E. coli* heat labile enterotoxins (LTs), pertussis toxin (PT), Shiga (ShT) and Shiga-like (ShLT) toxins (Fig. 8.5).

A third group of toxins are the products of separate genes giving rise to different proteins which are functionally equivalent to A and B

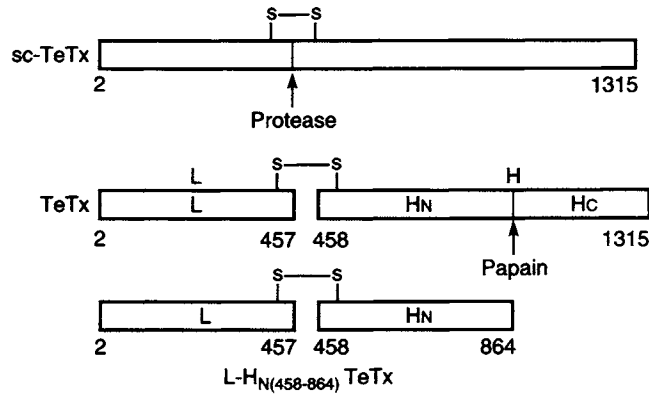


Fig. 8.6 Structure/nomenclature of tetanus and botulinum neurotoxins. sc-TeTx: single chain noncleaved tetanus toxin. TeTx: tetanus toxin after proteolytic activation. L and H: intact light and heavy chains, respectively. L-H_{N(458-864)} TeTx: intact L chain linked to a fragment of H chain (from residue 458 at the N-terminal end to residue 864). H_N and H_C: N-terminal and C-terminal parts, respectively, of the H chain. The corresponding nomenclature for botulinum toxin type A is BoNT/A, and for type B, BoNT/B, etc. (Reproduced with permission of the publisher (Gustav Fischer Verlag, Stuttgart, Germany) from 'Clostridial neurotoxins – proposal of a common nomenclature', in 'Bacterial Toxins, Fifth European Workshop', Veldhoven (B. Witholt *et al.*, eds, *Zentralbl. Bakteriol.*, 1992, Suppl. 23, p. 17.)

subunits. These proteins do not associate into stable complexes, but must act in concert to express toxicity and are known as binary (or bi-component) toxins. Examples include anthrax toxins and *Clostridium botulinum* C2 toxin (Fig. 8.5), *Clostridium perfringens* iota toxin, and *Clostridium spiroforme* toxin.

Translocation of toxiphore into the cytoplasm

Direct escape from endosome

DT B fragment binds to its receptor (a precursor of heparin-binding epidermal growth factor (EGF)-like growth factor), undergoes conformational change in the acidified endosome and inserts into the endosomal membrane, pulling the C terminus of the A fragment across the membrane. The -S-S- bridge is exposed to the cytosol, reduced, thereby freeing A to enter the cytosol (Fig. 8.7). A similar mechanism operates with anthrax LF and EF toxins but in this case a third protein, protective antigen (PA) acts as the functional equivalent of DT B (Fig. 8.8).

Route to endoplasmic reticulum

For most if not all other toxins the route to an intracellular target is much more complex. There is direct, or persuasive indirect evidence that the following scenario (based on Olsnes *et al.*, 1999; Hirst, 1999) applies to CT, LTs, ShT, ShLTs, PEA, and probably to many others (see

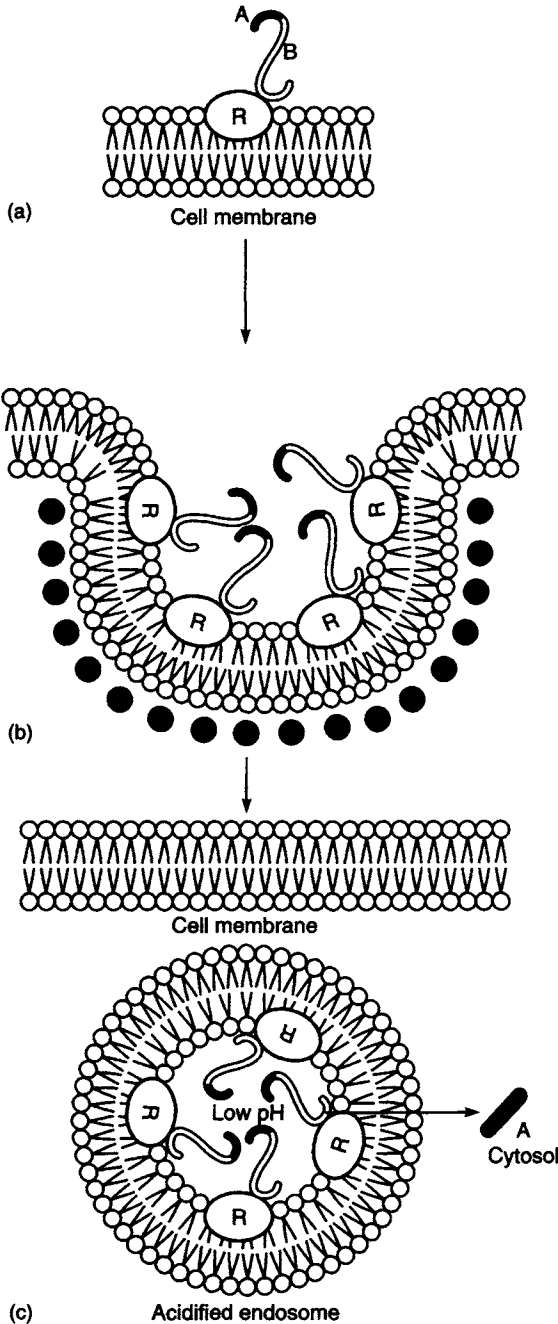


Fig. 8.7 Direct escape of DT A fragment and anthrax LF and EF toxins from acidified endosome. (a) DT binds via B fragment to its receptor (R) in the cell membrane. (b) These complexes migrate to clathrin-coated pits. (c) This gives rise to acidified endosomes which induces conformational changes in B, insertion of B into the membrane and escape of A fragments into the cytosol. The only other known examples of direct escape of toxin into the cytosol are anthrax EF and LF (see Fig. 8.8).

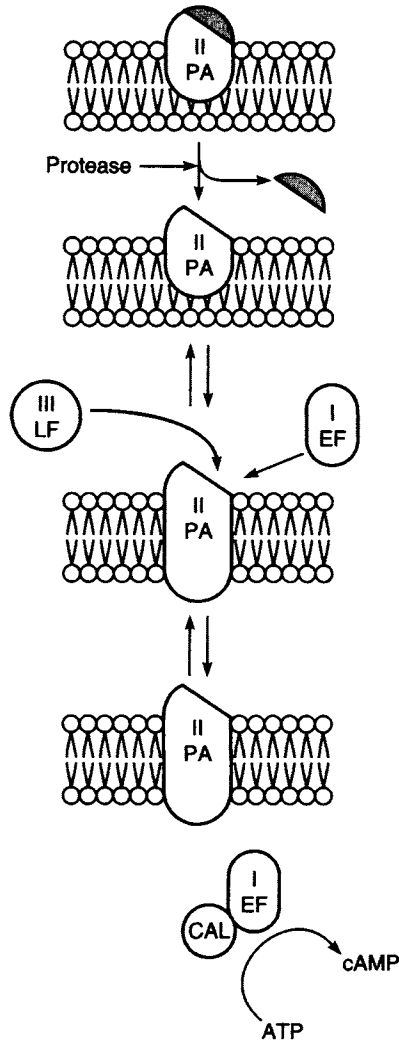


Fig. 8.8 Mode of entry and action of anthrax toxins. Protective antigen (PA) interacts with the cell membrane and forms heptameric oligomers. After proteolytic cleavage, PA sites are exposed which bind edema factor (EF), thereby facilitating translocation of EF into the cytosol directly from acidified endosomes as described for DTA (Fig. 8.7). EF must be rapidly inactivated since washing toxin-treated cells results in a rapid loss of adenylate cyclase activity. EF interacts with calmodulin (CAL) to become an active adenylate cyclase enzyme in nearly all cells. Interaction of PA with EF and subsequent internalisation of EF is blocked by prior binding of LF. In contrast to EF which is active in many cells, LF protease is only active in macrophages. This model explains the characteristic hypovolaemic shock syndrome (cAMP is a potent secretagogue), cytotoxicity to macrophages, and the immunogenicity of PA.

Fig. 8.9). The evidence is hardest for ricin, an A-B type toxic plant lectin (believed to have been located in the tip of an umbrella and used to poison a Bulgarian spy!). Newly formed toxin-containing endosomes enter those vesicular trafficking pathways which lead to the trans-Golgi network (TGN), through the Golgi and further into the endoplasmic reticulum (ER). This is the reverse of the normal secretory pathway and is therefore called 'retrograde transport'. This gives functional significance to the C-terminal sequence *lysine-aspartate-glutamate-leucine* (the KDEL motif) in the A subunit of cholera toxin and related sequences in LT and PEA. The KDEL motif is normally found in proteins which, having been processed in the Golgi, are returned to and are trapped by the ER which recognises the KDEL motif, thereby preventing such proteins being lost to the cell via exocytotic trafficking. The next part is speculative and described for CT. Either in the TGN or the ER, CTA is reduced freeing CTA₁, the CT toxiphore (Fig. 8.5). There is another ER pathway, the Sec61p secretion channel, through which aberrantly folded molecules are returned to the cytosol to proteasomes

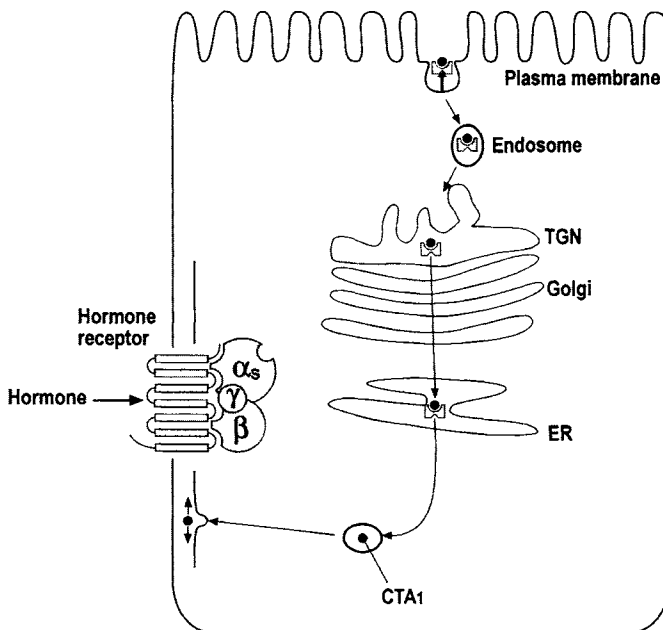


Fig. 8.9 Mode of entry of cholera toxin. This represents a much more complex route to an intracellular target. As described in the text it involves interaction of CTB subunits (shaded part of molecule) with ganglioside receptor GM1 (vertical arrow), receptor-mediated endocytosis, retrograde transport of the endosome to the trans-Golgi network (TGN), through the Golgi to the endoplasmic reticulum (ER), and anterograde transport of liberated CTA₁ from ER in vesicles directed to the basolateral membrane, the intracellular location of adenylyl cyclase. This mechanism is operative at least for several other toxins.

for degradation. Scrap proteins are normally ubiquitinated at lysine residues which targets them to proteasomes. Since CTA₁ is lacking in lysine and is hydrophobic, it may well exploit this secretory pathway but remain attached to the cytosolic face of the ER. The next stage is marginally less speculative. It is known that *in vitro* CT is activated by ADP-ribosylating factors (ARFs) which are also known to be involved in vesicular trafficking. Thus, by anterograde transport (the term given to secretory pathways from ER to the plasma membrane) CTA₁ may well be ferried in vesicles to the basolateral membrane with which they fuse thereby depositing CTA₁ near to the target, adenylate cyclase (Fig. 8.9).

Intracellular targets

The targets for some of the intracellular toxins are listed in Table 8.3, and illustrated in Figs 8.10–8.14.

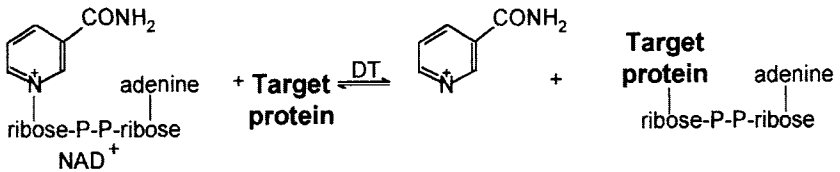


Fig. 8.10 ADP-ribosylation reaction. This enzymic reaction is common to a wide range of toxins with different target proteins (see Table 8.3).

Fig. 8.11 Inhibition of protein synthesis by diphtheria toxin (DT), *Pseudomonas aeruginosa* toxin A (PEA), Shiga toxin (ShT), Shiga-like toxins (ShLTs) and poliovirus. The schema shows a round of peptide elongation and illustrates the key role played by two enzymes, EF-1 and EF-2. EF-1-GTP interacts with aminoacyl-tRNA; this complex is docked into site A, EF-1-GTP becomes EF-1-GDP and is recycled as shown. After peptidyl transfer, EF-2-GTP catalyses transfer of the extended peptide to site P, and is itself autocatalytically converted to EF-2-GDP. DTA and PEA each ADP-ribosylates diphthamide (a modified histidine) in EF-2-GTP, which can no longer translocate the newly elongated peptide from the A site to the P site. The ShTA fragment is a specific *N*-glycosidase which cleaves an adenine residue from near the 3' end of the 28S ribosomal RNA. This depurination results in failure of EF-1-dependent binding of aminoacyl-tRNA to site A and hence inhibits protein synthesis. Poliovirus achieves selective inhibition of host protein synthesis at an earlier stage than is depicted here. Host mRNA is first modified (capped) then bound to the small ribosomal subunit; poliovirus mRNA is not capped. The function of a cap-binding protein, which recognises and binds host mRNA to the ribosome, is inhibited by a poliovirus virion protein thereby allowing differential translation of virus messenger RNA. EF-1 α , nucleotide-binding protein; EF-1 $\alpha\beta\gamma$, nucleotide exchange protein. (Modified with permission of authors and publisher (Elsevier Trends Journals, Cambridge, UK) from Fig. 1, Riis, B. *et al.*, 1990, *Trends Biochem. Sci.* **15**, 420–424.)

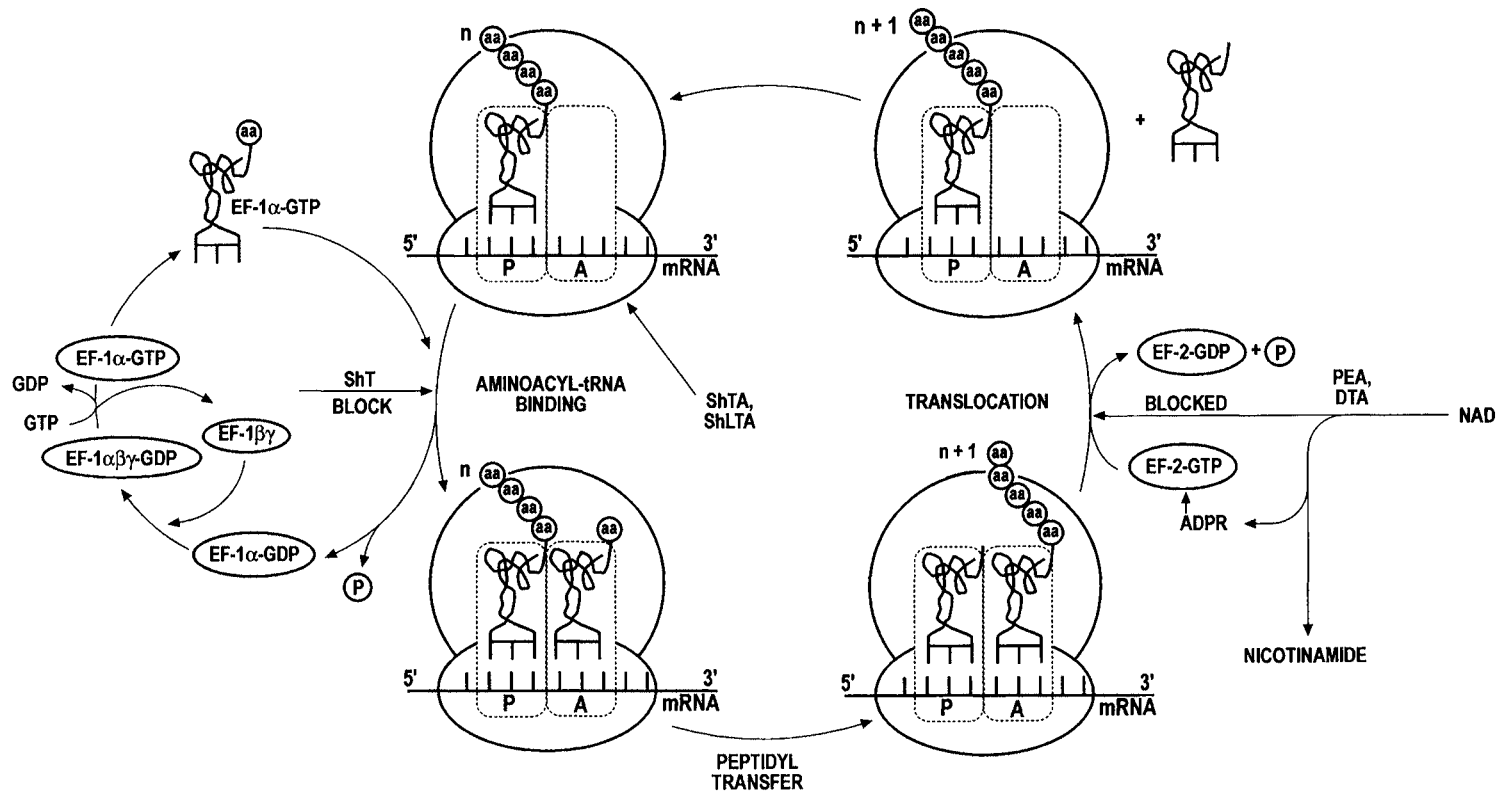


Table 8.3. Target proteins for intracellular toxins

| Toxin group Organism | Toxin | Preferred targets | | |
|--|--|--|---|-----------------------|
| | | GTP-binding proteins | ATP-binding proteins | Other targets |
| Ribosyltransferases (ADPRases) | | | | |
| <i>Corynebacterium diphtheriae</i> | Diphtheria toxin (DT) | Elongation factor 2 (EF2); see Figs 8.10 and 8.11 | | |
| <i>Pseudomonas aeruginosa</i> | Exotoxin A (PEA) | Elongation factor 2 (EF2); see Figs 8.10 and 8.11 | | |
| <i>Vibrio cholerae</i> | Exotoxin S (PES) Cholera toxin (CT) | Ras G-protein α_s subunit of G_s ($\alpha_s\beta\gamma$) regulator of adenylyl cyclase; ^b see Fig. 8.12 | | Vimentin ^a |
| <i>Escherichia coli</i> | Heat-labile toxins LTI and LTII | α_s subunit of G_s ($\alpha_s\beta\gamma$) regulator of adenylyl cyclase; see Fig. 8.12 | | |
| <i>Bordetella pertussis</i> | Cytotoxic necrotizing factor (CNF1) Pertussigen | Rho G-protein α_i subunit of G_i ($\alpha_i\beta\gamma$) regulator of adenylyl cyclase; ^c see Fig. 8.12 | | |
| <i>Clostridium botulinum</i> | C2 toxin | | Non-muscle actin, γ smooth muscle actin; see Fig. 8.14 | |
| Iota group ^d <i>Clostridium perfringens</i> <i>Clostridium spiroforme</i> <i>Clostridium difficile</i> | Iota toxin <i>C. spiroforme</i> toxin ADPRase | } | All mammalian actin isoforms | |
| <i>Clostridium botulinum</i> <i>Clostridium limosum</i> | C3 ADPRase ADPRase (similar to <i>C. botulinum</i> C3) | | Rho G-protein | |

**Glycosyltransferases
(large clostridial toxins)**

| | | |
|------------------------------|---------------|----------------------------|
| <i>Clostridium difficile</i> | TcdA and TcdB | Rho, Rac G-proteins |
| <i>Clostridium sordelli</i> | TcsL | Rac (and other) G-proteins |
| <i>Clostridium novyi</i> | Ten α | Rho, Rac G-proteins |

Shiga, Shiga-like toxins

| | | |
|-------------------------------|-------------------------|-----------------------------|
| <i>Shigella dysenteriae</i> 1 | Shiga toxin (ShT) | Ribosomes; see Fig. 8.11 |
| <i>Escherichia coli</i> | Shiga-like toxin (ShLT) | Ribosomes; see Fig. 8.11 |

**'Invasive' adenylate
cyclases**

| | | |
|-----------------------------|--------------------|---------------------------------|
| <i>Bacillus anthracis</i> | Oedema factor (EF) | Activated by calmodulin; ATP |
| <i>Bordetella pertussis</i> | AC-Hly | Activated by calmodulin; ATP |

Proteases

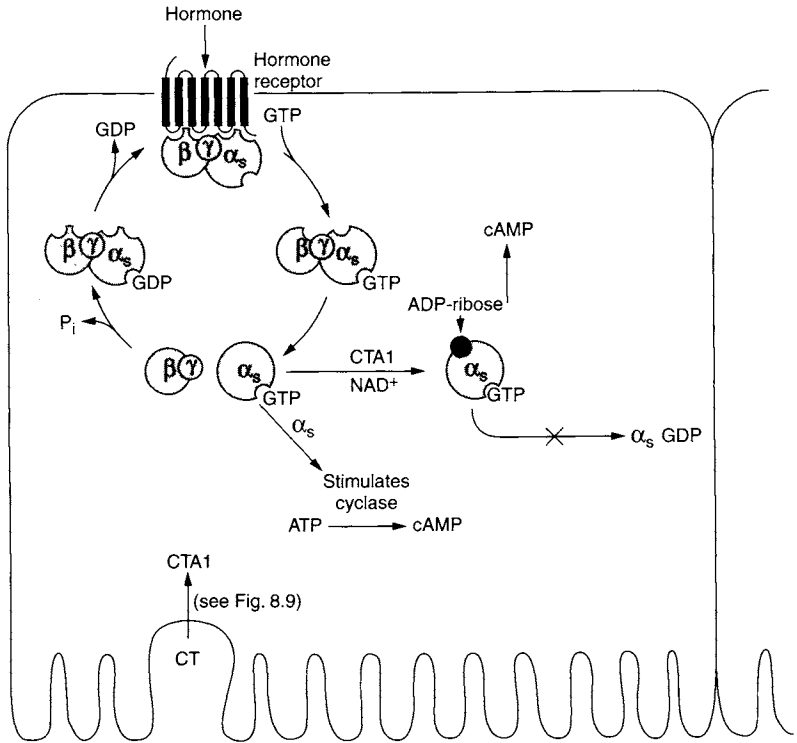
| | | |
|------------------------------|-------------------|---|
| <i>Clostridium botulinum</i> | Neurotoxin (BoNT) | } Proteins involved in release of neuro- transmitters; see Fig. 8.13 |
| <i>Clostridium tetani</i> | Neurotoxin (TeTx) | |
| <i>Bacillus anthracis</i> | Lethal toxin (LF) | Protein kinase kinases 1 and 2 |

^a Vimentin: intermediate filament protein.

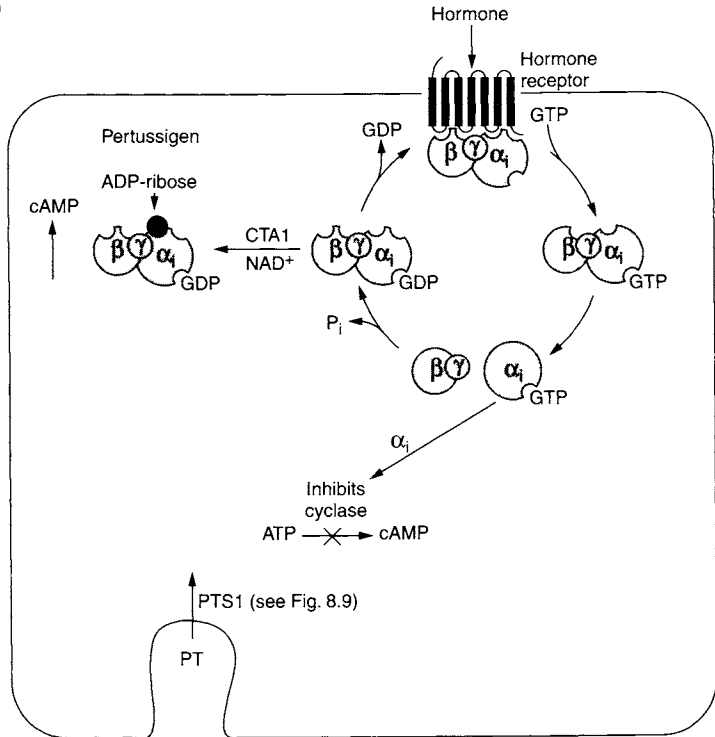
^b Cholera toxin A1–A2 will catalyse a range of reactions involving transfer of ADP-ribose to other substrates.

^c Pertussigen will catalyse the ADP-ribosylation of G proteins involved in several transmembrane signalling events. This would account for many of its biological effects.

^d *C. perfringens* and *C. spiroforme* toxins and *Clostridium difficile* ADP-ribosyltransferase form an iota subgroup, in that antibodies will cross-react within this group but not with *C. botulinum* C2 toxin. Only within the iota group are the binding components interchangeable. The iota group will also modify all mammalian actin isoforms.



(a)



(b)

Another important example concerns *Helicobacter pylori*, which produces, in addition to urease, a vacuolating cytotoxin (VacA) and a protein encoded by the cytotoxin-associated gene A(*cagA*), which are encoded in a pathogenicity island. Both VacA and CagA are nearly always associated with strains isolated from severe forms of disease. The precise role of CagA is unclear but VacA belongs to the category of toxins with an intracellular site of action. However, the exact details have yet to be worked out.

Superantigens: toxins with multiple biological activities

The recognition of superantigenicity and its molecular basis has allowed us to classify into one major (still expanding) group the

Fig. 8.12 Mode of action of cholera toxin (CT), *E. coli* LT toxins, and pertussigen (PT). There are five main features in this diagram.

1. The production of cAMP by adenylyl cyclase. Cyclic AMP is an important second messenger involved in the intracellular amplification of many cellular responses to external signals including hormones. The nature of the physiological response reflects the differentiation of the cell responding to the stimulus. For, example, in gut cells the response would be altered ion transport and hence fluid secretion; in muscle cells it would be glycogen breakdown in response to the call for more energy. The production of cAMP is controlled both positively (a) and negatively (b) at two levels. Interaction of hormone and receptor releases the heterotrimeric ($\alpha\beta\gamma$) G-protein regulator complex which, upon binding GTP, dissociates into α -GTP and $\beta\gamma$. The α -subunit may be stimulatory (α_s) and activate adenylyl cyclase (as in (a)) or inhibitory (α_i) and inhibit adenylyl cyclase (as in (b)); adenylyl cyclase is not shown structurally in the diagram. In gut cells the receptor would be on the nonluminal basolateral side enabling enterocytes to respond to stimuli from the circulation.

2. The second level of control involves endogenous GTPase properties of both α_s and α_i subunits of the G-protein regulator: α_s -GDP and α_i -GDP are inactive.

3. The level of cAMP may be affected by physiological stimuli or by perturbation of the normal regulatory cycle as illustrated, by CT and LTs in enterocytes (a) or PT in a pancreatic B cell (b).

4. CTA1 ADP-ribosylates α_s -GTP which promotes continued dissociation of the heterotrimer and also inactivates the endogenous GTPase activity. Hence stimulation of the cyclase continues. LTs act in a similar manner.

5. PTs ADP-ribosylates the α_i -GDP $\beta\gamma$ -heterotrimer which can no longer associate with the receptor or lose GDP to undergo another cycle of GTP activation; active cyclase can no longer be turned off. In pancreatic cells this results in loss of inhibition of insulin secretion.

Note: the α -GTP subunits are functionally analogous to the monomeric GTPases described in Fig.4.1. (Adapted with kind permission of the author (Gierschik, P.) and publisher (Springer-Verlag GmbH & Co. KG, Heidelberg, Germany) from Fig. 4 in 'ADP-ribosylation of signal-transducing guanine nucleotide-binding proteins by pertussis toxin', *Curr. Topics Microbiol. Immunol.*, 1992, **175**, 78, edited by K. Aktories.)

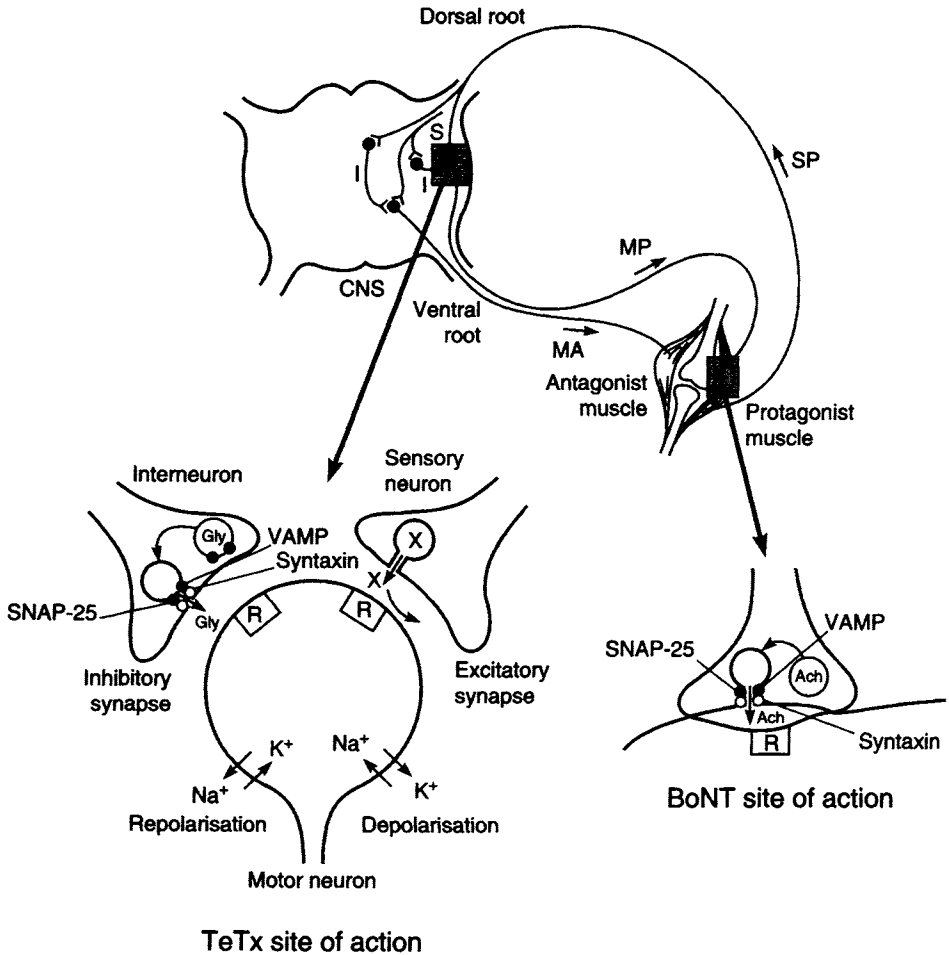


Fig. 8.13 Sites and mode of action of clostridial neurotoxins BoNT and TeTx. This figure has three main features.

1. Reflex arc (top). Mechanism for inhibiting the antagonists to a muscle contracting in response to stretch. Muscles are reciprocally innervated with sensory and motor neurons, although for clarity this is shown only for the protagonist muscle. On stretch, the stretch receptors generate an impulse which is transmitted along the afferent sensory (S) neuron of the protagonist (P) muscle. This SP neuron enters the spinal cord by the dorsal root and synapses with the motor neuron supplying the protagonist muscle (MP) and with an interneuron (I) which in turn synapses with the motor neuron supplying the antagonist muscle (MA); the efferent motor neurons leave the spinal cord by the ventral root. At the SP/MP synapse an excitatory transmitter is released which induces an impulse in MP which leads to contraction of protagonist muscle. However, excitation of I causes release of an inhibitory transmitter at the I/MA synapse which leads to relaxation of the antagonist muscle. Note that the basic reflex arc has been shown for simplicity but TeTx acts mainly on voluntary muscles.

2. A simplified version of the biochemical events occurring in synapses (lower left). Excitatory and inhibitory synapses, neurotransmitter release and

erythrogenic/pyrogenic toxins A and C* of *Streptococcus pyogenes* (SPEA, SPEC), staphylococcal enterotoxins (of which there are eight major serotypes, A to I, designated SEA, SEB, etc.) and staphylococcal toxic shock syndrome toxin (TSST-1 (human strains) and TSST_{ovine}). These proteins are superantigens by virtue of their ability to bind to major histocompatibility (MHC) class II molecules, outside the antigen-binding groove. They are presented as unprocessed proteins to certain T lymphocytes expressing specific T-cell receptor (TCR) motifs located in the variable domain of the β -chain ($V\beta$) of the TCR (see Chs 6 and 7). Nanogram to picogram quantities of superantigen will stimulate up to 20% of all T cells, compared with only 0.001 to 0.00001% T cells stimulated by conventional presentation of antigen to TCR. As a consequence of this huge proliferation of T cells and expression/release of aberrantly high levels of cytokines and other mediators, many biological systems are affected causing lethality/shock. This represents an important interference with a coordinated immune response, and the widespread polyclonal activation and cytokine release can be regarded as a microbial strategy, a 'diversion' of host immune defences. Ironically, the superantigen not only expands the circulating T-cell population, but also reacts with developing T cells in the thymus causing the same subpopulation to decline (see Ch. 7). It seems probable that these effects on immune cells represent a more important biological function of these toxins than the one responsible for the characteristics of disease; the latter may be no more than an 'accidental' phenomenon. It turns out that similar molecules are formed by mycoplasma and by certain retroviruses (e.g. the MIs antigen of mouse mammary tumour virus).

It has been shown experimentally (or proposed) that immune stimulation, cytokine release, induction of capillary leak, shock, and lethality are related to the superantigenicity of these proteins. However, there are other biological activities of these toxins which are not mediated by their superantigenicity. Some of these activities are common to all, and

* SPEB is a cysteine protease and is no longer classified as a superantigen.

action. Gly, glycine; R, receptors of neurotransmitters; X, hitherto uncharacterised (candidates include glutamate, dopamine, ATP, substance P, and somatostatin).

3. Sites of neurotoxin action (lower left and right). The predominant site of action of TeTx is the intermotor neuron synapse; the exocytotic machine is interfered with by the endopeptidase action of TeTx on VAMP. BoNT acts at the neuromuscular junction inhibiting the release of acetyl choline (Ach) by its proteolytic action on VAMP (types B, D and F), or SNAP (types A and E), or syntaxin (type C).

(Amplified from Figs 18 and 19, in 'Bacterial Toxins', 2nd edn, by J. Stephen and R. A. Pietrowski, 1986, pp. 60 and 62, Van Nostrand Reinhold (UK).)

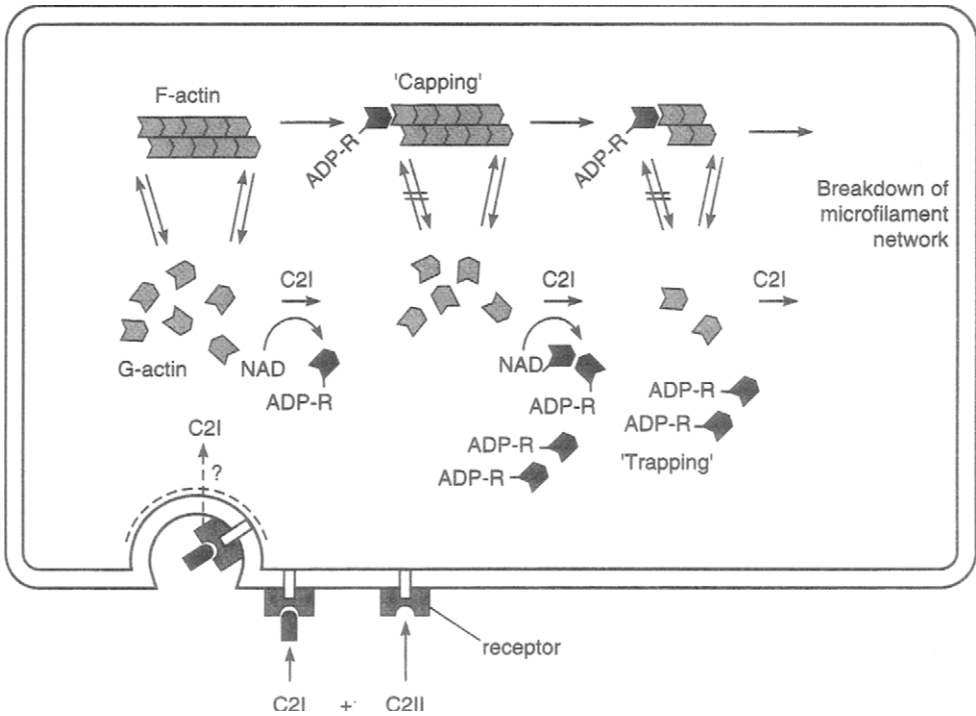


Fig. 8.14 Mode of action of actin-ADP-ribosylating toxins. *C. botulinum* C2 toxin component 2II binds to the cell membrane followed by C2I. The latter is internalised and upsets the equilibrium between polymerisation and depolymerisation of actin. ADP-ribosylation of actin inhibits its polymerisation and turns G-actin into a capping protein which binds to the fast-growing (concave) ends of actin filaments. Capping of the concave ends increases the critical concentration for actin polymerisation. Since the slow-growing (pointed) ends of actin filaments are free, depolymerisation of actin occurs at these ends. Released actin is substrate for the toxin and will be withdrawn from the tread-milling pool of actin by ADP-ribosylation, i.e. trapped. Both reactions will finally induce the breakdown of the microfilament network. (Reproduced with permission of the authors (K. Aktories *et al.*) and publisher (Springer-Verlag GmbH & Co. KG, Heidelberg, Germany) from Fig. 2 in 'Clostridial actin-ADP-ribosylating toxins', in *Curr. Topics Microbiol. Immunol.*, 1992 **175**, 107, edited by K. Aktories.)

others specific to certain members of this group. The red skin rash elicited by the streptococcal toxins (which gave rise to the original nomenclature 'erythrogenic' toxins) is regarded as a secondary hypersensitive effect, but pyrogenicity is the result of direct action on the hypothalamus as well as release of IL-1 and TNF- α from macrophages. Sensitivity to endotoxin shock can be increased up to 100 000 times in monkeys by SPEA/C and this may be due in part to an ability to impair

the reticuloendothelial system which would result in inefficient clearing of circulating endotoxin.*

Staphylococci cause food poisoning on a world-wide scale; infection rates are under-reported probably because it is normally a self-limiting gastrointestinal disease. Onset of disease is rapid after consumption of enterotoxin-containing food. The main features of the disease are diarrhoea and severe vomiting, the latter being due to enterotoxin stimulation of the vagus nerve.

In addition, some strains of staphylococci cause toxic shock syndrome (TSS), a multisystem disease. Originally, TSS was seen characteristically in menstruating women whose tampons harboured multiplying staphylococci. It is due to a toxin called toxic shock syndrome toxin 1 (TSST-1; originally recognised as SEF, one of the so-called staphylococcal enterotoxins). Toxic shock syndrome is characterised by sudden onset of fever, vomiting, diarrhoea, an erythematous rash followed by peeling of the skin, hypotensive shock, impairment of renal and hepatic functions and occasionally death. The main symptoms of the disease have been reproduced in rabbits by implanting chamber-enclosed TSS strains in the rabbit uterus or peritoneum or by injection of TSST-1 into rabbits. Complex changes are observed including haemorrhage in kidney and liver, congestion and haematomas in the lungs, leakage of blood into the thymus, and fluid in the pericardial sac and in the gut lumen. These effects in rabbits are very similar to those seen in humans and would certainly explain the shock and diarrhoeal syndrome so characteristic of the disease. The lethal effect of TSST-1 is enhanced considerably by endotoxin.

We now know that TSS is not confined to menstruating women. Non-menstrual TSS presents with essentially the same signs as menstrual TSS and is caused by other staphylococcal enterotoxins (SEs). TSST-1 is isolated only from menstrual cases of TSS: this toxin has the ability to cross the vaginal mucosal barrier whereas the other SEs do not. Moreover, TSS is no longer thought to be caused exclusively by staphylococci. Streptococcal TSS (STSS), a life-threatening disease caused by streptococci, is now a well-recognised clinical entity

* There are numerous examples of synergistic reactions between toxins of the same or different species. *Bacillus cereus* makes a phosphatidyl choline-preferring phospholipase and a sphingomyelinase which are separately nontoxic; in concert they are haemolytic and termed cereolysin A-B. Another entirely different type of synergistic interaction is that of the increase in toxicities of staphylococcal α - and γ -toxins, diphtheria toxin and endotoxin for neonatal ferrets preinfected with influenza virus. Increases were 14-, 3-, 219- and 84-fold respectively. No increase in viral replication was observed. Neonates died suddenly without clinical symptoms as in human babies dying from sudden infant death syndrome (SIDS). Pathological examination showed inflammation of the upper respiratory tract, lung oedema and collapse, and early bronchopneumonia in animals receiving the dual challenge but not those receiving either toxin or virus on their own. Thus, some bacterial toxins in conjunction with influenza virus could be one of the several causes of SIDS.

probably corresponding to the severe cases of scarlet fever described in the older literature.

There is another class of potent immunogens not normally classified under 'superantigens'. Cholera toxin (CT) and *E. coli* LTs – and their respective B subunits – are extremely potent antigens eliciting extraordinary high levels of antitoxin without the need for conventional adjuvants. The phenomenology is highly complex and under active study. Understanding of the basic immunological mechanisms could lead to important practical applications, since the inherent adjuvanticity of these potent immunogens is demonstrably effective in some settings with unrelated antigens.

Significance of toxins in disease

It is important to point out that, while the outstanding advances made in our knowledge of toxin structure and mode of action at the cellular level can be exploited in a remarkable way (see below), it is important to remember that such knowledge by itself does not tell the whole story of the pathogenesis of infectious disease. To illustrate this some examples are given below.

Cholesterol-binding cytolysins

It is obvious from our consideration of these toxins that the elucidation of their lytic activities towards red cells in terms of a fundamentally similar mechanism, by itself tells us nothing about their respective roles in disease. Moreover, as already outlined above, pneumolysin is now known to be a multifunctional molecule whose relevance in disease varies with the infection setting!

Corynebacterium diphtheriae

Corynebacterium diphtheriae produces diphtheria toxin which is of unquestionable importance in causing diphtheria. Sustained active immunisation with DT toxoid has made diphtheria a clinical rarity in advanced countries. Failure to continue this policy resulted in a huge diphtheria epidemic in the early 1990s in the states comprising the former USSR. *C. diphtheriae* organisms multiply on the epithelial surfaces of the body (nose, throat, skin) but do not penetrate deeply into underlying tissues. The infection on the body surface causes necrosis of mucosal cells with an inflammatory exudate and the formation of a thick 'membrane' (hence the name *C. diphtheriae*: Gr., *diphthera* = membrane) and if the infection spreads into the larynx there may be respiratory obstruction. The toxin probably assists colonisation of the throat or skin by killing epithelial cells and polymorphs. DT can also be disseminated from the infection site and has important actions, especially on the heart and nervous system. However, it is a fact that the strain used for commercial production of toxin for vaccine purposes is the avirulent PW8 strain. Five per cent of all the protein made by

this strain, and 75% of all protein secreted by this strain is DT and yet it is avirulent! It is the production of DT under *in vivo* conditions that matters. DT is encoded by a lysogenic corynephage β whose transcription is controlled by an iron-dependent repressor, emphasising the importance of *C. diphtheriae* Fe metabolism *in vivo*.

Pseudomonas aeruginosa

Pseudomonas aeruginosa, is common in soil and water and can occasionally be isolated from the faeces of normal, healthy individuals. It is virtually harmless for healthy adults, but its ability to multiply in almost any moist environment and its resistance to many antibiotics have made the bacterium a major cause of hospital-acquired infection. This is particularly so among patients with impaired host defence mechanisms such as those with chronic illness, genetic immunodeficiencies, those under treatment with immunosuppressive drugs, or patients suffering from extensive burns. *P. aeruginosa* causes localised infection in the urinary tract, respiratory tract, burns and wound infections. In severely debilitated patients these localised infections may develop into general septicaemia, with mortality in such cases approaching 100%. However, unlike *C. diphtheriae*, the organism elaborates several potentially toxic extracellular products, including a phospholipase, several proteases, lipase, haemolysin, enterotoxin, lipopolysaccharide endotoxin, elastase, exoenzyme S (PES), and exotoxin A (PEA). While there is definite evidence to implicate the last three, the roles of the others are less well defined; mechanisms of pathogenicity of *P. aeruginosa* are complicated and still unclear.

Shigella dysenteriae 1

Shigella dysenteriae 1 is the cause of bacillary dysentery. For a long time it was thought that Shiga toxin (ShT) was the principal cause of this disease. However, in Ch. 2, the importance of gut invasiveness in *Shigella* infections was emphasised. While it is not at all clear how ShT can be involved in the watery diarrhoea phase of dysentery, it is perceived as exacerbating the bloody diarrhoea phase rather than initiating it. In contrast there is now evidence that Shiga-like toxins (ShLTs) are important in haemorrhagic colitis (HC) and haemolytic urea syndrome (HUS) caused by ShLT-producing strains of enterohaemorrhagic (EHEC) *E. coli*: EHEC has the capacity to progress disease beyond a watery diarrhea stage to HC and HUS; the latter is characterised by renal failure, thrombocytopenia and microangiopathic haemolytic anaemia. This virulence attribute is due to possession of one or more of a family of ShLTs. As in dysentery, the role of ShLT in the causation of watery diarrhoea is controversial and indeed it may not be absolutely necessary. However, there is no doubt that ShLT is either responsible for, or severely exacerbates the bloody diarrhoea in HC. Recently the first histochemical demonstration was made

of ShLTs bound to renal tubules in the kidney of a child who died as a result of HUS associated with *E. coli* O157:H7 infection.

Vibrio cholerae

The classic paradigm for bacterial watery diarrhoea is cholera caused by *V. cholerae* in the small intestine. *V. cholerae* colonises the upper small intestine by adhering to epithelial cells. Water and electrolytes are lost through the intact epithelial cells into the small intestine. As the multiplying bacteria increase in numbers and more and more epithelial cells are affected, the absorptive capacity of the colon is overwhelmed and there is profuse watery diarrhoea, as much as 1 litre h⁻¹ in severe cases. The massive loss of isotonic fluid with excess of sodium bicarbonate and potassium leads to hypovolaemic shock, acidosis and haemoconcentration. Anuria develops, and the collapsed, lethargic patient may die in 12–24 h. Lives are saved by replacing the lost water and salts; but the patient recovers as affected cells are shed and replaced in the normal fashion. The infection is particularly severe in children who easily develop low levels of plasma potassium. However, on a global scale this greatly feared disease, cholera, is only responsible for less than 1% of the total deaths due to diarrhoea.

Despite being arguably the most studied pathogen over the last three decades, the basis of *V. cholerae* pathogenicity and the detailed mechanisms underlying the dramatic diarrhoeal secretion induced by this organism are still not fully understood. Very recently, spectacular advances have been made in the molecular biology of *V. cholerae*. Chromosomal DNA of virulent *V. cholerae* contains two essential genetic elements which are important in *V. cholerae* virulence: CTX ϕ (the genome of a filamentous bacteriophage) which encodes the cholera toxin (CT), and a large pathogenicity island VPI (for *Vibrio cholerae* pathogenicity island). VPI is now known to be the integrated genome of another large filamentous bacteriophage (VPI ϕ) and encodes the toxin co-regulated type IV pilus (Tcp). Of the numerous colonization factors known to be produced by *V. cholerae* only Tcp has been proven to be important in human disease. Tcp is a remarkable entity; its subunit TcpA is a coat protein of VPI ϕ , but it also acts as a receptor for CTX ϕ and mediates interbacterial adherence. Thus, as a result of sequential infection by two 'pathophages', *V. cholerae* acquires the ability to colonise the human gut and secrete classical cholera toxin, which is a potent enterotoxin. The integration into the chromosome of these phage genomes brings their expression under the control of regulatory genes in the ancestral chromosome, whilst the replication of phages enables their interbacterial spread. CT is an 'AB' type toxin in which the pentameric B subunit recognises and binds to its cell receptor (GM1 ganglioside) thereby initiating the internalisation of the active A subunit (CTA₁) and elevation of cAMP.

Such elegant work implies that cholera diarrhoea is a purely pathophysiological disease and that CT is the only determinant of disease;

that is too simplistic. CT may be the major diarrhoeagenic toxin but there are at least eight other toxins which have been potentially implicated in cholera diarrhoea, of which we have already mentioned one, ZOT (Fig. 8.1). Moreover, studies on human jejunal biopsies show that cholera is not a purely pathophysiological disease but a pathological one, involving changes in the microvasculature and enteric nerve fibres, degranulation of argentaffin cells, mucosal mast cells and eosinophils; the extent of these changes correlated with clinical severity of disease. A *V. cholerae* vaccine strain produced by the deletion/mutation of all known toxin genes yielded a vaccine strain which, although less reactogenic than wild-type virulent strains, still produced a significant diarrhoea suggesting the involvement of an inflammatory component (as yet undefined) in the causation of cholera diarrhoea. There is also experimental evidence to implicate the enteric nervous system (ENS) in cholera diarrhoea. It has also been shown that CT administered to rat jejunum elicited a secretory response in both the jejunum *and* colon, which suggests neurological transmission of the locally induced secretory stimulus to distal colon.

Despite the undoubted importance of CT in the causation of the disease, and the potent antigenicity of CT, it is now recognised that protective immunity is very largely antibacterial. It is stopping effective colonisation which is important rather than neutralisation of the toxin. This has been partially achieved by using killed whole cell vaccines. Several attempts have been made in the laboratory to manipulate virulent strains genetically (in practice this means deleting or inactivating the known toxin genes) such that the attenuated strain will colonise the gut and stimulate local immune responses, and thereby prevent colonisation of the gut by virulent strains. To date, attenuated strains have been developed which fulfil these criteria, but these induce a mild transient diarrhoea, which has prevented their adoption into vaccination programmes, and incidentally, reinforce the argument that cholera is not wholly about CT.

Bordetella pertussis toxin (pertussigen; PTx)

Whooping cough (pertussis) is a severe respiratory tract infection characterised by prolonged paroxysmal coughing, attacks of which continue long after infection has cleared. The disease is capable of striking all ages but is particularly prevalent and severe in young children, where hospitalisation is required in about 10% of cases. The causative agent, *B. pertussis*, is transmitted aerielly from the respiratory tract of an infected individual to that of a susceptible host. The organism attaches via several adhesins – filamentous haemagglutinin, fimbriae and the 69 kDa outer-membrane protein, pertactin – to the mucosal surface between cilia, and multiplies there during the incubation period of the disease, which is commonly around 7 days. The infection then manifests as a slight fever and catarrh which is often indistinguishable from a common cold. However, 1–2 weeks later bouts of uncontrollable

coughing begin. It is this paroxysmal coughing, along with the notorious 'whoop' as the child attempts to draw breath, which characterises the disease. The paroxysmal coughing stage often lasts for several weeks and no treatment is fully effective in controlling the symptoms. The only proven means of controlling whooping cough is vaccination but, in the UK at least, sporadic reports of vaccine-induced brain damage in infants has diminished public acceptance of the vaccine. However, it should be noted that permanent encephalopathy (brain damage) is a recognised though rare consequence of whooping cough infection.

Without doubt, PTx, whose biochemical mode of action is described above, is an exceedingly important virulence determinant of *B. pertussis*: PTx toxic activities including histamine sensitisation, hyperinsulinaemia followed by hypoglycaemia, induction of leukocytosis, IgE induction are all observed after infection and administration of PTx; these toxic properties of PTx are abolished when the ADPR activity of PTx is inactivated. PTx non-toxic activities – mitogenicity, haemagglutination, platelet activation, mucosal adjuvanticity – are triggered by PTx B subunits. Much current work is being devoted to producing immunogenic, completely nontoxic preparations of pertussis toxin by genetic manipulation of the gene encoding the S1 subunit (Fig. 8.5); in clinical trials in Italy, such engineered vaccines have been shown to be both safe and effective as judged by antibody titres to pertussis toxin.

However, *Bordetella pertussis* also produces other potentially important toxins including AC-Hly involved in colonisation (see above), dermonecrotic toxin (DNT; formerly known as heat-labile toxin), and two non-protein toxins – tracheal cytotoxin (TCT) and endotoxin. DNT is lethal for mice and causes skin lesions in rabbits and guinea-pigs. It is of doubtful significance in humans but important in atrophic rhinitis in pigs caused by *Bordetella bronchiseptica*.^{*} TCT is a small glycopeptide which destroys ciliated epithelial cells and is almost certainly responsible for some of the observed histopathological damage in *B. pertussis* infections.

Clostridial neurotoxins

Tetanus occurs in man and animals when *Clostridium tetani* spores germinate in an infected wound and produce their toxin; all strains of *C. tetani* produce the same toxin. Spores are ubiquitous in faeces and soil and require the reduced oxygen tension for germination provided locally in the wound by foreign bodies (splinters, fragments of earth or clothing) or by tissue necrosis as seen in most wounds, the uterus after septic abortion, or the umbilical stump of the newborn. The site of

^{*} DNT is similar to the dermonecrotic toxin of *Pasteurella multocida* also involved in porcine atrophic rhinitis. They inactivate the GTPase activities of Rho proteins resulting in cytoskeletal changes affecting osteoblasts.

infection may be a contaminated splinter just as well as an automobile or battle injury. It also reaches the central nervous system by travelling up other peripheral nerves following blood-borne dissemination of the toxin through the body. The motor nerves in the brain stem are short and therefore the cranial nerves are among the first to be affected, causing spasms of eye muscles and jaw (lockjaw). There is also an increase in tonus of muscles round the site of infection, followed by tonic spasms. In generalised tetanus there is interference with respiratory movements, and without skilled treatment the mortality rate is about 50%.

Botulism* is caused by *Clostridium botulinum*, a widespread saprophyte present in soil and vegetable materials. *C. botulinum* contaminates food, particularly inadequately preserved meat or vegetables, and produces a powerful neurotoxin. The toxin is destroyed at 80°C after 30 min – of great importance to the canning industry – and there are at least seven antigenically distinct serotypes (A–G) produced by different strains of bacteria but which have a pharmacologically similar mode of action. It is absorbed from the intestine and acts on the peripheral nervous system, interfering with the release of acetylcholine at cholinergic synapses of neuromuscular junctions. Somewhere between 12 and 36 hours after ingestion there are clinical signs suggesting an acute neurological disorder, with vertigo, cranial nerve palsies and finally death a few days later with respiratory failure. A less typical form of botulism occurs in small infants. The spores, present in honey applied to rubber teats, appear to colonise the gut, so that the toxin is produced *in vivo* after ingestion.

However, some puzzles remain to be resolved. Tetanus and botulinum toxins enter neuronal tissue preferentially at motoneuronal endplates, but the nature of the relevant toxin receptors is still not known. It remains unclear why botulinum toxin acts directly at the site of uptake and not, as observed with tetanus toxin, in the central nervous system, although a considerable amount of botulinum toxin (like tetanus toxin) is retrogradely transported. Botulinum toxin blocks the release of acetylcholine at neuromuscular junctions to cause flaccid paralysis. Likewise, one might ask why tetanus toxin fails to act at the motoneuronal junction at concentrations which would completely block release of neurotransmitter from GABAergic synapses. To reach inhibitory interneurons – its principal site of action – tetanus toxin must leave α -motoneurons after the primary uptake step, traverse the synaptic cleft to interneurons, leave those again in order to become finally internalised again from presynaptic membranes – a route identical to that of several neurotropic viruses. It

* *Botulus* (Latin) = sausage. In 1793 a large sausage was eaten by 13 people in Wildbad in Germany; all became ill, and six died. The disease was subsequently referred to as botulism.

acts by blocking the release of inhibitory transmitters (glycine or GABA) resulting in a failure to relax the affected muscle – pathophysiological ‘tetanus’. Only in rare cases does it act peripherally like botulinum toxin.

Anthrax toxin

Anthrax is a disease of animals, particularly sheep and cattle, and to a lesser extent man, caused by infection with *Bacillus anthracis*. Infection takes place following the ingestion of spores, the inhalation of spores, or in most cases by the entry of spores through abraded skin. The spores germinate inside macrophages and then the bacteria form a toxin which kills macrophages, increases vascular permeability and gives rise to local oedema and haemorrhage. Infection of the skin in man leads to the formation of a lesion (malignant pustule; a *black eschar*, hence *B. anthracis*; Gr. *anthrakos* = coal) consisting of a necrotic centre surrounded by vesicles, blood-stained fluid and a zone of oedema and induration. In severe infections (nearly all cases of inhalation anthrax are fatal) there is septicaemia with toxic signs, loss of fluid into tissues, with widespread oedema and eventually death. Anthrax in man occurs mainly in those whose work brings them into contact with infected animals. It is not a common disease in the UK, and the usual source of infection is imported bones, hides, skins, bristles, wool and hair, or imported fertilisers made from the blood and bones of infected animals.

The anthrax toxin complex consists of three components, factor I (oedema factor; EF), factor II (protective antigen; PA) and factor III (lethal factor; LF), none of which are toxic by themselves, but in binary combinations exhibit two types of activity. PA and LF form a binary proteolytic cytotoxin which kills macrophages (see Ch. 4) but not any other cell type, whereas PA and EF form a binary toxin which will elevate cAMP levels (Fig. 8.8) in nearly all types of cell.

These combined binary toxic activities explain much, but not quite all of the events which occur in anthrax infections. Virulent *B. anthracis* contains two plasmids: plasmid pXO1, which encodes the toxin genes, and plasmid pXO2, which encodes capsule genes. Loss of either results in a dramatic loss of virulence. Avirulence due to loss of toxin genes is understandable in terms of the above outline. The capsule contains poly-D-glutamic acid which renders the organism resistant to phagocytosis; loss of capsule renders the organism avirulent even though it still possesses toxin genes! The most effective field vaccine is the Sterne strain used to protect animals. This is a pOX2⁻ strain which cannot establish an infection focus due to the loss of the antiphagocytic capsular poly-D-glutamic acid. However, this strain manages to express sublethal doses of toxin, the PA component of which is the protective immunogen, a fact readily understood in terms of the model for cell entry described in Fig. 8.8. If you block attachment of PA with anti-PA antibodies, then EF and LF will be nontoxic.

Streptococcal and staphylococcal superantigens

Streptococci cause a wide spectrum of diseases including simple skin pimples, uncomplicated tonsillitis, scarlet fever, rheumatic fever, severe invasive infections like necrotising fasciitis and STSS. We have touched on a few of the known streptococcal virulence determinants, and sought to explain much of the pathology caused by streptococci in terms of these determinants, despite the fact that the severity of streptococcal disease can range from simple pimples to STSS! In addition to the status of the host, full explanations must clearly reflect the particular strain of the organism, its initial site of lodgement, and the efficiency with which it produces its particular combination of virulence determinants *in vivo*. The virulence determinants must include surface antiphagocytic M protein and maybe some of the huge number of other proteins known to be secreted by streptococci.

Exactly the same kind of considerations apply to staphylococci and the diseases they cause. Staphylococci also produce a plethora of putative virulence determinants in addition to those discussed here.

Clostridium difficile

Clostridium difficile represents a classic example of the difficulty in interpreting disease mechanisms in terms of characterised enzyme activities ascribed to toxins relevant in disease. *C. difficile* is now established as the most common nosocomial enteric pathogen causing pseudomembranous colitis, antibiotic-associated colitis and antibiotic-associated diarrhoea. The most important defence against this opportunistic pathogen is the normal colonic microflora, although the microbial species responsible for, and the mechanisms whereby they suppress the growth of *C. difficile* are still not understood. Disruption of the normal ecosystem by antibiotics can result in colonisation by *C. difficile* which, if of the right pathotype, will cause diarrhoea or, more seriously, pseudomembranous colitis. Production of proteolytic and hydrolytic enzymes and capsule, expression of fimbriae and flagella, chemotaxis and adhesion to gut receptors, may all play a part in the pathogenesis of *C. difficile*-induced disease by facilitating colonization or by directly contributing to tissue damage. However, toxins A and B (TcdA, TcdB) are thought to be the primary virulence determinants of this pathogen in the context of antibiotic-associated gastrointestinal disease. The toxins have identical enzyme specificities – they glucosylate the same serine residue in target proteins – but yet they have very different biological properties. B is *ca.* 1000-fold more cytotoxic to cultured cells than A, but does not cause fluid secretion in the gut on its own, whereas A does. A powerful case can be marshalled to implicate toxin A as the major effector in *C. difficile* diarrhoeal disease and of the colitis so characteristic of *C. difficile* infections. Several attempts have been made to explain these superficially discrepant data but perhaps

the most obvious explanation is that glucosyltransferase is not the primary mechanism of toxicity!

One alternative explanation is that the main effect of toxin A is to upregulate the secretion of IL-8 from colonocytes and downregulate the exocytosis of mucin. This would result in the recruitment of inflammatory and immune cells (seen in pseudomembranous colitis) with consequential indirect mucosal damage. The depression of stimulated mucin secretion (observed in toxin A treated cells *in vitro*) could well be explained as a secondary effect in terms of the enzymatic activity of the toxin which by disruption of the cytoskeleton would impair exocytosis.

Other clostridial toxins

There is a plethora of toxins produced by numerous clostridial pathogens important in both human and veterinary medicine. The clostridial genus comprises a large number of toxigenic species, some of which are known to produce several toxins, extracellular enzymes, and other factors which are as yet recognised only by a letter of the Greek alphabet. All the α -toxins are dermonecrotic and the others have haemolytic, enzymatic properties. Toxin production *in vitro* is used as the basis of typing clostridia, and the picture is complex. No attempt will be made to describe every disease in man or animals associated with clostridia; only those are selected which best serve to illustrate the involvement of some recognisable toxins. In the case of sheep diseases – lamb dysentery, struck, enterotoxaemia, black disease, braxy, black quarter – some of the best evidence for implicating relevant toxins comes from field studies using multivalent vaccines (based on toxoids of these toxins).

Clostridium perfringens type C causes pig bel in man, essentially due to the production of β -toxin. This is a rare disease in developed societies but a public health hazard in Papua New Guinea. Three factors are responsible: the ubiquity of *C. perfringens* type C in the soil and faeces of man and pigs; the high-carbohydrate, low-protein nature of the staple diet; and the sporadic consumption of large quantities of pork on occasions of celebration. The latter dietary change promotes a proliferation of clostridia in the intestine which may lead to intestinal gangrene and death. β -Toxin damages the mucosa, reduces mobility of villi and causes more bacteria to become attached to the villi. More toxin is absorbed and the mucosa and underlying intestinal wall become necrotic, leading to death in many cases. The influence of diet is additionally important in that low-protein diets cause decreased secretion of pancreatic proteolytic enzymes and sweet potato contains a trypsin inhibitor. These conditions promote the survival of β -toxin which is highly sensitive to proteolytic inactivation. Immunisation with β -toxoid preparations has dramatically lowered the incidence of this fatal disease in children.

Gas gangrene in man may be caused by several bacterial species

separately or in concert. These include *Clostridium perfringens* type A, *C. novyi* types A and B, and *C. septicum*; *C. perfringens* and its α -toxin have already been discussed. Far less is known about *C. novyi* and *C. septicum* and their toxins in gas gangrene in man. Much more is known about the role these organisms play in diseases of animals, and multivalent vaccines confer a very high degree of immunity (particularly to sheep) against several clinically identifiable but separate diseases. A few of these diseases are described below.

Clostridium perfringens type B causes lamb dysentery. This is an acute, fatal disease of young lambs occurring during the first week of life and caused by absorption of toxin(s) generated by *C. perfringens* type B in the small intestine.

Clostridium perfringens type C causes struck in sheep, a disease occurring in the Romney marshes of Kent, but rare in other areas in the world. The pathological changes observed differ markedly from other enterotoxaemias and include enteritis.

Clostridium perfringens type D enterotoxaemia in sheep is another acute fatal disease. The most constant lesion is subendocardial haemorrhage around the mitral valve. *C. perfringens* type D ϵ -toxin or its protoxin are recoverable from intestinal contents.

Clostridium novyi type B causes black disease of sheep or infectious necrotic hepatitis. This is an acute infectious disease of sheep (occasionally cattle) caused by the absorption of the α -toxin elaborated by the organism in necrotic foci in the liver, and is nearly always associated with invasion of the liver by immature liver flukes. How *C. novyi* gets to the liver in the first place is not known but it is readily demonstrable in livers of normal sheep in areas where the disease is prevalent. Experimental reproduction in guinea-pigs of a similar disease is possible by the combined action of *C. novyi* spores and liver fluke infestation.

Clostridium novyi type D causes a rapidly fatal disease in cattle (redwater disease) similar to, and regarded by some as an atypical manifestation of, black disease. The characteristic lesions include jaundice, various haemorrhagic manifestations, and anaemic infarcts in the liver; active liver fluke infestation may or may not be present. In culture this organism produces β -toxin, which explains the haemoglobinuria, but no α -toxin.

Clostridium septicum causes braxy in sheep. The role of *C. septicum* in this acute, fatal disease is assumed because of its association with the characteristic haemorrhagic inflammatory lesion in the abomasum. The disease has not been reproduced experimentally with *C. septicum* but can be prevented by immunisation with sterile toxoids derived from this organism.

Clostridium chauvoei causes black quarter in sheep and cattle. This is a gas gangrene-type infection of muscles and associated connective tissues in cattle and sheep; *C. chauvoei* is also the causative agent of parturient gas gangrene in sheep. The initial stimulus which activates

the infection in cattle is not known, since the disease is hardly ever associated with any overt wounding. Washed spores alone do not cause disease when injected, but do in conjunction with a tissue-necrotising agent. In sheep, wounding caused by parturition, castration, tailing, shearing, vaccination, as well as accidental damage, will create a focus within which *C. chauvoei* can multiply.

Exploitation of native toxins and toxin chimeras as therapeutic agents

Reference has already been made to the potential exploitation of non-toxic B subunits of CT and LT. However, the most toxic substances known to man are now being used as therapeutic agents to treat focal dystonias such as neck twists and eye squints, or eyelid closure and, more recently, some childhood palsies. The preparations are made from BoNT A and consist of toxin-haemagglutinin complex – the form in which the toxin is usually produced by the organism. This complex is less toxic than purified neurotoxin when administered parenterally but relatively more toxic when given orally; the haemagglutinin apparently protects the neurotoxin from proteolytic degradation in the gut. The effects of BoNT in relieving muscle spasms are not permanent but last for several months. Treatment has to be repeated. To date the successes significantly outweigh the failures and no long-term adverse effects have been reported. Antibody to the toxin has not so far been detected in the sera of the majority of patients.

Another exciting development concerns the development and use of modified toxins as therapeutic agents. For decades, attempts have been made to make immunotoxins by coupling native toxins to antibodies specific to some surface antigen on tumour cells, with little practical success, as yet. Several attempts have been made to modify diphtheria toxin (DT), adenylyl cyclase of *Bordetella pertussis*, *Pseudomonas aeruginosa* exotoxin A, cholera toxin and the LF anthrax toxin for selective intracellular delivery of extraneous proteins. The most successful attempt to date has been the development by Murphy and colleagues in the USA of an anticancer agent developed from DT. Their DT chimera has now been approved by the Food and Drug Administration for treatment of refractory cutaneous T-cell lymphoma (CTCL). DT comprises three functional domains: C-domain which is the toxiphore (A fragment), T-domain (in B fragment) involved in the translocation of C to the intracellular target, and R-domain (in B fragment) which recognises and binds to the receptor on sensitive cells. For DT, the receptor is an EGF-like precursor which happens to be widely distributed throughout all organ systems. DT was genetically engineered by substituting that portion of the DT structural gene encoding R domain with cDNA encoding IL-2, such that the hybrid toxin would target IL-2-receptor positive cells. The resultant fusion toxin bears a new 'cellular address', but retains all of the other biological properties of the native DT molecule as well as a three-dimensional structure nearly

identical with native DT. This chimeric toxin has been shown to be safe and well tolerated and is successful in treating CTCL which expresses the high-affinity receptor for IL-2. Such constructs are potent against human CTCL and have achieved partial to durable remission of this tumour. An era of new 'magic bullets'?

Fungal exotoxins

Many fungi contain substances that are harmful when taken by mouth, and there are two diseases that result from the ingestion of food containing preformed fungal toxins. As with *C. botulinum*, the disease is caused without the need for infection. *Aspergillus flavus* infects ground nuts (monkey nuts) and produces a very powerful toxin (aflatoxin). Contaminated (badly stored) ground nuts used to prepare animal feeds caused the death of thousands of turkeys and pigs in the UK in 1960 and the survivors of intoxication nearly all developed liver cancer. Human disease has not yet been associated with this toxin. *Claviceps purpurea* is a rust fungus affecting rye, and it produces toxins (ergotamine especially) that give rise to ergot poisoning when contaminated grain is eaten. Mushrooms and toadstools have long been recognised as sources of poisons and hallucinogens.

Cell-associated toxins

Unlike the toxins already discussed in this chapter, there is a group of toxins which are distinct structural components and are not released into the surrounding medium in any quantity except upon death and lysis of the bacteria. Several protein toxins (e.g. *Clostridium difficile* toxin A, tetanus toxin, pneumolysin) are released during the decline phase of batch culture – probably on autolysis – and these are classified as exotoxins. Here we deal with toxins which are known to comprise well-recognised structural entities which on a priori grounds must have key functions in the organism: they are found in the outer membranes of Gram-negative organisms. There are two chemically distinct types of toxin considered: lipopolysaccharide (endotoxin; LPS) and protein. The bulk of this section is taken up with endotoxin.

Many pathogenic organisms, however, are pathogenic by virtue of possessing various types of surface structure important in conferring virulence. These include, for example, adhesins which are important in colonising body surfaces or a variety of surface molecules (which may or may not be inside capsules) that render them resistant to phagocytosis. But the majority of adhesins and antiphagocytic determinants are themselves nontoxic.

The Gram-negative bacterial cell wall is subject to considerable variations in both the composition of LPS and in the number and nature of

the proteins found in the outer-cell membrane. Apart from the examples given in Ch. 7 in relation to the gonococcus, such phenotypic variation in LPS has rarely been examined in the context of pathogenicity. However, the examination of cell-bound proteins of *Yersinia pestis* from organisms grown *in vivo* led to the discovery of a toxin lethal for mice and guinea-pigs.

Protein toxins of *Yersinia pestis*

Plague is one of the most deadly diseases of man and has, over several thousands of years, claimed millions of lives. In the fourteenth century, 'the black death' wiped out a quarter of the population of Europe before spreading through the Middle East and Asia. Fortunately, however, the last 60 years or so have seen a drastic decrease in outbreaks of plague, although the threat of another epidemic is still with us.

The causative organism of plague, *Yersinia pestis*, is primarily a parasite of rodents in which it is endemic in many areas of the world. Only when man comes into close proximity with infected rodents do outbreaks of human plague occur. The disease is spread from rat to rat and from rat to man by fleas as already described in Chs 3 and 4. The principal features of human plague can be reproduced in guinea-pigs and mice. Monkeys show shock-like signs only during the terminal period of 6–10 h, when they become quiet, progressively weak, prostrate and hypothermic; for the previous 2–4 days infected animals are lively and vigorous. In the terminal stages blood pressure drops rapidly but there is no evidence of oligaemia (low blood volume) caused by haemorrhage, or of oedema, suggesting that vascular collapse must be associated with a vasodilatory factor(s), resulting in pooling of blood. In this respect monkeys differ from humans and guinea-pigs.

The symptoms of plague – high fever and vascular damage – are characteristic of intoxication with endotoxin. However, it is extremely unlikely that endotoxin alone is the main toxin involved in plague. It is much more likely to act in conjunction with one or more other potentially toxic fractions from *Y. pestis*. Plague murine toxin is a protein which, although highly lethal for mice and rats, is relatively nontoxic for guinea-pigs, rabbits, dogs and monkeys. A completely separate guinea-pig toxin complex exists comprising at least two cell wall/membrane protein components, one of which will kill mice, although both are needed to kill guinea-pigs. However, the nature of the toxin or toxins of *Y. pestis* and their role in the human disease syndrome are still far from clear. As has been the case with anthrax, it would be highly valuable to rework the early experimental pathology in the light of the wonderful molecular biological insights we now have of *Yersinia* as outlined in Chs 2 and 3. The classical approach to such a problem is to prepare a specific toxoid and to determine whether injection of this confers immunity to the disease. Needless to say, the severity of human

plague renders such experiments impossible. Until such questions can be answered, we are left to argue whether, in the context of plague, man resembles a mouse, a monkey or a guinea-pig.

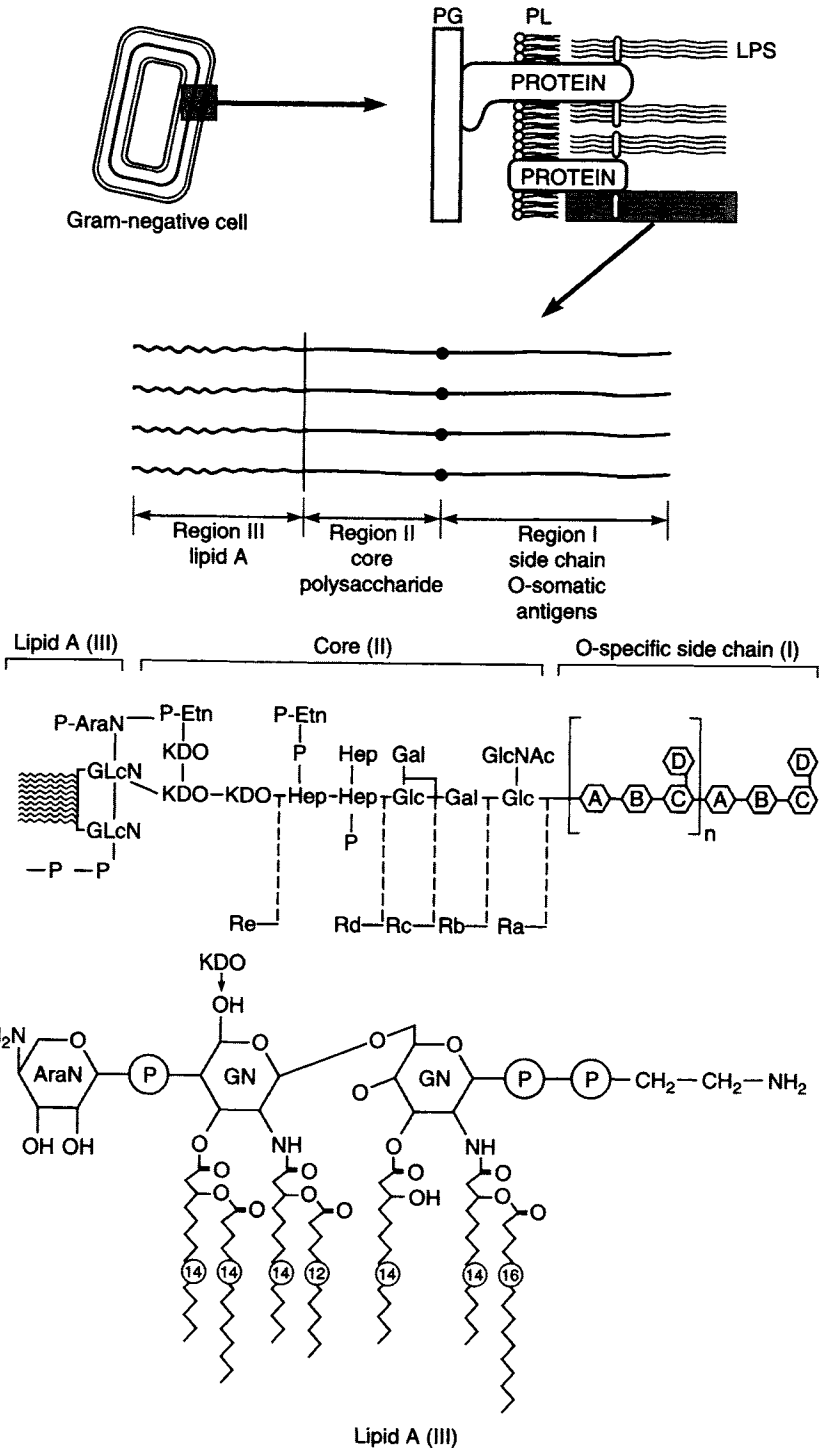
Endotoxins

Endotoxins are part of the outer membrane of Gram-negative bacteria. It has been known for many years that the cells (alive or dead) or cell extracts of a wide variety of Gram-negative bacteria are toxic to man and animals. The literature on this subject is vast, sometimes confusing and often controversial; here we can give no more than a brief outline. Some of the diseases in which endotoxin may play an important role include typhoid fever, tularaemia, plague and brucellosis, and a variety of hospital-acquired infections caused by opportunistic Gram-negative pathogens, which include *Escherichia coli*, *Proteus*, *Pseudomonas aeruginosa*, *Enterobacter*, *Serratia* and *Klebsiella*. In addition, endotoxin has been intensively studied as a possible causative agent of shock arising from postoperative sepsis or other forms of traumatic injury in which the normal flora of the gut is often the source of endotoxin.

The toxins we have considered so far have been protein (or at least part protein) in nature but, in contrast, endotoxin is a complex lipopolysaccharide. It is also much more heat stable than protein toxins and much less easily toxoided. In addition to lethality, endotoxin displays a bewildering array of biological effects.

Location in cell envelope

The complex nature of the multilayered Gram-negative bacterial envelope is shown in Fig. 8.15 (see also Fig. 4.3). The outer membrane is composed of a bimolecular leaflet arrangement as are other membranes but has a different composition from the cytoplasmic membrane. The lipopolysaccharide (LPS) is unique in nature, only found in Gram-negative bacteria, and is, or contains within it, what we designate endotoxin. Immunoelectron microscopy indicates that LPS exists in the outer leaflet of the membrane and extends outward up to 300 nm; it is on, rather than in, the cell. Thus it is evident that the term endotoxin is a misnomer which derives from the era when toxins were considered to be either exotoxins, which were synthesised and secreted by the viable organism, or endotoxins, which were intracellular and released only upon lysis. Moreover, extraction with EDTA shows that approximately 50% of LPS is held noncovalently linked in the membrane. Extraction with a variety of different solvents yields material which is highly heterogenous and of apparent molecular weight $1-20 \times 10^6$. However, treatment with pyridine or addition of detergents reduces the polydispersity. The endotoxic glycolipid from the rough



mutant of *Salmonella minnesota*, R 595, has an M_r of 5900 for the basic unit, from which complex aggregate structures are derived.

Structure

Lipopolysaccharide consists of three regions: polysaccharide side chains, core polysaccharide, and lipid A which consists of a di-glucosamine backbone to which long-chain fatty acids are linked (Fig. 8.15). The relationship of this type of molecule to the outer membrane is also shown in Fig. 8.15. The long-chain fatty acids interdigitate between the phospholipids in the outer leaflet and may also be linked (or interact) with lipoproteins, which in turn may or may not be covalently anchored to the rigid peptidoglycan (PG). The polysaccharide side chains project outwards.

This structure is not invariant. For example, many organisms when first isolated give rise to colonies with a smooth appearance on agar but on subculture produce colonies with a rough appearance. In general, 'smooth' strains of pathogenic species are more virulent than rough strains. This S \rightarrow R conversion is accompanied by a loss of region I side chains, which contain the deoxy and dideoxy sugars found in these LPS complexes. In addition to these somewhat drastic changes involving loss of side chains, it is possible to induce major compositional changes by manipulating the growth rate of these organisms in a chemostat. Thus the LPS of *Salmonella enteritidis*, when grown with a mean generation time of 20 min is nearly totally deficient in tyvelose (a dideoxy sugar), possesses 85% of the galactose and 150% of the glucose contents of LPS obtained when the generation time is 50 min. These genotypic S organisms exhibit an R-phenotype in terms of their vastly reduced O-agglutinability (see below); such observations are potentially very important in the context of the *in vivo* phenotype and pathogenicity, since it is well known that the growth rate of *Salmonella typhimurium* in mice is 10–20 times lower than *in vitro*. Examples have already been given in Ch. 7 of changes in LPS structures *in vivo* in relation to *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Fig. 8.15 General structure of *Salmonella* lipopolysaccharide. See text for fuller explanation. Abbreviations: PG, peptidoglycan; PL, phospholipid; A–D, sugar residues; Glc, D-glucose; Gal, D-galactose; GlcN, D-glucosamine; GlcNAc, N-acetyl-D-glucosamine; Hep, L-glycerol-D-manno-heptose; KDO, 2-keto-3-deoxy-D-manno-octonate; AraN, 4-amino-L-arabinose; P, phosphate; EtN, ethanolamine; ~~~~ hydroxy and nonhydroxy fatty acids; Ra–e, incomplete forms of lipopolysaccharides. The structures indicated are typical of the Enterobacteriaceae and the Pseudomonadaceae. *Haemophilus influenzae*, *Neisseria meningitidis*, *Bordetella pertussis*, *Acinetobacter calcoaceticus* and *Bacteroides fragilis* have less complicated LPS structures in that they do not possess the equivalent of the O-somatic side chains. *Chlamydiae* possess only lipid A and the inner core region comprising lipid A and KDO.

Immunochemistry and seroclassification

The extent to which lipid A is common between different genera is uncertain, but it is not likely to vary tremendously. The core polysaccharide structure is the same or very similar within groups of the Enterobacteriaceae. Thus polysaccharides from salmonellae are similar to each other, but differ from those of *E. coli* strains. However, within a group such as the salmonellae, there is a wide variation in the composition and detailed structures of the side chains, a fact which is exploited in the Kauffman–White scheme for classifying salmonellae, giving rise to several thousand serotypes.

The side chains carry the O-somatic antigen specificities of which there are far more than can readily be accounted for on the basis of the known number of sugars involved in the basic repeating units. In the side chains are found a range of deoxy and dideoxy sugars. The general principles governing the relationship between the various chemotypes and serotypes are now well understood; the multiplicity of antibody specificities evoked may be explained in terms of antibodies which can recognise different aspects of one three-dimensional structure.

Biological properties

There is now direct evidence that lipid A is the primary toxiphore, but the polysaccharide plays an important part in conferring solubility upon, and optimising the size of micellar aggregates of LPS, hence affecting biological activity. However, the immune status of the test animal may affect toxicity: as normal animals produce antibodies to the antigenic determinants on the surface of normal gut organisms (including O-somatic antigens), some of the biological effects of endotoxin may be mediated by hypersensitivity mechanisms.

The range of biological properties of endotoxin is quite bewildering and the mode(s) of action very complicated. Included among those effects which might play a role in Gram-negative bacterial infections are abortion, pyrogenicity, tolerance (not immune tolerance), the Schwartzmann phenomenon, hypotension and shock, and lethality, but the precise part played by LPS in these phenomena in Gram-negative infections is far from clear. LPS causes the release of vasoactive substances, activates the alternative pathway of the complement cascade, and also activates factor XII (Hageman factor), the first step of the coagulation cascade, which sometimes results in disseminated intravascular coagulation (p. 287). Many, perhaps nearly all, the actions of LPS are due to the stimulation of cytokine release from macrophages and other cells. There is an effect on the circulation, leading ultimately to vascular collapse. The vascular regions most affected differ from species to species; in man and sheep the main changes are found in the lungs. LPS has powerful immunological actions, which is surely no accident; as well as activating the comple-

ment system, it induces IL-1 production and is a potent B-cell mitogen. Man is one of the most sensitive of all species to the pyrogenic action of endotoxin. A dose of 2 ng kg^{-1} of body weight injected intravenously into man causes the release of the endogenous pyrogen IL-1 and TNF from macrophages, which act on the hypothalamus to give an elevation of body temperature within an hour. It is possible that the pyrogenic action of LPS helps to generate fever in Gram-negative bacterial infections, but LPS is not the only bacterial factor capable of inducing a febrile response. For example, recall the streptococcal and staphylococcal superantigens discussed earlier in this chapter.

In spite of all these toxic actions, there have been suggestions that some of the responses to LPS (by macrophages, polymorphs) could be advantageous to the host, possibly assisting in the recognition and destruction of bacteria. Could it be that host responses to LPS are, like the complement or the clotting systems, useful in moderation but harmful in excess? There are reports that, when animals with less vigorous responses to LPS are infected, they suffer fewer symptoms, but permit greater growth of bacteria.

Very large numbers of Gram-negative bacteria are normally present in the intestines (see Ch. 2), their continued death and exit in the faeces being balanced by multiplication in the lumen. There is a continuous, inevitable low-grade absorption of endotoxin from the intestine.* Absorbed (endogenous) endotoxin enters the portal circulation and is taken up and degraded by reticuloendothelial cells, mainly Kupffer cells in the liver. Continuous exposure to endotoxin probably has profound effects on the immune system and on the histology of the intestinal mucosa, stimulating development of the immune system in the immature individual, but there are no obvious pathogenic consequences. Normal people have low levels of antibody to endotoxin as a result of this continuous exposure. The sick individual may be much more susceptible to endogenous endotoxin, perhaps because of defects in removal by Kupffer cells.

After trauma or after genito-urinary instrumentation, endotoxin is detectable in peripheral blood but this leads to no particular signs or symptoms. When large amounts of endotoxin enter the blood there are profound effects on blood vessels with peripheral vascular pooling, a drastic fall in blood pressure, collapse and sometimes death. Thus, if enough endotoxin enters the blood during massive Gram-negative bacterial sepsis, the vasomotor action of endotoxin becomes important

* In addition, various antigens are absorbed in small quantities from the intestine, and in normal individuals antibodies are formed against various food proteins and to some extent against resident intestinal bacteria (see Ch. 2). Kupffer cells remove any antigen-antibody complexes formed locally in the intestine and prevent them from entering the systemic circulation.

and shock intervenes.* In experimental animals endotoxin also causes vasodilation and haemorrhage into the intestinal mucosa, and sometimes haemorrhage into the placenta with abortion, but these actions do not appear to be important in all Gram-negative bacterial infections.

To summarise, endotoxin, although studied so carefully and for so long, has not yet been shown to play a definitive role as a toxin in the pathogenesis of any infectious disease. But, in spite of its effects on various host defence systems including polymorphs, lymphocytes, macrophages, complement, and on endothelial cells and platelets, its overall role in *infection* is still not clear. It can, however, cause shock when Gram-negative bacteria invade the blood. It is for this reason that considerable effort in recent years has gone into the development of antilipid A antibodies for use as therapeutic agents to combat shock in such situations; the success rate is only partial and the expense enormous. For that reason several groups are seeking to exploit the wealth of chemical and biophysical information available on LPS in attempts to develop synthetic derivatives which would neutralise the biological activity of lipid A. We await the outcome of such research. However, the characteristics of the O-antigen polysaccharide are sometimes important in determining virulence: certain chemotypes are important in resisting phagocytosis.

General observations on toxins

Considerable space has been given to toxins because they are being intensively investigated as possible virulence determinants. The account illustrates the complexity of host-microbe interactions when analysed at the molecular level. Most toxins are liberated from the microbial cell and can be studied with greater facility than many of the more elusive determinants of pathogenicity. But remember that microbes that replicate inside host cells are less likely to form powerful toxins because they cannot afford to damage at too early a stage the cell in which they are multiplying. Thus, toxins are not prominent products in intracellular infections due to mycobacteria, *Brucella*, *Rickettsiae*, *Mycoplasma* or *Chlamydia*, and viruses do not form toxins.

Although a single molecule of a toxin-like diphtheria toxin is enough to kill a cell, other toxins may do no more than impair cell function when present in sublethal concentrations. This can lead, for instance, to defective function in immune or phagocytic cells. Low concentrations of the streptococcal streptolysins will inhibit leucocyte chemotaxis. At even lower concentrations the toxins can be potent inducers of

* It must be remembered that endotoxin is only one of the pathways to shock in infectious diseases. Shock is also seen for instance in leptospiral and rickettsial infections, in gas gangrene, and in sepsis due to Gram-positive bacteria (see above).

cytokines. The pneumolysin of *S. pneumoniae* and anthrax lethal toxin make monocytes release IL-1 and TNF- α at 10^{-15} and 10^{-18} molar concentrations, respectively.

The ability to form toxins, whether encoded by plasmids or the microbial genome, is subject to selective forces. If toxin production puts a microorganism at a serious disadvantage, it will tend to disappear. If it is advantageous it will be maintained, and will spread through the microbial population, just as the genetic changes that confer resistance to antimicrobial drugs are selected for when these drugs are widely used. It is therefore not unreasonable to ask how many of the well-known toxins are actually useful to the microbe as well as being important in causing disease in the host (Table 8.4). However, microbes that multiply extracellularly must produce a variety of enzymes and other molecules involved in nutrition, adherence to substratum, and so on. In the case of free-living microbes, these substances, as well as substances that damage or interfere with competing organisms, are of major importance. Probably they cannot all be discarded when the parasitic mode of life is adopted. Many will have a toxic action. Yet, for the infecting microbe, these substances remain as unfortunate necessities, of no particular advantage and perhaps a disadvantage, in the parasitic way of life.

Indirect Damage via Inflammation

In infectious diseases there is nearly always a certain amount of direct microbial damage to host tissues, as discussed above. Host cells are destroyed or blood vessels injured as a direct result of the action of microbes or their toxins. Blood vessel injuries account for much of the disease picture in rickettsial infections (see above). Inflammatory materials are liberated from necrotic cells, whatever the cause of the necrosis. Also many bacteria themselves liberate inflammatory products and certain viruses cause living infected cells to release inflammatory mediators. Therefore it is not always clear how much of the inflammation is directly microbial rather than host in origin.* But inevitably the host (see Ch. 3) generates inflammatory and other tissue responses, and these responses sometimes account for the greater part of the tissue changes. Pathological changes can then be regarded as occurring indirectly as a result of these responses to the infection. Inflammation causes redness, swelling, pain and sometimes loss of

* For instance, peptidoglycan of *H. influenzae* type b causes acute inflammation when introduced into the cerebrospinal fluid of adult rats. Probably much of this is caused by inflammatory mediators from the host; TNF- α is detectable in cerebrospinal fluid of most cases of purulent bacterial meningitis in humans and there are raised concentrations in the serum of patients with *P. falciparum* malaria.

Table 8.4. Examples of possible usefulness of toxins to microorganisms

| Microorganism | Toxin | Disease production by toxin | Value of toxin to microorganism |
|---|---|--|---|
| <i>C. diphtheriae</i> | Diphtheria toxin | Epithelial necrosis Heart damage Nerve paralysis | Epithelial cell and polymorph destruction assists colonisation |
| <i>Clostridium tetani</i> <i>Clostridium botulinum</i> | Tetanus toxin Neurotoxin | Muscle spasm, lockjaw Paralysis | } Could killing the host be worthwhile? A dead, putrefying corpse is a fine growth medium for these anaerobic, basically saprophytic bacteria |
| <i>Shigella</i> spp. | Shiga toxin | Exacerbates diarrhoea, dysentery Neurological effects | |
| <i>V. cholerae</i> | Cholera toxin | Diarrhoea | Diarrhoea aids transmission |
| <i>B. anthracis</i> | Anthrax toxin(s) | Oedema, haemorrhage Circulatory collapse | Kills phagocytes. Also a dead host, teeming with spores, can be a good source of infection |
| <i>Legionella pneumophila</i> | Proteases etc. | Contribute to lung pathology | Possible role in resisting phagocytic destruction by free-living amoebae |
| <i>Staphylococcus pyogenes</i> | TSSS-1 Enterotoxins | Toxic shock Diarrhoea, vomiting | } All are powerful T-cell mitogens (superantigens, see Chs 7 and 8). Possible role in diverting T cells from antibacterial activity |
| <i>Streptococcus pyogenes</i> | 'Erythrogenic toxin' (SPEA) | Scarlet fever | |
| <i>Pseudomonas aeruginosa</i> | Exotoxin A Proteases, elastase, etc. | Various clinical diseases | Possible role in free-living existence |
| <i>Bordetella pertussis</i> | Pertussigen | Whooping cough | Cough aids transmission; interferes with T-cell migrations? |
| <i>Streptococcus pneumoniae</i> | Pneumolysin | Promotes bacteraemia Sensorineural deafness | Weakens host defences (polymorphs, complement) |
| <i>Yersinia pestis</i> | Endotoxin Other toxins | Severe systemic disease | Kills phagocytes |
| Various Gram-negative bacteria | Endotoxin | Contributes to disease, septic shock | LPS acts as B-cell mitogen. Possible role in diverting B cells |

function of the affected part (see Ch. 6), and is generally a major cause of the signs and symptoms of disease. Indirect damage attributable to the host immune response is discussed separately below. In most diseases direct and indirect types of damage both make a contribution to pathological changes, but in a given disease one or the other may be the most important.

In a staphylococcal abscess the bacteria produce inflammatory materials, but they also kill infiltrating polymorphs whose lysosomal enzymes are thereby liberated and induce further inflammation. This type of indirect nonimmunological damage is sometimes important in streptococcal infections. Virulent streptococci produce various toxins that damage phagocytes, and also bear on their surfaces substances that impede phagocytosis (see Ch. 4). Nevertheless, with the help of antibody, all streptococci are eventually phagocytosed and killed and the infection terminated. Unlike the staphylococci, however, killed group A streptococci pose a digestive problem for phagocytic cells. The peptidoglycan component of the streptococcal cell wall is very resistant to digestion by lysosomal enzymes. When streptococci are injected into the skin of a rabbit, for instance, streptococcal peptidoglycans persist in macrophages for as long as 146 days. Hence macrophages laden with indigestible streptococcal cell walls tend to accumulate in sites of infection. Lysosomal enzymes, including collagenase, leak from these macrophages, causing local destruction of collagen fibres and the connective tissue matrix. Macrophages secrete many other substances some of which may contribute to cell and tissue damage (see also pp. 95–96). Many macrophages eventually die or form giant cells, sometimes giving rise to granulomatous lesions (see p. 322). In this way persistent streptococcal materials sometimes cause chronic inflammatory lesions in the infected host. An additional immunopathological contribution to the lesions is to be expected if the host is sensitised to peptidoglycan components. Other pathogenic microorganisms that are digested with difficulty by phagocytes include *Listeria*, *Shigella*, *Candida albicans* and, of course, *Mycobacteria*, but the importance of this in the pathogenesis of disease is not generally clear.

Indirect Damage via the Immune Response (Immunopathology)

The expression of the immune response necessarily involves a certain amount of inflammation, cell infiltration, lymph node swelling, even tissue destruction, as described in Ch. 6. Such changes caused by the immune response are classed as immunopathological. Sometimes they are very severe, leading to serious disease or death, but at other times they play a minimal part in the pathogenesis of disease. With the possible exception of certain vertically transmitted virus infections and the

transmissible 'prion' dementias (see Ch. 10), there are signs of an immune response in all infections. Therefore it is to be expected that there will nearly always be some contribution of the immune response to pathological changes.* Often the immunological contribution is small, but sometimes it forms a major part of the disease. For instance, in tuberculosis the pathological picture is dominated by the operation of a strong and persistent cell-mediated immunity (CMI) response to the invading bacillus. In the classical tubercle a central zone of bacilli with large mononuclear and giant cells, often with some necrosis, is surrounded by fibroblasts and lymphocytes. Mononuclear infiltrations, giant cells and granulomatous lesions (see p. 322) are characteristic pathological features of tuberculosis. When macrophages are killed by intracellular mycobacteria the lysosomal enzymes and other materials released from the degenerating cell contribute to chronic inflammation as in the case of the streptococcal lesions referred to above. There are no recognised toxins formed by tubercle bacilli, and there seems to be no single antigen or other component that accounts for virulence. Bacterial glycolipids (e.g. 'cord factor'), resistance to H_2O_2 (see pp. 91–92) and ability to utilise host Fe (see p. 387) have been correlated with pathogenicity, and inhibition of phagosome–lysosome fusion in macrophages (see p. 106) by release of unidentified bacterial components would also contribute to pathogenicity. However, none of these factors is by itself absolutely necessary for virulence, which in such a complex, ancient parasite is likely to be multifactorial. Now that the genome of *M. tuberculosis* has been completely sequenced, there will be opportunities for clearer definition of virulence determinants.

The mere enlargement of lymphoid organs during infectious diseases is a morphological change that can often be regarded as pathological. The lymph node swelling seen in glandular fever, for instance, is an immunopathological feature of the disease, and the same can be said of the striking enlargement of the spleen caused by chronic malaria and other infections in the condition known as tropical splenomegaly.

As often as not the relative importance of direct microbial damage as opposed to immune and nonimmune inflammatory reactions have not yet been determined, but the picture is clearer in most of the examples given below.

In one important human disease, pathological changes are certainly immunopathological in nature, but not enough is known about it to classify the type of reaction (see Table 8.5). This disease is rheumatic fever, which follows group A streptococcal infections of the throat. It is the commonest form of heart disease in many developing countries,

* A number of different microbial antigens are produced during most infections (see Ch. 6) and the possible immunological reactions are therefore numerous. For instance, at least 18 types of circulating malarial antigen are found in heavily infected individuals.

Table 8.5. Immunopathological reactions and infectious diseases

| Reaction | Mechanism | Result | Example from infectious disease |
|-----------------------------------|--|--|--|
| Type 1 Anaphylactic | Antigen + IgE antibody attached to mast cells → histamine, etc. release | Anaphylactic shock Bronchospasm Local inflammation | Contribution to certain rashes? Helminth infections |
| Type 2 Cytotoxic | Antibody + antigen on cell surface → complement activation or ADCC | Lysis of cell bearing microbial antigens | Liver cell necrosis in hepatitis B? |
| Type 3 Immune complex | Antibody + extracellular antigen → complex | <i>Extravascular complex</i> Inflammation ± tissue damage <i>Intravascular complex</i> Complex deposition in glomeruli, joints, small skin vessels, choroid plexus → glomerulonephritis, vasculitis, etc. | Allergic alveolitis Glomerulonephritis in LCM virus infection (mice) or malaria (man) Prodromal rashes Fever |
| Type 4 Cell-mediated (delayed) | Sensitised T lymphocyte reacts with antigen; lymphokines liberated; cytotoxicity triggered | <i>Extracellular antigen</i> Inflammation, mononuclear accumulation, macrophage activation Tissue damage <i>Antigen on tissue cell</i> T lymphocyte lyses cell | Acute LCM virus disease in mice Certain virus rashes Tuberculosis, leprosy (granulomas) An <i>in vitro</i> classic, but difficult to demonstrate <i>in vivo</i> |

where it currently affects 30 million children. Antibodies formed against a streptococcal cell wall or membrane component also react with the patient's heart muscle or valves, and myocarditis develops a few weeks later. Many strains of streptococci have antigens that cross-react with the heart, and repeated infections with different streptococci cause recurrent attacks of rheumatic fever. There is genetic predisposition to the disease, based either on a particular antigen present in the heart of the patient or on a particular type of antibody response. Chorea, a disease of the central nervous system, is a rare complication of streptococcal infection and antistreptococcal antibodies have been shown to react with neurons in the caudate and subthalamic nuclei of the brain.

A number of microorganisms have antigens similar to host tissue components (p. 187) so that in the course of responding immunologically to such infections the host is vulnerable to autoimmune damage (see ankylosing spondylitis, p. 220). The antibodies to host components such as DNA, IgG, myofibrils, erythrocytes, etc. that are seen in trypanosomiasis, *Mycoplasma pneumoniae*, and Epstein–Barr virus infections appear to result from polyclonal activation of B cells (see p. 199). It is not clear how important these autoimmune responses are in pathogenesis, but they reflect fundamental disturbances in immunoregulation.

Four types of immunopathology can be distinguished according to the classification of allergic reactions by Coombs and Gell, and microbial immunopathology will be described under these headings (see Table 8.5).

Type 1: anaphylactic reactions

These depend on the reactions of antigens with reaginic (IgE) antibodies attached to mast cells via the latter's Fc receptors. The reaction takes place mostly at the body surfaces, resulting in the release of histamine, eosinophil and neutrophil chemotactic factors, leukotrienes (see p. 76) and heparin from mast cells, and the activation of serotonin and plasma kinins. If the antigen–antibody interaction takes place on a large enough scale in the tissue, the histamine that is released can give rise to anaphylactic shock, the exact features depending on the sensitivity and particular reaction of the species of animal to histamine. Guinea-pigs suffer from bronchospasm and asphyxia, and in man there are similar symptoms, sometimes with a fall in blood pressure and shock. This type of immunopathology, although accounting for anaphylactic reactions to horse serum or to penicillin, is not important in infectious diseases. When the antigen–IgE antibody interaction takes place at the body surface there are local inflammatory events, giving rise to urticaria in the skin, and hay fever or asthma in the respiratory tract. This local type of anaphylaxis may play a part in the pathogenesis of virus infections of the upper respiratory tract (e.g. common cold, respiratory syncytial virus infections of infants), or in skin rashes in infectious diseases.

Type 1 reactions are common in helminth infections perhaps because IgE antibodies have an important role in protection against these parasites. The IgE–antigen reaction, by causing inflammation, summons up from the blood antimicrobial forces such as polymorphs, antibodies, and complement components. A dramatic Type 1 reaction can follow rupture of a hydatid cyst of *Echinococcus granulosus* (the dog tapeworm). Slow leakage of worm antigens means that mast cells are sensitised with specific IgE antibody, and the sudden release of antigen can cause life-threatening anaphylaxis. When the larvae of *Ascaris lumbricoides*

coides pass through the lung on their journey from blood to intestine, they can give rise to IgE-mediated respiratory symptoms, with infiltration of eosinophils.

Type 2: cytolytic or cytotoxic reactions

Reactions of this type occur when antibody combines with antigen on the surface of a tissue cell, and either activates the complement sequence whose membrane attack complex (see p. 177) kills the cell, or triggers cytotoxicity by K cells (NK cells or phagocytes with Fc receptors). K (killer) cell cytotoxicity is referred to as antibody-dependent cellular cytotoxicity (ADCC). The antibody-coated cell is destroyed. As discussed in Ch. 6, the same reaction on the surface of a microorganism (e.g. enveloped virus) constitutes an important part of antimicrobial defences, often leading to the destruction of the microorganism. Cells infected with viruses and bearing viral antigens on their surface are destroyed in a similar way.

Clearly the antibody-mediated destruction of infected cells means tissue damage, and it perhaps accounts for some of the liver necrosis in hepatitis B, for instance, and probably in yellow fever. Infected cells can also be destroyed by sensitised lymphocytes or NK cells independently of antibody (see below).

In certain infections antibodies are formed against host erythrocytes and these cells are particularly sensitive to lysis. The haemolysis in malaria is caused by antibodies to parasite-derived antigens that have attached to red cells, rather than by autoantibodies to red cells themselves. In pneumonia due to *Mycoplasma pneumoniae* (atypical pneumonia), antibodies (cold agglutinins) are formed against normal human group O erythrocytes. Haemolytic anaemia is occasionally seen, and there is reticulocytosis (see Glossary) in 64% of patients. The lesions in the lungs are perhaps based on cell-mediated immunopathological reactions.

Type 3: immune complex reactions

The combination of antibody with antigen is an important event, initiating inflammatory phenomena that are inevitably involved in the expression of the immune response. In the infected host, these inflammatory phenomena are most of the time of great antimicrobial value (see Ch. 6). But there are nevertheless immunopathological features of the infection, and immune complex reactions sometimes do a great deal of damage in the infected individual. The mechanisms by which antigen-antibody reactions cause inflammation and tissue damage are outlined in Fig. 8.16. IgA immune complexes are generally less harmful. Antigens absorbed from the intestine can combine locally with IgA

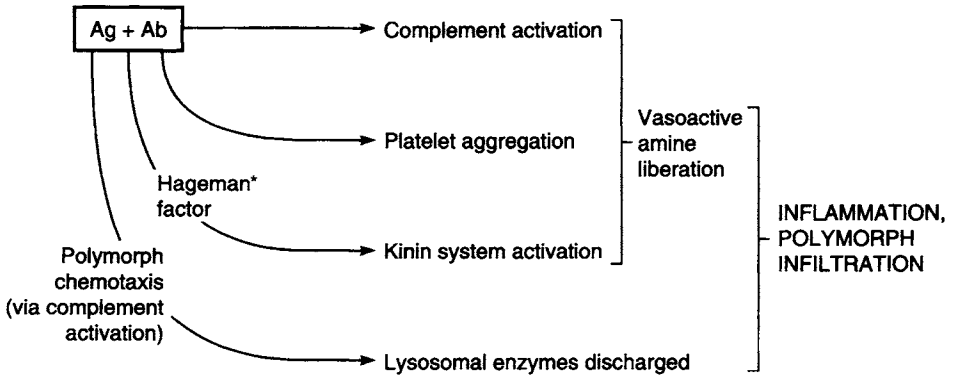


Fig. 8.16 Mechanisms of inflammation and tissue damage induced by antigen-antibody reactions. *Activation of Hageman factor can lead to blood coagulation.

antibody and the complex then enters the blood, to be filtered out in the liver and excreted harmlessly in bile (see p. 160).

When the antigen-antibody reaction takes place in extravascular tissues, there is inflammation and oedema with infiltration of polymorphs. If soluble antigen is injected intradermally into an individual with large amounts of circulating IgG antibody, the antigen-antibody reaction takes place in the walls of skin blood vessels, and causes an inflammatory response. The extravasating polymorphs degenerate and their lysosomal enzymes cause extensive vascular damage. This is the classical Arthus response. Antigen-antibody reactions in tissues are not usually as serious as this, and milder inflammatory sequelae are more common as in the case of allergic alveolitis (see below).

Glomerulonephritis and vasculitis

When the antigen-antibody reaction takes place in the blood to give circulating immune complexes, the sequelae depend to a large extent on size and on the relative proportions of antigen and antibody. If there is a large excess of antibody, each antigen molecule is covered with antibody and is removed rapidly by reticuloendothelial cells, which have receptors for the Fc portion of the antibody molecule (see Ch. 4). When equal amounts of antigen and antibody combine, lattice structures are produced, and these form large aggregates whose size ensures that they are also rapidly removed by reticuloendothelial cells. If, however, complexes are formed in antigen excess, the poorly coated antigen molecules are not removed by reticuloendothelial cells. They continue to circulate in the blood and have the opportunity to localise in small blood vessels elsewhere in the body. The mechanism is not clear, but complexes are deposited in the glomeruli of the kidneys, the

choroid plexuses, joints and ciliary body of the eye. Factors may include local high blood pressure and turbulent flow (glomeruli), or the filtering function of the vessels involved (choroid plexus, ciliary body). In the glomeruli the complexes pass through the endothelial windows (Fig. 8.17) and come to lie beneath the basement membrane. The smallest-sized complexes pass through the basement membrane and seem to enter the urine. This is probably the normal mechanism of disposal of such complexes from the body.

Immune complexes are formed in many, perhaps most, acute infectious diseases. Microbial antigens commonly circulate in the blood in viral, bacterial, fungal, protozoal, rickettsial, etc. infections. When the immune response has been generated and the first trickle of specific antibody enters the blood, immune complexes are formed in antigen excess. This is generally a transitional stage soon giving rise to antibody excess, as more and more antibody enters the blood and the

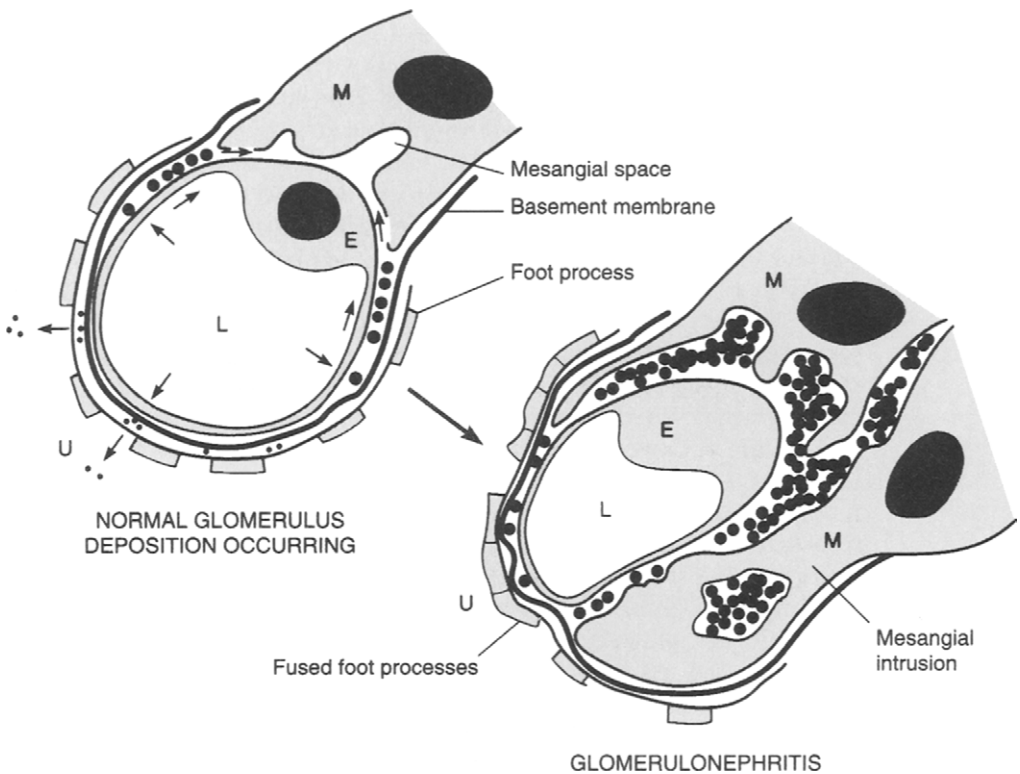


Fig. 8.17 Immune complex glomerulonephritis. Arrows indicate the movement of immune complex deposits, some moving through to the urine and others (larger deposits) being retained. M, mesangial cell; U, urinary space; L, lumen of glomerular capillary; E, endothelial cell (contains 100 nm pores or windows; see Fig. 3.2b).

infection is terminated. Sometimes the localisation of immune complexes and complement in kidney glomeruli* is associated with a local inflammatory response after complement activation. There is an infiltration of polymorphs, swelling of the glomerular basement membrane, loss of albumin, even red blood cells, in the urine and the patient has acute glomerulonephritis. This is seen following streptococcal infections, mainly in children (see below). As complexes cease to be formed the changes are reversed, and complete recovery is the rule. Repeated attacks or persistent deposition of complexes leads to irreversible damage, often with proliferation of epithelial cells following the seepage of fibrin into the urinary space.

Under certain circumstances complexes continue to be formed in the blood and deposited subendothelially for long periods. This happens in certain persistent microbial infections in which microbial antigens are continuously released into the blood but antibody responses are only minimal or of poor quality (see below). Complexes are deposited in glomeruli over the course of weeks, months or even years. The normal mechanisms for removal are inadequate. The deposits, particularly larger complexes containing high molecular weight antigens or antibodies (IgM) are held up at the basement membrane and accumulate in the subendothelial space together with the complement components. As deposition continues, they gradually move through to the mesangial space (Fig. 8.17) where they form larger aggregates. Mesangial cells, one of whose functions is to deal with such materials, enlarge, multiply and extend into the subepithelial space. If these changes are gradual there are no inflammatory changes, but the structure of the basement membrane alters, allowing proteins to leak through into the urine. Later the filtering function of the glomerulus becomes progressively impaired. In the first place the glomerular capillary is narrowed by the mesangial cell intrusion. Also, the filtering area is itself blocked by the mesangial cell intrusion, by the accumulation of complexes (Fig. 8.17), and by alterations in the structure of the basement membrane. The foot processes of epithelial cells tend to fuse and further interfere with filtration. The pathological processes continue, some glomeruli ceasing to produce urine, and the individual has chronic glomerulonephritis.

Circulating immune complex deposition in joints leads to joint swelling and inflammation but in choroid plexuses there are no apparent pathological sequelae. Circulating immune complexes are also deposited in the walls of small blood vessels in the skin and elsewhere, where they may induce inflammatory changes. The prodromal rashes seen in exanthematous virus infections and in hepatitis B are probably caused in this way. If the vascular changes are more marked, they give rise to the condition called erythema nodosum, in which there

* Cells in kidney glomeruli, in joint synovium and in choroid plexuses bear Fc or C3b receptors. This would favour localisation in these tissues.

are tender red nodules in the skin, with deposits of antigen, antibody and complement in vessel walls. Erythema nodosum is seen following streptococcal infections and during the treatment of patients with leprosy. When small arteries are severely affected, for instance in some patients with hepatitis B, this gives rise to periarteritis nodosa.

Immune complex glomerulonephritis occurs as an indirect immunopathological sequel to a variety of infections. First there are certain virus infections of animals. The antibodies formed in virus infections generally neutralise any free virus particles, thus terminating the infection (see Ch. 6), but the infection must persist if antigen is to continue to be released into the blood and immune complexes formed over long periods. Non-neutralising antibodies help promote virus persistence because they combine specifically with virus particles, fail to render them noninfectious, and at the same time block the action of any good neutralising antibodies that may be present. Immune complexes in antigen excess are formed in the blood when the persistent virus or its antigens circulates in the plasma and reacts with antibody which is present in relatively small amounts. Virus infections with these characteristics are included in Table 8.6. In each instance complexes are deposited in kidney glomeruli and sometimes in other blood vessels as described above. In some there are few if any pathological changes (LDV and leukaemia viruses in mice) probably because there is a slow rate of immune complex deposition, whereas in others glomerulonephritis (LCM virus in mice, ADV in mink) or vasculitis (ADV in mink) is severe.

A persistent virus infection that induces a feeble immune response forms an ideal background for the development of immune complex glomerulonephritis, but there are no known viral examples in man.

Table 8.6. The deposition of circulating immune complexes in infectious diseases

| Microbe | Host | Kidney deposits | Glomerulonephritis | Vascular deposits |
|--|------------|-----------------|--------------------|-------------------|
| Leukaemia virus | Mouse, cat | + | ± | — |
| Lactate dehydrogenase virus (LDV) | Mouse | + | ± | — |
| Lymphocytic choriomeningitis virus (LCM) | Mouse | ++ | + | ± |
| Aleutian disease virus (ADV) | Mink | + | + | ++ |
| Equine infectious anaemia virus | Horse | + | + | + |
| Hepatitis B virus | Man | + | — | + |
| <i>Streptococcus pyogenes</i> | Man | + | + | — |
| Malaria (nephritic syndrome) | Man | + | + | — |
| <i>Treponema pallidum</i> (nephritic syndrome in secondary syphilis) | Man | + | + | ? |
| Infectious causes of chronic glomerulonephritis ^a | Man | ++ | ++ | — |

^a Nephrologists and pathologists distinguish ten different types of glomerulonephritis, some of them infectious in origin, the immune complexes being deposited directly from blood or formed locally in glomeruli.

There are one or two other microorganisms that occasionally cause this type of glomerulonephritis, and it is seen, for instance, in chronic quartan malaria and sometimes in infective endocarditis. In both these examples microbial antigens circulate in the blood for long periods. However, immune complex deposition does not necessarily lead to the development of glomerulonephritis, and immune complexes are detectable in the glomeruli of most normal mice and monkeys. Even in persistent virus infections the rate of deposition may be too slow to cause pathological changes as with LDV and leukaemia virus infections of mice (see Table 8.5). During the acute stage of hepatitis B in man, when antibodies are first formed against excess circulating viral antigen (hepatitis B surface antigen), immune complexes are formed and deposited in glomeruli. However, the deposition is short-lived and there is no glomerulonephritis. Persistent carriers of the antigen do not generally develop glomerulonephritis, because their antibody is usually directed against the 'core' antigen of the virus particle, rather than against the large amounts of circulating hepatitis B surface antigen.

Immune complex glomerulonephritis occurs in man as an important complication of streptococcal infection, but this is usually acute in nature with complement activation and inflammation of glomeruli, as referred to above. Antibodies formed against an unknown component of the streptococcus react with circulating streptococcal antigen, perhaps also with a circulating host antigen, and immune complexes are deposited in glomeruli. Streptococcal antibodies cross-reacting with the glomerular basement membrane or with streptococcal antigen trapped in the basement membrane may contribute to the picture. Deposition of complexes continues after the infection is terminated, and glomerulonephritis develops a week or two later. The streptococcal infection may be of the throat or skin, and *Streptococcus pyogenes* types 12 and 49 are frequently involved.

Kidney failure in man is commonly due to chronic glomerulonephritis, and this is often of the immune complex type, but the antigens, if they are microbial, have not yet been identified. It is possible that the process begins when antigen on its own localises in glomeruli, circulating antibody combining with it at a later stage. The antibody is often IgA ('IgA nephropathy') which could be explained as follows. Antigen in intestinal or respiratory tract combines locally with IgA, and the complex enters the blood. Here, for unknown reasons, it is not removed in the normal way by the liver, and thus has the opportunity to localise in glomeruli.

Allergic alveolitis

When certain antigens are inhaled by sensitised individuals and the antigen reaches the terminal divisions of the lung, there is a local

antigen–antibody reaction with formation of immune complexes. The resulting inflammation and cell infiltration causes wheezing and respiratory distress, and the condition is called allergic alveolitis. Persistent inhalation of the specific antigen leads to chronic pathological changes with fibrosis and respiratory disease. Exposure to the antigen must be by inhalation; when the same antigen is injected intradermally, there is an Arthus type reaction (see p. 282), and IgG rather than IgE antibodies are involved.

There are a number of microorganisms that cause allergic alveolitis. Most of these are fungi. A disease called farmer's lung occurs in farm workers repeatedly exposed to mouldy hay containing the actinomycete *Micromonospora faeni*. Cows suffer from the same condition. A fungus contaminating the bark of the maple tree causes a similar disease (maple bark stripper's disease) in workers in the USA employed in the extraction of maple syrup. The mild respiratory symptoms occasionally reported after respiratory exposure of sensitised individuals to tuberculosis doubtless have the same immunopathological basis.

Other immune complex effects

In addition to their local effects, antigen–antibody complexes generate systemic reactions. For instance, the fever that occurs at the end of the incubation period of many virus infections is probably attributable to a large-scale interaction of antibodies with viral antigen, although extensive CMI reactions can also cause fever. The febrile response is mediated by endogenous pyrogen IL-1 and TNF liberated from polymorphs and macrophages, as described on p. 329. Probably the characteristic subjective sensations of illness and some of the 'toxic' features of virus diseases are also caused by immune reactions and liberation of cytokines.

Systemic immune complex reactions taking place during infectious diseases very occasionally give rise to a serious condition known as disseminated intravascular coagulation. This is seen sometimes in severe generalised infections such as Gram-negative septicaemia, meningococcal septicaemia, plague, yellow fever and fevers due to hantaviruses (see Table A.5). Immune complex reactions activate the enzymes of the coagulation cascade (Fig. 8.16), leading to histamine release and increased vascular permeability. Fibrin is formed and is deposited in blood vessels in the kidneys, lungs, adrenals and pituitary. This causes multiple thromboses with infarcts, and there are also scattered haemorrhages because of the depletion of platelets, prothrombin, fibrinogen, etc. Systemic immune complex reactions were once thought to form the basis for dengue haemorrhagic fever. This disease is seen in parts of the world where dengue is endemic, individuals immune to one type of dengue becoming infected with a related strain of virus. They

are not protected against the second virus, although it shows immunological cross-reactions with the first one. Indeed the dengue-specific antibodies enhance infection of susceptible mononuclear cells, so that larger amounts of viral antigen are produced (see p. 173). It was thought that after virus replication, viral antigens in the blood reacted massively with antibody to cause an often lethal disease with haemorrhages, shock and vascular collapse. However, it has proved difficult to demonstrate this pathophysiological sequence, and the role of circulating immune complexes and platelet depletion remains unclear. Perhaps in this and in some of the other viral haemorrhagic fevers the virus multiplies in capillary endothelial cells. Disease seems due to cytokines liberated from infected mononuclear cells.

Immune complex immunopathology is probable in various other infectious diseases. For instance, the occurrence of fever, polyarthritides, skin rashes and kidney damage (proteinuria) in meningococcal meningitis and gonococcal septicaemia indicates immune complex deposition. Circulating immune complexes are present in these conditions. Certain African arthropod-borne viruses with exotic names (Chikungunya, O'nyong-nyong) cause illnesses characterised by fever, arthralgia and itchy rashes, and this too sounds as if it is immune complex in origin. Immune complexes perhaps play a part in the oedema and vasculitis of trypanosomiasis and in the rashes of secondary syphilis.

Sensitive immunological techniques are available for the detection of circulating complexes and for the identification of the antigens and antibodies in deposited complexes. The full application of these techniques will perhaps solve the problem of the aetiology of chronic glomerulonephritis in man.

Type 4: cell-mediated reactions

Although antibodies often protect without causing damage the mere expression of a CMI response involves inflammation, lymphocyte infiltration, macrophage accumulation and macrophage activation as described in Ch. 6. The CMI response by itself causes pathological changes, and cytokines such as TNF play an important part. This can be demonstrated, as a delayed hypersensitivity reaction by injecting tuberculin into the skin of a sensitised individual. The CMI response to infection dominates the pathological picture in tuberculosis, with mononuclear infiltration, degeneration of parasitised macrophages, and the formation of giant cells as central features. These features of the tissue response result in the formation of granulomas (see Glossary) which reflect chronic infection and accompanying inflammation. There is a ding-dong battle as the host attempts to contain and control infection with a microorganism that is hard to eliminate. The

granulomas represent chronic CMI responses to antigens released locally. Various other chronic microbial and parasitic diseases have granulomas as characteristic pathological features. These include chlamydial (lymphogranuloma inguinale), bacterial (syphilis, leprosy, actinomycosis), and fungal infections (coccidiomycosis). Antigens that are disposed of with difficulty in the body are more likely to be important inducers of granulomas. Thus, although mannan is the dominant antigen of *Candida albicans*, glucan is more resistant to breakdown in macrophages and is responsible for chronic inflammatory responses.

The lymphocytes and macrophages that accumulate in CMI responses also cause pathological changes by destroying host cells. Cells infected with viruses and bearing viral antigens on their surface are targets for CMI responses as described in Chs 6 and 9. Infected cells, even if they are perfectly healthy, are destroyed by the direct action of sensitised T lymphocytes, which are demonstrable in many viral infections. In spite of the fact that the *in vitro* test system so clearly displays the immunopathological potential of cytotoxic T cells, this is not easy to evaluate in the infected host. It may contribute to the tissue damage seen, for instance, in hepatitis B infection and in many herpes and poxvirus infections. In glandular fever, cytotoxic T cells react against Epstein–Barr virus-infected B cells to unleash an immunological civil war that is especially severe in adolescents and young adults. Antigens from *Trypanosoma cruzi* are known to be adsorbed to uninfected host cells, raising the possibility of autoimmune damage in Chagas' disease, caused by this parasite.* It is also becoming clear that cells infected with certain protozoa (e.g. *Theileria parva* in bovine lymphocytes) have parasite antigens on their surface and are susceptible to this type of destruction. Little is known about intracellular bacteria.

The most clearly worked out example of type 4 (CMI) immunopathology is seen in LCM virus infection of adult mice. When virus is injected intracerebrally into adult mice, it grows in the meninges, ependyma and choroid plexus epithelium, but the infected cells do not show the slightest sign of damage or dysfunction. After 7–10 days, however, the mouse develops severe meningitis with submeningeal and subependymal oedema, and dies. The illness can be completely prevented by adequate immunosuppression, and the lesions are attributable to the mouse's own vigorous CD8⁺ T-cell response to infected cells.

* Chagas' disease, common in Brazil, affects 12 million people, and is transmitted by blood-sucking bugs. After spreading through the body during the acute infection, the parasitaemia falls to a low level and there is no clinical disease. Years later a poorly understood chronic disease appears, involving heart and intestinal tract, which contain only small numbers of the parasite but show a loss of autonomic ganglion cells. An autoimmune mechanism is possible (see p. 188), because a monoclonal antibody to *T. cruzi* has been obtained that cross-reacts with mammalian neurons.

These cells present processed LCM viral peptides on their surface in conjunction with MHC I proteins, and sensitised CD8⁺ T cells, after entering the cerebrospinal fluid and encountering the infected cells, generate the inflammatory response and interference with normal neural function that cause the disease. The same cells destroy infected tissue cells *in vitro*, but tissue destruction is not a feature of the neurological disease. In this disease the CD8⁺ T cells probably act by liberating inflammatory cytokines. It may be noted that the brain is uniquely vulnerable to inflammation and oedema, as pointed out earlier in this chapter. The infected mouse shows the same type of lesions in scattered foci of infection in the liver and elsewhere, but they are not a cause of sickness or death. LCM infection of mice is a classical example of immunopathology in which death itself is entirely due to the cell-mediated immune response of the infected individual. This response, although apparently irrelevant and harmful, is nevertheless an 'attempt' to do the right thing. It has been shown that immune T cells effectively inhibit LCM viral growth in infected organs. However, a response that in most extraneural sites would be useful and appropriate turns out to be self-destructive when it takes place in the central nervous system.

Another type of T cell-mediated immune pathology is illustrated by influenza virus infection of the mouse. When inoculated intranasally, the virus infects the lungs and causes a fatal pneumonia in which the airspaces fill up with fluid and cells. The reaction is massive and the lungs almost double in weight. Effectively the animal drowns. The cause is an influx of virus-specific CD8⁺ T cells. Normally when an appropriate number of T cells had entered the lungs, the T cells would issue a feedback response to prevent such overaccumulation, but it is thought that influenza virus infects the T cells and inhibits this control process, so that the lungs are eventually overwhelmed. The virus does not multiply in or kill the infected T cells, and it is presumed that it undergoes limited gene expression.

One human virus infection in which a strong CMI contribution to pathology seems probable is measles. Children with thymic aplasia show a general failure to develop T lymphocytes and cell-mediated immunity, but have normal antibody responses to most antigens. They suffer a fatal disease if they are infected with measles virus. Instead of the limited extent of virus growth and disease seen in the respiratory tract in normal children, there is inexorable multiplication of virus in the lung, in spite of antibody formation, giving rise to giant cell pneumonia. This indicates that the CMI response is essential for the control of virus growth. In addition there is a total absence of the typical measles rash, and this further indicates that the CMI response is also essential for the production of the skin lesions. Cell-mediated immune responses also make a contribution to the rashes in poxvirus infections.

Other Indirect Mechanisms of Damage

Stress, haemorrhage, placental infection and tumours

Sometimes in infectious diseases there are prominent pathological changes which are not attributable to the direct action of microbes or their toxins, nor to inflammation or immunopathology. The stress changes mediated by adrenal cortical hormones come into this category. Stress is a general term used to describe various noxious influences, and includes cold, heat, starvation, injury, psychological stress and infection. An infectious disease is an important stress, and corticosteroids are secreted in large amounts in severe infections (see also Ch. 11). They generally tend to inhibit the development of pathological changes, but also have pronounced effects on lymphoid tissues, causing thymic involution and lymphocyte destruction. These can be regarded as pathological changes caused by stress. It was the very small size of the thymus gland as seen in children dying with various diseases, especially infectious diseases, that for many years contributed to the neglect of this important organ, and delayed appreciation of its vital role in the development of the immune system.

Appreciation of the effects of stress on infectious diseases and the immune response in particular has led to the establishment of the science of neuroimmunology. Properly controlled experiments are difficult to mount but it is clear that the nervous system affects the functioning of the immune system. The pathways of this communication are still poorly understood, but there is a shared language for immune and neural cells. For example, neural cells as well as immune cells have receptors for interleukins, and lymphocytes and macrophages secrete pituitary growth hormone. Work on *Mycobacterium bovis* grew out of observations from the turn of the century that stress appears to increase the death rate in children with tuberculosis (TB). In one type of experiment mice were stressed by being kept in a restraining device where movement was virtually impossible. This resulted in the reduction of expression of MHC class II antigens on macrophages, which correlated with increased susceptibility to infection. Similarly stressing mice infected with influenza virus caused several immunosuppressive events including reduction of inflammatory cells in the lung, and decreased production of IL-2. Suppression of antibody responses is found in people suffering a type of stress familiar to students – examinations! The best responses to hepatitis B vaccine in students immunised on the third day of their examinations were found in those who reported the least stress. Finally, in a double-blind trial at the Common Cold Research Unit in England with five different respiratory viruses, it was ascertained in human volunteers that stress gave a small but statistically significant increased likelihood of an individual developing clinical disease.

Pathological changes are sometimes caused in an even more indirect way as in the following example. Yellow fever is a virus infection trans-

mitted by mosquitoes and in its severest form is characterised by devastating liver lesions. There is massive mid-zonal liver necrosis following the extensive growth of virus in liver cells, resulting in the jaundice that gives the disease its name. Destruction of the liver also leads to a decrease in the rate of formation of the blood coagulation factor, prothrombin, and infected human beings or monkeys show prolonged coagulation and bleeding times. Haemorrhagic phenomena are therefore characteristic of severe yellow fever, including haemorrhage into the stomach and intestine. In the stomach the appearance of blood is altered by acid, and the vomiting of altered blood gave yellow fever another of its names, 'black vomit disease'. Haemorrhagic phenomena in infectious diseases can be due to direct microbial damage to blood vessels, as in certain rickettsial infections (see p. 140) or in the virus infection responsible for haemorrhagic disease of deer. They may also be due to immunological damage to vessels as in the Arthus response or immune complex vasculitis, to any type of severe inflammation, and to the indirect mechanism illustrated above. Finally there are a few infectious diseases in which platelets are depleted, sometimes as a result of their combination with immune complexes plus complement, giving thrombocytopenia and a haemorrhagic tendency (see also disseminated intravascular coagulation, p. 287). Thrombocytopenic purpura is occasionally seen in congenital rubella and in certain other severe generalised infections.

Infection during pregnancy can lead to foetal damage or death not just because the foetus is infected (p. 333), but also because of infection and damage to the placenta. This is another type of indirect pathological action. Placental damage may contribute to foetal death during rubella and cytomegalovirus infections in pregnant women.

Certain viruses undoubtedly cause tumours (leukaemia viruses, human papillomaviruses, several herpes viruses in animals – see Table 8.1) and this is to be regarded as a late pathological consequence of infection. As was discussed in Ch. 7 the tumour virus genome can be integrated into the host cell genome whether a tumour is produced or not, so that the virus becomes a part of the genetic constitution of the host. Sometimes the host cell is transformed by the virus and converted into a tumour cell, the virus either introducing a transforming gene into the cell, activating expression of a pre-existing cellular gene, or inactivating the cell's own fail-safe tumour suppressor gene. The transforming genes of DNA tumour viruses generally code for T antigens which are necessary for transformation, and the transforming genes of RNA tumour viruses are known as *onc* genes.*

* *Onc* genes (oncogenes) are also present in host cells, where they play a role in normal growth and differentiation, often coding for recognised growth factors (e.g. human platelet-derived growth factor). They can be activated and the cell transformed when tumour viruses with the necessary 'promoters' are brought into the cell. The *onc* genes of the RNA tumour viruses themselves originate from cellular oncogenes which were taken up into the genome of infecting viruses during their evolutionary history.

Transformation has been extensively studied *in vitro*, and the features of the transformed cell described (changed surface and social activity, freedom from the usual growth restraints).

Dual infections

Simultaneous infection with two different microorganisms would be expected to occur at times, merely by chance, especially in children. On the other hand, a given infection generates antimicrobial responses such as interferon production and macrophage activation which would make a second infection less likely. Dual infections are commonest when local defences have been damaged by the first invader. The pathological results are made much more severe because there is a second infectious agent present. This can be considered as another mechanism of pathogenicity. Classical instances involve the respiratory tract. The destruction of ciliated epithelium in the lung by viruses such as influenza or measles allows normally nonpathogenic resident bacteria of the nose and throat, such as the pneumococcus or *Haemophilus influenzae*, to invade the lung and cause secondary pneumonia. If these bacteria enter the lung under normal circumstances, they are destroyed by alveolar macrophages or removed by the mucociliary escalator. In at least one instance the initial virus infection appears to act by interfering with the function of alveolar macrophages. Mice infected with parainfluenza 1 (Sendai) virus show greatly increased susceptibility to infection with *Haemophilus influenzae*, and this is largely due to the fact that alveolar macrophages infected with virus show a poor ability to phagocytose and kill the bacteria. Specialised respiratory pathogens such as influenza, measles, parainfluenza or rhinoviruses damage the nasopharyngeal mucosa and can lead in the same way to secondary bacterial infection, with nasal catarrh, sinusitis, otitis media or mastoiditis. The normal microbial flora of the mouth, nasopharynx or intestine are always ready to cause trouble if host resistance is lowered, but under normal circumstances they hinder rather than help other infecting microorganisms (see Ch. 2).

One interesting example of exacerbation of infection occurs in mice dually infected with influenza virus and microorganisms such as *Streptococcus aureus* or *Serratia marcescens*. Under these conditions animals suffer a more severe viral infection. This results from the need to proteolytically cleave the viral haemagglutinin protein which is done by a cellular enzyme. If the appropriate protease is in short supply or lacking completely, virions are formed but they are not infectious. Under these circumstances the haemagglutinin can be cleaved extracellularly by microbial proteases with resulting increased amounts of infectious virus and disease.

As a final example of dual infections, microorganisms that cause

immunosuppression can activate certain pre-existing chronic infections. In measles, for instance, there is a temporary general depression of CMI; tuberculin-positive individuals become tuberculin negative, and in patients with tuberculosis the disease is exacerbated. In the acquired immunodeficiency syndrome (AIDS; see p. 191) immunosuppression by HIV activates a variety of pre-existing persistent infections.

Diarrhoea

Diarrhoea deserves a separate section, since it is one of the commonest types of illness in developing countries and a major cause of death in childhood. Particularly in infants, who have a very high turnover of water relative to their size, the loss of fluid and salt soon leads to life-threatening illness. In 1998, diarrhoea was responsible for 2.2 million deaths world-wide in children under 5 years old. In villages in West Africa and Guatemala, the average 2–3-year-old child has diarrhoea for about 2 months in each year.* Diarrhoea also interacts with malnutrition and can cause stunted growth, defective immune responses and susceptibility to other infections (pp. 377–379). Fluid and electrolyte replacement is a simple, highly effective, life-saving treatment that can be used without determining the cause of the diarrhoea. Oral rehydration therapy (ORF) means giving a suitable amount of salt and sugar in clean water, and this is something that can be done by the mother. Diarrhoea is also a common affliction of travellers from developed countries, and business deals, athletic successes and holiday pleasures can be forfeited on the toilet seats of foreign lands. The most reliable prophylaxis is to 'cook it, peel it, or forget it'. Most attacks of diarrhoea are self-limiting. Diarrhoea means the passage of liquid faeces,† or faeces that take the shape of the receptacle rather than have their own shape. This could arise because of increased rate of propulsion by intestinal muscles, giving less time for reabsorption of water in the large bowel, or because there was an increase in the amount of fluid held or produced in the intestine. In many types of infectious diarrhoea the exact mechanism is not known. Diarrhoea, on the one hand, can be

* Diarrhoea on a massive scale is not always confined to developing countries. There was a major outbreak of *Cryptosporidium* infection in Milwaukee, USA, in 1993 with more than 400 000 cases; 285 of these were diagnosed in the laboratory and they suffered watery diarrhoea (mean 12 stools a day) for a mean of 9 days. The small (4–5 mm) oocysts, probably from cattle, had entered Lake Michigan, and then reached the community water supply because of inadequate filtration and coagulation treatment.

† Liquid faeces are not abnormal in all species. The domestic cow experiences life-long diarrhoea, but presumably does not suffer from it.

regarded as a microbial device for promoting the shedding and spreading of the infection in the community, or, on the other hand, as a host device to hasten expulsion of the infectious agent. Diarrhoea is a superb mechanism for the dissemination of infected faeces (see p. 58) and there is no doubt that strains of microbes are selected for their diarrhoea-producing powers. The advantages to the host of prompt expulsion of the infectious agent was illustrated when volunteers infected with *Shigella flexneri* were given Lomotil, a drug that inhibits peristalsis. They were more likely to develop fever and had more difficulty in eliminating the pathogen.

Before attempting to explain the pathophysiology of diarrhoeal disease, the normal structure and function of gut will be considered. The main function of the gut is the active inward transport of ions and nutrient solutes which is followed by the passive movement of water (Fig. 8.18). The driving force is the Na^+/K^+ ATPase situated in the basolateral membrane of enterocytes on the villus (Fig. 8.18), which maintains a low intracellular $[\text{Na}^+]$, thus creating the electrochemical gradient favourable for Na^+ entry and a high regional $[\text{Na}^+]$ in the intercellular spaces; Cl^- follows Na^+ . A similar situation exists in crypt cells: Na^+/K^+ ATPase drives secretion. The key difference is the location of the carrier systems responsible for the facilitated entry of the actively transported species. In villus cells the carriers are present in the brush border, whereas in crypt cells they are located in the basal membrane: this is responsible for the vectorial aspects of ion/fluid traffic in villus/crypt assemblies. However, it is clear that several factors in addition to enterocytes are involved in regulating fluid transport in the gut; these include the enteric nervous system and the anatomy of the microcirculation. The latter plays a profoundly important role in the uptake of fluid. This is illustrated in Fig. 8.19, which shows the existence of zones of graded osmotic potential. At the tips of villi in adult human gut, osmolalities range from 700 to 800 $\text{mOsm kg}^{-1} \text{H}_2\text{O}$, which would generate huge osmotic forces. Thus, current perceptions are that enterocytes are responsible for generating this gradient and the blood supply acts as a countercurrent multiplier which amplifies the gradient in a manner analogous to the loops of Henle in the kidney. The hypertonic zone has been demonstrated directly in whole villi of infant mice in terms of the changing morphology of erythrocytes: in the lower regions of villi they show characteristic discoid morphology, whereas in the upper region they are crenated, indicating a hyperosmotic environment. The hypertonicity is dissipated if the blood flow is too slow and washed out if too fast. It is the villus unit rather than enterocytes by themselves that is responsible for fluid uptake. Another consequence of the microcirculatory anatomy is that villus tip regions are relatively hypoxic. In addition, neonatal brush borders contain disaccharidases (principally lactase) which break down nonabsorbable disaccharides (e.g. lactose) into constituent absorbable monosaccharides.

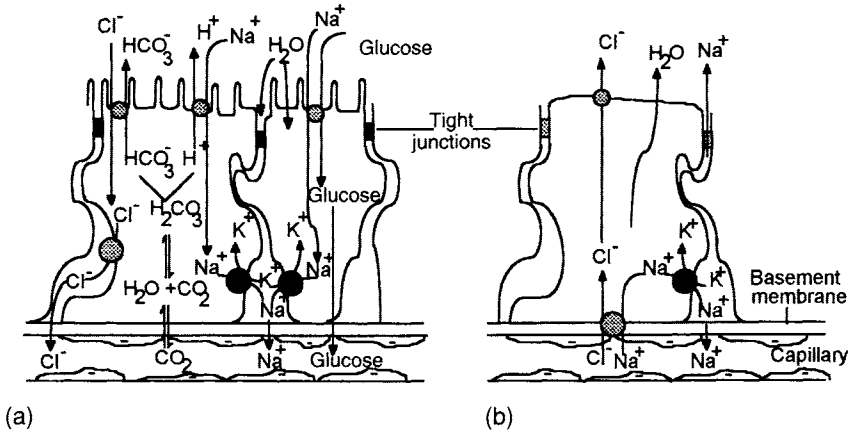


Fig. 8.18 Simplified schematic representation of electrolyte transport by ileal mucosal tissue and its consequence for (a) absorption and (b) secretion. Active processes involve the movement of ions and nutrient solutes; water follows passively.

(a) Two methods of Na^+ co-transport are shown involving a glucose-linked symport and two coupled antiports; the latter results in the co-transport of Cl^- . The coupled antiports are functionally linked via H^+ and HCO_3^- , the relative concentrations of which are a reflection of metabolic activity. These processes occur within the same cells but are shown separately for clarity. The driving force for Na^+ uptake is the low Na^+ concentration maintained by the Na^+/K^+ pump (ATPase) which creates the electrochemical gradient that promotes the inward movement of Na^+ ; Cl^- follows Na^+ by diffusion. Water is drawn osmotically across the epithelium paracellularly (i.e. across tight junctions) and/or transcellularly, the former pathway accounting for approximately 80% of fluid movement.

(b) Secretion is the result of the coupled entry of Na^+ and Cl^- across the basolateral membrane. Na^+ is recycled by the Na^+/K^+ pump and Cl^- exits by diffusing down an electrochemical gradient and across the undifferentiated crypt cell apical membrane; Na^+ follows Cl^- and water follows passively.

Note: (i) The driving force results from the same mechanism that powers absorption, i.e. the Na^+/K^+ pump located in the basolateral membrane; it is the location of the 'port' 'diffusion' systems that determines the vectorial aspects of ion movement. (ii) The tight junctions are less tight in the crypts than villi. (iii) The apical membrane of the crypt cell is undifferentiated and only acquires microvilli during ascent into villous regions. ●, Na^+/K^+ pump; ⊙, symport, antiport or diffusion channel.

Villus tips and crypts are regarded as the anatomical sites of physiological absorption and secretion respectively. Fluid transport is a bi-directional process in the healthy animal with net absorption in health and net secretion in disease. The balance between absorption and secretion is poised at different points throughout the intestinal tract reflecting differences in both structure and function. Proximal small intestine is relatively leaky; in contrast the colon is a powerfully absorptive organ.

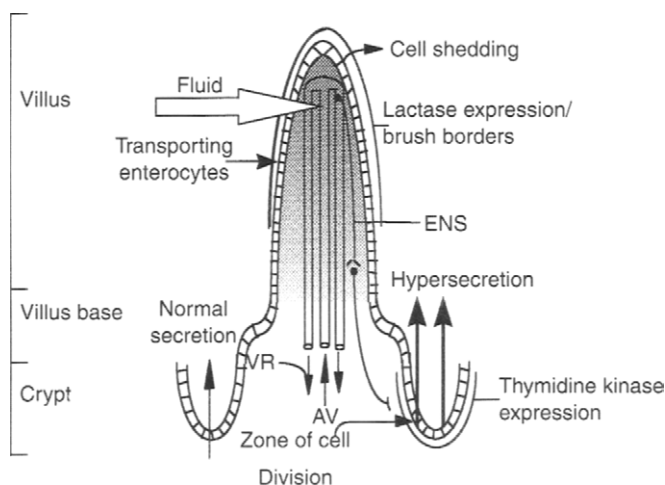


Fig. 8.19 Small intestinal villus: simplified schema of integrated structure and function. Note the central arterial vessel (AV) which arborizes at the tip into a capillary bed drained by a subepithelial venous return (VR). Movement of sodium into VR creates a concentration gradient between VR and AV, causing absorption of water from AV and surrounding tissue. This results in a progressive increase in the osmolarity of incoming blood moving into the tip region through to VR. Tip osmolarity is about three times higher than normal. Hyperosmolarity has been demonstrated in man and can be inferred in mice from the morphology of erythrocytes which changes during ascent of the same vessel from base to tip regions of villi. The intensity of shading indicates a vertical increase in osmolarity. The left crypt represents normal physiological secretion and the right crypt hypersecretion. ENS, the enteric nervous system, is depicted schematically and not anatomically.

Finally, crypts are the principal sites of cell regeneration, replacing cells which migrate up the epithelial escalator. The epithelium is renewed in approximately 3–5 days. At villus tips senescent cells are shed.

Diarrhoeal disease can result from interference with almost any one, or combination of these systems. The range of intestinal pathogens and the types of disease they cause is illustrated in Tables 8.7 and 8.8. The pathological/pathophysiological nature of some pathogen/host interactions is illustrated in Fig. 8.20. Noninvasive pathogens like *V. cholerae* and enterotoxigenic *E. coli* (ETEC) secrete toxins which perturb the ion transport systems. Invasive nonhistotoxic pathogens, such as some *Salmonella* strains (see Ch. 2) and rotavirus, invade villus tip cells which are then shed into the intestinal lumen. Invasive histotoxic pathogens, such as some strains of *Salmonella* (see Ch. 2), cause rapid toxin-mediated detachment of epithelial cells. Experimental rotavirus infections have been studied in great detail allowing us to delineate

Table 8.7. Production of diarrhoea by microorganisms shed in faeces

| Infectious agent | Diarrhoea | Site of replication |
|--|-----------|--|
| Rotaviruses | + | Intestinal epithelium |
| Parvoviruses (dogs) | + | Intestinal epithelium (crypt cells) |
| Intestinal adenoviruses (types 40, 41) | + | Intestinal epithelium |
| Intestinal coronaviruses ^a | + | Intestinal epithelium |
| Norwalk virus group (caliciviruses) | + | Intestinal epithelium |
| Toroviruses (calves, horses, humans) | + | Intestinal epithelium and M cells (see Table A.5) |
| <i>Vibrio cholerae</i> | + | Intestinal lumen |
| <i>Clostridium difficile</i> | + | Intestinal lumen |
| <i>Campylobacter jejuni</i> | + | Intestinal epithelium |
| <i>E. coli</i> | + | Varies ^b |
| <i>Shigella</i> | + | Intestinal epithelium |
| <i>Salmonella</i> sp. | ± | Intestinal epithelium (varies) |
| <i>Salmonella typhi</i> | + | Intestinal lymphoid tissue, liver, biliary tract |
| <i>Cryptosporidium</i> | + | Intestinal epithelium |
| <i>Giardia lamblia</i> | + | Attached to intestinal epithelium |
| <i>Entamoeba histolytica</i> | + | Invasion of intestinal epithelium |

^a Described for pigs, foals, calves, sheep, dogs, mice, man and turkeys; maximum susceptibility in the first few weeks of life.

^b Strain ETEC remains in the lumen; EIEC is similar to *Shigella*, EHEC reaches subepithelial tissues.

intermediate stages between initial infection, through clinical diarrhoea to recovery from infection. We either do not know or can only infer what the intermediate stages are for the other examples alluded to – signified by broken arrows (Fig. 8.20) – leading to a return to normal in those cases in which disease is self-limiting.

Campylobacter jejuni does not figure in our treatment so far despite the fact that *C. jejuni* and related species are the most common bacterial cause of diarrhoea in many industrialised countries. This is because of a severe lack of relevant 'mechanistic' information due to the lack of good experimental models; hence we know very little about the detailed mechanisms of pathogenicity of this hugely important pathogen. The clinical picture of the pathogenesis of *C. jejuni* infection may be summarised as follows. In developing countries the most common clinical presentation is mild watery diarrhoea, whereas in developed countries disease often manifests as a severe inflammatory diarrhoea. No evidence has yet been found to suggest that the watery type and severe bloody type of diarrhoeas can be explained in terms of a *C. jejuni* equivalent of the ETEC and EHEC mechanisms described above. Current thinking proposes that the different disease patterns reflect the immunological status of the host. Those with full immunity experience no clinical disease, whereas those with no pre-immunity experience the full-blown bloody diarrhoea and those with partial

Table 8.8. Types of intestinal infection

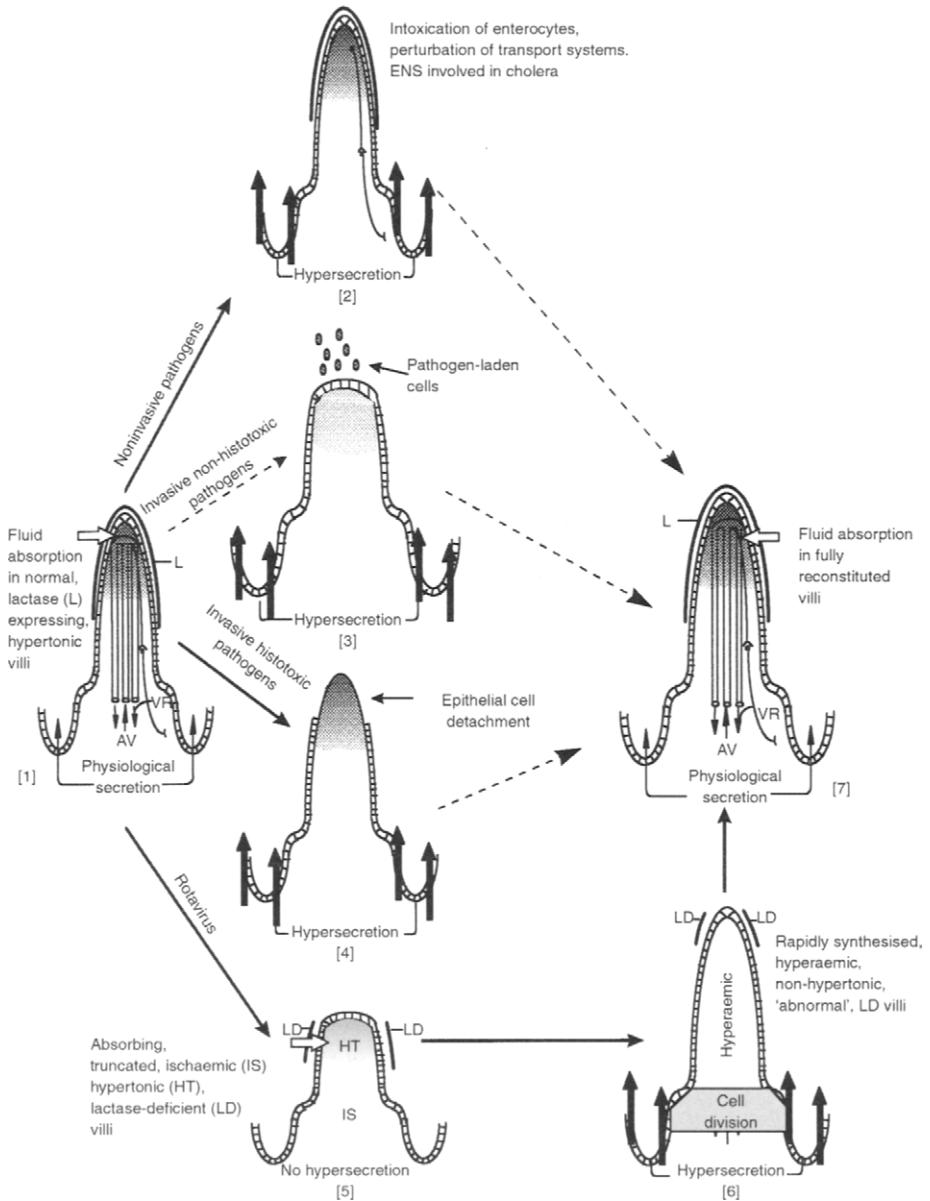
| Types of infection | Microorganism | Disease |
|--|--|---|
| Microorganism attaches to epithelium of small intestine, rarely penetrates and causes disease (diarrhoea) often by forming a toxin(s) which induces fluid loss from epithelial cells | <i>Vibrio cholerae</i> <i>E. coli</i> (certain strains) | Cholera Infantile gastroenteritis (certain types) or mild cholera-like disease in adults (travellers' diarrhoea) Calf diarrhoea |
| | <i>Giardia lamblia</i> | Giardiasis |
| Microorganism attaches to and penetrates epithelium of large intestine (<i>Shigella</i>) or ileum (<i>Salmonella</i>), causing disease by shedding/killing epithelial cells (exotoxin?) and inducing diarrhoea. Subepithelial penetration uncommon | <i>Shigella</i> spp. | Bacillary dysentery |
| | <i>Salmonella</i> (certain species) ^a | Salmonellosis |
| | <i>E. coli</i> (certain strains) | Coliform enteritis or dysentery Piglet diarrhoea |
| | <i>Campylobacter jejuni</i> | Diarrhoea, enteritis in man ^b |
| | Human diarrhoea viruses | Gastroenteritis |
| Microorganism attaches to and penetrates intestinal wall. Also invades subepithelial tissues, sometimes (typhoid, hepatitis A) spreading systemically | <i>Eimeria</i> spp. | Coccidiosis in domestic animals (may cause diarrhoea and blood loss) |
| | <i>Entamoeba histolytica</i> | Amoebic dysentery |
| Microorganism attaches to and penetrates intestinal wall. Also invades subepithelial tissues, sometimes (typhoid, hepatitis A) spreading systemically | <i>Salmonella typhi</i> and <i>paratyphi</i> | Enteric fever (typhoid) |
| | <i>Salmonella</i> (certain species) | Salmonellosis (severe form) |
| | <i>E. coli</i> (certain strains) | Calf enteritis |
| | Hepatitis A virus | Varied |
| | Reoviruses, enteroviruses | Hepatitis |

^a There are more than 1000 serotypes of *Salmonella*, distinct from *Salmonella typhi* and *Salmonella paratyphi*. They are primarily parasites of animals, ranging from pythons to elephants, and their importance for man is their great tendency to colonise domestic animals. Pigs and poultry are commonly affected, and human disease follows the consumption of contaminated meat or eggs.

^b Other campylobacters cause sepsis, abortion and enteritis in animals.

immunity, watery diarrhoea. The incubation period can range from 1 to 7 days and acute diarrhoea can last for 1–2 days with abdominal pain which may persist after diarrhoea has stopped. Diarrhoeal stools often contain fresh blood, mucus and an inflammatory exudate with leucocytes; bacteremia may also occur though it is rarely reported. Infected mucosae may be oedematous and hyperaemic with petechial haemorrhages. The disease, even its severe form, tends to be self-limiting,

despite the fact that organisms may be isolated for several weeks after resolution of the symptoms. We do, however, know that there is a strong correlation between infection with *C. jejuni* and Guillain-Barré syndrome which is the most notable complication of *C. jejuni* infection. Guillain-Barré syndrome is a peripheral neuropathy, and one possible cause may be an autoimmune phenomenon arising from molecular



mimicry between the polysaccharide side chains of *C. jejuni* and neural gangliosides.*

While there are reasonable models for studying colonisation and initial invasion, there is a problem regarding experimental animal models in which to reproduce the extreme form of bloody diarrhoea seen in humans. However, the situation concerning *C. jejuni* is probably about to change dramatically. New strategies based on the use of the new technology of 'microarrays'† are now being used. By this means, and by reference to the genomic atlas, it is theoretically possible to identify which genes are expressed under different sets of experimental conditions including those which mimic the infection environment. Doubtless a plethora of new data is about to be generated from which we hope to learn more of the disease-conferring attributes of *C. jejuni* and related species.

Rotaviruses are known to invade intestinal epithelial cells and cause diarrhoea in man, foals, dogs, pigs, mice, etc. Extensive multiplication takes place and very large amounts of virus (10^{11} particles g^{-1}) are shed in faeces. The conventional wisdom is that tips of villi especially are

* Guillain-Barré syndrome is also associated with certain virus infections, and 'flu vaccination (see Ch. 12).

† Microarrays: see Ch. 1.

Fig. 8.20 Diarrhoeal mechanisms: initial stages and (for rotavirus) some intermediate stages in disease progression. This represents a schematic summary of the text on diarrhoeal mechanisms. In all cases, broken arrows indicate uncertainty about the number and nature of intermediate steps in the return to normality of affected villi in self-limiting diarrhoeal disease. For clarity, the blood supply in [2] and both blood supply and enteric nervous system (ENS) in [3], [4], [5] and [6] have been omitted.

[1] represents a normal villus; the shading intensity (as in Fig. 8.19) represents the magnitude of osmolarity. [2] Intoxication of villi by noninvasive pathogens such as *V. cholerae* and ETEC. The main diarrhoeal determinant is CT in *V. cholerae* and LT and ST in ETEC. However, as discussed in the text, toxins are not the whole story, hence the broken arrows. [3] Represents disease caused by invasive pathogens such as nonhistotoxic *S. typhimurium* and rotavirus. Villi are shortened with presumed loss of absorption and observed increase in secretion. Again the mechanistic pathway for return to normality is not known for bacterial infections. [4] Loss of epithelia due to a histotoxin seen in some strains of *S. typhimurium*. Clearly loss of enterocytes will affect absorption and open up other routes for progressive invasion. Again note the broken arrow. [5] A more complete experimentally based understanding of the pathophysiological mechanisms is possible in rotavirus infection of neonatal mice ([5], [6] and [7]). The main point is that conventional wisdom is not sustained: maximum diarrhoea occurred during the resynthesis of truncated villi and villus shortening was preceded/caused by ischaemia. Prolongation of diarrhoea coincided with non-hypertonic villi; diarrhoea ceased on reconstitution of hypertonic villus tip regions. It is possible to infer that some of these intermediate steps take place in other gut infections.

affected, leading to reduced absorption of fluid from the lumen. In addition destruction of enterocytes leads to a loss in lactase resulting in an accumulation of lactose in the gut causing an osmotic flux of fluid into the intestine. A major study of rotavirus-induced diarrhoea in neonatal mice provides a different model of this important disease of children. The main features of this model are summarised in Fig. 8.20. Oral infection of the gut induces ischaemia in villi, followed by hypoxia, enterocyte damage, and shortening of villi. The perception is that it is the induction of ischaemia and not viral replication *per se* that results in these changes. It is during rapid resynthesis of the atrophied villi that maximum diarrhoea occurs due to the transient accumulation of excess NaCl in dividing cells. Prolongation of diarrhoea is seen to be due to the hyperaemic state of the newly reconstructed villi which reduces the hypertonicity of villi. Resolution of the diarrhoea occurs when microcirculation is restored to normal with concomitant restoration of hypertonic tip zones in villi.

The preceding description of the self-limiting diarrhoea induced by rotavirus in neonatal mice is that of a basic response probably applicable to many diarrhoeas since the features of the post-peak phase have often been reported or can be inferred in other infections. However, the observed pathology will be different according to age, host species, or the inducing pathogen. For example, in rotavirus-infected lambs, villus atrophy and crypt hypertrophy occur (the latter indicative of crypt cell division) but as in mice, infected lambs are not lactose intolerant. In rotavirus-infected swine piglets, crypt hypertrophy occurs but villus atrophy is severe, the animals are lactose intolerant and mortality is high; a similar situation exists for the coronavirus, transmissible gastroenteritis (TGE) virus of swine. The latter has often been used as the model for infantile diarrhoea but the question is whether human infants are more like piglets or lambs. Clinical studies have shown that recovery from *mild*, acute gastroenteritis of rotavirus origin occurs within 2 weeks irrespective of the carbohydrate ingested. Clearly, the severity of disease and the clinical outcome will depend on the extent of 'vertical' villus/crypt involvement and the regions of intestine infected. When villus erosion is severe, then lactose may cause an 'osmotic' purge or be fermented by intestinal bacteria to short-chain fatty acids which stimulate secretion in the colon. Astroviruses, Norwalk virus, caliciviruses and certain adenoviruses all cause gastroenteritic disease by infecting enterocytes. However, parvoviruses cause severe intestinal disease in dogs by virtue of their predilection for the mitotically active crypt cells which is the cause of the near-complete erosion of villi similar to that seen after exposure to sublethal doses of irradiation.

Can we be more specific about the viral determinants responsible for triggering these complex host reactions? It has recently been shown that a non-structural rotavirus protein, NSP4, induces diarrhoea in mice when introduced into the ileum, by causing increased Cl⁻ secre-

tion. An apparent exception to the 'rule' that viruses do not form toxins!

Entamoeba histolytica causes lysis of target cells apparently by direct contact with the cell membrane. This pathogen produces under *in vitro* conditions a spectacular array of potential (but as yet unproven) virulence determinants including: proteases that round up cells, pore-forming proteins, collagenases and oligosaccharidases and neurotransmitter-like compounds; the latter can induce intestinal fluid secretion. Some of these factors have been implicated as the determinants responsible for liver abscess formation.

Although much research has been focused on toxins, their mode of action, and their role in disease, it is useful to compare different types of intestinal infection and to refer to the concept of *food poisoning*. Types of intestinal infection are set out in Table 8.8. Food poisoning is a loosely used term, and usually refers to illnesses caused by preformed toxins in food, or sometimes to illnesses that come on within a day or so after eating contaminated food. Food may be contaminated with plant poisons, fungal poisons (e.g. poisoning due to *Amanita phalloides*), fish poisons,* heavy metals, as well as with bacterial toxins or bacteria.

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* Ingestion of scombroid fish (mackerel, etc.) containing large amounts of histamine or similar substances leads to headache, flushing, nausea and vomiting within an hour.

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