



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Recovery from Infection

OUTLINE

Immunological Factors in Recovery	233	Temperature	249
Inflammation	242	Tissue Repair	250
Complement	243	Resistance to Re-Infection	253
Interferons	245	Bibliography	255
Multimechanistic Recovery: An Example	247		

If there is to be recovery from an infection, it is first necessary that the multiplication of the infectious agent is brought under control. The microbe must decrease in numbers and cease to spread through the body or cause progressive damage. This is accomplished by immunological and other factors whose action is now to be described. The average multiplication rate of various microorganisms in the infected host as shown by doubling times is nearly always longer than in artificial culture under optimal conditions. This in itself reflects the operation of anti-microbial forces. In the process of recovery from an infectious disease, damaged tissues must of course be repaired and reconstituted. Sometimes the microorganism is completely destroyed and tissues sterilised, but often this fails to take place and the microorganism persists in the body, in some instances continuing to cause minor pathological changes. The individual is nevertheless said to have recovered from the acute infection and is usually resistant to re-infection with the same microorganism. Persistent infections are dealt with in Chapter 10.

IMMUNOLOGICAL FACTORS IN RECOVERY

The mechanisms of recovery from a primary infection are not necessarily the same as those responsible for resistance to re-infection (see below). For instance, antibody to

measles is of prime importance in resistance to re-infection and susceptible children can be passively protected by the antibody present in pooled normal human serum. But, compared with T-cell-mediated immunity (CMI), antibody plays only a small part in the recovery from initial infection with measles virus. Antibody, T cells, NK cells, complement, phagocytes and cytokines such as interferon are involved in the response to nearly all infections and, without any doubt, are together responsible for recovery. They constitute a formidable anti-microbial force, and the relative importance of the individual components in recovery is now becoming understood. The application of the 'omic' technologies, i.e. high throughput proteomics, and transcriptome analysis using microarray analysis, or more recently RNAseq, coupled with the use of gene 'knockout' mice, has been tremendously important in evaluating, for example, the role of cytokines and chemokines in the recovery process. This has led to understanding that the immune response is a very plastic system where the loss of one gene can invariably be compensated for by other gene products.

If there is one cell that can be labelled with a multifunctional role in recovery from infection, then that cell is the macrophage (see Chapters 3, 4, 6 and 8). They play a pivotal role in both the induction and the expression of CMI responses; they are at the centre of the inflammatory responses and are key cells in the repair of tissue damage.

One major difficulty in assessing the importance of immune responses is that nearly all microorganisms are very complex, with large numbers of antigens. Various tests for antibody and T cells are carried out, but it is not always possible to test the response to a defined antigen, or to know precisely which antigens are important for infection and pathogenicity.

Antibody

The different types of antibody and the ways in which they have an anti-microbial action are listed in Chapter 6. Antibody actions against microorganisms are further discussed at the end of this chapter under the section 'Resistance to Re-infection'.

In some infections, antibody plays a major part in the process of recovery. For instance, viruses producing systemic disease, with a plasma viraemia, are controlled primarily by circulating antibody. This seems to be so in yellow fever and poliomyelitis virus infections. Children with severe agammaglobulinaemia are unable to form antibodies to poliovirus, and are about 10,000 times more likely than normal individuals to develop paralytic disease (which is generally of a chronic type) after live virus vaccination.¹ They have normal CMI and interferon responses, normal phagocytic cells and complement, but lack the specific antibody which must be produced if virus multiplication and spread to the central nervous system are to be inhibited.

¹Agammaglobulinaemics are also susceptible to pneumococcal infections. Theoretically, opsonisation of these bacteria should occur after activation of the alternative complement pathway (Chapter 6), but antibody appears to be needed for optimal uptake and killing by phagocytes. Antibody may also be needed for lysis of virus-infected host cells after complement pathway activation.

Antibody on its own can neutralise virus infectivity and inhibit the activity of bacterial toxins. Virus neutralising antibodies can be of the IgG, IgA or IgM immunoglobulin isotypes. The amount of antibody required to induce neutralisation is unclear. Some early studies on the kinetics of neutralisation by high affinity antibodies suggested a single hit mechanism, meaning that one virus particle is neutralised by one molecule of antibody. The single hit hypothesis has recently been challenged in favour of multiple hit kinetics with estimates of 10 antibody molecules required to neutralise HIV-1. Multiple hit kinetics are more likely, and in fact are essential, if aggregation of virions by bivalent antibodies is to occur.

Neutralising antibodies can act by blocking the interaction between a cell receptor and the virus attachment proteins present on the virion surface. For non-enveloped viruses, such as the picornaviruses, attachment takes place via interaction with a limited number of structures present on the capsid. For rhinoviruses this is a narrow surface depression (or 'canyon') that surrounds each of the 12 capsid vertices. In contrast there are hundreds of virus glycoproteins present on the surface of an enveloped virus and it is likely that at any one time (i) not all of these are recognised by antibody and (ii) that only one antibody is bound per virus protein, i.e. for the flu HA spike, which has five sites recognised by neutralising antibody, only one of these sites may be occupied on a single HA molecule. The size of an antibody molecule and the distribution of these sites on HA would support this view. In addition to sterically hindering the interaction between virus and receptor, antibodies can interfere with uncoating, by either triggering a stage of uncoating prematurely or preventing uncoating by cross-linking surface structures on the virion. Some antibodies inhibit fusion (the first stage of uncoating) probably by preventing the conformational change in the virus proteins required to allow fusion to occur. Rhinovirus capsids are known to undergo a pH-mediated conformational change within the endosome, allowing the virus genome to escape the endosome. It is possible that antibodies can prevent this change, so blocking genome release.

Antibody also promotes the uptake and digestion of virus by phagocytic cells, so that the virus-antibody complex is finally taken up and disposed of. As antibodies are bivalent (IgG) they can bind to two virions simultaneously, cross-linking the virions and so creating large aggregates or clumps of virus. This decreases the ability of the virus to interact with cells and targets the virus for phagocytosis. In all the mechanisms outlined above, complement can act to increase the effectiveness of antibody. Non-neutralising antibodies are also produced during the immune response and are likely to contribute to recovery by activating complement or by interacting with Fc receptors on NK cells/macrophages.

As discussed above, antibodies neutralise free virus particles liberated from cells, but, despite the help of complement and of phagocytes with Fc receptors, are less able to influence events in infected cells. Action on the infected cell seems necessary for recovery from the above virus infections. The destruction of cells infected with viruses takes place in various ways but depends on the mechanism of virus maturation in the cell. The non-enveloped viruses, such as poliovirus or papilloma viruses, replicate and produce fully infectious particles inside the cytoplasm. These particles consist of the nucleic acid with its protein coat (capsid) and are exposed to antibody when liberated from the cell (Figure 9.1). Enveloped viruses are liberated by a process of budding from cell

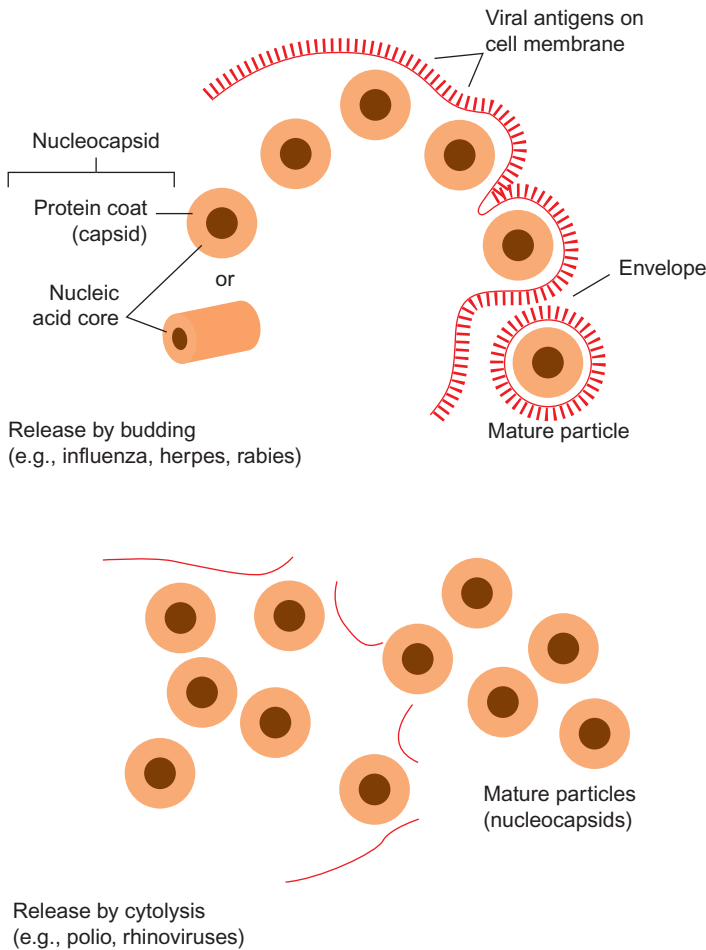


FIGURE 9.1 Diagram to illustrate mechanisms of virus release from infected cell. Nucleocapsids may be spherical (herpes viruses) or tubular (influenza). Budding may also take place from nuclear membrane (herpes viruses) or from the membrane lining cytoplasmic vacuoles (coronaviruses, flaviviruses).

membranes. The viral genome and nucleoproteins or capsids become closely associated with the cell membrane, either the cytoplasmic membrane or in some cases (e.g. herpes, pox) with an internal membrane and acts as an *initiation* point for the viral envelope proteins (Figure 9.1). The virus particle matures by budding through the altered membrane, acquiring an envelope as it does so. There are two important consequences of this mechanism of virus maturation. First, virus can be released even though the cell remains alive and intact. Second, the foreign viral glycoproteins appearing on the cell surface are recognised by host antibody and an immune response is generated with the infected cell as the target. The significance of this is that the infected cell can be destroyed before virus has been liberated as viral glycoproteins often appear on the cell surface early in the replication process, before progeny virus particles have been formed.

Various bacteria have been shown to make specific attachments to epithelial surfaces and here secretory IgA antibodies are significant. IgA antibodies are formed in most

infections of mucosal surfaces whether bacterial, viral or due to other microorganisms. They tend to prevent re-infection, but if formed early enough in the primary infection they could block the attachment of the microorganism to susceptible cells or cell surfaces and thus interfere with the spread of infection. Their actual function in recovery, however, is doubtful. As was pointed out earlier, virus infections that are limited to epithelial surfaces and do not have a time-consuming spread of infection through the body have incubation periods of no more than a few days. There is little opportunity for the slowly evolving immune response to play an important role in recovery, and virus replication is often inhibited before there has been a detectable IgA response. On the other hand, it must be remembered that antibodies (IgG or IgA) can be produced locally within two days after experimental respiratory tract infections, for instance, and they would not be detected routinely when bound to viral antigens at this stage. But interferon is produced by the first infected cell and is likely to have an important local antiviral action. If the process of infection takes longer, then secretory IgA antibodies have more opportunity to aid recovery. When the intestinal protozoan *Giardia lamblia* causes symptoms, these are not seen until 6–15 days after infection. A role for secretory IgA antibodies is indicated because patients with a shortage of these antibodies show troublesome and persistent giardial infection.

The antibody response to streptococci, staphylococci and various encapsulated bacteria such as the pneumococcus is of particular importance. These are the common pyogenic (pus-forming) infections. For its anti-bacterial function, antibody needs to operate together with phagocytic cells and complement and, if either of these are missing, resistance to pyogenic infections is impaired. Children with agammaglobulinaemia suffer repeated infections with pyogenic bacteria. The spleen is an important site of antibody formation, and when the spleen has been removed surgically, or rendered incompetent in children with sickle cell disease, there is increased susceptibility to such infections. On the other hand, many bacterial infections (tuberculosis, syphilis, typhoid, gonorrhoea) can persist or can re-infect in spite of the presence of large amounts of antibody. This is discussed more fully in Chapter 7, and it is a reminder of the frequent inability of antibodies to ensure recovery.

Antibodies are vital in recovery from diseases caused by toxins, such as diphtheria and tetanus. As soon as antibodies have been formed to neutralise the powerful toxins and prevent further tissue damage, recovery is possible; without antibodies the other anti-bacterial forces may operate in vain. In diphtheria the patient often recovers and is immune to the toxin without having controlled the infection itself, and remains a carrier.

In the case of protozoa, such as malaria, antibody must be directed against the relevant stage of the microorganism (especially the merozoite) and also against the relevant antigen on the microorganism. Merozoites are the forms that specifically enter red blood cells and parasitise them, and protective antibodies coat the merozoite surface and inhibit this absorption, at the same time promoting phagocytosis by the reticuloendothelial system (RES).

Host defences against fungi are less clearly defined but there are indications that CMI is more important than antibody. Disseminated infection with certain fungi (*Coccidioides*, *Histoplasma*) occurs even in the presence of high antibody titres, and in such cases there is usually no CMI demonstrable by skin tests, suggesting that T-cell responses matter most. Local infections with fungi elicit good CMI responses but poor antibody responses, and

the patient recovers. Severe mucocutaneous candidiasis is seen in those with defective CMI, in spite of normal antibody production. Although CMI is central for recovery from the majority of fungal infections, antibody with anti-fungal properties are produced during infection as determined by monoclonal antibodies against membrane antigens of *Cryptococcus neoformans* and *Candida albicans*. Such antibodies when injected into infected mice protect against infection. Therapeutic antibodies are being used to control a number of infectious diseases.

Small microorganisms such as viruses may have no more than one (HIV, human immunodeficiency virus) or two different proteins on their surface. The surface of influenza virus, for instance, consists of 500 or more haemagglutinin trimers, interlaced with about 100 neuraminidase tetramers. Antibodies to either antigen protect against infection, although the haemagglutinin contains the major neutralisation sites. Antibody to the neuraminidase inhibits its enzymatic activity. It does not prevent infection of the cell but prevents the dissemination of newly formed virus and thus hinders the spread of infection. This occurs because neuraminidase is required to detach haemagglutinin from sialic acid at the point on the cell membrane where the virus emerges. One can begin to work out the mechanisms of antibody protection in a relatively simple microorganism of this sort. Larger microorganisms, however, generally have many different proteins and carbohydrates on their surface. Some of these will be concerned with vital steps in the process of infection, and antibodies to specific neutralisation sites on these will be protective. Antibodies to other antigenic sites on these structures and even to some complete structures will not be protective, and when they are attached to the microbial surface may even physically interfere with (block) the action of protective antibodies. In addition, a large assortment of irrelevant antibodies are produced to internal components of the microorganism. Antibodies themselves differ in the firmness of the combination they make with antigens and may be of high or low avidity (see Glossary). Thus the quality of the antibody also matters. Protection by antibody is therefore a complicated matter, and if there is no protection in spite of the presence of large amounts of antibody, one has to ask first what components of the microbe these antibodies are combining with, and whether these antibodies have the relevant specificity for the job. Second, one needs to ask whether the antibody itself is of sufficient quality and of the appropriate isotype.

Cell-Mediated Immunity

As presented in Chapter 6, T cells are a heterogeneous population of cells as reflected by cytokine profiles and effector function. In particular, CD4 T cells assume a central role in coordinating cells from the innate response in recovery from infection. This is reflected in tissue responses in the host that bear the hallmarks of T-cell involvement, with the infiltrating cells consisting primarily of lymphocytes and macrophages. The nature of the particular T-cell subset will depend on the pathogen. For example, Th17 cells are important in recovery from extracellular bacteria and fungal infections including *Staphylococcus aureus*, whereas intra-cellular bacteria such as tuberculosis, brucellosis, listeriosis, tularaemia, syphilis and tuberculoid leprosy are controlled by Th1 cells. In *Leishmania* infection,

recovery is associated with the development of a Th1 response. This is orchestrated by the production of interleukin-12 (IL-12) from infected macrophages which acts on either natural killer (NK) cells or CD4⁺ T cells to produce interferon- γ (IFN- γ) and tumour necrosis factor (TNF), which in turn feeds back on macrophages to induce nitric oxide, an important molecule in controlling this parasite. The blockade of IL-12 activity *in vivo*, either by neutralising antibodies or the use of IL-12-deficient mice, leads to the development of a Th2 response which fails to protect the host from a generalised parasite infection, through a lack of nitric oxide production. Similar mechanisms operate in recovery from *Listeria* infection, illustrating the central role of IL-12 and IFN- γ in the evolution of Th1-protective immune responses. In some situations persistence of antigen, as with *Mycobacterium tuberculosis*, can lead to protracted Th1 responses resulting in chronic inflammation. These responses are characteristic of delayed-type hypersensitivity which can be demonstrated in a specific manner by skin testing.

As pointed out earlier, CMI develops in many other infections but is not very clearly associated with recovery. On infection with *Streptococcus pyogenes*, for instance, delayed hypersensitivity develops to the streptococcal products streptokinase and streptodornase, but it is less important than antibody in recovery from infection.

The clearest picture about CMI in recovery comes from certain virus infections, particularly herpes viruses, poxviruses, influenza virus. All viruses utilise the cellular translation machinery to produce virus proteins and so a proportion of these proteins enter the MHC I processing pathway in a manner similar to all other cellular proteins. It can be argued that MHC I/CD8⁺ T-cell responses evolved primarily to combat virus infections, as destruction of infected host cells has long been considered a feature of viral rather than other infections. There is now, however, evidence for this occurring with other infections. Host cells infected *in vitro* with protozoa (*Plasmodia* and *Theileria*), rickettsia (*Coxiella burnetii*) and with certain bacteria (*Listeria*) can present pathogen derived peptides in the context of MHC I. This is achieved as a natural consequence of degradation of intracellular antigens by the proteolytic machinery of the infected cell and the presentation of antigenic peptides by MHC class I. Consequently, whether a cell is infected by an enveloped or non-enveloped virus, a bacterium or a parasite, antigens can be recognised by cytotoxic T cells (CTLs).

The immune mechanisms for the destruction of cells bearing foreign antigens on their surface can be summarised as 'burns', 'pores' or 'poisons' and are as follows:

1. As mentioned above (Chapter 6), T-cell receptors only recognise peptides in association with MHC class I proteins (CD8 T cells) or class II proteins (CD4 T cells) on the target cell surface. Any viral protein can be processed in this way and usually internal virion proteins or non-virion (e.g. nuclear-transcription factors) proteins provide the major target for T cells. In order to destroy a target cell, T cells must become activated. Once this is achieved, the activated CTL makes intimate contact with the target cell membrane and delivers a lethal hit. The T cell then disengages and moves to another target. Killing of target cells occurs by one of two mechanisms. One mechanism involves releasing the contents of cytotoxic granules containing perforin, which deposits 'pores' in the membrane of the target cell, and granzyme B, which enters the

cell through the pores to act as a 'poison' in triggering apoptosis. Perforin is homologous to C9, the pore-forming component of complement. Both proteins polymerise on contact with the target cell breaching the membrane and producing pores through which electrolytes and other molecules flow causing cell damage. In contrast to apoptosis, cellular necrosis may occur (possibly as a result of large amounts of perforin being deposited) in which there is a leakage of cell components and K^+ ions, an influx of water and Na^+ ions, and the target cell swells up and dies. NK cells also use the perforin lytic mechanism to kill target cells. A second method that triggers cell death is the interaction of Fas (a TNF-like receptor on target cells) with the Fas ligand (on T cells), said to be the 'kiss of death', due to the activation of the 'death' domain in the cytoplasmic tail of Fas. This results in the initiation of a cascade of cellular proteases leading to apoptosis. An additional cytolytic mechanism involves the cytokine IL-7. This cytokine mediates killing of persistent virus-infected cells involving interaction with the intracellular protein arih2. IL-7 is presently undergoing clinical trials against a variety of persistent viral and bacterial infections.

2. Macrophages, polymorphs (neutrophils, basophils and eosinophils) and NK cells have the ability to destroy target cells with the assistance of specific antibody (antibody-dependent cell-mediated cytotoxicity (ADCC)). Antibody combines with antigen on the infected cell surface, and the killer cell attaches to the antibody-coated cells via the Fc receptor. The process is enhanced when complement is activated, the C3b molecules deposited on the cell surface being recognised by mononuclear and phagocytic cells that bear C3b receptors. The final killing mechanism is not clear, but the killer cell releases oxygen radicals and hydrogen peroxide (see p. 92) which 'burn' the target cell. The Fc receptors for IgG and IgE on eosinophils enable them to kill multicellular parasites such as schistosomes after adhering in large numbers to the antibody-coated surface of these parasites. This involves releasing toxic proteins (e.g. major basic protein and eosinophil cationic protein) directly onto the parasite surface to 'burn' holes in the tegument, enabling eosinophils to enter the parasite to deliver the *coup de grace*. These proteins are so toxic they can also damage mammalian cells, so the eosinophil carefully seals the area on the parasite where the proteins are delivered. Why the eosinophil is not destroyed is a mystery.

Destruction of infected cells is not the only mechanism available to T cells in controlling a virus infection. In hepatitis B virus (HBV) infection of the liver and in herpes simplex virus infection of neurons, $CD8^+$ T cells act to 'cure' the infection rather than kill the cells. This has been demonstrated in a transgenic mouse model of HBV infection in which every hepatocyte expresses viral genomes. By delivering immune T cells to these mice, the infection is readily controlled, but widespread destruction of hepatocytes is not observed. The key protective mechanism is $IFN-\gamma$, released by the activated $CD8^+$ T cells, which blocks virus replication and rids the cells of the viral genome. A similar mechanism operates in HBV infected chimpanzees, the other natural host for this virus. In neurons infected with herpes simplex virus, it is likely that similar $CD8^+$ T-cell control mechanisms occur, since neurons expressing late virus proteins (an indicator of the late stages of virus replication) can be prevented from cell death and 'cured' of this productive infection. However, the virus may persist in a latent form. This strategy benefits the virus in terms of its survival

as a latent infection and also the host in retaining the function of these irreplaceable cells. A decision on whether HBV or herpes virus-infected cells are killed or cured could be related to the amount of MHC class I expression on the infected cell. When MHC expression is high, these cells can be targeted by CTLs and killed. When MHC expression is low, CTLs may have difficulty directly engaging the target cell but can still influence virus replication through the local release of IFN- γ . This further illustrates the diversity of antiviral mechanisms at the disposal of the host.

The sequence of events with herpes virus, poxvirus and measles virus infections appears to be as follows: at sites of virus multiplication, T lymphocytes in the course of their normal movements through the body encounter viral peptides that are complexed with MHC proteins on the surface of a dendritic cell or other antigen-presenting cell. When a T cell encounters the antigen to which it is specifically sensitised, it becomes activated and divides to give fresh supplies of specifically sensitised T cells. These can react with any cell presenting the relevant peptide in association with a MHC molecule. Cytokines are liberated to attract macrophages and other leucocytes and focus them onto the site of infection. Infected cells are destroyed or cured by cytotoxic T lymphocytes and other cells, and virus material and cell debris is phagocytosed and disposed of by activated macrophages. Similar events occur in lymph nodes to which virus or virus antigens have been brought by lymphatic drainage.

The best way of discovering the function of a bodily mechanism or organ is to see what happens when it is removed. In experimental infections, CMI can be inactivated without affecting antibody or interferon responses, and changes in the disease are then studied.

A defined depletion of T cells can be achieved by treatment with monoclonal antibodies specific for CD4 or CD8 proteins. This approach enables T cells or other cells to be depleted at any stage in the immune response to infective agent. An alternative method is to use transgenic 'knockout' mice in which the gene encoding the protein of interest is inactivated at the DNA level. This powerful technology enables selected defects in host defence to occur, resulting in deficiencies of, for example, IFN- γ , TNF, IL-2, IL-4, IL-10 or their receptors; CD8 and CD4 T-cell function (disrupt CD8 or CD4 genes) and B-cell function (disrupt expression of IgM). Most of these gene knockout mice develop normally and remain well but show increased susceptibility to intra-cellular infections caused by various viruses, bacteria or protozoa. The picture is however complex. Deleting one cytokine or cell function upsets a delicate network of anti-microbial forces. Often a different defence mechanism takes over the function of the one that has been deleted. In other words, there is a redundancy in host defence mechanisms, as might be expected as an evolutionary response to infectious agents that often evade or interfere with these mechanisms.

At the clinical level, albeit without the precise focusing achieved in the knockout mice, evidence for the importance of CMI in the control of infections comes from studies on patients with defective CMI. Very rarely, infants are born with an absent or poorly developed thymus gland (thymic aplasia or hypoplasia). Their T lymphocytes fail to differentiate and develop, giving rise to severe CMI deficiency. Although their T-cell-dependent antibody response is also defective, they make a normal T-cell-independent antibody response (mainly IgM) (see Chapter 6 and Glossary). Thymic aplasia gives some insight into the importance of CMI in infectious diseases. Affected infants show a normal ability to control most bacterial infections, but a greatly increased susceptibility to infections with various viruses and certain other

intra-cellular microorganisms. After measles infection, for instance, there is no rash in these children, but an uncontrolled and progressive growth of virus in the respiratory tract, leading to fatal giant cell pneumonia. Evidently the CMI response controls the infectious process and at the same time plays a vital role in the development of skin lesions. In the days when affected children were vaccinated against smallpox with vaccinia virus, the virus grew as usual in epidermal cells at the inoculation site to give an increasing zone of skin destruction. In normal infants there was an inflammatory response at the edges of the lesion after 6–8 days and this led to inhibition of virus growth, then scabbing and healing of the lesion. The infant with thymic aplasia, however, did not show this response and the destructive skin lesion continued to enlarge, occupying an ever-increasing area of the arm and shoulder. The infection could be controlled by local injection of immune lymphocytes from a closely related donor, but not by antibody. Infants with this type of immune deficiency also tend to suffer severe generalised infections with herpes simplex virus. In addition, they show increased susceptibility to other intra-cellular microorganisms. When they are vaccinated against tuberculosis with live BCG vaccine, the attenuated bacteria (instead of undergoing limited growth with induction of a good CMI response) multiply in an uncontrolled fashion and may eventually kill the patient. The CMI response is therefore necessary for the control of infection with intra-cellular bacteria of this type.

The CMI response may have additional anti-microbial effects in chronic infections with certain intra-cellular organisms. When the microorganism persists as a source of antigenic stimulation and the CMI-induced influx of mononuclear cells continues, a granuloma may be formed (see below). The focus of infection tends to be walled off, and this is often associated with the inhibition of microbial growth. Granulomas are a feature of respiratory tuberculosis, contributing to pulmonary fibrosis. Granulomas, however, can result from chronic accumulation of immune complexes as well as from chronic local CMI reactions (see Chapter 8).

INFLAMMATION

Inflammation, whether induced by immunological reactions, tissue damage or microbial products, plays a vital role in recovery from infection (see also Chapters 3 and 6). Inflammation is necessary for the proper functioning of the immune defences because it focuses all circulating anti-microbial factors onto the site of infection. The circulating anti-microbial forces that arrive in tissues include polymorphs, macrophages, lymphocytes, antibodies, activated complement components and materials like fibrin that play a part in certain infections. The increased blood supply and temperature in inflamed tissues favour maximal metabolic activity on the part of leucocytes, and the slight lowering of pH tends to inhibit the multiplication of many extracellular microorganisms. The prompt increase in circulating polymorphs during pyogenic infections is caused in the first place by the release of cells held in reserve in the bone marrow, but there is also an increase in the rate of production. Monocyte release and production is controlled independently. At least four colony-stimulating factors, all glycoproteins, control the mitosis of polymorph and macrophage precursors, and their final differentiation and activity. They are present in increased amounts in serum during infection, and in animals the serum levels are dramatically raised by the injection of endotoxin.

Circulating polymorphs show increased functional activity during pyogenic infections and readily take up and reduce a certain yellow dye (nitroblue-tetrazolium), forming dark blue deposits in the cytoplasm. An increase in the proportion of polymorphs showing this reaction reflects their increased activity, but the test is of no value in the diagnosis of pyogenic infections because of false-positive and false-negative results. In any case, increased reduction of the dye is not necessarily associated with increased bactericidal activity.

When inflammation becomes severe or widespread, there is a general body response with the appearance of acute-phase proteins in the blood. As a result, two classical changes can be detected in the blood. The first is an increase in the erythrocyte sedimentation rate (ESR), and this is a clinically useful indication that inflammation or tissue destruction is occurring somewhere in the body. The exact mechanism of the increase is not understood. The second change is the appearance in the blood of increased quantities of a β -globulin synthesised in the liver and detected by its precipitation after the addition of the C carbohydrate of the pneumococcus. It is therefore called C-reactive protein and other acute-phase proteins are formed as a result of the action of mediators such as IL-1, IL-6 and TNF. Very small amounts are present in the blood of normal individuals, but there is a 1,000-fold increase within 24 h of the onset of inflammation. After binding to substances derived from microorganisms and from damaged host cells, it activates the complement system, acts as an opsonin, and possibly serves a useful function. Both the ESR and C-reactive protein changes are non-specific sequelae to inflammation of any sort, whether infectious or non-infectious.

When the infection is persistent, inflammation may become chronic, lasting weeks or months. Infections do not generally last for long periods if they induce acute polymorphonuclear inflammation; the battle between host and microbe is decided at an early stage. Chronic inflammation depends on a constant leakage of microbial products and antigens from the site of infection. The type of infection that persists and causes chronic inflammation is generally an intra-cellular bacterial or fungal or chlamydial infection. In these infections there is a chronic CMI response, with proliferation of lymphocytes and fibroblasts in infected areas, a steady influx of macrophages, and the formation of giant and epithelioid cells. Episodes of tissue necrosis alternate with repair and the formation of granulation tissue, then fibrous tissue. It is a ding-dong battle between microorganisms and host antimicrobial forces. The resulting granuloma (see also above) can be regarded as an attempt to wall off the infected area. Chronic infections with chronic inflammation and granuloma formation include tuberculosis, syphilis, actinomycosis, leprosy, lymphogranuloma inguinale and coccidioidomycosis. Chronic viral infections are not associated with chronic inflammatory responses, probably because virus growth is often defective and no more than minute amounts of antigen are liberated.

COMPLEMENT

Complement has been discussed and invoked on many occasions in Chapters 6–8 and in this chapter. It should be remembered that some of the complement components are quite large molecules and do not readily leave the circulation except where there is

local inflammation. Complement can carry out anti-microbial activities in the following ways:

1. *Complement lysis*. Complement reacts with antibody (IgG and IgM) that has attached to the surface of infected cells or to the surface of certain microorganisms, and destroys the cell or microorganism after making holes in the surface membrane. Gram-negative bacteria are killed in this way and also enveloped viruses such as rubella and parainfluenza (although as mentioned above, lack of complement does not exacerbate these virus infections). Because of the amplification occurring in the complement system (see Chapter 6), especially when the alternative pathway is also activated, antibody attached to the surface of a microorganism is more likely to induce complement lysis than it is to neutralise it. Complement lysis is therefore perhaps more important when antibody molecules are in short supply, early in the immune response. Bacteria with surface polysaccharide components can activate complement without the need for antibody (see 5, below), as can host cells infected with viruses such as measles. In the latter case, alternative pathway activation by itself does not do enough damage to kill the cell. Presumably, less severe membrane lesions can be repaired; antibody as well as complement must be present for lysis.
2. *Complement opsonisation*. Complement reacts with antibody attached to the surface of microorganisms, providing additional receptor sites for phagocytosis by cells bearing the appropriate complement receptors, like polymorphs or macrophages. Phagocytosis is also promoted by antibody attached to the microorganism because of the Fc receptors on phagocytes, but when complement is activated there are many more molecules of C3b present as a result of the amplification phenomenon. Therefore complement often has a more pronounced opsonising effect than antibody alone and for some bacteria, such as the pneumococcus, opsonisation actually depends on complement. Complement opsonisation is important when the antibody is IgM, because human phagocytes do not have receptors for the Fc region of IgM. Complement can also act as an opsonin though not always so effectively, in the absence of antibody (see 5, below).
3. *Complement-mediated inflammation*. Specific antibodies react with microbial antigens that are either free or on the surface of microorganisms. Following this antigen–antibody reaction, complement is activated, with generation of inflammatory and chemotactic factors (C3a and C5a). These substances focus anti-microbial serum factors and leucocytes onto the site of infection.
4. *Complement-assisted neutralisation of viruses*. In the case of viruses coated with antibody, complement adds to the mass of molecules on the virus surface and may hinder attachment of virus to susceptible cells. In some situations complement can mediate neutralisation of virus coated with a non-neutralising antibody. This will depend on the antibody isotype and presumably the density of antibody on the surface of the virus. Some viruses (murine leukaemia virus, Sindbis virus) can directly activate the complement system by interaction of virion envelope proteins with Clq or C3, resulting in the neutralisation of infectivity.
5. *Complement-assisted cell lysis*. C3b deposition on infected host cells not only opsonises (see above) but also augments ADCC and antibody-dependent complement-mediated lysis of cells.

6. *Complement opsonisation via alternative pathway.* Complement reacting with endotoxin on the surface of Gram-negative bacilli, with capsular polysaccharide of pneumococci, etc., or with *Candida*, is activated via the alternative pathway and C3b-mediated opsonisation takes place. It seems likely that this is important in natural resistance to infection.

Unfortunately, there is little direct evidence that the above anti-microbial activities of complement are in fact important in the body. The rare patients with C3 deficiency develop repeated pyogenic infections, and C3-deficient mice show increased susceptibility to plague and to staphylococcal infections. Mice with C5 deficiency (controlled by a single gene) are more susceptible to *Candida* infection, probably because of inadequate opsonisation. Patients with C5–C8 deficiencies, however, are often particularly susceptible to disseminated or recurrent neisserial infection. In this case the bactericidal rather than the opsonising action of complement seems important. But observations on complement deficiencies are probably too limited to draw firm conclusions and there have been few clearly defined deficiencies. The system is a highly complex one, with alternative pathways, positive feedback amplification and multiple inhibitors. A similar complement system occurs in a wide range of vertebrates and it must be assumed that such a complex, powerful system confers some biological advantage, presumably by giving resistance to microbial infections.

INTERFERONS

The interferons are cytokines, members of a family of cell-regulatory proteins produced by all vertebrates. There are several types of interferon, but only four relevant to studies on infectious diseases: alpha (α), beta (β), gamma (γ) and lambda (λ). Alpha and beta interferons are very similar and are made by nearly all cells in the body, including epithelial cells, neurons and muscle cells in response to viral and other infections (bacteria, mycoplasma, protozoa). There are 12 human alpha interferon genes and one beta interferon gene encoded on the short arm of chromosome 9. Gamma interferon is produced by NK cells and by T lymphocytes following antigen-specific stimulation. Only one gene exists for gamma interferon, encoded on chromosome 12. There are three lambda interferons ($\lambda 1$ or IL29, $\lambda 2$ or IL28a, $\lambda 3$ or IL28b). These interferons are very similar to IFN α/β in that they are induced upon virus infection, but differ in their tissue distribution. They are particularly active in the respiratory system as potent inhibitors of influenza virus. All are cytokines with immunoregulatory functions as well as the anti-microbial action described below.

Viruses are the most important inducers of IFN- α and - β , the stimulus to the cell being the double-stranded RNA formed during virus replication (Figure 9.2). Interferons act on uninfected cells, binding to a cell surface receptor and activating a number of genes involved in immunity to viruses. Some of these gene products (e.g. 2'5'A synthetase/RNaseL) target viral messenger RNA and others (e.g. PKR) inactivate polypeptide chain elongation, blocking viral protein synthesis. Interferons are exceedingly potent *in vitro*, being active at about 10^{-15} M. They have no direct action on virus itself and do not interfere with viral entry into the susceptible cell. The interferons produced by different species of animals are to a large extent species specific in their action. Interferon liberated from

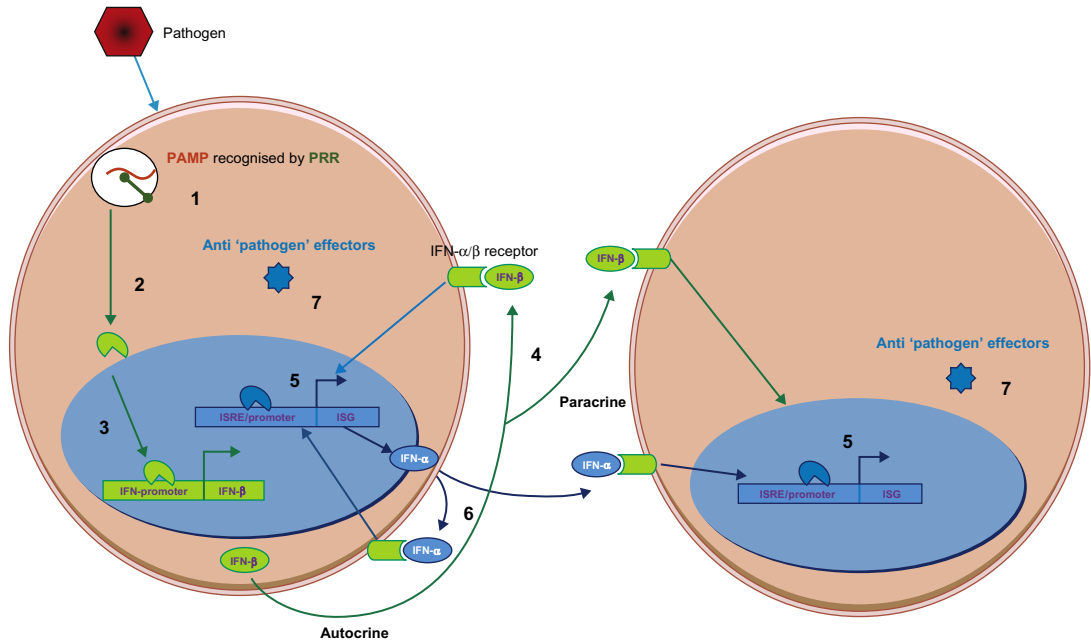


FIGURE 9.2 Mechanism of induction and expression of α and β interferon. Following infection with a pathogen, PAMPs (e.g. LPS) are recognised by cellular PRRs (e.g. TLR4) (step 1). This triggers a signal transduction cascade (e.g. involving MyD88 and NF- κ B) (step 2) which results in the stimulation of IFN- β gene expression (step 3). IFN- β is released from the cell and binds to the IFN- α/β receptor on both the infected cells (autocrine) and adjacent uninfected cells (paracrine) (step 4). This receptor binding stimulates a further signal transduction event (mediated in part by the JAK/STAT pathway) which in turn activates expression of interferon-stimulated genes (ISGs) via recognition of interferon-stimulated response elements (ISRE) (step 5). This also induces IFN- α expression which acts on the infected and uninfected cells to potentiate the response (step 6). The ISGs (e.g. PKR, RNaseL and Mx) act to inhibit pathogen replication (step 7).

infected cells can reach other cells in the vicinity by diffusion and establishes an antiviral state which protects them from infection. A cell is thus protected from infection with all viruses for a period of up to 24 h. It would seem inevitable that interferon is important in recovery from virus infections, whether on epithelial surfaces or in solid tissues. Interferons are also induced by non-viral agents such as rickettsiae and other bacteria, and will protect cells from various non-viral intra-cellular microorganisms and have other effects on host resistance. They activate NK cells and control T-cell activity by upregulating the expression of MHC proteins and thus the concentration of available peptide antigen.

It is now clear that interferon plays a central role in limiting virus infection *in vivo*. Evidence for this comes from various experimental approaches. The most dramatic are mice lacking the receptor for IFN- α/β (deleted by transgenic 'knockout' technology) which means they are unable to respond to interferon produced during a virus infection. Such animals are highly susceptible to many virus infections, including herpes simplex, MHV-68 and Semliki forest virus. Alpha/beta interferon can also be selectively inhibited in mice

by treatment with antibody to interferon. When this is done, enhanced susceptibility to certain virus infections is observed. Interferon has also been given passively to experimental animals and can be effectively induced by the administration of a synthetic ds-RNA preparation (poly I: poly C). Antiviral results are demonstrable in experimental infections, and are most clearly seen in infections of epithelial surfaces such as the conjunctiva or respiratory tract, and when treatment is begun before rather than after infection.

In humans, naturally occurring deficiencies in IFN are rare, partly because, for IFN- α at least, there are so many different genes involved. A study of 30 children who suffered from recurrent respiratory tract infections identified four with impaired interferon production. When these particular children were infected with common cold viruses, IFN- α could not be detected in nasal washings. Their peripheral blood leucocytes also failed to produce IFN- α on repeated testing *in vitro*, although INF- γ production was normal.

Interferon would seem to be the ideal antiviral chemotherapeutic agent for use in man, being produced naturally by human cells, non-immunogenic and active against a broad spectrum of viruses. However, it does cause influenza-like symptoms. So far, results in human patients have not been dramatic. For instance, volunteers infected intranasally with rhinoviruses and other respiratory viruses have been given either poly(I:C) or repeated very large doses of purified human interferon by the same route, but with only slight protection. However, it has proved useful in clearing up some cases of chronic hepatitis B infection and is being used to treat hepatitis caused by the flavivirus, hepatitis C. HBV downregulates the expression of MHC class I proteins on infected hepatocytes, thus preventing CD8⁺ T cells from destroying infected cells. By treating with IFN- γ or IFN- α , expression of MHC class I proteins is upregulated and CTLs can act. Initially the patient may become ill from the effects of interferon, but eventually a virus-free liver regenerates. Here interferon is exercising its regulatory function as well as its antiviral effect. A strain of mice can be created in which the IFN- γ gene or its receptor has been inactivated or 'knocked-out' transgenically. In the absence of pathogens, mice developed normally, but they were more susceptible to the intra-cellular bacteria *Mycobacterium bovis* and *Listeria monocytogenes*, and to vaccinia virus (but not to influenza virus). The multiplicity of effects of this interferon was demonstrated by impairment in these mice of the functions of macrophages and NK cells, reduction of macrophage MHC class II proteins, uncontrolled proliferation of splenocytes and a reduction in the amount of antigen-specific IgG2a. Further support for the importance of interferons in anti-microbial defences comes from the discovery that certain viruses have gene products that interfere with the antiviral action of interferon (see Chapter 7).

MULTIMECHANISTIC RECOVERY: AN EXAMPLE

Although the host factors responsible for recovery have been described separately, they generally act together. Recovery is multimechanistic. *Salmonella typhimurium* infection of mice is the most widely used model for typhoid-like disease caused by *Salmonella typhi* in man. The system involves intravenous injection of mice with organisms and, over a period of several days, estimation of the bacterial populations present in liver and spleen (whose mononuclear cells represent the main battleground in this infection). Sublethal infection

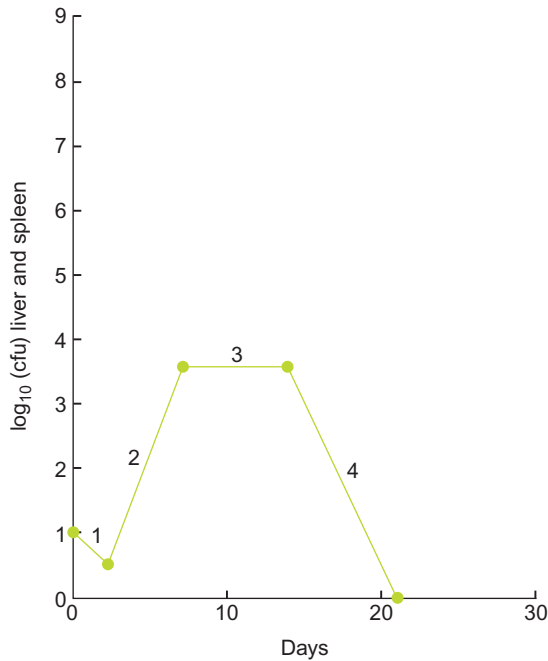


FIGURE 9.3 The four phases of a sublethal *Salmonella* infection in mice. Phase 1: initial inactivation of a large fraction of the challenge inoculum. Phase 2: exponential growth in the RES over the first week. Phase 3: plateau phase in which growth is suppressed. Phase 4: clearance of the organisms from the RES. cfu, colony-forming units.

proceeds in at least four distinct phases, schematically depicted in Figure 9.3, and with some *Salmonella* this confers solid immunity to re-challenge.

Phase 1: Initial inactivation of the inoculum. This is a constant finding representing the transition from the *in vitro* to the *in vivo* phenotype. The decline is due to immunologically non-specific uptake and destruction in macrophages of the RES. It is enhanced when animals are pre-treated with opsonising antibody, as would be expected.

Phase 2: Exponential growth in the RES. This occurs during the first week with an estimated doubling time for *Salmonella* of ca. 2–5 h; killing rates are also slow. Three factors can affect phase 2: (i) Inoculum dose. By increasing the dose the pattern of phases 1 and 2 remains the same but raised to a higher level. When the inoculum reaches LD₅₀ or higher (see Glossary) no phase 3 is observed; phase 2 continues till lethal numbers (10^8 – 10^9) are reached. With very high doses, the slope of phase steepens and the time to death shortens. (ii) Virulence of the bacteria. Increase in the slope of phase 2 is also a function of the virulence of the strain. (iii) Innate resistance of the host. A gene *ity* (immunity to typhimurium) expressed through macrophages controls phase 2. A similar situation exists for *Leishmania donovani*, *M. tuberculosis* BCG, and *Mycobacterium lepraemurium*.

Phase 3 is essential for the host to survive. It is not mediated by T cells but requires continued production of TNF- α , which stimulates the production of IFN- γ . Studies with the *Listeria* model indicate that TNF- α is produced by macrophages which stimulate NK cells to release IFN- γ , which in turn activates newly recruited macrophages. It is of interest that, during the plateau phase, mice show a manifest macrophage-mediated immunosuppression towards other antigens.

Phase 4 is the clearance phase that does require the presence of T cells, causing macrophage activation. CD8 as well as CD4 T cells are involved. Again host genes play an important role in this phase.

This example illustrates the importance of a coordinated multimechanistic approach by the innate and adaptive immune responses in resolving a complex host–pathogen interaction.

TEMPERATURE

In man, the mean daily body temperature is 36.8°C with a daily variation of only 1.3°C, the maximum being at about 18.00 h, the minimum at about 03.00 h. This almost constant body temperature, like the almost constant level of blood sugar, illustrates Claude Bernard's dictum that '*La fixité du milieu intérieur est la condition de la vie libre*'. If the individual is to function steadily in spite of changes in the external environment, the internal environment must remain constant. The brain is one of the most sensitive parts of the body to departures from normality. At body temperatures below 27.7–30°C people become unconscious, at 40.5°C or above they become disoriented and may be maniacal; above 43.3°C they are comatose. A rise in body temperature is one of the most frequent and familiar responses to infection, whether the infection is largely restricted to body surfaces (common cold, influenza) or is obviously generalised (measles, typhoid, malaria). During fever the appetite is often lost and headache may result from dilation of meningeal blood vessels. The temperature rise is largely due to an increase in heat production, and the raised metabolic rate, together with reduced food intake, results in a high excretion of nitrogen in the urine. There is rapid wasting of body fat and muscles if the fever is prolonged.

A common mediator of the febrile response is IL-1 and, in addition, IL-6 causes fever by acting on the hypothalamus, whereas TNF (in the LPS fever model in rats) tends to reduce an already elevated temperature.

IL-1 is present in inflammatory exudates and in the plasma during fever and acts on the temperature-regulating centre in the anterior hypothalamus, resetting the body thermostat. IL-1 is produced by macrophages and certain other cells, and as little as 30–50 ng causes fever in rabbits. Fever is a common accompaniment of generalised antigen–antibody reactions. For instance, rabbits immunised with bovine serum albumin develop fever when injected with this antigen. Systemic virus infections such as the exanthems (see Glossary) are characterised by an asymptomatic incubation period during which virus replicates and spreads through the body, followed by a sudden onset of illness with fever. The febrile reaction is mainly due to the immune response to the virus; hence its relatively sudden onset a week or two after infection. The CMI response as well as the antibody response is involved. Antigen–antibody reactions, in addition to causing fever, can also give rashes, joint swelling and pain, even glomerulonephritis (see Chapter 8). The first signs of illness in hepatitis B, before jaundice, are often 'allergic' in nature and mediated by antigen–antibody interactions, with fever, joint pains and fleeting rashes.

The generalised CMI response in the infected host is also a cause of fever, e.g. in tuberculosis, brucellosis and perhaps staphylococcal and cryptococcal infections. Tuberculin added to alveolar macrophages from an immunised animal induces IL-1, and patients with chronic brucellosis develop fever when injected with 10 g of purified brucella.

Certain bacterial products are pyrogenic. The peptidoglycan in the cell wall of staphylococci causes monocytes to liberate IL-1. More importantly, endotoxins from Gram-negative bacteria also have this effect, as little as 2 ng of *Salmonella* endotoxin kg^{-1} causing fever in man. Endotoxin is present in the circulation during systemic infection with Gram-negative bacteria, but tolerance to endotoxin-induced fever develops quite rapidly, and endotoxin itself probably makes no more than a partial contribution to the febrile response, even in infections such as typhoid and dysentery. There is no good evidence that other microbial products or toxins cause fever other than by immunological mechanisms. In the old days before penicillin, pneumococcal pneumonia used to give one of the highest fevers known in man with dramatic and severe onset, the temperature often rising to 40°C within 12 h. These bacteria, however, have no endotoxin or other pyrogens and the mechanisms were presumably immunological.

When human volunteers were infected with influenza virus, those with the most IL-6 and IFN- γ in nasal fluids had higher temperatures, as well as more virus, more mucus production and more symptoms. In the case of influenza virus infection of ferrets, which is the best animal model for transmission of human influenza, there is a direct correlation between virulence and viral pyrogenicity. IL-1 is released locally in the respiratory tract as a result of virus-phagocyte interaction. Since fever is such a constant sequel of infection, it is natural to suppose that it has some anti-microbial benefit. Thomas Sydenham in the seventeenth century wrote that 'Fever is a mighty engine which nature brings into the world for the conquest of her enemies'. Bodily functions are profoundly disturbed by fever. Metabolic activity is increased in phagocytic cells, and studies *in vitro* show that there are large increases in T-cell proliferation and in antibody production at febrile temperatures. The evidence, however, is disappointing. Temperature-sensitive mutants of certain viruses are often less virulent, and experimental virus infections can sometimes be made more severe by preventing fever with antipyretic drugs. When fever is induced in infected animals by raising the environmental temperature, there are also other complex physiological changes, making it difficult to interpret such experiments. In two bacterial infections, gonorrhoea and syphilis, the microbes themselves are actually killed by febrile temperatures, but in the natural disease these temperatures are rarely reached. Before the introduction of antibiotics, patients with these two diseases were infected with malaria in order to induce body temperatures high enough to eradicate the infection (following which the malaria was treated with quinine).

If fever is of value to the host, one might expect microbes to attempt to prevent it. Vaccinia virus, which normally fails to cause fever in mice, produces a soluble receptor for IL-1 β (the fever mediator) and virus strains lacking the gene for this receptor do cause fever. We may ask whether *T pallidum* actively inhibits the fever response.

Fever is costly in energy and is an ancient bodily response, having evolved with the vertebrates over hundreds of millions of years. Perhaps one day some more convincing evidence will emerge to give substance to Sydenham's eloquent convictions.

TISSUE REPAIR

Once the multiplication of the infecting microorganism has been controlled, and the microorganism itself perhaps eliminated from the body, the next step in the process of recovery is to tidy up the debris and repair the damaged tissues. In other words

pathogenesis is followed by 'pathoexodus'. Four examples will be given, in the skin, respiratory tract, liver and the foetus. At the molecular level a profusion of mediators are involved. Cytokines, because of their effects on cell growth and differentiation, play a part at all stages in the repair process.

In the skin

During recovery from a boil, for instance, the sequence of events is as follows. Superficial tissue debris, including necrotic epidermis, inflammatory cells and plasma exudate, dries off as a scab. This gives mechanical protection, acts as a barrier to further infection, and can be shed to the exterior after repair is completed. Below the scab, phagocytic cells clear up the debris and fibroblasts move in, multiply and lay down a mucopolysaccharide matrix over the underlying intact tissues. New blood vessels are formed, and later on lymphatics, by the sprouting of the endothelial cells of neighbouring vessels into the fibroblast matrix. The newly formed capillaries advance into the damaged zone at 0.1–0.6 μm a day. They are fragile and leaky, and there is a continuous extravasation of polymorphs, macrophages and fibroblasts into the matrix. As seen from the surface, each collection of capillary loops in the fibroblast matrix looks like a small red granule and this soft vascular material is therefore called granulation tissue. It bleeds easily and with its rich blood supply and abundant phagocytic cells is well protected against infection. Meanwhile, epidermal cells at the edges of the gap have been multiplying. The newly formed layer of cells creeps over the granulation tissue, and the epidermis is thus reconstituted. Fibroblasts in the granulation tissue lay down reticulum fibres and later collagen. If the area of epidermal cell destruction is large, and when underlying sebaceous glands, hair follicles, etc. are destroyed, a great deal of collagenous fibrous tissue is formed to repair the gap. The newly formed collagen in fibrous tissue contracts and tends to bring the skin edges together. Contracting collagen can strangle an organ like the liver, but in the skin it merely forms a scar. A scar is a characteristic sequel to vaccination with BCG, or to a bacterial infection involving sebaceous glands, as seen in severe acne.

In the Respiratory Tract

After infection with a rhinovirus or influenza virus, there are large areas where the epithelial cells are destroyed, mucociliary transport is defective and the underlying cells are vulnerable to secondary bacterial infection. Phagocytic cells must now ingest and dispose of tissue debris, and the epithelial surface must be reconstituted by a burst of mitotic activity in adjacent epithelial cells. To some extent, pre-existing cells can slide across the gap but repair depends on mitosis in cells at the edges. The process of repair takes several days, and the mechanism is the same whether the damage is caused by viruses, bacteria or chemicals. Epithelial regeneration is particularly rapid in respiratory epithelium, and also in conjunctiva, oropharynx and mucocutaneous junctions, but it is delayed if the infection continues. After chronic bacterial or chemical damage, there is an increase in mucus-producing goblet cells in the respiratory epithelium, and sometimes impairment of

mucociliary mechanisms, resulting in the condition called chronic bronchitis. As a rule, however, recovery is complete.

In the Liver

During recovery from focal hepatitis, polymorphs and macrophages are active in areas of tissue damage, phagocytosing dead and damaged hepatic cells, Kupffer cells, biliary epithelial cells, inflammatory cells and microorganisms. As this proceeds, neighbouring hepatic cells and bile duct epithelial cells divide to replace missing cells. This, together with cell movement and rearrangement, leads to remodelling of the lobules and the restoration of normal appearances. If supporting tissues have been significantly damaged, and particularly if there are repeated episodes of necrosis, healing involves scar formation. When this is widespread it is referred to as cirrhosis, the bands of fibrous tissue dividing up the organ into irregular islands. The regenerating islands enlarge to form nodules, the fibrous tissue thickens and contracts and there is obvious distortion of structure, with circulatory impairment, biliary obstruction and liver dysfunction.

In most tissues, repair with restoration of structural integrity can be achieved by fibrous tissue formation. Recovery of function depends more on the ability of differentiated cells in damaged tissues to increase their numbers again and thus restore functional integrity. Liver cells or epithelial cells have a great capacity for mitosis, and the intestinal epithelium, respiratory epithelium or liver can be restored to normal without great difficulty. In the case of cardiac muscle, striated muscle or brain, the differentiated cells show little if any mitotic capacity and destruction in these tissues results in a permanent deficit in the number of cells. This may be of no consequence in a muscle as long as firm scar tissue repairs the damage, but it may be important in the central nervous system. Anterior horn cells destroyed by poliovirus cannot be replaced, and if enough are destroyed there will be a permanent paralysis, although some restoration of function takes place by learning to use muscles more effectively and by the recovery of damaged anterior horn cells.

In the Foetus

Tissue repair in the foetus is in some ways easier and in others more difficult. In general there is a very great capacity for repair and reconstitution of damaged tissues. Primitive mitotic cells abound, organs are in a state of plasticity, and in the developmental process itself tissue destruction and repair accompanies mitosis and construction. On the other hand, at critical times in foetal life, there is a programmed cell division and differentiation in the course of constructing certain major organs. If one of these organs is damaged at this critical time, the developmental process is upset and the organ is malformed. This is what happens when rubella virus infects the human foetus during the first three months of pregnancy. Depending on the exact organ system being formed at the time of foetal infection, there may be damage to the heart, eyes, ears or brain, resulting in congenital heart disease, cataract, deafness or mental retardation in the infant. Other infections (see Table 5.3) affect particularly the central nervous system of the foetus (toxoplasmosis, cytomegalovirus, syphilis) and sometimes bones and teeth (syphilis).

If the foetal infection is severe, as is the rule with vaccinia virus or with most bacteria, foetal death and abortion is the inevitable consequence. There are only a small number of microorganisms that infect the foetus and interfere with development without proving fatal. This type of nicely balanced pathogenicity is needed if the infected foetus is to survive and be born with a malformation. Even the infections that cause malformations (teratogenic infections) are sometimes severe enough to kill the foetus. In most congenital infections the microorganism remains present and is detectable in the newborn infant (cytomegalovirus, rubella, syphilis, etc.), often persisting for many years. It is a striking feature of most teratogenic foetal infections (rubella, cytomegalovirus, toxoplasmosis) that the mother suffers a very mild or completely inapparent infection.

Certain microorganisms infect the foetus and damage developing organs, but are then eliminated from the body. The damaged organs are formed as best as possible, and at birth there are no signs that the malformation was caused by a microorganism. Tissues are sterile, and no inflammatory responses are visible histologically. Thus when a pregnant hamster is infected with K virus (a polyomavirus), there is infection of the dividing cells that are to form the molecular layers constituting the bulk of the cerebellum. These cells are destroyed, the cerebellum therefore fails to develop normally, and the newborn hamster shows severe signs of cerebellar dysfunction, although it is perfectly well in every other way. The affected cerebellum is small and greatly depleted of cells, but there is no evidence of past microbial infection.

RESISTANCE TO RE-INFECTION

Resistance to re-infection depends on the immune response generated during primary infection. Passive immunisation with antibody is known to protect humans against measles, hepatitis A, hepatitis B, rabies, etc., and the passively acquired (maternal) immunity of the newborn child or calf to a great variety of infections is another example of the resistance conferred by specific antibody. Most resistance to re-infection is antibody-mediated. IgG antibodies generally continue to be formed in the body many years after the initial infection; IgA antibodies are less persistent than IgG antibodies. Even if antibody levels are undetectable, memory B cells from the initial infection are often present in large enough numbers to give an accelerated (anamnestic) response within a few days of re-infection. This is especially important in infectious diseases with incubation periods measured in weeks because there is time enough for the anamnestic response to operate and terminate the infection during the incubation period, before production of clinical disease. Sometimes resistance to re-infection is maintained by repeated subclinical infections, each of which boosts the immune response. For instance, children catching rubella at school can re-infect their immune parents sub-clinically and this is detected by a rise in antibody levels. Resistance to rubella, diphtheria and perhaps other infectious diseases is maintained in this way.

Antibodies protect against infection in a number of ways. For instance, they attach to the microbial surface and promote its uptake by phagocytic cells, acting as opsonins. Other antibodies protect against re-infection by combining with the microbial surface and blocking attachment to susceptible cells or body surfaces. Microorganisms that need

to make specific attachments are listed in Table 2.1. However, circulating IgG or IgM antibodies coat polioviruses, coxsackie viruses or adenoviruses, and act by interfering with viral uncoating rather than by blocking attachment to susceptible cells. Secretory IgA antibodies are particularly important because they can act on the microorganism before its attachment to a body surface. They do not act as opsonins, and they do not lyse microorganisms because there is no complement on body surfaces, and in any case they fix complement poorly. But by preventing the attachment of microorganisms such as *Vibrio cholerae* to intestinal epithelium, the gonococcus to urethral epithelium, or *Chlamydia* to the conjunctiva, IgA antibodies can ensure that these microorganisms are carried away in fluid secretions rather than initiate infection. Acquired resistance to infection of the surface of the body is often of short duration. For instance, resistance to gonorrhoea or parainfluenza viruses following natural infection seems to last only for a month or so, and in childhood repeated infections with respiratory syncytial virus and *Mycoplasma pneumoniae* are common. Presumably the IgA antibodies that mediate resistance are short-lived and IgA memory cells do not generate a good enough or rapid enough secondary response.

Resistance to re-infection, since it is immunological in nature, refers especially to the antigenic nature of the original infecting microorganism. Resistance to measles or mumps means resistance to measles or mumps wherever or whenever they occur, because these viruses are essentially of only one type (monotypic) immunologically. Resistance to the disease influenza or poliomyelitis, however, depends on the separate acquisition of resistance to a number of distinct antigenic types of influenza or polioviruses. Resistance to streptococci depends on the acquisition of antibodies to the M protein in the bacterial cell wall, and since there are at least 10 types of M protein that circulate quite commonly in communities (40–50 types of M protein altogether), repeated infections with *S. pyogenes* occur as antibodies are gradually developed against the various types. Often, however, different serological types of a given microorganism show some overlap so that antibodies to one type can confer partial resistance to another.

When resistance to a disease appears not to develop, the possibility of multiple antigenic types must be considered. There are multiple antigenically distinct types of gonococcus, for instance, a fact that helps account for successive attacks of gonorrhoea. Numerous attacks of non-specific urethritis are to be expected because of the variety of microorganisms that cause this condition. In one study, 40% of attacks were due to *Chlamydia*, but there are 12 known antigenic types.

Resistance to re-infection can also be mediated by CMI. The CMI response generated on primary infection lasts for weeks or perhaps months rather than years, and there is an accelerated CMI response on re-infection, although less vigorous than in the case of antibodies. Nearly always, a persistent infection is needed to give continued CMI resistance and infections showing this are usually intra-cellular in nature. For instance, resistance to re-infection with tuberculosis, syphilis and possibly malaria depends on the active presence of the microorganism in the body, with continuous stimulation of the antibody and CMI responses. In most of these instances, resistance to re-infection is CMI-mediated. There are a few examples, however, such as measles, in which recovery from primary infection is largely due to CMI, but resistance to re-infection is attributable to antibody.

Bibliography

- Akdis, M., Burgler, S., Cramer, R., Eiwegger, T., Fujita, H., et al., 2011. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J. Allergy Clin. Immunol.* 127, 701–721.
- Biron, C.A., Sen, G.C., 2007. Innate responses to viral infections. In: Knipe, D.M. (Ed.), *Fields Virology*, fifth ed. Lippincott Williams and Wilkins, Philadelphia, PA, pp. 249–278.
- Baumann, H., Gauldie, J., 1994. The acute phase response. *Immunol. Today* 15, 74–80.
- Bogdan, C., Gessner, A., Solbach, W., Rollinghoff, M., 1996. Invasion, control and persistence of *Leishmania* parasites. *Curr. Opin. Immunol.* 8, 517–525.
- Bouabe, H., Okkenhaug, K., 2013. Gene targeting in mice: a review. In: Bailer, S.M., Lieber, D. (Eds.), *Virus–Host Interactions: Methods and Protocols*. Humana Press Inc., Totowa, pp. 315–336.
- Chisari, F.V., Ferrari, C., 1995. Immunopathogenesis of viral hepatitis. *Annu. Rev. Immunol.* 13, 29–60.
- de Jong, H.K., Parry, C.M., van der Poll, T., Wiersinga, W.J., 2012. Host–pathogen interaction in invasive salmonellosis. *PLoS Pathog.* 8.
- Dimmock, N.J., 1993. Neutralization of animal viruses. *Curr. Top. Microbiol. Immunol.* 183, 1–149.
- Dinarello, C.A., 1996. Biologic basis for interleukin-1 in disease. *Blood* 87, 2095–2147.
- Hormaeche, C.E., Villarreal, B., Mastroeni, P., Dougan, G., Chatfield, S.N., 1993. Immunity mechanisms in experimental salmonellosis. In: Cabello, F.C., Hormaeche, C.E., Mastroeni, P., Bonina, L. (Eds.), *Biology of Salmonella*. pp. 223–235. NATO ASI Series, Series A: Life Sciences 245.
- Kaufmann, S.H.E., Ladel, C.H., 1994. Application of knockout mice to the experimental analysis of infection with bacteria and protozoa. *Trends Microbiol.* 2, 235.
- Law, M., Hengartner, L., 2008. Antibodies against viruses: passive and active immunization. *Curr. Opin. Immunol.* 20, 486–492.
- Morgan, B.P., Walport, M.J., 1991. Complement deficiency and disease. *Immunol. Today* 12, 301–306.
- Pellegrini, M., Calzascia, T., Toe, J.G., et al., 2011. IL7 engages multiple mechanisms to overcome chronic viral infection and limit pathology. *Cell* 144, 607–613.
- Randall, R.E., Goodbourn, S., 2008. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. *J. Gen. Virol.* 89, 1–47.
- Ravetch, J.V., Clynes, R.A., 1998. Divergent roles for Fc receptors and complement *in vivo*. *Annu. Rev. Immunol.* 16, 421–432.
- Unanue, E.R., 1997. Inter-relationship among macrophages, natural killer cells and neutrophils in early stages of *Listeria* resistance. *Curr. Opin. Immunol.* 9, 35–43.
- Van den Broek, M.F., Muller, U., Huang, S., Zinkernagel, R.M., Aguet, M., 1995. Immune defence in mice lacking type I and/or type II interferon receptors. *Immunol. Rev.* 148, 5–18.
- Wuethrich, M., Deepe Jr., G.S., Klein, B., 2012. Adaptive immunity to fungi. *Annu. Rev. Immunol.* 30 (30), 115–148.