Infection: Immunological Barriers

Introductory article

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Microorganisms can infect a host through various portals of entry. The host attempts to counter microbial infection and dissemination using many physical, chemical and immunological weapons.

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Routes of Infection

The potential portals of entry that can be exploited by microbes can be divided into two categories: first, the skin, and second, mucosal surfaces. The two are, of course, contiguous, with skin covering the external surfaces, and mucosa lining the inner sancta.

Infection in or through the skin

The skin can be thought of as a conveyor belt, in which living cells from the innermost layer travel towards the skin's surface; during this process, they die; and hence the surface of the skin comprises dead cells. These cells are relatively resistant to microbial insult, but their removal, for example if the skin is abraded, exposes the underlying live cells, which may be more sensitive to infection. The skin can also be breached by a cut, through which the microbe might gain access to underlying tissues, and even to the blood. Furthermore, certain pathogenic organisms are inoculated into or through the skin. For example, rabies virus is transmitted by the bite of a rabid animal; malaria and yellow fever are delivered when a carrier mosquito feeds on blood, by puncturing the skin using its needle-like proboscis; and tick bites transfer the bacteria causing Lyme disease

Infection of mucosal surfaces

In contrast to the dead cells on skin's surface, mucosa (also called mucous membranes) have live cells at their surfaces, and the areas are rich in cells that secrete mucus, which covers the surfaces, keeping them moist. There are many anatomically distinct mucosal surfaces, but only three shall be considered here: the respiratory tract, the gastrointestinal tract and the genitourinary tract.

Respiratory infections

Respiratory infections such as the common cold, influenza and bacterial pneumonia are transmitted when an infected person coughs or sneezes, propelling into the surrounding air a spray of very small water droplets in which the microbes are suspended; these droplets are inhaled by an unfortunate neighbour, who a few days later develops symptoms of the infection. This aerosol transmission explains the increased frequency of 'colds' in inclement weather – when people are often crowded together, and humidity is high (prolonging the 'lifespan' of the aerosolized droplets). Many respiratory infections are limited to the upper part of the respiratory tract, resulting in a cough and sore throat. More serious infections reach into the lower respiratory tract, infecting the lungs themselves, and reducing the gas exchange that is necessary for our survival; such infections may be life-threatening or fatal.

Gastrointestinal infections

Many microbes infect and replicate in the gastrointestinal (GI) tract. Massive numbers of these organisms, and of several others which replicate in GI-related organs, such as the liver, may be shed in the faeces; and poor hygiene leads to contamination (e.g. of handled foods), and consequent transmission of the infection to a new host. Many infections are spread by this faecal–oral route, including the bacterial diseases typhoid and cholera, and the viral diseases poliomyelitis and hepatitis A. Some infections remain largely localized to the GI tract (e.g. *Helicobacter pylori*, which infects approximately half of the world's population, and causes, among other things, peptic ulcer disease) while others can spread to distant tissues, via the blood, the lymphatic system, or in nerves; for example, poliovirus replicates in the GI tract, and spreads via the blood to infect distal sites including the central nervous system, where it may destroy nerve cells, causing paralysis and sometimes death. While the acidic environment of the upper GI tract is inimical to many microbes (see below), several viruses have evolved to exploit the proteases which digest our foods; for example, reoviruses in the intestinal lumen are cleaved by host proteases, and the resulting particles have greatly enhanced infectivity.

Genitourinary (GU) infections

Bacterial infections of the urinary tract are more frequent in females, mainly for anatomical reasons, and invasion into the bladder, ureters and kidney is not uncommon. Infections of the genital tract are usually sexually transmitted. A multitude of microbes have exploited sexual activity as a means to ensure their own dissemination.

Among these are fungi (thrush), the bacteria causing syphilis and gonorrhoea, and the viruses responsible for AIDS, hepatitis B, genital herpes and genital warts.

Immune Responses

The immune response can be subdivided in several ways. In this brief review, it is divided into nonspecific immunity and antigen-specific immunity. Nonspecific immune responses are the first line of defence against most infections. Some of the nonspecific barriers are constantly active, while others are activated by infection. However, once the infection has been eradicated, the nonspecific barriers return to their previous states – they are not fundamentally altered by the infection; for this reason, they are often termed 'innate' immunity. The responses are nonspecific in that they do not recognize individual microbial antigens, although, as will be seen later, some of them may be recruited into antigen-specific responses.

Nonspecific Defences

Physico-chemical barriers

The skin acts as a surprisingly effective barrier, repelling many dangerous microbes to which it is exposed. The surface of skin comprises mainly dead cells, which are unable to support virus infection (because viruses need living cells in which to grow). Furthermore, these superficial cells are constantly shed and replaced by cells from the lower layers; this constant release and replenishment makes it difficult for microbes to establish a strong 'foothold'. The rapid cell turnover is a relatively effective deterrent, and is repeated in many mucosal linings (e.g. the lining of the GI tract also turns over very rapidly). In addition, normal skin is populated by bacteria which, under normal circumstances, are not harmful to the host; in fact, these bacteria are beneficial, and if they are removed (e.g. by frequent application of bactericidal soaps, or by the indiscriminate use of antibiotics), other potentially pathogenic bacteria are given the opportunity to establish infection, and then to cause disease. Similarly, the normal GI tract is heavily populated with bacteria (bacteria represent some 25% of faecal mass), and antibiotic treatment may eradicate these organisms, leading to colonization by pathogens, with disease ranging from mild diarrhoea to life-threatening necrotizing enterocolitis.

One physical defence not available to skin, but by definition present in the mucosae is mucus, which is constantly shed, trapping microbes and reducing their ability to establish infection. In the GI and GU tracts this mucus is usually shed with the excreta; since this method of elimination is not available to the respiratory tract, another method of mucus removal must be employed – the ciliary system. Cilia are small, 'hairlike' appendages on the surface of cells lining the airways; these cilia 'wave', moving the mucus towards the upper regions of the tract, where it can be expelled by coughing. The GI tract is home to several additional physico-chemical defences. Although our mouths support much bacterial growth – hence the high incidence of dental caries (tooth decay) – things could be worse; saliva contains lysozyme, an enzyme that degrades bacterial cell walls. Furthermore, the acid environment of the stomach ($\sim$pH 2) discourages many microbes, as does the presence of a plethora of proteases secreted by the stomach and pancreas; and bile salts, delivered to the GI lumen from the liver, can inactivate most viruses that are enshrouded in lipid coats. Consequently, the viruses that replicate effectively in the GI tract (e.g. the enteroviruses) for the most part lack such lipid coats, instead being enclosed in protein shells

Phagocytes

Several cell types, mainly white blood cells and their relatives, can engulf microbial invaders, killing them (although some microbes have evolved to survive within their predators). White blood cells can be broadly subdivided into three groups: polymorphonuclear leucocytes (polymorphs), monocytes and lymphocytes. The first two are important in nonspecific defences, while lymphocytes confer antigen-specific immunity, and are described below. Polymorphs play a major role in containing many infections, travelling rapidly to the affected site, assisting in the recruitment of other immune responses, and engulfing the microbes and other debris. Monocytes are the source of tissue macrophages which, as their name suggests, are phagocytic, and hence contribute to nonspecific responses; in addition, they can present microbial antigens to lymphocytes, alerting them to the presence and nature of the invading microbe (see below). Macrophages are central to many aspects of the nonspecific and antigen-specific immune responses.

Natural killer cells

Natural killer (NK) cells are important in controlling several viral infections, and may play a role in tumour eradication. These cells can be considered complementary to cytotoxic T lymphocytes (CTLs). As described below, CTLs are triggered when a microbial peptide is displayed on the infected cell's surface by the major histocompatibility complex (MHC) proteins. Many viruses attempt to circumvent CTL recognition by preventing the MHC molecule from reaching the cell surface – and here natural killer (NK) cells step into the breach. These cells do not recognize specific foreign antigen, instead being activated by the absence of MHC molecules on a cell's surface;

activated NK cells destroy susceptible target cells by inoculating a protein named perforin into the target cell membrane; perforin molecules assemble in the membrane to form a pore, through which other toxic molecules can flow into the target. NK cells are also prolific producers of the antiviral cytokine interferon γ.

Complement

The complement system was discovered, and named, because it could assist (or 'complement') antibodies in their neutralization of bacteria. The human complement system contains more than 20 proteins, arrayed such that activation of one component leads to a cascade effect in which the entire complement system is activated. There are three pathways of activation. The first, the 'classical' pathway, is activated by antibody–antigen complexes; the second, 'lectin' pathway is activated by lectin binding to pathogen surfaces; and the third 'alternative' pathway is triggered by direct binding of a complement component to a pathogen's surface. The second and third pathways are clearly nonspecific, but the first pathway appears to have been exploited by antibodies to introduce complement's effector functions into the antigen-specific response. Although triggered by different events, and initially employing different components of the complement system, all three activation pathways converge to a single point, the production of a protein named C3 convertase. This leads to the activation of all three effector arms of the complement cascade. The first effector mechanism (and probably the most important) is coating (or 'opsonization') of pathogens with the C3b complement component; this interacts with receptors on the surface of phagocytes (polymorphs, macrophages – see above), encouraging pathogen engulfment. The second effector mechanism is production of a 'membrane attack complex', in which a monomeric protein undergoes assembly followed by insertion into the lipid membrane of the pathogen, or of the infected cell, generating a membrane-spanning pore, similar to that formed by perforin, which disrupts homeostasis. The third aspect of complement's effect is release of peptide inflammatory mediators which can aid in the recruitment of phagocytes and monocytes to the site of infection.

Cytokines and chemokines

Cytokines comprise a wide variety of mainly soluble factors produced in response to microbial challenge. Most of these factors act locally, their effects being mediated through high concentration; for example, they may limit microbial replication (e.g. interferons) or destroy targeted cells. Some act over short distances (e.g. chemokines set up a concentration gradient which attracts immune effector cells to the source), while a few may act more distantly. Many cytokines act to regulate cells participating in immune responses. For example, tumour necrosis factor alpha (TNFα) is produced by T cells, and activates macrophages. However, additional study has shown that cytokines may be produced by non-immune cells; and they may also act on non-immune cells, often altering the outcome of infection. For example, the interferon-β is produced by fibroblasts, and acts on somatic cells to increase MHC expression.

Chemokines are a subset of cytokines that are related in amino acid sequence and by the receptors to which they bind; the three categories of chemokines are defined by specific patterns of cysteine residues. They are produced not only by immune cells (monocytes, macrophages, T cells) but also in some cases by cells not normally considered immune-related (fibroblasts, keratinocytes, endothelial cells). All chemokines are chemoattractants; the cells attracted vary between individual chemokines, but usually are polymorphs, monocytes and/or T lymphocytes.

Antigen-specific Immunity

Antigen-specific immune responses are vital to our survival. Upon exposure to an antigen, specific molecules capable of recognizing only that antigen are activated, to eradicate the foreign material. One could argue that that is similar to nonspecific responses, which also can be activated, or upregulated, by microbial infection. However, unlike nonspecific responses, the specific response has 'memory'–when the antigen (e.g. a microbe) is encountered for a second time, the antigen-specific host response is much faster, and much more extensive. This is how vaccines work; they induce antigen-specific memory cells which, upon exposure to the pathogenic organism, rapidly expand to counter the challenge. For this reason, in contrast to nonspecific innate immunity, antigen-specific responses are said to be 'adaptive'. Antigen-specific immunity is the domain of lymphocytes, white blood cells recognizable by their large nuclei. In the adult mammal, most haematopoiesis occurs in the bone marrow, and hence all lymphocytes have a common origin, from bone marrow haematopoietic progenitor cells. However, during development, lymphocytes divide into two groups; one undergoes maturation in the thymus (and hence these *t*hymus-derived lymphocytes were named T cells), while the other was initially discovered in an avian organ, the *b*ursa of Fabricius, and hence these lymphocytes were named B cells (there is no bursal equivalent in mammals, but B cells have retained the name). In short, T cells recognize antigens only when displayed on the surface of cells in association with MHC molecules, while B cells produce antibodies, which can recognize intact microbes as well as intact proteins on cell membranes or in soluble phase.

The MHC and antigen presentation pathways

To understand antigen-specific responses it is imperative first to understand the role of the MHC. The MHC comprises a collection of genes discovered during studies on skin grafting (the locus was so named because it determined whether or not graft and recipient tissues were compatible), but its function is absolutely central to antigen-specific immunity. In summary, there are two major classes of MHC molecule, termed class I and class II; class I molecules are present on almost all nucleated cells, while class II expression is restricted to specialized antigen-presenting cells (e.g. macrophages, dendritic cells). The two MHC classes are similar in crystallographic structure; both are dimeric proteins, present on the cell membrane, and both contain a 'groove', exposed to the extracellular environment, and containing a short peptide. One picturesque analogy is a 'Venus fly trap', with a peptide caught in the lips; the peptide, which is recognized by T cells, is said to be 'presented' by the MHC molecule. What is the nature of the peptide, and why did evolution provide us with two such apparently similar MHC molecules? It was first hypothesised that the two MHC classes might present physically distinct peptides – perhaps class I would present hydrophobic peptides, while class II presented hydrophilic ones. This, however, is not the case; indeed, some studies showed that the same peptide sequence could be presented by either MHC class, deepening the conundrum. The answer is straightforward, and delightfully elegant: the two MHC classes present peptides originating from different sources. MHC class I presents peptides generated from proteins made inside the cell, while MHC class II presents peptides derived from proteins taken up from outside the cell. Thus, for the same protein, synthesis within the cell will drive peptides into the class I pathway, while delivery from outwith the cell will drive the peptides into the class II pathway. The class of MHC displaying the peptide therefore informs the perusing T cells whether that protein is being made inside the cell (in which case the cell is probably infected) or whether it came from outside the cell (in which case the cells may not be infected

T lymphocytes

There are two classes of T cell, originally characterized by surface proteins, and now known to differ in function, and in the nature of the antigens they recognize. Both classes of T cell recognize the peptide via antigen-specific T-cell receptors (TCRs) on their cell membranes. On any one cell, all TCRs will be identical; but TCRs on different cells will differ (hence ensuring different antigen specificities). One T-cell class carries the CD8 molecule, while the other carries the CD4 molecule. The CD8 molecule binds to MHC class I, while CD4 binds to class II; because of these interactions, CD8+ T cells are activated only by peptides presented by MHC class I, while CD4+ T cells are activated only by peptides presented by MHC class II. Therefore, because of the differing nature of the two MHC pathways, CD8+ T cells recognize endogenously synthesized peptide (and hence CD8+ T cells recognize infected cells) while CD4+ T cells recognize exogenously-derived peptide (and so the antigen-presenting cells seen by CD4+ T cells may not be infected). Evolution has ensured that the function of the two T-cell classes is appropriate to the types of antigen recognized. Thus, CD8+ T cells usually lyse target cells, and hence are named 'killer' T cells, or CTLs; their lytic activity, mediated by perforin, is an appropriate response to the target cells they recognize, since most cells recognized by CD8+ T cells will be microbially infected. In contrast, CD4+ T cells are usually not lytic, instead being activated by antigen to produce cytokines which help in maintaining CTL memory, and in permitting B cell maturation; CD4+ T cells are therefore usually 'helper' cells. Again, this appears appropriate; the cells which activate CD4+ T cells are specialized antigen-presenting cells (APCs) which usually are not infected by the microbe, but are instead signalling that a problem exists elsewhere in the host; their destruction would not benefit the host, and indeed would often be deleterious, since it would diminish the help that could be provided.

In addition to lysing target cells, CTLs carry and express an array of cytokines and chemokines, which may directly or indirectly limit microbial pathogenesis. At this point it is important to note that, as with many attempts to rigidly categorize biological topics, our scission of the immune response into antigen-specific and nonspecific is imperfect. For example, interferon γ is categorized above as a component of nonspecific immunity, since it has no intrinsic capacity for antigen specificity; however, its secretion by CTLs will occur at or near the site of infection (since that is where the peptide-class I stimulus is located), and under these circumstances this cytokine is being delivered in an antigen-specific manner. Conversely, CTLs are most certainly antigen-specific, but the cytokines they release can have nonspecific effects; for example, if two different viruses infect adjacent cells, cytokines released by a CTL responding to the first virus may also eradicate the second virus.

Antibodies

Unlike T cells, which recognize antigens as short peptides presented on the cell surface by the MHC, antibodies recognize proteins without any requirement for 'presentation' by host molecules. Antibodies are therefore more catholic in their recognition; they can recognize free proteins, in solution; proteins displayed on cell walls or membranes; and proteins within higher-order structures, such as viral capsids. Therefore antibodies can bind to

intact microbes, and this binding may lead to the inactivation ('neutralization') of the microbe.

Antibodies, or immunoglobulins (Ig), can be thought of as Y-shaped molecules, each containing four proteins, two 'light' chains and two 'heavy' chains. One light and one heavy chain together define the antigen-binding site of the antibody, so each antibody molecule has two antigen-binding sites (at the ends of each arm of the Y). The effector function of the molecule is defined by the heavy chain alone. There are five distinct classes of antibody, based on the type of heavy chain involved; these classes are IgM, IgD, IgG, IgA and IgE. In addition, some classes have subclasses; for example, IgG subdivides (in humans) into IgG1 to IgG4. IgM and IgD are expressed as monomers on the surface of B lymphocytes. Upon interaction with the appropriate antigen, a B cell is stimulated to release soluble IgM (which is released as a pentamer, i.e. the soluble IgM molecule has ten antibody-binding sites). In addition, upon receipt of help from CD4+ T cells, the B cell undergoes 'class switching', in which the class of heavy chain used is changed, while the antigen specificity remains unaltered. As a result, the initial antibody response to a microbe will be IgM, but as the B cells mature this will change to, for example, IgG; and upon re-exposure to antigen, the secondary response will be the accelerated and enhanced production of (in this example) IgG. Antigen-stimulated B lymphocytes differentiate to produce the mature antibody-producing cells (plasma cells) as well as memory B cells.

As stated above, the heavy chain determines the effector function of the antibody. Class switching therefore is accompanied by changes in the effector functions of the antibody response. Antibodies can have three main effector functions – complement activation, opsonization and neutralization. IgM is effective in activating the complement system, with its attendant effector functions, but it is not especially effective in opsonization, relying on complement to provide this effector mechanism. IgM can neutralize virus infections, perhaps by 'aggregating' the viral particles. However, IgM cannot effectively diffuse into extravascular sites, nor can it be transported across the placenta or epithelia. Class switching delivers these important attributes. For example in humans, when compared to IgM, IgG1 is a less potent activator of complement, but is remarkably effective in opsonization; furthermore, in common with all four IgG subclasses, it can diffuse into extravascular sites and be transported across the placenta. IgA is important in protecting mucosal surfaces, since it can be transported across epithelia, and is frequently found in mucus. IgE sensitizes specialized 'mast' cells, important in protecting against parasitic infections. The function of IgD remains unclear, and mice lacking this molecule have essentially normal immune responses.

With antibodies, too, the boundary separating antigen-specific and nonspecific effects can be flexible. For example, antibody binding to microbial surfaces can greatly enhance their phagocytosis by neutrophils and macrophages; in this case, nonspecific effector cells are being activated by antigen-specific events. Similarly, in the classical complement pathway, nonspecific effector mechanisms have been recruited into the antibody-mediated antigen-specific response.

Antigen-specific immunity: conclusions

Antibodies recognize, and contribute to the destruction of, free microbes, thus diminishing the number of infected cells; CTL kill cells soon after infection, thus limiting microbial multiplication, and hence reducing the number of free microbes released. Therefore antibodies and T cells operate in a complementary fashion, each alleviating the workload of the other. Note that the antigen presentation pathways play a central role. For example, bacteria are usually extracellular; they will induce antibodies, as well as helper T cells (via the MHC class II pathway), but minimal CTL induction will occur. In contrast, viruses are invariably intracellular; they too will induce antibodies and helper T cells, but in addition their antigens will enter the MHC class I pathway, inducing CTL, which can recognize and lyse infect cells. Thus, the conflict between microbes and host has led to the evolution of an elegant system in which the antigen presentation pathways serve to regulate the immune response produced, tipping the balance between antibodies and CTL, and thus ensuring that the response will be optimized for the specific organism encountered.

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Further Reading

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