Immune responses to infectious agents

The immune Response to Viral Infection

Viruses constitute some of the most successful pathogens responsible for significant morbidity and mortality in animal and human populations. This is possible because these organisms have the potentials to evolve a range of strategies to circumvent or inhibit the host immune response. Some these viruses (e.g retroviruses such as feline leukaemia virus, Fel V) have the ability to integrate their genetic material into the host genome, others are able to alter their antigenic appearance to produce repeated epidemics or pandemics of disease (e.g human and animal influenza viruses) and yet other viruses are able to capture host genes and express host-related proteins that interfere with development of the protective immune response (e.g the capture of the human IL-10 gene by Epstein-Barr virus).

In an attempt to discuss immune response to virus infection, we shall focus on how the immune system might handle a viral infection of the enterocyte lining of the intestinal tract, as might be seen, for example, an intestinal rotavirus of domestic livestock. Upon arrival of infectious virus particles at their target surface, they are often confronted by a myriad of innate immune defences relevant to that surface as the first line of defence. In the intestinal mucosa these will include the enterocyte barrier, luminal secretions coating surface of that barrier (including mucus, antimicrobial enzymes and defensins and poly-reactive immunoglobulins) and other innate immune cells that normally populate the epithelial compartment (e.g the TCR T cells) and the underlying lamina propria (e.g macrophages, dendritic cells and NK cells).

Infection of host cells by virus particles generally begins by binding to a receptor molecule expressed on the surface of the target cell. This receptor molecule is a normal host cell surface protein that the virus employs as a receptor or co-receptor to access target cell. In our model example, the virus interacts with receptor on the enterocyte surface to gain access to the host cell. Once inside the cell, the virus is to replicates itself by producing new virions that might eventually exit the infected cell (after which it will have been destroyed) to infect new targets. In a bid for host cells to defend themselves and ultimately the host, most virus-infected cells begin to secrete the antiviral cytokines IFN-α and IFN-β. These antiviral interferon transmits messages to uninfected adjacent cells by binding to their receptors and stimulating the uninfected cell to produce a legion of other proteins that aid in resisting the invading viral particle. The antiviral

cytokines (interferons) may also positively induce local NK cells to act. Alternatively, the infected cell may process and present virus antigen in the context to MHC class I and II molecules for other immune cells like macrophages to act.

Following viral infection, antigen presenting cells (APC) like dendritic cells may sample virus antigen or even become infected by virus particles, allowing classic processing and presentation by these APCs. The interaction between virus and APCs involves viral Pathogen Associated Molecular Patterns PAMPs (often of nucleic acid origin) and dendritic cell PRRs (pattern recognition receptor) that occur in the cytoplasm. These interactions lead to selective gene activation in the APC.

Once the antigen-bound APC has entered the lymph node, it will locate and activate recirculating antigen-specific naïve peptides. The interaction between Th0 cell and APC will be influenced by the range of co-stimulatory surface molecules and cytokines that have been activated within the APC following PRR-PAMP interaction. Since the most 'relevant' type of adaptive immune response for viral infection is the Thl-regulated cytotoxic effector response, it is often expected that APC will activate clones of ThI CD4- T cells and CD8 cytotoxic T cells. Recalling that Thl cells also provide stimulatory help for those B cells committed to producing the subclass of IgG antibody able to opsonize and destroy viruses.

Thl, Tc and B cells generated must then leave the mesenteric lymph node in efferent lymph to enter the bloodstream and invade the anatomical site to viral infection (the intestinal mucosa). Involved in this interaction is homing receptors such as the α_4-β_7 integrin and vascular addressin MAdCAM (Mucosaladdressin cell adhesion molecule). Once adaptive immune cells arrival the mucosa is achieved, the effector phase of adaptive immunity commences. Thl-derived IFN-y will amplify the effects of NK cells and Tc cells. The Thl cell also stimulates B-Cell transformation to plasma cells which secretes IgG subclass that contributes to the cytotoxic process. Antibody bound to infected cells may also mediate the activation of the classical pathway of complement which results in cell lysis. Although in a protective anti-viral immune response the Thl arm of adaptive immunity is more prominent, Th2 effectors also plays a passive role of stimulating the local production of anti-viral IgA that could be secreted across the mucosal barrier to bind virus particles and block their interaction with receptors. Locally secreted IgG may act in a similar fashion. Success of the adaptive immune response could lead to a late

stage immunosuppression (induced T-Cell receptors TCRs) and the development of T-and B-cell memories.

The Immune Response to Bacterial Infection

We shall remain with the intestinal model by considering immune response that might be generated in response to an enteric bacterial pathogen such a *Escherichia coli* or *Salmonella spp*. in the intestinal tract. On arrival, these pathogenic organisms are confronted by a range of innate immune defences. However, of note in this context is the presence of the endogenous intestinal bacterial microflora, which will compete with the pathogen for necessities of life such as space and nutrient thereby, making colonization much more tedious. Another interaction of innate immunity we will be discussing in this class of infection is the $_{\gamma}{}^{\delta}$T cell occurring inside the enterocyte layer. These cells are anatomically well cited for early interaction with bacterial pathogens and are thought to be primarily activated in response to this type of organism.

Just like for viruses, bacteria most often require an initial receptor-mediated interaction with target host cells. For example, the K88 and K99 pili of *E.coli* permit attachment to receptors at the enterocyte interface between these bacteria and host tissue. Enteric pathogens, such as *E.coli* or *Salmonella* spp., utilize a variety of mechanisms to induce disease, dependent on the genetic strain of the bacterium. While some may secrete locally active enterotoxin to help bind toxin receptors and result in osmotic imbalance and metabolic diarrhea, others attach to and disrupt the epithelial surface or invade the intestinal mucosa and regional lymph nodes, resulting in local pyogranulomatous inflammatory response. Such gram-negative rods, are also characterized by the ability to produce severe generalized disease (endotoxaemia).

Once the innate immune response is breached and mucosal surface is colonized, the adaptive immune will be called on to resolve the infection. Also, mucosal APCs like dendritic cells carry out bacterial antigen screening and the process involves the interaction of PRRs with a range of bacterial PAMPs. The activated dendritic cells migrate to the regional mesenteric lymph nodes in orders in order to enlist and activate paracortical T cells and, in turn, follicular B cells for response. The effector immune response phase here is one dominated by the production of antigen-specific immunoglobulin. Hence, APC signalling of the Th0 cell leads to production of Th2 effector which combine with antigen-specific B cells and then leave the mesenteric lymph node to home back to the mucosal surface.

The most beneficial effector immune activity is the synthesis of specific IgA and IgG antibodies. For those organisms mediating pathology via toxin production, IgG neutralization of toxin will be important, IgG antibodies may also opsonize invasive organisms for phagocytosis or permit the complement-mediated lysis of the bacteria. Bacterium-specific IgA antibodies will be secreted to the luminal surface, where they may interfere with the interaction of organism with receptor molecules. Again, in a successful immune response, final down-regulation of the effector populations will be required together with the generation of immunological memory.

The Immune response to fungal infection

Fungal pathogens often provide challenges to immune system because of the relative size of the colonies of organisms. In this discourse, we shall consider an example of the immune response of the dog to colonization of the nasal sinuses and nasal cavity by the organism *Aspergilus fumigatus*. This fungus produces large colonies over the mucosa of the nasal tissues with the colonies comprising tangled mass of fungal hyphae.

To get fungal colonies established, the organism must overcome the normal innate immune barriers of the upper respiratory tract, including the antimicrobial substances found within nasal secretions. Although innate phagocytic cells such as neutrophils and macrophages are capable of phagocytosing fungal spores, they fail to do so, simply because fungal hyphae are large and massive.

APCs carrying fungal antigen induce response in regional lymphoid tissue such as the nasopharyngeal tonsil or retropharygeal lymph nodes. The effector mechanism involves infiltration by CD4+ Thl and probably Thl7 cells, as determined by up-regulation of gene expression for IFN-y and IL-23 in inflamed tissue. Thl-derived IFN-y likely stimulate macrophages to induce their destruction of any phagocytosed fungal spores. Antibody and complement molecules also coat hyphal elements and form a bridge to FcR-bearing granulocytes thereby subjecting them to destruction. Similar to helminth infection, these cells may degranulate locally and induce focal damage to the hyphae. Infected dogs generally mount a strong serum IgG antibody response to the organism. The inflammatory response itself it likely responsible for the extensive tissue and bone destruction that may occur in this disease. Similar to observations in leishmaniosis, there is an additional regulatory element to the response, as there is concurrent up-regulation of lL-10 gene expression. Again, this is interpreted as an attempt by the adaptive

immune of systemic sequelae, but at the same time allows persistence of the infection and the development of chronic sinonasal disease.

IMMUNOLOGICAL TOLERANCE

I. Neonatal tolerance

This a phenomenon whereby exposure of the developing immune system to foreign antigen either *in utero* or during early neonatal life leads to the induction of tolerance to that antigen such that antigenic challenge in life fails to induce an immune response. This effect has been widely carried out experimentally by immunizing neonatal laboratory rodents with antigen and demonstrating tolerance in later life.

A good veterinary example of neonatal tolerance is that which develops to infection with bovine viral diarrhoea virus (BVDV), the aetiology of 'mucosal disease' in cattle. If a foetal calf is infected between days 42 and 125 of gestation (i.e before commencement of immunocompetence in the last trimester), that animal will become persistently infected (PI) as it develops immune tolerance to that particular strain of the virus. These PI animals are viraemic and continually shed the virus, thereby acting as reservoir of infection within the heard. The PI animals remain sero-negative because of the tolerant state, but other animals in the heard will develop high-titre virus neutralizing antibodies. The PI animals remain tolerant to the specific strain of virus that it carries, but may it may be superinfected with a cytopathic biotype of BVDV to which it is not tolerant and this may result in fatal mucosal disease.

II. Adult tolerance

Induction of tolerance has be shown experimentally in adult laboratory animals (**adult tolerance**). This effect is very much dependent on the experimental protocol employed and the dose of antigen given. Two fundamental protocols for tolerance induction are;

a. 'High-zone' tolerance which involves injecting the animal with a single very high dose of antigen that induces paralysis of both T and B cells. In contrast,

b. 'Low –zone' tolerance, involves repeated injections of a low dose of antigen which induce T-cell tolerance. As most antigens are T dependent, induction of T-cell tolerance generally leads to concomitant B-cell tolerance.

III. Oral tolerance

The mechanism underlying oral tolerance is well elucidated. At one level the phenomenon may relate to the route by which the tolerizing antigen is absorbed across the intestinal mucosa. **Particulate antigens** to which an active immune response is induced are more likely to be absorbed by **M cells** overlying the Peyer's patches. In contrast, **tolerated antigens** are more likely to be **soluble** and absorbed directly across the **enterocyte surface**. This tolerance may not be absolute, as most normal individuals have detectable serum IgG or IgA antibody specific for dietary antigens.

It is now known that oral tolerance is probably an active immunological event. The tolerizing antigen must be processed and presented by dendritic cells , but the consequence of such presentation may be variable. Some T cells that recognize processed antigen may undergo **apoptosis** (clonal deletion) and others might recognize antigen but fail to become fully activated, as not all three signals required for T-cell activation are received. Such T cells are not deleted, but remain non-functional or **anergic**.

IV. Self tolerance

The final form of tolerance is self-tolerance (the ability of the immune system to tolerate the self antigens that comprise the tissues of the body). Failure of self-tolerance leads to autoimmune diseases. In order to achieve self-tolerance, potentially autoreactive T and B lymphocytes must be brought under control.

Knowledge abounds on how self-tolerance is achieve for T-cells. One mechanism involves elimination of T-cells by **negative selection** during intrathymic maturation. However, if this process was full-proof, there would be no such thing as autoimmune disease, so a proportion of autoreactive T cells must 'escape' clonal deletion and be allowed to enter the peripheral T-cell pool. Circulating autoreactive T-cells are readily identified in man and have also been demonstrated in the dog. These cells must clearly be controlled in order to prevent autoimmunity and a range of mechanisms are probably employed to achieve this aim. Some autoreactive T-cells may recognize antigen presented to them in peripheral lymphoid tissue. This cells may either undergo apoptosis ('**peripheral deletion'** as opposed to 'central' intrathymic deletion) or may become **anergic** if they fail to receive appropriate costimulatory signals. Other stimulatory T cells may be kept away from their target autoantigens in a process known as '**immunological ignorance'**. This may work at different levels; for example, some body tissues are normally kept at distance from the adaptive system behind a 'blood-brain barrier' or 'blood-testis barrier', so it

is relatively difficult to induce autoimmunity to brain or testicular tissue. Alternatively, this may work at the level of the APC, which processes self-antigen but fails to present it. Although all of these mechanisms might be at play, the single most important means of controlling autoreactive T cells is via regulatory T cells.

Autoreactive B cells must also be kept in check in order to prevent those autoimmune diseases caused by autoantibody production. The development of B lymphocytes is less well defined than that for T cells, but also involves a form of clonal deletion. The control of autoreactive B cells within the periphery likely relies on the regulation of those autoreactive T cells that would normally be requires to provide help for activation of the B-cell response. Lack of T-cell help renders the autoreactive B cell anergic. Autoreactive B cells within the periphery may also be 'ignorant' of their cognate antigens if these antigens are normally kept sequestered away from the immune system.

IMMUNOSUPRESSION AND IMMUNODEFICIENCY STATES

Immunodeficiency is defined as the presence of impairement in function any part or parts of the immune system that results in the immunodeficient individual being vulnerable to infectious disease. Two broad types of immunodeficient states are recognized;

a. Primary immunodeficiency – this occurs when immunodefeiciency is occassioned by a mutation in a gene encoding a molecule of the immune system. Such diseases are inherited and congenital, with clinical signs manifesting early in life.

b. Secondary immunodeficiency- this occurs in adults that have previously had normal immune function and may be related to age, infection, medical therapy or the presence of chronic disease. Causes of secondary immunodeficiency are discussed below;

MEDICAL IMMUNOSUPPRESSION

Secondary immunodeficiency can be deliberately induced by veterinarians when **immunosuppressive drugs** are used to control autoimmune disease or when chemotherapy is used to control autoimmune disease or when **chemotherapy** is used in managing cancer. The

ultimate side-effect of these drugs is secondary immunosuppression and increase susceptibility to infection.

i. Specific Infections

The best example to illustrate infection associated secondary immunodeficiency is feline Immuniodeficiency Virus (FIV) infection. FIV is a **T lymphotropic retrovirus** that infects lymphocytes and APCs and has been extensively investigated as an animal model of human immunodeficiency virus (HIV). Infected cats have an acute phase of mild illness during which there is progressive decline in blood $CD4^+$ T cells. The cat will then become asymptomatic, but during this second phase of disease there is a continued decline in circulating $CD4^+$ T cells that may occur over several years. During the third stage of the disease, there is recurrence of mild illness that progresses to a more severe terminal stage 4-5 disease. The terminal illness is similar to human AIDS and is a **chronic , multisystemic disease** that may include gingivostomatitis, respiratory tract infection, enteritis, dermatitis, weight loss, pyrexia and lymphoadenomegaly. Neurological disease and lymphoma may also develop and a range of secondary infections have been reported. Concurrent Feline Leukaemia virus (FeLV) should also be considered and FeLV may be immunosuppressive in its own right due to depletion of infected T cells.

ii. Chronic disease

Animals afflicted by chronic infectious, inflammatory or neoplastic disease will likely have a degree of secondary suppression of the immune system and increased susceptibility to infection. Some infectious agents (e.g canine distemper virus [CDV], canine and feline parvovirus, FIV, and FeLV, porcine circovirus-2 as the cause of postweaning multisystemic wasting syndrome in this species, equine herpesvirus-1, bovine viral diarrhoea virus) may cause **direct depletion of lymphoid tissue**. Other infections are associated with the production of circulating immunosuppressive factors that appear to inhibit lymphocyte blastogenic responses. Such inhibition of lymphocytes function has been demonstrated in diseases such as demodicosis, deep pyoderma, pyometra and disseminated aspergillosis in the dog.

iii. Stress

Chronic stress is also immunosuppressive and follows elevation in endogenous glucocorticoid production. A similar effect is seen in hyperadrenocorticism, in which there is circulating lymphopaenia and increased susceptibility to secondary infection. Stress-induced immune suppression is likely to play a major role in susceptibility to infectious disease in intensively

reared livestock. Animals housed indoors in high density rearing units or animals transported for long distances in close confines are considered at risk for such immune suppression. High-intensive exercise is also immunosuppressive, although milder exercise can enhance a range of immune functions.

iv. Malnutrition

Severe malnutrition leads to increased susceptibility to infection due to **impairement of T-cell function**, but with sparing of B-cell activity and immunoglobulin production. These effects are thought to be related to **leptin,** adipokine (cytokine produced by adipocytes) related to body fat mass. An animal suffering malnutrition will have loss of body adipose tissue reserve and reduced concentrations of leptin. Leptin is also immunostimulatory (macrophages and Th1 function) and proinflammatory, starvation is associated with immune suppression.

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I will like to acknowledge the use of the book 'VETERINARY IMMUNOLOGY PRINCIPLES AND PRACTICE'. It is also recommended for this class.

1. VETERINARY IMMUNOLOGY PRINCIPLES AND PRACTICE

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