Chapter III: Veterinary Immunology

Section 1. Immunity & Pathology

The body’s defences work on 3 different levels: physical barriers, innate immunity and acquired immunity. Physical barriers, though very effective, cannot entirely exclude invaders. The second level consists of preexisting or rapidly responding chemical and cellular defense mechanisms such as macrophages, dendritic cells, neutrophils and Natural Killer (NK) cells. Innate immunity relies on the fact that invading microorganisms are chemically very different from normal body components thus recognition of foreign antigens is simplified. It is characterized by carrying out a rapid although scarcely specific response.

Inflammation is a key aspect of innate immunity where defense mechanisms are focused on sites of microbial invasion. During inflammation, the blood flow is increased and there is a local accumulation of cells ready to attack and destroy invading microbes. In addition to the cells mentioned above, animals have carbohydrate-digesting enzymes such as lysozyme and other carbohydrate-binding proteins which are natural antimicrobial molecules that digest bacterial cell walls by coating them in order to hasten their destruction. Lysozyme is especially effective against Gram positive bacteria. However, innate immunity lacks memory thus cannot offer the ultimate solution to the defense of a body.

Acquired immunity on the other hand, is characterized by its capacity in recognizing, destroying and remembering the invaders so that when it encounters them in another occasion, it responds more rapidly and more effectively. Its constituents are mainly T and B cells. The acquired response takes several days to form but is remarkably effective. As opposed to the innate immune system, this particular one does not rely on preexisting receptors but on the ability of its structurally unique receptors in binding to an enormous array of foreign molecules. The acquired immune system is divided in two branches on the basis of what type of invader they defend the organism against. One branch is directed against the extracellular or exogenous invaders, mainly thanks to antibodies which make up the “humoral immune response”. The other branch is directed against the intracellular or endogenous invaders that invade cells and this type of response is called “cellular-mediated response” because carried out by specialized cells (Tizard, 2004).

It is practically impossible to draw a dividing line between the innate and acquired immune systems due to the fact that they are closely intertwined. Infact, the binding of certain receptors on the surface of macrophages to microbial molecules triggers inflammation but also begins the process of “turning on” the acquired immune system. These particular receptors are called Toll-like receptors and are found not only on the surface of macrophages but also on mast cells, dendritic cells, eosinophils and epithelial cells, which all fall into the category named Sentinel Cells. The binding of the TLRs on the surface of sentinel cells to a microbe or microbial product initiates the secretion of a mixture of cytokines from these cells. Clearly, different TLRs trigger the production of a combination of different cytokines and different microbes trigger different responses within a cell. In this way, TLRs that recognize bacteria will trigger the production of cytokines optimized to combat bacteria and so forth (Tizard, 2004).

Pathology is the detrimental derangement of molecules, cells and functions that occurs in living organisms in response to injurious agents or deprivations. Pathogens in general, elicit a range of responses in animals, including a set of immunological responses which include
proliferation and activation of: (i) B-cells synthesizing antibodies which facilitate the ingestion of antigens carried out by granulocytes and macrophages or destruction by complement, (ii) T-cells which destroy cells with foreign antigens on their surface assisted by T-helper cells or (iii) carried out by T-Helper cells; (iv) NK-cells which destroy cells that lack normal antigens, such as tumour cells or those containing viruses and finally (v) memory cells which increase humoral- and cell-mediated responses. These and other defense mechanisms interact against pathology. The wide range of responses to pathology includes behavioural changes, physiological changes in the body such as the production of acute-phase proteins in body fluids and productions of cytokines in the brain, as well as immunological changes. Short-term responses to pathological effects include vomiting, which helps get rid of toxins and is mediated by interferons whereas diarrhea is mediated by interleukin-2. Long-term responses include malaise or sickness behaviour which is linked to immunological changes. Immune system responses may need much energy whilst pathogens may take energy directly from their host. Hence, some sickness behaviour results in energy saving, some promotes body defense mechanism, and all is adaptive (Broom, 2006).

Sickness behaviour is due to three major cytokines (IL-1, IL-6 and TNF-α) secreted by infected cells, endothelial cells, phagocytes, fibroblasts and lymphocytes so there are many peripheral sources as well as brain-mediated sources. The subjective feeling of sickness-malaise, lassitude, fatigue, loss of appetite, and muscle and joint pains, along with fever, are part of a highly organized strategy to fight infection. They reflect a change in the body’s priorities as it seeks to fight off invaders. IL-1, IL-6 and TNF-α will diffuse into the brain where they cause an increase in body temperature, induce sleep and suppress appetite. Fever is promoted by IL-1 and IL-6, and it promotes dendritic cells to mature, enhance the circulation of lymphocytes and promote the secretion of the key cytokine IL-2. There is an energetic cost associated with fever but in many cases it is life-saving. The most dramatic sickness behaviour manifestations are represented by reduced activity, tiredness and sleepiness; all adaptive mechanisms because sleep deprivation and too much activity when fatigued can lead to a reduction in NK cell activity and IL-2 response to antigen challenge. IL-2 also promotes a second kind of reduced activity which is caused by the feeling of pain. The feeling of pain being mediated also by products of damaged tissue, such as nitric oxide, which tend to promote the action of inflammatory agents such as bradykinin and prostanoids. IL-1 is known to lead to more non-REM (rapid eye movement) sleep. It is also responsible for the reduction in social behaviour seen in sickness which is a very important adaptive mechanism because an isolated, infected individual whose activity is reduced, is less likely to transmit infection to other individuals. IL-1 is also responsible for the loss of appetite by suppressing the hunger center in the brain. It is thought that this saves energy in the short-term because gut activity does not occur. It may also permit the animal to become more selective about its food. Fasting however, can harm immunological defences if prolonged and can have an adverse effect on growth (Tizard, 2004).

These three major cytokines, in addition to the effects mentioned above, act on skeletal muscle to enhance protein catabolism and thus mobilize a pool of available amino acids which are now available for antibody synthesis. Another effect can be seen in the liver, where hepatocytes are stimulated to synthesize and secrete proteins. These proteins are called acute-phase proteins which play an important part in coping with pathology. This response begins within a few hours of injury and subsides within 24 to 48 hours after. Many of the acute-phase
proteins are important components of the innate immune system and they include complement components, clotting molecules, protease inhibitors and metal-binding proteins. In sheep, the major acute-phase protein is Haptoglobin (Tizard, 2004).

In order to fully generate an acquired immune response, sentinel cells must bind to microbial products, invade and destroy them, but a fundamental process is that of representing the broken down antigen using another surface receptor called the antigen-presenting receptor or histocompatibility molecules, located among a gene cluster called the Major Histocompatibility Complex (MHC). The receptors are thus called MHC molecules. If this is not fulfilled, an acquired immune response will not be generated. The genes located within the MHC are divided into three major classes where Class I codes for molecules found on the surface of almost all nucleated cells, although mostly on macrophages and lymphocytes; Class II codes for MHC molecules found only on the surface of the professional antigen-presenting cells (dendritic cells, macrophages and B cells) and Class III codes for a wide variety of molecules many of which belong to the innate immunity (Tizard, 2004).

Lymphocytes are cells responsible for mounting the acquired immune response but given that there is the humoral and the cell-mediated immune response, they are divided into two large populations-T and B cells. Among these two large groups, many diverse subsets exist, each with different characteristics and functions. When exposed to an antigen that binds to their receptors, lymphocytes will initiate either a cell-mediated or an antibody-mediated immune response. There are three major populations of antigen-sensitive lymphocytes which are T-helper cells that regulate immune responses; T-cytotoxic cells that destroy endogenous antigens and B cells which synthesize and secrete antibodies to destroy exogenous antigens. The identification of the surface molecules present on lymphocytes has allowed the characterization of these distinct subpopulations, where each of these has a name and a cluster of differentiation (CD) designation. The same surface molecule can have two names where one is correlated to the CD designation and the second one describes its function (for e.g. IL-6R is the same as CD126) (Poli, unpublished material).

CD4 is only found on T cells that recognize exogenous antigens processed by MHC Class II molecules, which are specifically the T-helper cells. Hence CD4 is a receptor for MHC class II molecules and it allows T-helper cells to recognize exogenous antigens. In contrast, CD8 is only found on T cells that attack and kill abnormal cells-T-cytotoxic cells or T-suppressor cells. CD8 is a receptor for MHC class I molecules and allows T-cytotoxic cells to recognize endogenous processed antigens. The ratio of CD4⁺ to CD8⁺ cells in blood may be used to estimate lymphocyte function. An elevated CD4 count implies increased lymphocyte reactivity because helper cells predominate, whereas a high CD8 count implies decreased lymphocyte reactivity. Normally in sheep, the relative proportions in percentage of blood lymphocytes are the following:

<table>
<thead>
<tr>
<th>T cells</th>
<th>B cells</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>CD4/CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>56-64%</td>
<td>11-50%</td>
<td>8-22%</td>
<td>4-22%</td>
<td>1.55</td>
</tr>
</tbody>
</table>

Table 1. Relative percentages of blood lymphocytes in sheep (Tizard, 2004).
CD4 is not necessarily found only on T-helper cells but can also be found on macrophages, monocytes, neutrophils and eosinophils, depending on the species (Tizard, 2004).

Dendritic cells trap and process exogenous antigens and in secondary lymphoid organs they present them to T-helper cells. If the antigen binds in a correct manner to the T-helper antigen receptor (TCR), an immune response is initiated by proliferating and differentiating. B cells and cytotoxic T cells cannot respond optimally if not stimulated by T-helper cells which undoubtedly have a central role in the acquired immune response. CD4 and CD8 are closely associated to the T-cell antigen receptor (TCR) and clearly play a key role in the immune system in that they are the receptors for MHC molecules. Their presence thus determines the class of MHC molecules recognized by a T cell. Therefore, CD4+/CD8- T-helper cells are responsible for the recognition of MHC class II molecules which are specific receptors for exogenous antigens found only on antigen-presenting cells. On the other hand, CD4-/CD8+ T-cytotoxic cells are responsible for the recognition of MHC class I molecules which are specific receptors for endogenous antigens found on abnormal cells. When they bind to an MHC molecule on an antigen-presenting cell, TCR signal transduction is stimulated 100-fold. This statement can be explained by underlining the fact that when an antigen binds to the TCR, a signal must be sent to the T-cell in order to trigger an immune response. Furthermore, this emphasizes the importance of these two surface molecules, CD4 and CD8 (Tizard, 2004).

Section 2. Immunity to Helminths

It is not surprising that the immune system is relatively inefficient against helminth parasitism. Infestations occur usually causing a wide range of parasite load within an animal population showing a heterogeneous distribution. Most animals harbor a few worms but a few animals harbor a lot of worms. This difference is clearly related to the different genetic predisposition of the host and of the immune response effectuated. Predisposition is a result of genetic, behavioural, nutritional or environmental factors (Tizard, 2004).

Innate immunity has less of an important role in controlling helminth infestations. The innate factors which influence helminth burden include age, sex and genetic background of the host. The first two aspects are largely hormonal where in animals whose sexual cycle is seasonal, parasites tend to synchronize their own with that of the host. As mentioned in Chapter II, ewes show a spring rise or PPRI rise of fecal egg counts, which is caused by the onset of pregnancy and especially lactation (Houdijk, 2008).

Acquired immunity must be put into action in controlling helminthes seeing as they present a particular challenge. Unlike bacteria or protozoa, worms have a thick extracellular cuticle which cannot be penetrated by the complement or by T cell perforins (Tizard, 2004). Bacteria and protozoa normally stimulate the development of a distinct Th1-type response, where IFN-γ is produced and macrophages, CD8+ cells, NK cells and neutrophils are primary players. In contrast, large multi-cellular parasites require a different set of immune effectors for their clearance compared with responses required to control bacteria, protozoa and viruses. The array of control mechanisms designed to sequester and kill parasites may include: (i) walling off the parasite from the surrounding tissue by creating an immune cell and/or
connective tissue barrier; (ii) impairing the ability of the parasite to migrate to or remain in its preferred tissue destination; (iii) release of toxins or other factors that may directly damage or stress the parasite. In recent studies, it has been seen that wound-healing mechanisms are largely regulated by a Th2-type response (Patel et al., 2009).

Thus the immune system must either destroy the intact cuticle or attack the parasites through their weak spots such as the digestive tract. In fact, adult worms in the intestine are coated by effector cells, cytokines, antibodies and complement (Tizard, 2004). Mast cells and eosinophils are the chief effector cells implicated in parasite rejection (Meeusen et al., 2005). In fact, in the mucosa of resistant lambs, a higher density of mast cells and eosinophils can be found than those of random-bred lambs (Gill et al., 2000). This can be clarified by the fact that generally worms elicit a very strong Th2 response, which is characterized by the production of the two effector cells mentioned and IL-4, and IgE antibodies. Th2 response and IgE synthesis is triggered by the binding of the immune-dominant nematode antigens which are lipid-binding proteins (NPAs) (Tizard, 2004). The Th2 profile includes induction of IL-5 and IL-13 cytokines as well (Meeusen et al., 2005). The Th2 response is associated with the production of IL-4, IL-10 and IL-13, which all lead to mobilization of eosinophils, intestinal mast cell infiltration, elevated serum IgE and elevated parasite-specific IgG1 levels. This panel of cytokines and IgE antibodies manifests a typical “allergic” reaction or what is more precisely called Type I Hypersensitivity; characterized by eosinophilia, edema, asthma and urtical dermatitis (Tizard, 2004).

### Table 2. Th1- and Th2-type cytokine secretions (Tizard, 2004).

<table>
<thead>
<tr>
<th>Th1 products of secretion</th>
<th>Th2 products of secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1, IL-2, IL-8, IL-12, TNF-α, IFN-γ, MCP-1 (monocyte chemo-attractant protein-1), GM-CSF, down-regulation of IL-4 and IL-5 and up-regulation of IFN-γ.</td>
<td>IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13, GM-CSF (granulocyte macrophage colony-stimulating factor), up-regulation of IL-4 and IL-5 and down-regulation of IFN-γ.</td>
</tr>
</tbody>
</table>

The Th2-type response, as opposed to the Th1-type response, is known to assist the collaboration of cell-mediated with humoral immunity. In fact, a Th2-type response is characterized by high levels of IgG1, IgE antibodies and mast cells (Bricarello et al., 2008). Th2-mediated IgE production is essential in controlling worm burdens. This has been well observed in sheep that perform self-cure against H. contortus (Tizard, 2004). NPAs bind with mast cell-bound IgE complexes and this generates mast cell degranulation and consequent release of vasoactive molecules and proteases (Tizard, 2004). Adult worms in the intestinal lumen induce changes in the gut physiology characterized by increased mucous secretion into the gut (leaky gut), increased luminal flow and increased smooth muscle contractility, which together contribute to making the environment inhospitable or inimical for the worms (Patel et al., 2009). These mechanisms can result in dislodgement and expulsion of worms (Tizard, 2004).
<table>
<thead>
<tr>
<th>Cell type</th>
<th>Mediators of eosinophil degranulation (of our interest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>IgE-coated parasites, IL-13, IL-5, GM-CSF, many chemokines, PAF and C5a.</td>
</tr>
</tbody>
</table>

Table 3. Regulators of eosinophil degranulation (Tizard, 2004).

IgE-coated parasites can bind to mast cells, macrophages and platelets using FceR-specific IgE antibody receptor- which will then become activated and able to kill the parasites (Tizard, 2004). The degranulation of mast cells releases chemotactic factors that attract eosinophils to sites of helminth invasion. Moreover, IL-5 produced from Th2 lymphocytes, is known to mobilize eosinophils from the bone marrow pool contributing to eosinophilia. Eosinophils which encounter IgE-coated parasites, can also bind using Fc receptors, which in turn leads to degranulation onto the worm cuticle. These granules contain oxidants, nitric oxide and lytic enzymes, capable of damaging the outer cuticle (Tizard, 2004). An IgE eosinophil-mediated response is by far the most significant mechanism of resistance to larval helminths (Tizard, 2004).

In sheep genetically resistant to H. contortus, protective immune response involves the expansion of Th2 cells with minimal activation of a Th1-type response. If an inappropriate Th1-type response predominates, the capability of expelling infection is compromised (Bricarello et al., 2008). In Bricarello’s experiment, it was found that there was an interesting difference between the cytokine gene expression ratio in the small intestine tissue of the two groups of cattle studied. IL-4 and IL-13 were upregulated in the resistant group whilst IL-2, MCP-1 and IFN-γ were upregulated in the susceptible group. As a consequence of the Th2-type response, higher levels of IgA immunoglobulin levels were found in mucus among the resistant animals. The higher levels of local IgA in resistant animals revealed its possible effector role in expelling parasites at the mucosal surface. Moreover, both IFN-γ and IL-12 increased as an effect of positive feedback in the susceptible group, which probably contributed to the inhibition of Th2 response, leading to the maintenance of the worm population in the small intestine.

CD4+ T cells have been shown to be essential against H. contortus for immunity even though results from various other experiments are disaccording however, they are always present in a greater number than CD8+ cells (Halliday, 2009). The expulsion of intestinal nematodes is indeed largely dependent upon CD4+/CD8- Th2 cells, among other components of the immune refection process such as parasite-specific antibodies, effector cells, cytokines and other novel molecules expressed by epithelial cells in the gut (Meeusen et al., 2005).

Epithelial cells themselves could be regarded as immune effector cells against intestinal nematode parasites seeing as the balance between their proliferation and death is critical in maintaining epithelial integrity and tissue morphology in the gut. In resistant mouse strains, the rate of epithelial cell migration up the crypt column during worm expulsion occurred much more rapidly. On the contrary, during nematode chronic infection, this migration was reduced where the slowing of the rate of migration may be a mechanism utilized by nematodes to enhance their own survival. Thus during chronic infection, there is an accumulation of proliferating epithelial cells which is driven by a Th1-type response (Artis, 2006).
As previously established, CD4+ T-helper cells contribute to resistance by releasing a variety of cytokines which amplify and regulate the recruitment, proliferation and differentiation of effector cells, such as mast cells, eosinophils and B cells (Gill et al., 2000). But whether or not a Th1- or a TH2-type response prevails is a curious concept, which can be explained by analyzing the different relationship these two cells have with dendritic cells. It is known that there are two populations of dendritic cells-DC1 and DC2- which respectively activate Th1 and Th2 cells (Liew, 2002). Which dendritic cell population processes the antigen depends on the way in which it encounters the antigen, and on the set of TLRs that are activated. Different parasite strains differ in their ability to trigger Th1 and Th2 responses, which could be one explanation. Alternatively, differences may be due to parasite dose (Tizard, 2004).

Even though it has been stated that cytotoxic T cells promote a T cell response against abnormal cells such as tumour cells or cells with endogenous antigens, nevertheless, CD4-/CD8+ T cells may attack helminths that are embedded in the intestinal mucosa or undergoing tissue migration and in this site may cause larval destruction (Tizard, 2004).

Section 3. The cost of immunity towards GIN

Colditz has identified six costs of the immune system which are: (i) increased metabolic activity that occurs during fever and locally during activation of the immune cells; (ii) reduced nutrient availability due to anorexia and sickness behaviour; (iii) altered priorities for nutrient utilization that reduce the capacity of non-immune tissue to utilize nutrients; (iv) change in the size and rate of turnover cell and protein pools of the immune system; (v) immunopathology as a result of inappropriate or excessive activity of the immune system; and (vi) genetic costs which are changes in the capacity of offspring to express production traits following selective breeding for natural disease resistance. By analyzing step by step these cited points, there are several statements which are important to consider in understanding the pathological state of an organism during nematode parasitism (Colditz, 2008):

(i) Increased metabolic activity is caused naturally by the production of cytokines which induce fever, by the greater requirements of leukocytes and by increased consumption of oxygen, glucose utilization and glutamine utilization. It is also noteworthy that in sheep gut bacteria increase in number during infection with GIN in sheep acting as a concurrent stressor.

(ii) Anorexia brought about during sickness behaviour reduces voluntary feed intake but on the other hand forces the animal in becoming more selective over the food it ingests.

(iii) The production of acute-phase proteins leads to an altered capability of tissue in utilizing available nutrients. During this phase, increased gluconeogenesis can lead to hyperglycemia.

(iv) Pools of immune cells and proteins such as globulins and albumins in plasma can both decrease with correspondent lowering of the Body Condition Score in sheep.
(v) Excessive and ineffectual activity of the immune system can result in damage to host tissues. In some cases, this is a predominant feature of parasite infection and may pose a limit to the evolution of immunological resistance to parasites. This occurs more frequently during primary infection in lambs.

(vi) Genetic resistance can interfere with the expression of economic traits in farm animals and when this occurs it represents a cost of immunity to parasites.