Structure and function of the bovine immune system

Cattle, like other mammals, possess a complex immune system which helps protect them against invasion by viruses, bacteria or other parasitic micro-organisms. Stated in the simplest terms, the immune system produces different types of lymphocytes (white blood cells) which are programmed to recognize specific ‘foreign’ substances, including antigens displayed by the micro-organisms which cause disease. When lymphocytes encounter a foreign antigen, those with specific receptors for this antigen proliferate and give rise to effector cells. The responses which follow are of two main types—humoral and cell-mediated. In a humoral response, the effector cells produce antibody which reacts with the foreign antigen or micro-organism and results in its elimination. In a cell-mediated response, the effector cells kill the cells bearing the foreign antigen, either directly or by recruiting other accessory cells.

The mammalian immune system consists of specialized lymphoid organs, plus a diffuse network of lymphatic vessels which collect lymph and cells from tissues in almost every part of the body, and a pool of lymphoid cells which recirculate continuously between the lymphatic system and the bloodstream. The primary lymphoid organs are the bone marrow, the thymus and, in ruminants, probably the Peyer’s patches in the intestine. These organs produce lymphocytes and different types of accessory cells which play a role in the immune response. Cells produced in the primary, lymphoid organs join the recirculating pool of lymphocytes and populate the spleen and lymph nodes, secondary lymphoid organs which are adapted to trapping and responding to foreign antigens. Each of the different lymphoid organs has a distinct structural arrangement adapted to its specific function.

ILRAD was established to develop improved methods for the control of two important livestock diseases caused by protozoan parasites: East Coast fever, caused by *Theileria parva*, and African animal trypanosomiasis, caused by several species of trypanosome. In both diseases, profound changes can occur in the organization and cell content of the lymphoid organs, resulting in impaired immune responses. One important task of ILRAD scientists is to obtain a complete understanding of the structure and function of the normal immune system in livestock, in order to understand the effects of infection and develop interventions which might lead to improved disease control.

Over the past 20 years, numerous studies have been carried out *in vitro* on cells taken from lymphoid organs. A great deal of information has been obtained on the different types of
cells produced by the lymphoid system, how they are produced and how they interact
during the generation of an immune response. Yet the immune reactions obtained with
isolated cell populations may not always reflect what occurs in the animal. Furthermore,
most immunological research has focused on laboratory rodents. ILRAD scientists have
obtained considerable information on bovine cell populations and their role in immune
responses from *in vitro* studies. This work is now being extended to studies of the normal
bovine immune system and immune responses as they occur in cattle.

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>B5/4, B4/27</td>
<td>B lymphocytes</td>
</tr>
<tr>
<td>P5</td>
<td>T lymphocytes and monocytes</td>
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<tr>
<td>K1</td>
<td>most if not all T lymphocytes</td>
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<tr>
<td>T1/D8</td>
<td>Subpopulation of T lymphocytes</td>
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<tr>
<td>BT3/8</td>
<td>Subpopulation of T lymphocytes, monocytes,</td>
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<td></td>
<td>granulocytes</td>
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<tr>
<td>P13</td>
<td>some T lymphoblasts</td>
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<tr>
<td>P8</td>
<td>Monocytes/macrophages and granulocytes</td>
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<tr>
<td>P12, B4/18, P3, M4, M5, M7, T1/C6</td>
<td>class I MHC</td>
</tr>
<tr>
<td>R1, P2</td>
<td>class II MHC</td>
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Reactivities of monoclonal antibodies with bovine cell populations.

Progress in this area has accelerated over the past few years thanks to the development of
several new research tools. Perhaps the most important breakthrough has been the
development of techniques to produce highly specific monoclonal antibodies which
recognize antigens on the surface of different populations of cells. These antibodies can be
used to identify different types of white blood cells, cells at different stages of maturity and
functionally important molecules on cell surfaces. They are the products of hybrid cells
created in the laboratory by a fusion of mouse spleen cells, which produce antibodies, and
tumour cells, which are capable of growing and multiplying in culture. A number of
monoclonal antibodies have been produced at ILRAD which recognize populations of cells
that play a role in the immune responses of cattle. These monoclonal antibodies and the
cells they recognize are listed in the table, although some of the specificities given are still
tentative. The development over the past few years of highly sensitive immuno-fluorescence
and immunohistochemical techniques for the detection of cells in tissues has made it
possible to use these monoclonal antibodies—together with more traditional histological,
ultrastructural and enzyme histochemical techniques—to study the cellular components and
organization of the bovine immune system in more detail than was possible in the past.

This discussion will concentrate on findings related to two important organs of the bovine
immune system, the thymus and the lymph nodes, as examples of primary and secondary
lymphoid organs. The information presented is based on detailed studies carried out at
ILRAD on tissues from over 40 cattle.
The bovine thymus

A great deal of experimental evidence derived from studies in laboratory animals indicates that the lymphocytes which give rise to cell-mediated immune responses (T lymphocytes) are produced in the thymus. In mice without a thymus, the lymph nodes and spleen contain many fewer T lymphocytes than those of normal mice. Athymic mice are less capable of mounting cell-mediated immune responses and are highly susceptible to many infectious diseases. When the thymus is removed from foetal or newborn calves or lambs, they also produce many fewer T lymphocytes, but their ability to mount cell-mediated immune responses is not completely abolished. This suggests that ruminants may not be completely dependent on the thymus for production of T lymphocytes.

In cattle, the thymus is composed of two lobes made up of multiple lobules. Each lobule consists of an outer layer, the cortex, and an inner region, the medulla. The arterioles and venules conveying the blood supply to and from the thymus are located in the outer medulla of each lobule. These perivascular areas contain a network of reticular fibres and prominent bundles of collagen. The main structural framework of the rest of the thymus is composed of epithelial cells.

Cell suspensions prepared from bovine thymus contain 98% or more T lymphocytes, with about 85% of these located in the cortex. Most lymphocytes in the thymus are smaller and denser and have a lower cytoplasm-to-nucleus ratio than peripheral T lymphocytes found in the lymph nodes and bloodstream. Lymphocytes in the cortex do not express either class I or class II major histocompatibility (MHC) antigens, while lymphocytes in the medulla express class I MHC antigens. These antigens are cell-surface molecules which show marked differences between individual animals and appear to play an important role in recognition events during the generation of immune responses. Most peripheral T lymphocytes are also positive for class I MHC antigens.

Lymphocytes in the cortex of the thymus bind peanut agglutinin (PNA) with high avidity, while lymphocytes in the medulla bind PNA with low avidity. The medullary lymphocytes appear to be the more mature population, as their phenotype is similar to that of peripheral T lymphocytes.

The cortex

The lymphocytes in the cortex of the bovine thymus are densely packed within a fine network of epithelial cells. These cells are difficult to distinguish in histological sections, but
they can be recognized at the ultrastructural level by their euchromatic nucleus, relatively sparse perinuclear cytoplasm and delicate cytoplasmic processes extending between the surrounding lymphocytes. The cytoplasm of the epithelial cells contains numerous vacuoles and moderate numbers of small, electron-dense granules. They stain strongly for class II MHC antigens, but very weakly or not at all for class I MHC antigens.

Small numbers of macrophages, which stain strongly for non-specific esterase activity, are scattered throughout the cortex, but are most numerous in the inner cortex. Many of these macrophages have voluminous cytoplasm containing lymphocytes in varying stages of degeneration, which the macrophages have absorbed by phagocytosis.

The medulla

The medulla has a more complex structure and contains a greater variety of cell types than the cortex. Epithelial cells are more numerous and more varied in shape and there is also a population of interdigitating cells which is not found in the cortex.

In the outer medulla surrounding the arterioles and venules, there are distinct perivascular regions which vary in width from about 3 to 10 cell diameters. The outer boundary of these areas is delineated by a basement membrane-like structure on which lies a layer of epithelial cells linked by desmosomal junctions. These epithelial cells are cuboidal in some areas and extremely flattened in others. There are large gaps at intervals in this epithelial cell layer which theoretically would permit the free movement of cells between the medulla and the perivascular areas. Epithelial cells do not extend into the perivascular areas. Instead, these areas contain moderate numbers of fibroblasts and extensive bundles of collagen. Apart from this difference, the cell types found in the perivascular areas are apparently similar to those in the surrounding medulla. Small numbers of lymphocytes can be observed traversing the endothelium of the postcapillary venules in the medulla, but it is not possible to ascertain the direction in which they are moving. The perivascular areas may represent a discrete compartment of the thymus in which lymphocytes complete their maturation, or they may merely be a holding area for cells in the process or leaving or entering the thymus.

The epithelial cells within the medulla vary in size and shape, ranging from large cells up to 20 microns in diameter with a euchromatic nucleus and abundant discrete cytoplasm to smaller, spindle- or star-shaped cells. These resemble more closely the epithelial cells of the cortex. There are numerous desmosomal junctions between the cytoplasmic processes of adjacent cells. Prominent bundles of keratin are present in the cytoplasm of medullary epithelial cells; they are particularly abundant in some of the large cells. The Hassal's corpuscles in the central medulla are composed of accumulations of these large cells with a central area of keratin deposit.

The other main cell type found in the medulla is the interdigitating cell. This is a large cell with a characteristically irregular-shaped nucleus and voluminous pale staining cytoplasm, with relatively few organelles and processes. Similar cells are found in the thymus-dependent areas of lymph nodes and spleen. Evidence has been obtained in rats that these cells are derived from the bone marrow. Small numbers of macrophages and mast cells and occasional neutrophils are also present in the medulla.
Epithelial cells (E) in the thymic medulla.

Staining of tissue sections by the immunoperoxidase technique using monoclonal antibodies indicates that the lymphocytes and most of the non-lymphocytic cells in the medulla express class I MHC antigens. A large proportion of non-lymphocytic cells also appear to express class II MHC determinants, including almost certainly both epithelial and interdigitating cells.

How does the thymus function?

We do not yet know just how the thymus functions to produce immunocompetent T lymphocytes. It has been shown in mice that the entire lymphocyte population of the thymus turns over every 3 to 5 days. There is a high rate of cell death, and only about one % of the lymphocytes produced ever leave the thymus.

Most mature T lymphocytes possess receptors for foreign antigen plus self class I or class II MHC antigens. It is generally believed that T lymphocytes become capable of mounting an immune response when they acquire these receptors in the thymus. Studies in mice have provided evidence that the specificity of some T lymphocyte populations for foreign antigen plus MHC antigen is determined by the microenvironment in the thymus: the T lymphocytes recognize foreign antigen when it is presented on cells with the same MHC antigen as the epithelial cells of the thymus. These observations imply that the thymus has a role in selecting T lymphocytes of particular recognition specificities. Thus the epithelial cells, which are rich in MHC antigens, must play an important role in thymic function.

The lymph nodes

Lymph nodes are found throughout the body. They act as filters for foreign materials which enter the lymph and provide a favourable environment for the interaction of different cell types in the generation of immune responses to lymph-borne antigens. A lymph node is in a highly dynamic state, with large numbers of lymphocytes entering continuously from the blood and afferent lymph and leaving in efferent lymph. Lymphocytes from the bloodstream enter the paracortical areas of the lymph node cortex through specialized post-capillary venules. The output of lymphocytes from the prefemoral lymph node (weighing approximately 3 to 5 grams) of a 6-month-old calf is in the region of 2 to 5 billion cells a day.
In sheep, approximately 90% of the cells entering the peripheral lymph nodes come from the blood, with the remaining 10% arriving in afferent lymph. The afferent lymph contains not only lymphocytes, but also up to 20% large macrophage-like cells. These large cells are absent or present in extremely small numbers in efferent lymph. The arrangement of a dual circulation of cells through the lymph nodes from blood and lymph provides maximum opportunity for lymphocytes to come into contact with antigen or antigen-bearing cells arriving from the tissues.

Normal bovine thymus stained by the immunoperoxidase technique with monoclonal antibody R1, which recognizes class II MHC antigen on epithelial cells in the cortex (C) and the medulla (M).

Different compartments of the lymph node are populated predominantly by different types of lymphocytes, and each compartment also possesses its own specialized accessory cell population. Thus different types of immune responses involve different compartments of the lymph node to a greater or lesser extent.

**Reticulin/ reticulum cell network**

The structural framework of the lymph node is a network of reticulum cells and reticulin. Reticulin is a rather poorly-defined, extracellular, connective-tissue substance which is identified histologically by its affinity for silver stains. At the ultrastructural level, reticulin is seen to consist of amorphous or fine fibrillar material containing variable numbers of fibres which show distinct banding, similar to collagen fibres. Within the lymph nodes, there is a network of reticulin enveloped by reticulum cells. These are elongated or spindle-shaped,
electron-dense cells, and it is generally assumed that they produce reticulin. The reticulin network is present throughout the lymph node except within the germinal centres. The reticulum cells abutt and overlap each other and are linked by desmosomal junctions. They form a continuous layer around blood capillaries and the perivascular connective tissue of larger blood vessels and line the lymph node sinuses.

The sinus walls consist of two layers of reticulum cells with an intervening layer of reticulin. Bundles of reticular fibres which traverse the lumena of the sinuses are enveloped in reticulum cells. The reticulum cells and reticulin within areas of solid lymphoid tissue are attached to the outer aspect of the sinus walls, thus providing continuity of the network throughout the entire node. The reticulin and other areas of connective tissue are always separated from the lymphocytes by a layer of reticulum cells.

In laboratory animals, it has been shown that the reticulum cells which line the lymph node sinuses form a continuous layer except in the inner lining of the subcapsular sinus, where there are small gaps which enable cells to cross the sinus wall. This is thought to be the major route by which cells and antigens in afferent lymph enter the substance of the lymph node. The sinus reticulum cells, unlike those elsewhere in the node, possess a well-developed lysosomal apparatus and have considerable phagocytic capacity. In sheep, the lymph nodes are capable of trapping 75% or more of bacteria or other foreign cells introduced into the afferent lymph. By contrast, only about 1% of soluble antigen is retained in the node.

**Follicular areas and germinal centres**

The follicular areas in bovine lymph nodes are found in the superficial cortex and in the deeper cortex adjacent to the sinuses which extend inwards around strands of connective tissue. Primary follicles are composed almost entirely of small lymphocytes which express surface immunoglobulin (sIg), while secondary follicles contain a germinal centre surrounded by a mantle of small sIg-positive lymphocytes. Small numbers of T lymphocytes are found within both primary and secondary follicles. In the peripheral lymph nodes of calves, germinal centres first appear in the first week after birth; in healthy 6- to 12-month-old animals 30 to 70% of the follicles within a lymph node may contain germinal centres.

These germinal centres contain dense granular deposits of immunoglobulins which represent immune complexes on the surface of follicular dendritic cells. Active germinal centres are usually demarcated into two distinct zones: the inner pole distal from the adjacent sinus contains mainly B lymphoblasts, lymphocytes which are multiplying rapidly, while the outer, less active cap region contains fewer lymphocytes and more numerous non-lymphocytic cells. The significance of this zonal arrangement is not known. The granular deposits of immunoglobulin are usually much more abundant in the outer cap region, suggesting that this area is rich in follicular dendritic cells. The high level of lymphocyte proliferation is associated with lymphocyte death, and macrophages are detected which contain dead and dying lymphocytes.

Unlike the small lymphocytes elsewhere in the follicles, many of the multiplying lymphoblast cells in the germinal centres do not appear to express surface IgM, though in the cap region this is difficult to observe because of the presence of immunoglobulin on the follicular dendritic cells. This finding would agree with studies in other species which have shown that germinal centre cells do not express surface IgM, but express low levels of other surface immunoglobulins (IgG or IgA).

In suspensions of bovine lymph node cells, approximately 10 to 18 are positive for class II MHC determinants. Staining of tissue sections has confirmed that these positive cells are B lymphocytes found in the follicular areas. It is difficult to determine whether the follicular dendritic cells also express class II MHC antigens because of the staining of other cells in the area. Cells in the germinal centre express much lower levels of class I MHC antigens than cells in other parts of the lymph node. The germinal centre cells bind PNA (peanut agglutinin) with high avidity, as has been observed in other species.
Normal bovine lymph node stained by the immunoperoxidase technique, with (a) monoclonal antibody B54 which identifies B lymphocytes and (b) monoclonal antibody K1 which identifies T lymphocytes.

The function of the germinal centres is thought to relate to the properties of the follicular dendritic cells. These cells, which form a network within the germinal centre, have relatively small amounts of perinuclear cytoplasm, but have long slender cytoplasmic processes extending between the surrounding lymphocytes. It is not known whether they are modified reticulum cells or are derived from the bone marrow. Follicular dendritic cells isolated from mice express receptors for the constant (Fc) region of immunoglobulin and for the third component of complement (C3); they do not adhere to glass, are not phagocytic, contain only weak lysosomal enzyme activity and do not express detectable levels of class II MHC antigens. They are, therefore, distinct from macrophages and from the interdigitating or dendritic cells found in the paracortical areas of the lymph node.

The two main functions which have been proposed for germinal centres are the generation of memory B cells (responsible for secondary antibody responses) and the regulation of the level and duration of the antibody response. There is considerable evidence that the initial production of antibody during a humoral immune response does not occur within the follicles of the lymph node, but in the perifollicular areas or medullary cords prior to germinal centre formation. It is believed that the stimulus for germinal centre formation is the trapping by follicular dendritic cells of antigen-antibody complexes formed once antibody production has commenced. Such complexes may be retained within the germinal centres for many months. There is evidence to suggest that this retention of antigen-antibody complexes may serve to maintain levels of circulating antibody: if the level of circulating antibody falls, antibody breaks off from the complexes in the germinal centres and moves into the circulation, resulting in the exposure of free antigenic determinants in the germinal centres, which stimulates further antibody production. The production of memory B cells has also been shown to depend on the trapping of immune complexes by follicular dendritic cells and the formation of germinal centres. The localization of antigen-antibody immune complexes in germinal centres can lead to the generation of memory cells with specificity for the antibody in the complexes: this has led to the suggestion that the germinal centres may be involved in the negative regulation of antibody responses by the production of antibodies against antibody.

**Paracortical areas**

The paracortical areas are populated by lymphocytes which do not express surface immunoglobulin, but stain with several T lymphocyte-specific monoclonal antibodies. The paracortex also contains small numbers of macrophages and numerous interdigitating cells.
The interdigitating cells contain few or no lysosomal granules and do not appear to be actively phagocytic. They express class II MHC antigens. These interdigitating cells in cattle are probably the same as the dendritic cells observed in cell suspensions prepared from mouse lymphoid tissues. Present evidence suggests that the dendritic/interdigitating cells are important accessory cells in T lymphocyte-mediated immune responses.

Most macrophages and dendritic/interdigitating cells probably enter the lymph nodes in the afferent lymph, rather than in the blood. Numerous large frilly cells are found in afferent lymph, some of which are phagocytic and have Fc and C3 receptors. In rabbits, it has been shown that a large percentage of these frilly cells enter the paracortical areas of the lymph node. Thus, it is believed that the population of interdigitating cells in the paracortical areas has a finite lifespan and is continually being replenished by cells arriving in the afferent lymph. These cells probably play a role in the transportation of antigen into the paracortical areas.

Interdigitating cells (I) in the paracortex of a normal bovine lymph node. These cells have a characteristic irregular-shaped nucleus and voluminous translucent cytoplasm, which shows numerous peripheral cytoplasmic processes but is relatively devoid of organelles.

**Conclusion**

A cow’s normal immune responses are often not sufficient to combat the parasites which cause trypanosomiasis and East Coast fever. If immunological studies are to lead to improved control of these two important diseases, they must provide a detailed, step-by-step account of exactly what happens in the immune system from the moment an animal becomes infected. The research findings on normal cell distributions and functions presented here provide an essential baseline for current studies on the disruptions which occur in the immune system of infected animals. A thorough understanding of immune responses in cattle infected with trypanosomiasis or East Coast fever may indicate how these responses might be enhanced.