

Adaptive Immune Responses in Cattle

Mini
Review

Bovine Passive and Adaptive Immunity

This article gives an overview of the passive and adaptive immunity of the bovine immune system, looking at aspects of the roles played by T and B cells, and making comparisons to human and murine adaptive immunity.

Adaptive immunity

The adaptive and innate immune systems include humoral and cell-mediated immunity components. However, unlike the innate system, the adaptive system is antigen specific and provides a long-lasting protection against specific pathogens.

In adaptive immunity, cell-mediated immune responses involve T cells (CD4 Helper Th1 and Th2 cells, cytotoxic CD8, and gamma delta ($\gamma\delta$) T cells) whereas humoral immune responses involve B cells. The adaptive immune system can be modulated, through vaccination, to become quicker, better and stronger in response to a specific pathogen with repeated exposure.

Passive immunity

The fetus does not receive maternal immune protection via the placenta; passive transfer of immunity is therefore dependent upon receiving colostrum. Without an adequate consumption of good quality colostrum within 24 hours of birth, the health and productivity of dairy calves is affected, resulting in increased morbidity and mortality rates and economic losses to the industry.

Passive immunity (short term lasting weeks to months) occurs when antibodies are passed on from another animal that has already been exposed to and developed antibodies against the pathogen, as in the case of neonatal calves acquiring passive immunity through transfer of three types of immunoglobulin (IgM, IgA and IgG) via bovine colostrum.

Each type of immunoglobulin (Ig) has a specific role and function:

IgM is the largest Ig and restricted to the bloodstream and is therefore important in the defense against septicemia.

Feeding colostrum to newborn calves for the first few days provides IgA which protects against invading pathogens by attachment to the mucosal cells lining the intestines.

IgG is the smallest of Igs and the most abundant, moving from the blood into tissues where it can interact with invading pathogens. Its absorption is the highest immediately after birth, but rapidly declines to almost nothing within 24 hours as cells from the small intestine rapidly mature and lose their ability to absorb IgG. As newborns have not been exposed to pathogens, they have no memory cells or antibodies and are therefore vulnerable to infections. Passive immunity via the colostrum provides them with a “borrowed memory” and protection from pathogens until their immune system is mature enough to make their own antibodies.

Passive transfer of immunity is generally considered to be adequate if the serum levels of IgG in neonatal calves are $\geq 1,000$ mg/dL (Poulsen et al. 2010). Shortage of colostrum can be the result of mastitis, leakage or problems during calving. Colostrum is a carrier for *Mycobacterium paratuberculosis* and can't be used from cows testing positive for infection with *Mycobacterium paratuberculosis*, *Salmonella dublin*, *Mycoplasma bovis*, bovine leukosis virus, bovine viral diarrhea virus, or *Neospora caninum*.

When colostrum can't be used, an adequate passive immunity can be achieved (as indicated by sIgG concentration $\geq 1,000$ mg/dL) by sequential feeding of neonatal calves with a bovine serum-based colostrum replacement (CR) product followed by a bovine serum-based colostrum supplement (CS) product (Poulsen et al. 2010). Calves that have not received enough colostrum or are sick can be passively immunized by administering specific antisera or antitoxins.

Vaccination is a form of acquired immunity

Active immunity, also called acquired immunity (involves T and B cells), is developed after exposure to a pathogen with the adaptive immune system creating a long term immunological “memory”. Subsequent contact with the specific pathogen results in a more rapid and stronger immune response which eliminates disease and the pathogen.

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Adaptive immunity - bovine T cells

Th1 and Th2 responses

Cattle Th1/Th2 responses to antigens are similar to human and mouse (Magombedze et al. 2014). Johne's disease (JD) is caused by gut macrophages being infected with the *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP infection has a long incubation period (several years) and is therefore difficult to detect at an early stage of infection. During the initial stage the infected animal mounts a strong cell mediated CD4 T cell response with production of interferon gamma (IFN- γ) (Th1 response). This Th1 response is lost over time and replaced with a Th2 response (production of antibodies) which is driven by interleukin-4 (IL-4) and IL-10 CD4⁺ T cells. In MAP infected cattle the Th2 response produces ineffective antibodies. The loss of Th1 response and production of ineffective antibodies indicates that the cattle response to MAP infection is dysfunctional (Begg et al. 2011).

Th17 response

IL-17 is a pro-inflammatory cytokine with both a protective role against infection (in particular parasitic infections) and on the other hand a promoter of inflammation in autoimmunity.

Two separate populations of bovine T cells (CD4⁺ and WC1⁺ $\gamma\delta$ T-cell) are capable of secreting IL-17 under appropriate cytokine stimulation (TGF- β 1, IL-6 and/or IL-1 β). Th17 cells are also known to be negatively regulated by IFN- γ and not to produce IL-17 and IFN- γ simultaneously (Peckham et al. 2014).

Gamma delta ($\gamma\delta$) TCR⁺ T cells regulate and suppress T cells

In contrast to humans and mice, cattle (in particular calves) have high levels of circulating $\gamma\delta$ TCR⁺ T cells (15-60%). The majority of cattle $\gamma\delta$ T cell receptor (TCR)⁺ T cells are CD2⁻ CD4⁻ CD8⁻ and some express the workshop cluster 1 (WC1 also known as CD163L1) glycoprotein, which is homologous to the scavenger receptor cysteine-rich (SRCR) family, closely related to CD163 (Guzman et al. 2014).

Cattle have cells with a T regulatory cell (Treg) phenotype (CD4⁺CD25^{high} Foxp3⁺ T cells) but, in contrast to humans and mice, these cells are neither suppressive nor anergic.

Bovine $\gamma\delta$ TCR⁺ T cells are the major regulatory T cells (Hoek et al. 2009). They spontaneously secrete IL-10 and proliferate in response to IL-10, IL-4 and TGF- β , which initiates further production (positive feedback) of IL-10 by the proliferating $\gamma\delta$ TCR⁺ T cells. IL-10 expressing $\gamma\delta$ TCR⁺ T cells suppress nonspecific and antigen specific proliferation of CD4⁺ and CD8⁺ T cells. The majority of IL-10 expressing $\gamma\delta$ TCR⁺ T cells express CD45RO of which 50% express IL-7R (Guzman et al. 2014).

There are two types of bovine IL-10⁺ $\gamma\delta$ TCR⁺ T cell: WC1⁺

(WC1.1 and WC1.2 subtypes) and WC1⁻. Depending on the expression of the WC1 molecule, $\gamma\delta$ TCR⁺ T cells can be divided into two distinct groups: WC1⁺/CD3⁺/CD5⁺/CD2⁻/CD6⁻/CD8⁻ expressing cells or WC1⁻/CD3⁺/CD5⁺/CD2⁺/CD6⁺/CD8⁺ bearing cells.

The WC1⁺ population has two subtypes, WC1.1 and WC1.2. Only the WC1.2 subpopulation can proliferate and express IL-10, whereas the WC1.1 subpopulation expresses IFN- γ when stimulated by mitogen or cytokines (Rogers et al. 2005). For $\gamma\delta$ TCR⁺ T cells to survive they need to express IL-10 and proliferate. This is dependent upon soluble IL-10 and to a lesser extent TGF- β and physical contact with CD14⁺ cells (monocytes). Unlike human and mouse Tregs, which require engagement of the TCR for expansion and function, bovine IL-10⁺ $\gamma\delta$ TCR⁺ T cells do not proliferate when cultured with anti-CD3 and anti-CD28 monoclonal antibodies (Hoek et al. 2009; Guzman et al. 2014).

Several labs have shown that within 1-3 days of infection with, for example foot-and-mouth disease virus (FMDV), or bovine herpes virus WC1⁺ $\gamma\delta$ T cells show an increased expression of CD25 indicating activation of these cells; a loss of CD45RO (lowest expression between day 2-5) and CD62L expression (lowest expression by day 2). CD62L is a homing receptor that directs T cell trafficking into lymph nodes.

CD335 is a typical marker for NK cells (including bovine NK cells) where it functions as a natural cytotoxicity-triggering molecule. Healthy animals do not express CD335 on WC1⁺ $\gamma\delta$ T cells. However, following infection with FMDV a transient expression of CD335 is induced (as is the presence of intracellular perforin) which would indicate a cytolytic function of WC1⁺ $\gamma\delta$ T cells (Toka et al. 2011).

B cell responses

Unlike humans and mice, cattle (and other species e.g. chicken, sheep, pig, rabbit and horse) have a limited germline repertoire. In cattle, the repertoire can be expanded and differentiated in the gut-associated lymphoid tissue (GALT). Antibody diversity is dependent upon the enzyme activation-induced cytidine deaminase (AID). The enzyme converts cytosines (C) to uracils (U) and its function is restricted to single stranded DNA exposed during transcription. The expression of AID⁺ B cells can be detected in fetal liver and thymus using the B cell marker CD79 (Liljvirta 2014).

Cattle also have unique Fc receptors directed at IgG2b to protect the tissues and a secreted form of IgG1.

Human and mouse have different immunoglobulins. Cattle have features similar to mouse and human and features unique to them (see Table 1).

Like mice, cattle have a high percentage of antibodies (about 95%) that use a single light chain, but unlike mice they use the lambda chain instead of kappa. Another difference in cattle is the secretion of monomeric IgG1, in addition to the conventional soluble IgA.

Table 1: Comparison of the immunological features of cattle, human and mouse

Feature	Cattle	Human	Mouse
Percent neutrophils in blood	50	45	10
Percent lymphocytes in blood	30	40	75
Percent monocytes in blood	5	10	10
Percent gamma-delta in blood	15	2	2
CD4:CD8 in adult	3:1	3:1	2:1
Polarization of Th1/Th2	weak	moderate	very strong
NO production to LPS	moderate	moderate-weak	very strong
ROS production to LPS	moderate	moderate	weak
MHC class II expressed on T cells	activated	activated	never
Epithelial cells present AG	probably	yes	no
Predominant pan-T cells CD	2, 6	2, 3	3
Trans-placental immune transfer	none	moderate	major
Immunoglobulin classes	IgG1, 2a, 2b, M, E, A	IgG1, 2, 3, 4, A1, A2, M, E, D	IgG1, 2a, 2b, 3, M, E, A
Light chain use	lambda	both used	kappa
Secretory Ig	G1 and A	A	A
Lymph	cellular	cellular	cellular
Lymph nodes	normal	normal	normal

{Modified from: The Immunology of large animal, by David Hurley: <http://www.vet.uga.edu/lam/teaching/woolums/5160/Lecture%20One/immunology.doc>}

Induction of IgA can be T cell dependent or T cell independent, and mediated by dendritic cells (DCs) and/or epithelial cells. Activation of bovine B cells via surface IgM crosslinking results in the expression of CD5. However, in the presence of CD40 ligand, CD5 expression is blocked. Bovine B cells constitutively express tumor necrosis factor- α (TNF- α) and interleukin 1 (IL-1).

Bovine leukemia virus (BLV) is associated with enzootic bovine leucosis (EBL), which is the most common neoplastic disease of cattle. BLV has been identified in B cells, CD2⁺ T cells, CD3⁺ T cells, CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ T cells, monocytes, and granulocytes in infected cattle that do not have tumors; although the most consistently infected cell is the CD5⁺ B cell. Although CD5⁺ IgM⁺ B cells are the main cell type targeted in BLV-infected but clinically normal cattle, CD5⁻ IgM⁺ B cells, CD4⁺ cells, and CD8⁺ T cells are infected to a greater extent than previously thought (Panei et al. 2013).

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To help you find the antibodies needed to investigate the adaptive side of the bovine immune system, visit bio-rad-antibodies.com/cow-bovine-antibodies.html

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