Innate Immune Responses in Cattle

Mini Review

This article gives an overview of the bovine innate immune system, examining the roles played by natural killer cells, dendritic cells, and macrophages. Extra attention is given to dendritic cells, including a comparison to human and mouse models.

The bovine immune system is made up of two arms: an innate (native) response which produces an immediate reaction and the slower adaptive (acquired) response which occurs 10-14 days post exposure.

The innate system functions through a combination of natural defensive barriers - mainly skin, phagocytes and neutrophils, natural killer cells, cytokines, complement and anti-microbial peptides. The sections below will cover natural killer cells, macrophages, and dendritic cells making comparisons to human and mouse models.

Innate immunity

After microbial invasion, sentinel cells, such as macrophages and dendritic cells, secrete cytokines – among them interleukin-1 (IL-1) and IL-6, tumour necrosis factor alpha (TNF-α) and high-mobility group protein B1 (HMGB1). In the case of a weak response, the immune reaction is local at the site of infection. A stronger stimulus leads to systemic effects in the liver, brain and bone marrow. The results include increased white cell production to help fight the infection.

The innate immune system is often referred to as an ancient defense system structured around cellular pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) found in prokaryotes but not eukaryotes.

Toll-like receptors (TLRs) bind microbial associated molecular patterns (MAMPs) and trigger innate immune responses (Akira and Takeda 2004). TLR4 was the first functional mammalian PRR to be identified on macrophages. It binds the lipopolysaccharide (LPS) cell wall component of gram-negative bacteria. Engagement of TLRs leads to secretion of cytokines such as IL-1β, IL-6, interferon gamma (IFN-γ) and TNF-α, and chemokines such as IL-8.

Innate immunity is not antigen specific, but does show molecular specificity. Contrary to adaptive immunity, innate immunity does not depend on encountering a pathogen; it is not augmented by repeated exposures to the same pathogen as it has no memory (Rainard and Riollet 2006).

There are two arms to innate immunity; the sensing arm (deals with how the host perceives infection) and the effector arm (deals with how the host combats infection) (Beutler 2004). Each arm has a humoral and a cellular component.

Delineating the innate immune system from the adaptive immune system is very difficult as they share many effector mechanisms (Rainard and Riollet 2006). The innate immune system is made up of monocytes, macrophages, dendritic cells, natural killer cells, neutrophils and eosinophils.

Natural Killer Cells

Natural killer (NK) cells are among the first cells of the innate immune system to respond during infection or inflammation through secretion of immunoregulatory cytokines and cellular cytotoxicity. In humans, NK cells are identified as $CD56^{+}/CD3^{-}$. NK cells in cattle can be characterized by their expression of NKp46 (CD335); IL-15 is an essential cytokine in NK cell development and survival.

Bovine NK cells, defined as $NKp46/NCR1^{+}$ $CD3^{-}$ cells, can be subdivided into two (Boysen et al. 2006):
- a $CD2^{+}$ subset dominating in peripheral blood and
- a $CD2^{-/low}$ subset dominating in lymph nodes.

The latter subset, expressing higher levels of CD16 and CD8α, as well as the activation markers CD25 and CD44, produce high levels of IFN-γ and are the main population following *in vitro* stimulation with IL-2 or IL-15 (Lund et al. 2013). Although cattle are not directly comparable to humans, bovine $CD2^{-/low}$ NK cells are similar to the human NK $CD56^{bright}$ phenotype found in human lymphoid tissues. Lund and colleagues investigated the phenotype and cytokine production of NK cells in skin-draining afferent lymph (AL).

Since the activated AL NK cells had a similar phenotype to the lymph node residing NK cells, it is possible that these cells recirculate to the lymph nodes. The recirculation patterns of NK cells in AL is thought to be relevant to the role of NK cells in vaccine responses.

Dendritic Cells

The key function of dendritic cells (DCs) is to present antigen to T and B cells. They have the ability to prime naïve T cells, initiate a primary T cell-mediated response and induce a Th1 or Th2 response. Within DCs there are some differences in the morphology, phenotypes and functions between subtypes. DCs link the adaptive and innate immune systems.

Dendritic cells (DC) are a heterogeneous population making up less than 1% of the lymphoid compartment. Because DCs are heterogeneous, it is difficult to group them; DC subtypes can be categorized by their location, phenotype, and their immune function. DCs do not express an exclusive marker, so their identification relies on the use of a combination of different markers. Human DCs can be identified by:

(a) the presence of high levels of major histocompatibility class II (MHC II) HLA-DR expression (higher than other professional antigen presenting cells (APCs));
(b) the absence of lineage markers for B cells (CD19/20), T cells (CD3), monocytes (CD14), NK cells (CD56), and granulocytes (CD66b);
(c) the expression of a variety of adhesion molecules using antibodies directed against CD11c, LFA-1 (CD11a), LFA-3 (CD58), ICAM-1 (CD54), ICAM-2 (CD50), and ICAM-3 (CD102).

Finally, the presence and up regulation of co-stimulatory molecules CD80 (B7.1) and CD86 (B7.2) helps to differentiate immature DCs (CD86) from mature DCs (CD80).

Human DCs express other lineage markers which enable them to be sub-typed as:

(a) conventional DCs (cDCs) also referred to as myeloid/classical, which also contain a minor cross-presenting DC subset;
(b) plasmacytoid DCs (pDCs); and
(c) monocyte related DCs (Mo-DCs)
(Collin et al. 2013; see Table 1 comparing human, mouse and cattle).

Human myeloid DCs express CD11c, CD13, CD33 and CD11b (equivalent to the mouse classical $CD11c^+$ DC). They can lack CD14 or CD16 and be further subtyped into $CD1c^+$ (equivalent to mouse CD11b) or $CD141^+$ (equivalent to mouse CD8/CD103) DCs. Plasmacytoid DCs lack myeloid markers and are identified by their expression of CD123 (IL-3R), CD303 (CLEC4C; BDCA-2) and CD304 (neuropilin; BDCA-4). Human monocyte-related $CD14^+$ DCs (equivalent to mouse $CD11b^+$) represent a third subset of $CD11c^+$ myeloid cells, also called interstitial DCs, that are more monocyte/macrophage like than $CD1b^+$ or $CD141^+$ myeloid DCs.
DCs can also be functionally classified based on their anatomical location. In the lymphoid tissue of mice there are five main subgroups of murine DC (Shortman and Liu 2002), which are classified by the presence or absence of CD4, CD8α, CD11b, and the interdigitating DC marker CD205: cDCs ($CD8a^+$), CD11b type, pDCs, Langerhans cells in the skin, and Mo-DCs.

DCs located in the epidermis (Langerhans cells) can also be identified by the expression of CD207, interstitial DCs by the expression of CD209, interdigitating DCs (located in the T cell areas of secondary lymph nodes) by the expression of CD208 (DC-LAMP) and the costimulatory molecules CD40, CD80, and CD54. A more recently defined subtype is the pDC. Unlike cDCs they do not express CD11c, or secrete IL-12. They express CD303 and CD123 and produce high levels of type 1 interferons (IFN-α/β) in response to viral infection and their function is to link the innate and adaptive immune systems.

Research on human and murine DCs has led to a better understanding of the role of dendritic cells in the pathogenesis of infectious disease. Similar phenotypes of DCs have been found in cattle (Bajer et al. 2003).

Although not as well characterized as mouse and human, bovine DCs have, to date, been divided into three groups:

(a) bone marrow derived (BMDC/myeloid/conventional derived),
(b) monocyte related (Mo-DC, $CD14^+$) and
(c) tissue resident (anatomical location).

Similar to human and mouse cDCs, bovine monocyte derived DCs express myeloid markers (CD11a, CD11b, CD14, and CD172a (SIRPa)) and when activated the expression levels of CD40, CD80, and CD86 are up-regulated.

Bovine DCs differ phenotypically based on their tissue distribution. MHC II expression detects DCs of hematopoietic origin; $CD208^+$ DCs are found in lymphoid tissues and $CD1b^+$ expressing DCs are mainly found in the thymic medulla (Romero-Palomo et al. 2013).

Bovine pDCs are still not as well characterized as human and mouse ones but researchers have shown that they express MHC II, CD4, CD172a, CD32, and CD45RB and produce very high levels of type 1 interferons in response to viral infections (e.g. foot and mouth disease (FMDV)) (Reid et al. 2011).

Expression of the myeloid specific marker CD172a has also been used to identify the tissue distribution of bovine DCs (Sei et al. 2014; Miyazawa et al. 2006). MHC II, CD11c, and CD172a being expressed on peripheral blood DCs; CD1 and CD172a on DCs in the thymic medulla and CD11b and CD172a on DCs within the Peyer's patches (Brimczok et al. 2005).

The finding that some of the Peyer's patches DCs express the prion protein (PrP) could indicate that these DCs may be involved in the transmission of bovine spongiform encephalopathy (BSE) (Rybner-Barnier C et al. 2006).

The differential expression of MHC II, CD208 (DC-LAMP), CD1b, CD205 (DEC-205), CNA.42 and S100 protein on DCs further helps to define DC subtypes. CD208 is expressed on interdigitating DCs present in the thymic medulla and within the Peyer's patches follicles. CD1b identifies thymic DCs and CD205 is strongly expressed on afferent lymph DCs (ALDC). Bovine follicular dendritic cells (FDA DC) can be identified by their expression of both CNA.42 and S100.

Bovine peripheral blood DCs express CD80 and have been shown to consist of three different subsets (Sei et al. 2012):

(a) immature $CD4^+MHC\ II^-$ pDCs that upon TLR stimulation up regulate their MHC II and CD80 expression and produce large amounts of type I interferons; (dendritic cells express TLR7 and TLR9) and
(b) two cDC types that express MHC II but have different functional markers, cytokine profiles and antigen processing abilities: $CD11c^+MHC\ II^+CD80^{hi}$ DCs (may be specialized in naïve T cell activation expression) that produce TNF-α and have antigen processing abilities; and $CD11c^-MHC\ II^+$ DCs which are precursors of $CD11c^+$ DCs (Sei et al. 2014).

Table 1: Human, mouse and cattle DC subtypes and markers.

	Human	Mouse	Cattle
Myeloid/Classical			
Major subset	$CD1c^+$ *Dectin 1 (CLEC7A)* *Dectin 2 (CLEC6A)*	$CD11b^+$ (tissues) $CD4^+\ CD11b^+$ (lymphoid) *ESAM*	CD1b CD11b CD172a
Cross presenting	$CD141^+$ *CLEC9A* *XCR1*	$CD103^+$ (tissues) $CD8^+$ (lymphoid) *CLEC9A* *XCR1* *Langerin*	
Plasmacytoid	*CD303 (CLEC4C)* *CD304 (neuropilin)* *CD123 (IL-3R)*	B220 *Siglec H*	CD4
Monocyte-related	CD14 CD209 (DC-SIGN) Factor XIIIA	$CD11b^+\ CD64^+$ (tissue) *CX3CR1, CD14*	CD14 CD11c
	$CD16^+$ monocyte *$CX3CR1^{hi}$* *SLAN (subset)*	Gr-1/Ly6C low monocyte $CX3CR1^{hi}$ *$CCR2^{neg}$*	
	Inflammatory DC *CD1c* *$CD16^{neg}$*	Monocyte-derived DC *CD209 (DC-SIGN)* *CD206*	

Data for human and mouse reproduced from Collin et al. (2013)

Macrophages

The macrophage is the innate immune cell that mediates the immune response following infection. Macrophages express PRRs such as TLRs which recognize microbial PAMPs (Rue-Albrecht et al. 2004).

The macrophage is the host cell that *Mycobacterium* infects, reproduces and resides in causing the development of chronic diseases such as Bovine tuberculosis (BTB) caused by *Mycobacterium bovis* and Johne's disease (JD) caused by *Mycobacterium avium paratuberculosis* (MAP).

PRRs (e.g. TLRs) on the macrophage recognize mycobacterial PAMPs on, for example, *Mycobacterium bovis*. As a result of PRR activation, subsequent activation signals result in the production of cytokines and chemokines driving an early protective Th1 response (i.e. $CD4^+$ T cells produce IFN-γ; and cytotoxic $CD8^+$ T cells lyze the infected macrophages). As the disease becomes more active the initial and strong Th1 response declines and is converted to a non-protective Th2 response (IL-4 and IL-10 driven) that does not control infection (Rue-Albrecht et al. 2014).

In MAP infected animals the switch from a Th1 to a Th2 response occurs at the same time as clinical signs of the disease show. The persistent MAP infection in and outside the gut macrophage results in an exhausted immune response and the loss of a Th1 response (Magombedze et al. 2014).

Bovine mastitis is caused by *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* infections. The response to *E. coli* infection is quick and strong and cleared in a few days. In contrast, infection by *S. aureus* results in a moderate immune response and the establishment of a chronic infection which is difficult to cure as the bacteria survive inside the tissue macrophage, neutrophils and epithelial cells (Nogueira de Souza et al. 2012).

CD163 is a monocyte/macrophage restricted, cysteine rich protein (a member of the scavenger receptor cysteine rich (SRCR) superfamily) that binds and internalizes circulating haptoglobin-hemoglobin (Hp-Hb) to produce an IL-10 driven anti-inflammatory response (Philippidis et al. 2004). Unlike human macrophage scavenger receptor (CD163), bovine CD163 is not restricted to monocytes and macrophages; it is also found on bovine γδ T cells (Herzig et al. 2010). CD163 expression is increased during macrophage differentiation.

During pregnancy, cattle M2 activated macrophages accumulate in the interplacental endometrium. These macrophages are $CD68^+CD14^+MHC\ II^-$ and often $CD11b^+$ and are thought to play an important role in the growth (angiogenesis and tissue remodeling) and survival of the conceptus. Fewer macrophages are found in the caruncular septa of the placentomes and express $CD68^+CD14^+MHC\ II^+$ and $CD11b^{lo/-}$ (Oliveira et al. 2010).

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

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