ISSN 1466-2523

Animal Health Research Reviews **16**(1); 4–14
doi:10.1017/S1466252315000110

Immune response to bovine viral diarrhea virus—looking at newly defined targets

Christopher C. L. Chase[1]*, Neelu Thakur[1], Mahmoud F. Darweesh[1,2], Susan E. Morarie-Kane[1,3] and Mrigendra K. Rajput[1,4]

[1] *Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD, USA*
[2] *South Valley University, Qena, Egypt*
[3] *Wenatchee Valley College, Wenatchee, WA, USA*
[4] *Medgene LLC, Brookings, SD, USA*

Received 31 December 2014; Accepted 18 March 2015

Abstract

Bovine viral diarrhea virus (BVDV) has long been associated with a wide variety of clinical syndromes and immune dysregulation, many which result in secondary bacterial infections. Current understanding of immune cell interactions that result in activation and tolerance are explored in light of BVDV infection including: depletion of lymphocytes, effects on neutrophils, natural killer cells, and the role of receptors and cytokines. In addition, we review some new information on the effect of BVDV on immune development in the fetal liver, the role of resident macrophages, and greater implications for persistent infection.

Keywords: bovine viral diarrhea virus, BVDV, macrophages, dendritic cells, neutrophils, natural killer cells, Kupffer cells, persistent infection, liver tolerance.

Introduction

Bovine viral diarrhea virus (BVDV) is considered one of the most important viruses infecting cattle worldwide. BVDV infections can cause multiple forms of disease with variable and complicated symptoms, such as growth retardation, persistent infection, hemorrhagic symptoms, respiratory and enteric infections, reproductive disease, and lethal mucosal disease (MD). This virus continues to baffle and batter the ruminant immune system.

Our knowledge continues to grow incrementally about BVDV and the immune system. Peterhans *et al.* (2003) provided an extensive review on BVDV and its effects on macrophages. Innate and adaptive immune responses to BVDV were reviewed by Brackenbury *et al.* (2003), and the immunosuppressive effects and the interaction of *in vivo* BVDV infection on interferon, antigen presentation and T-cell activation were reviewed. In our 2004 review of bovine immunity and BVDV (Chase *et al.*, 2004), innate and adaptive immunity (both cellular and humoral) were reviewed. In their 2010 review, Peterhans and Schweizer (2010) provided a great overview of common mechanisms that pestiviruses use to 'outmaneuver' their hosts, in particular the unique roles that the pestivirus-specific proteins, N^{pro} and E^{rns}, have with innate immunity. In 2013, we published an updated review examining the adaptive response to BVDV (Chase, 2013). Also Peterhans and Schweizer (2013) published an exceptional review expanding on their 2010 review on the effect of N^{pro} and E^{rns} to include 'tolerance of innate immunity'. In this review, we look at the latest information on immunological mechanisms that may contribute to immunosuppression and persistent infection, particularly as initiated by the innate immune system.

BVDV and the cells of the immune system

Does BVDV use the macrophage/dendritic cell as a 'Lymphocyte Hitman'?

One of the early hematological hallmarks of BVDV is leukopenia, primarily lymphopenia, affecting T-helper cells, cytotoxic T cells and gamma–delta T cells *in vivo* (Bolin *et al.*, 1985;

*Corresponding author. E-mail: Christopher.Chase@sdstate.edu

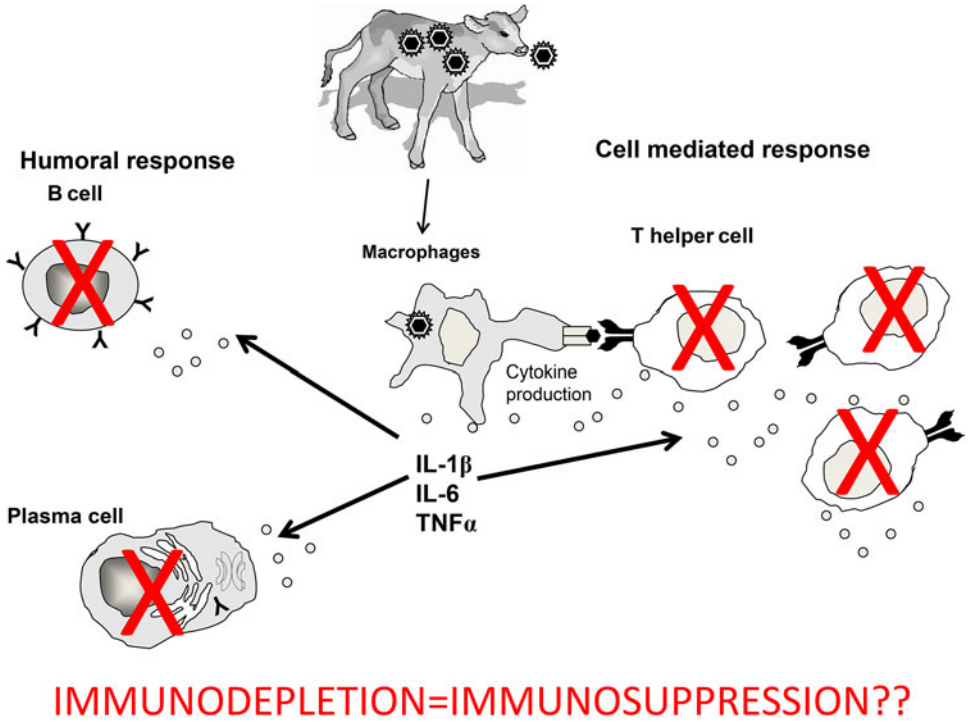

Fig. 1. B cells, T cells and plasma cells are all depleted by BVDV infection.

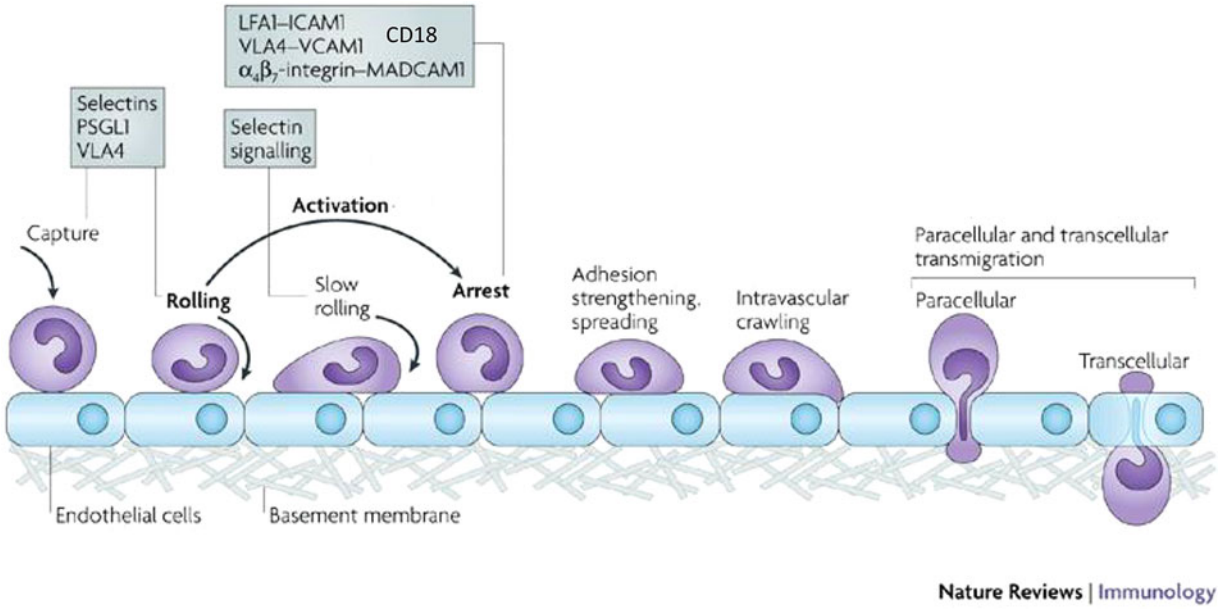

Fig. 2. CD18 (Integrin) and L-selectins are essential for adhesion and extravasation of neutrophils. Adapted from Ley *et al*. (2007).

Rebhun *et al*., 1989; Gånheim *et al*., 2005). These peripheral blood lymphocytes undergo apoptosis *in vitro* (Ridpath *et al*., 2006), resulting in lymphoid depletion of B cells, T helper cells, cytotoxic T cells, and gamma–delta T cells to variable degrees depending on the virulence of the strain of BVDV (Ridpath *et al*., 2006; Chase, 2013). B-cell levels in circulation are relatively normal (Gånheim *et al*., 2005) but B-cell depletion occurs extensively in the germinal centers of the lymph node (Liebler-Tenorio *et al*., 2002, 2003). Interestingly, there was no effect on circulating monocytes (Gånheim *et al*., 2005); although, *in vitro* infection of monocytes results in BVDV production (Brackenbury *et al*., 2003; Rajput *et al*., 2014). Likewise, during maturation from monocytes to monocyte-derived dendritic cells (MDDC), monocytes cultured in the presence of IL-4 and GM-SCSF initially produce virus, but within 48 h after *in vitro* culture, the virus yield is reduced and ceases altogether by 120 h in culture (Rajput *et al*., 2014). Interestingly, monocyte-derived macrophages (MDM) also fail to produce progeny virus

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

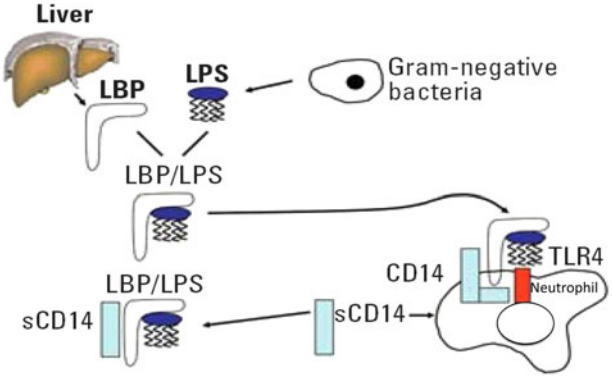

Fig. 3. The interaction of LPS and CD14 in the neutrophil. Adapted from Zeldin *et al*. (2006). *Environmental Health Perspectives,* 620–626 open access.

despite the presence of viral proteins and progeny viral RNA (Chase *et al.*, 2004; Rajput *et al.*, 2014). There is no effect on MDDC or MDM viability following BVDV infection (Chase *et al.*, 2004; Rajput *et al.*, 2014). In BVDV-infected lymphoid tissue, macrophages and dendritic cells (DCs) appear to be uninfected although they are surrounded by apoptotic lymphocytes (Liebler-Tenorio *et al.*, 2002, 2003). The cytokines released by macrophages can vary, but have the potential to induce lymphoid apoptosis in certain circumstances; this mechanism may be employed by BVDV infection and the macrophage may be functioning in a 'hitman' capacity, eliminating lymphocytes that would otherwise be activated (Chase *et al.*, 2004; Sánchez-Cordón *et al.*, 2005) (Fig. 1). Lymphoid depletion with macrophage and DC survival is also observed following infection with another pestivirus, classic swine fever virus (CSFV). Work with CSFV indicates that the likely cytokines involved in CSFV lymphoid apoptosis are interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) (Choi *et al.*, 2004; Sánchez-Cordón *et al.*, 2005). Interestingly, studies with cultured macrophages infected with BVDV (Peterhans *et al.*, 2003) or CSFV (Borca *et al.*, 2008) had increased IL-1 and IL-6 but did not have increased TNF-α production; however, *in vivo* infection with CSFV clearly demonstrated TNF-α at high levels in macrophages in both lymph nodes and spleen (Choi *et al.*, 2004; Sánchez-Cordón *et al.*, 2005). These macrophages were surrounded by apoptotic lymphocytes. Similar work needs to be done with BVDV.

What about other innate 'killers' – neutrophils and natural killer cells?

Neutrophils

Probably no cell population has been more understudied for viral diseases than the neutrophils – the ultimate 'bacteria killer' (Gabriel *et al.*, 2013). Neutrophils comprise from 20 to 70% of blood leukocytes depending on the age of animal (Menge *et al.*, 1998). Although blood neutrophils decline following BVDV infection, the decrease is much less than that observed with lymphocytes (Roth *et al.*, 1981; Gånheim *et al.*, 2005). BVDV causes a dramatic decrease in phagocytosis and killing that can last up 2 weeks following infection or vaccination (including two vaccine strains Singer and NADL) (Roth *et al.*, 1981; Roth and Kaeberle, 1983). Recently, we determined a direct *in vitro* effect of BVDV on bovine neutrophils (Thakur *et al.*, 2014). Exposure of neutrophils to either non-cytopathic or cytopathic BVDV *in vitro* did not result in apoptosis but did decrease CD18 and CD62L (L-selectin) expression (Thakur *et al.*, 2014). These two receptors are important for adhesion to endothelial cells, which is essential for neutrophil diapedesis (Fig. 2), indicating that BVDV could inhibit neutrophil migration. CD18 is the well-characterized leukocyte adhesion receptor associated with bovine leukocyte adhesion deficiency (BLAD) (Shuster *et al.*, 1992). Curiously, neutrophils exposed to BVDV also had increased CD14 expression (Thakur *et al.*, 2014). In contrast, CD14 was downregulated in BVDV-infected bovine macrophages in a strain-dependent manner (Chase *et al.*, 2004). CD14 is a polyspecific receptor, which recognizes predominately lipopolysaccharide (LPS) from the cell wall of Gram-negative bacteria (Fig. 3). CD14 is part of the toll-like receptor-4 (TLR-4) complex, which is an important receptor for pathogen recognition. Unlike monocytes/macrophages, neutrophil CD14 is not constitutively expressed but is induced by exposure to pathogen associated molecular patterns (PAMP) particularly LPS (Paape *et al.*, 2003). Binding to neutrophil CD14 receptor results in production of the pro-inflammatory cytokines TNF-α and IL-1 (Finlay and Hancock, 2004). Like monocytes/macrophages, neutrophil CD14 can also be secreted (sCD14; Sohn *et al.*, 2007) and the sCD14 has been shown to downregulate the inflammatory response. Several papers examining the effect of BVDV on immunoresponsiveness have indicated that BVDV infection results in an early proinflammatory response with often a corresponding decrease in anti-inflammatory response which overlays quite remarkably to the increased expression of neutrophil CD14 and the shift to sCD14; therein, this may be a target of interest for future studies (Risalde *et al.*, 2011; Molina *et al.*, 2014; Risalde *et al.*, 2014). Tempered inflammatory response and reduced diapedesis is likely to exacerbate any disease following viral infections, and is likely part of the immune dysregulation seen with BVDV (Fig. 4). Furthermore, while apoptosis was not noted *in vitro*, neutrophil decline has been observed *in vivo* (Roth *et al.*, 1981; Gånheim *et al.*, 2005). CD14 expression on neutrophils may be recognized and serve as a target for induction of apoptosis by macrophages (Sladek and Rysanek, 2006). Future neutrophil research investigating the interaction and cytokine profile between BVDV-infected macrophages and neutrophils *in vitro* would be of great interest and potentially the 'the lymphocyte hitman' function of macrophages might be expanded to include neutrophils.

Natural killer (NK) cells

NK cells are an important cog of innate anti-viral defense, and their characteristics were well-summarized in an excellent review

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

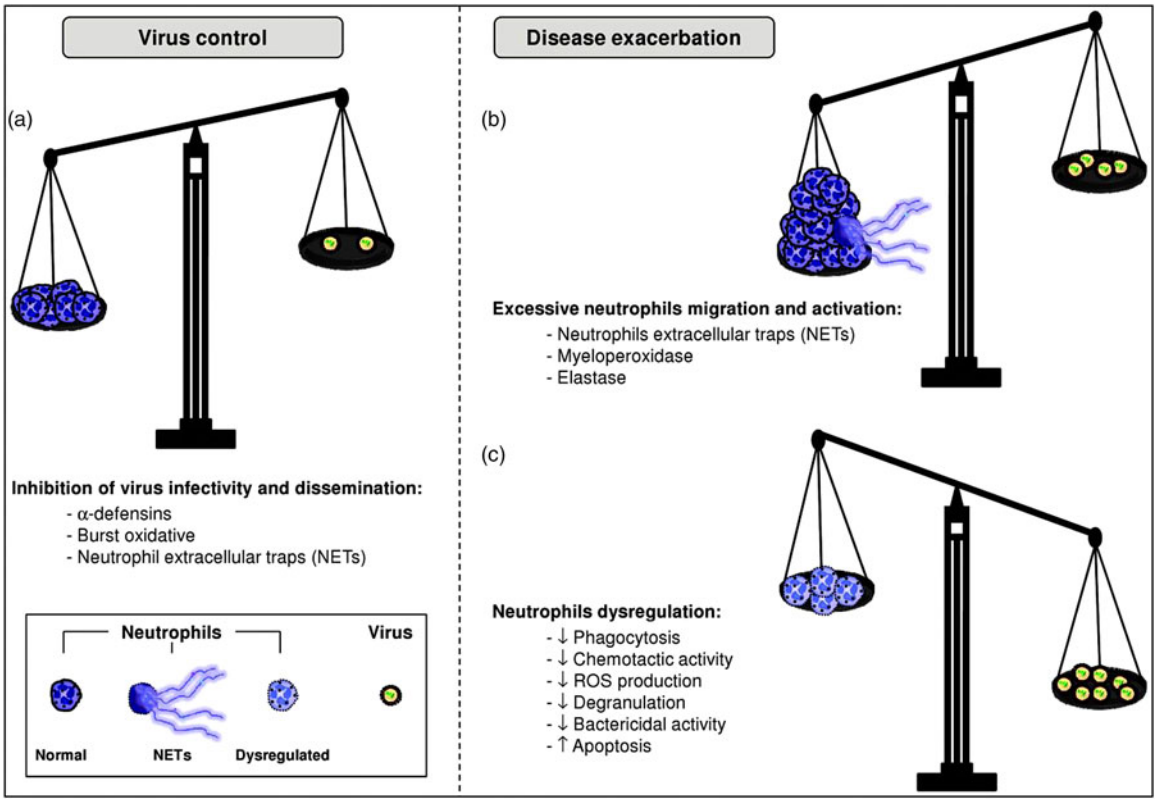

Fig. 4. Contradictory roles of neutrophils. Neutrophils can (a) control viral infections by releasing antimicrobial components and reactive oxygen species (ROS), and produce neutrophil extracellular traps (NETs) leading to the inhibition of virus infectivity, viral RNA degradation, and restrict virus dissemination. However, (b) excessive migration and overactivation of neutrophils can be detrimental to the host and exacerbate disease manifestation. (c) On the other hand, viruses can dysregulate neutrophil functions and alter phagocytosis, migration, ROS release, and degranulation. These often lead to secondary bacterial infections. Viruses can also induce apoptosis in neutrophils. Adapted from Gabriel *et al*. (2013).

by Boysen and Storset (2009) (Figs. 5–7). However, there are very little data on NK cell phenotype or function following viral infection particularly in cattle (Boysen and Storset, 2009). NK cells were first characterized in cattle in 2004 (Storset *et al*., 2004). NK cells represent 1–10% of blood mononuclear cells (Storset *et al*., 2004) and the percentage of NK cells in the blood is age dependent (Graham *et al*., 2009). Like lymphocytes, NK cells are produced in the bone marrow and then populate lymphoid tissue after birth and following exposure to microbial populations (Fig. 5). Despite low relative numbers in the lymphoid tissue, it is estimated that there are 10X as many total NK cells in the lymphoid tissue as compared to the peripheral blood (Boysen *et al*., 2008). NK distribution in lymph nodes varies depending on the type of lymph node with 2–4% NK cells in mucosal lymph nodes vs. 6–10% NK cells in spleen and non-mucosal lymph nodes (Boysen *et al*., 2008). NK cells are present in the paracortical as well as in the medullary areas of bovine lymph nodes and are evenly distributed except in germinal centers where only a few NK cells are seen. In mucosal lymph nodes, lymphoid follicles are more numerous and large, while non-mucosal lymph nodes had smaller and fewer follicles. NK cells in lymphoid follicles can express interleukin-2 receptor (IL-2 receptor; CD25) and become lymphokine-activated killer cells (LAK) (Figures 6 and 7b), resulting in the killing of infected somatic cells. These cells also produce gamma interferon (IFN-γ), which further activates DC, T-helper cells and macrophages (Boysen *et al*., 2008) (Figs. 5 and 8). Unlike other lymphocytes, NK immune suppression may result from a lack of mature NK cells rather than elimination of the population. NK cells are particularly dependent on IL-15 for their development and maturity (Endsley *et al*., 2006; Lund *et al*., 2012, 2013). A recent study in cattle infected with highly acute 1373 BVDV strain had decreased levels of IL-15 in bronchial lymph nodes, suggesting lower NK activation (Palomares *et al*., 2014). We found that like macrophages and DCs (Chase *et al*., 2004; Rajput *et al*., 2014), BVDV infection of NK cells with either highly acute strain BVDV-2 1373 or mild strain BVDV-2 28508-5 had no effect on bovine NK cell viability *in vivo* (Darweesh, 2013).

NK cells also present a new target for understanding the greater immunological dysfunction that results following BVDV infection. The formulation of the 'missing self' hypothesis by Ljunggren and Kärre (1990) early on, shaped perspectives regarding the importance of NK cells by suggesting that a principle function of NK cells is to eliminate cells that lack MHC class I (MHCI) or other 'self-markers' (Kärre *et al*., 1986). Such situations occur in cancer, viral infections and transplantation, indicating a prominent role for NK cells in these

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

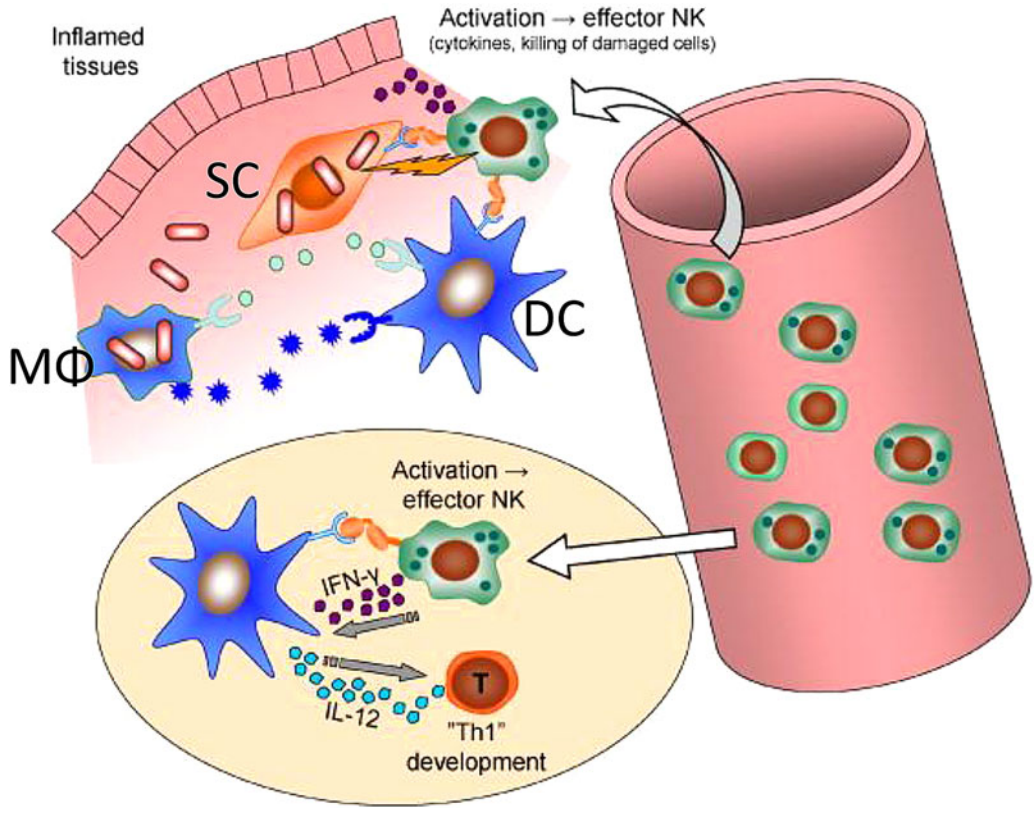

Fig. 5. Body compartments for NK cell priming of human/murine NK cells. Upon significant microbial attack, primed NK cells re-enter the lymph nodes, where they are activated by DCs to produce interferon-gamma, which participate in the polarization of Th1 cells. In addition, primed NK cells are recruited into inflamed tissues, where they are activated by damaged/infected cells as well as accessory cells, triggering effector functions such as killing of damaged cells and release of cytokines. Unlike T-cells, these NK effector responses are not restricted by microbe-specific antigens. In individuals or species heavily exposed to microbes, this may be illustrative of a 'steady state' situation, and comparably more primed NK cells will be present in lymph nodes and circulation (DC, dendritic cell; MΦ, macrophage; SC, somatic cells). Adapted from Boysen and Storset (2009).

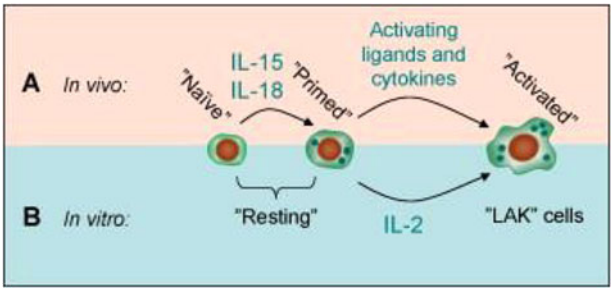

Fig. 6. Relationship between NK cells *in vivo* and experimental LAK cells. (A) Recent evidence has shown that in the living organism, NK cells are initially naïve and poorly responsive, but following microbial exposure, priming by cytokines (IL-15 and IL-18) render the NK cells responsive. Primed NK cells become activated and fully functional in response to activating cytokines and ligands on the target cells. (B) 'Resting' isolated NK cells normally show poor or moderate cytotoxic capacity in *in vitro* assays, and it may not be clear whether these are naïve or primed. Stimulation of resting NK cells with IL-2 for several days result in LAK cells, which are strongly cytotoxic. Adapted from Boysen and Storset (2009).

conditions. BVDV down-regulates MHCI in a strain-dependent manner (Chase *et al.*, 2004; Rajput *et al.*, 2014), which is a compelling reason to look closer at the phenotypical impact of this change on NK cells.

In general, NK cell receptor biology is more complicated than other lymphocytes (Boysen and Storset, 2009). NK cells have a number of cell receptors; some that are activating (triggering) and some that are inhibitory (Moretta *et al.*, 2001; Boysen and Storset, 2009) (Fig. 7a, b) and their activation is likewise variable (Figs. 7 and 8). The major NK phenotypic marker is the natural cytotoxicity activating (triggering) receptor (NCR1), which is also known as NKp46 (natural killer cell 46-kDa cell surface protein; CD335). The exact ligand for NKp46 is not specifically known, but is likely a tumor or viral protein (Boysen and Storset, 2009) (Fig. 7a). NK cells are also marked by granulysin; the antimicrobial protein contained in the NK and cytotoxic T-cells granules (Endsley *et al.*, 2004) and the IgG Fc receptor IIIA (FcgRIIIA; CD16), which binds the constant region of the IgG molecule and is important for NK antibody-dependent cell-mediated cytotoxicity (ADCC) function (Fig. 7c).

There are phenotypic differences with adhesion marker CD2 (found also on T cells) between NK cells in circulation (CD2+)

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

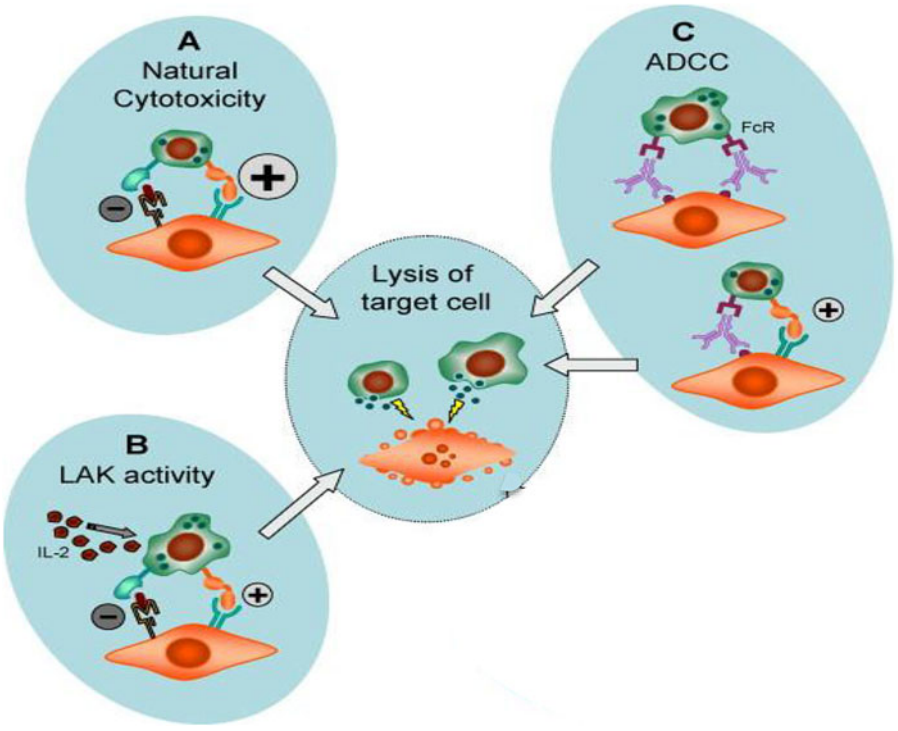

Fig. 7. NK cytotoxic phenomena. The release of cytotoxic granula and subsequent lysis of a target cell may occur from different experimental settings: (A) natural cytotoxicity is the spontaneous lysis of a target cell by the NK cell. This results when inhibiting signals (−) to the NK cell are absent or insufficient to overrule activating (+) signals. (B) LAK activity is the result of pre-activation of the NK cell by cytokines, most often IL-2. (C) ADCC (antibody-dependent cell-mediated cytotoxicity) involved the cross-linking of Fcg receptors on the NK cell, by antibodies directed to structures on the target cell. This reaction may also involve other recognition events between NK cells and target cells. Adapted from Boysen and Storset (2009).

and those found in lymphoid tissue (CD2−) (Boysen *et al.*, 2006; Lund *et al.*, 2013). In addition, bovine NK cells have another family of activation/inhibitory receptors, killer immunoglobulin-like receptors (KIRs) (Boysen and Storset, 2009). Although KIRs have been defined genetically in cattle, there is no functional work that has identified their roles in bovine NK activation/inhibition (Dobromylskyi and Ellis, 2007). Likewise, other NK activating/inhibitory receptors have been identified in human and murine models; however, homologues have yet to be identified in ruminants (Boysen and Storset, 2009). Therefore, there is still much to characterize with respect to the normal NK phenotype, and also then further understanding the interaction between BVDV and NK cells.

Recently, the effect of BVDV strains on NK activation marker CD25, phenotype markers CD2, NKp46 and the granule protein, granulysin were investigated in IL-2 stimulated NK cells *in vitro* using the type cell depicted in Fig. 6. BVDV infection of NK cells with either highly virulent strain BVDV-2 1373 or mild strain BVDV-2 28508-5 decreased NKCD2+ and MHCI and had no significant effect on NK activation as measured by expression of CD25 (Darweesh, 2013). BVDV-2 1373 strain increased the number of NKp46+ NK cells. Also both strains reduced NK cell killing, with BVDV-2 28508-5 mild strain having the largest reduction (Darweesh, 2013). These studies indicate that BVDV infection resulted in NK phenotypic and activation changes that are strain dependent *in vitro*, and this may result in immune dysfunction *in vivo*. Therein, this work presents yet another immunological target that must be critically evaluated to gain insight into the BVDV immune dysfunction.

Persistence and innate immunity – a role for the liver and Kupffer cells?

For many years, veterinarians and researchers have worked under the assumption that BVDV persistent infection developed as a result of the immune incompetence of the fetus in the late first-early second trimester of pregnancy; however, many immune components are in place early in the second, if not the first trimester. Bovine fetuses have lymphoid development of the thymus, spleen and some peripheral nodes by the middle of the first trimester, day 42, day 55, and day 60, respectively (Schultz *et al.*, 1973). The gut-associated lymphoid tissue (GALT), however, was not noted in this study until later in the second trimester (175 days) in gestation. Germinal centers as well as some IgM in antigenically stimulated fetuses were present in the mid-first trimester as well at day 59, and IgG was in circulation by the mid-second trimester at 145 days (Schultz *et al.*, 1973). Bovine fetal immunocompetence is established around mid-second trimester of gestational (day 150) (Brock, 2003). Understanding how BVDV evades and even

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

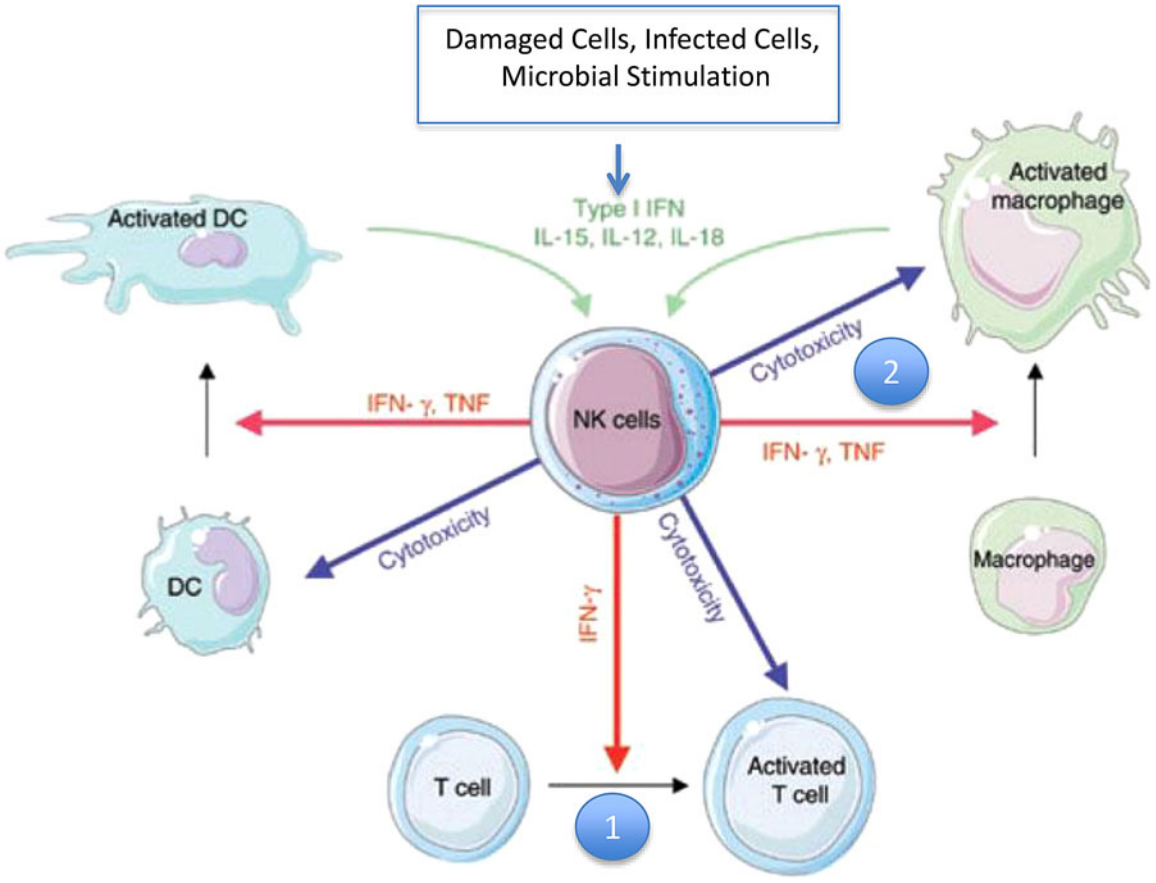

Fig. 8. Cytokine production by NK cells. Several types of stimuli result in the NK cell production of cytokines such as IFN-γ and TNF. Such stimuli can be damaged or infected cells display altered cellular ligands and release cytokines like IFN-α/β, both recognized by NK cells. Microbial stimulation of accessory cells leads to their production of IL-12 and IL-18 that work in synergy to activate the NK cell. The cytokines released from NK cells trigger several mechanisms of cellular immunity, most importantly: (1) Assistance to DCs to produce 'Th1' cytokines, which promote cellular T-cell responses. (2) Prompting of macrophages to eliminate microbes (DC, dendritic cell). Adapted from Vivier *et al.* (2008).

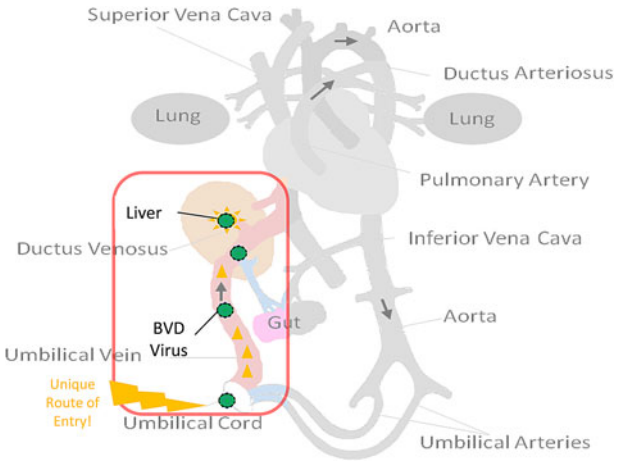

Fig. 9. Unique route of BVDV entry may be in part responsible for the development of BVDV persistent infection.

alters these early immune parameters seems critical to gaining insight into the mechanism of the development of BVDV persistent infection.

The liver is an immunologically important organ with a particularly significant role in a developing fetus. The liver is the primary site of hematopoiesis and immune development at 100 days of gestation and studies have demonstrated that the liver, like a lymph node, is competent in primary activation of naive T cells (Bertolino *et al.*, 2002; Bowen *et al.*, 2004). Interestingly, the location where a lymphocyte is educated has tremendous implications on the outcome of its response to antigen presentation, because lymphocytes activated in the lymph node differentiate in distinctly different ways than those activated in the liver. Lymphocyte primary education in the hepatic tissues has been shown to provide a strong bias toward immune tolerance (Bowen *et al.*, 2004). Overall, this prevailing response of the liver ensures the immune system does not react inappropriately to digestive antigens (Knolle and Gerken, 2000). Early studies showed that intraportal application of antigens induced antigen-specific systemic tolerance (Cantor and Dumont, 1967; Triger *et al.*, 1973; Li and Tian, 2013), suggesting that the immune response established in the liver can pilot the response in other immunological tissues as well.

The structural architecture of the liver allows it a pivotal role in the balance of a host immune response (Knolle and Gerken,

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

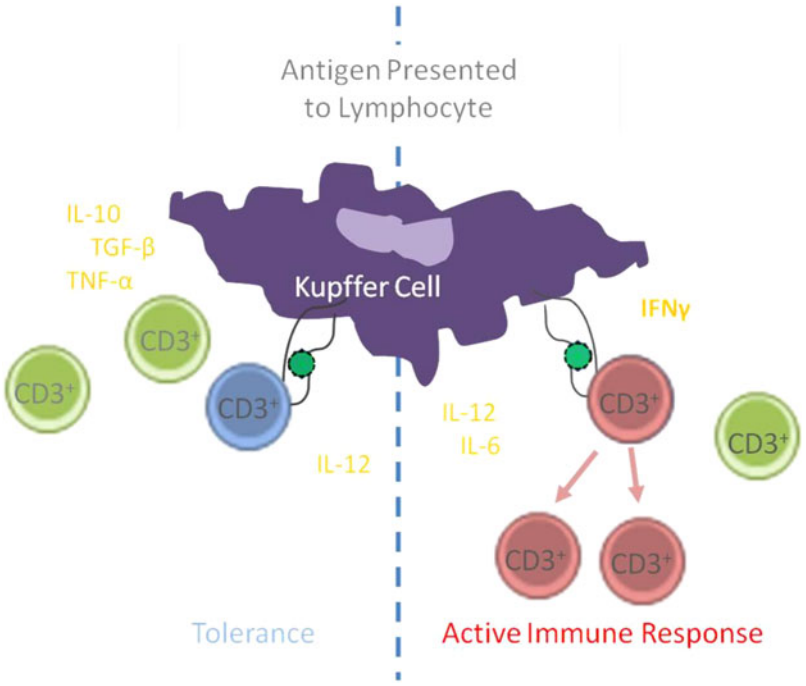

Fig. 10. Immunological switch that KCs make between tolerance and active immune response.

2000). Antigen-rich blood from the gastrointestinal tract in adults, and nutrient dense blood in the fetus, reaches the liver via the portal, or umbilical circulation, respectively, providing nutrients for digestion and storage (Fig. 9). The blood is then infused across a network of small diameter, high-pressure sinusoidal vessels. High resistance slows the flow significantly and allows maximal exposure to the non-parenchymal cells that comprise 20–40% of the liver. This population includes: endothelial cells, Kupffer cells (KCs), lymphocytes including B cells, and T-cell subsets: T regulatory, T helper, cytotoxic T cells, and DCs; gamma/delta cells, biliary cells and stellate cells.

KCs are recognized as the liver's resident macrophages and have many macrophage-type functions, including cytokine secretion, MHC presentation, and microbial killing by production of oxygen-derived free radicals (Decker, 1990, 1998; Laskin and Pendino, 1995). KCs exhibit both excitatory and tolerant measures in their interactions with leukocytes and are capable of secreting inflammatory mediators such as IL-1, IL-6, and TNF-α (Risalde *et al.*, 2011) as well as tolerogenic cytokines like IL-10 (Fig. 10) (Knolle *et al.*, 1995). The immune response of the KCs begins with the production of IL-12 and IL-18 in response to viral infection that activates local NK cells to produce IFN-γ. Following this activation, however, KCs release IL-10, which down regulates inflammatory mediators IL-6 and TNF-α and restores the more tolerant nature of the hepatic microenvironment (Tiegs and Lohse, 2010).

KCs also mediate local and peripheral immune suppression for specific antigens by expressing a negative co-stimulator for T-cell activation alongside the antigen they are presenting on either MHC I or MHC II (Knolle and Gerken, 2000). This negative co-stimulator is programmed cell death ligand 1 (PD-L1), which localizes circulating CD8+ and CD4+ T cells. CD8+ T cells in the liver begin to express cytotoxic lymphocyte antigen-4 (CTLA-4). CTLA-4 contributes to the suppression of immune response by inducing a T-regulatory cell profile (Tregs: CD4+CD25+FoxP3+) from naive and effector CD4+ T cells, converting them to Tregs, which induces tolerance (He *et al.*, 2009). All of these responses, including portal tolerance, are lost if KCs are depleted in hepatic tissues (Roland *et al.*, 1993; Rai *et al.*, 1997).

With Todd Hansen's group at Colorado State, as part of a larger study (Smirnova *et al.*, 2012, 2014), we analyzed fetal liver and isolated KCs from fetal calves whose dams were infected with BVDV at ~75 days of gestation followed by collection of fetuses at 82 and 89 days of gestation (7 and 14 days post-maternal infection). BVDV antigen was not detected in the fetuses at 82 days of gestation; however, BVDV was detected by immunohistochemistry in fetal liver at 89 days of gestation (14 days post-maternal infection) (Morarie *et al.*, 2012). Notably, within the fetal liver, only KCs were positive for BVDV antigen by IHC (Morarie *et al.*, 2012). The number of KCs was increased in the fetal liver at 89 days, and this is consistent with what was reported with acute BVDV liver infections (Risalde *et al.*, 2011). MHCI was expressed on 3X as many KCs in BVDV-infected calves (51 vs. 17%) as in control KCs from uninfected study fetuses at 89 days of gestation (Morarie *et al.*, 2012). MHC II was expressed on 2X as many BVDV-infected KCs (38 vs. 16%) as uninfected KCs from fetal calves (Morarie *et al.*, 2012). Cytokine analysis of supernatant collected from the BVDV-infected isolated KCs cultured for 24 h demonstrated lower levels of proinflammatory cytokines (TNF-α, IL-6, and IL-1β) than control KC supernatant (Morarie, 2012). There were also large aggregates of CD3+ lymphocytes noted in the fetal liver at gestational day 89 (Morarie, 2012). Taken together,

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

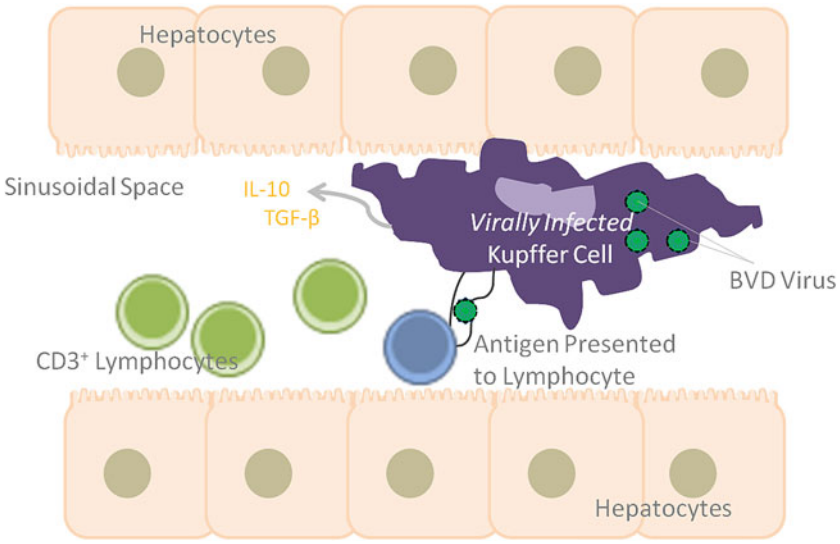

Fig. 11. Model for the induction of BVDV tolerance by KCs.

this provides one plausible model for induction of tolerance of BVDV that results in BVDV PI and identifies yet another new target for research and better understanding into the development of persistent BVDV infection in calves (Fig. 11). BVDV-infected KCs in the tolerance phenotype may play a critical role in the forthcoming immunosuppression and persistent infection (Fig. 11). Once more, further *in vitro* study and analysis will be essential to unravel this mechanism in greater detail.

Closing thoughts

BVDV continues to be a major problem for bovine health. With everything that we know about BVDV, there is still so much to learn. BVDV immune dysregulation can occur at many levels. New insight and perspectives have improved our understanding and shone light on several new areas to guide future research. The effect of BVDV infections on macrophages, NK cells, and neutrophils, and the mechanism of immune evasion in BVDV persistent infection, are targets that are critical to understanding BVDV pathogenesis. A greater insight in these innate immune responses will aid in the development of the next generation of BVDV prevention and control measures.

References

Bertolino P, McCaughan GW and Bowen DG (2002). Role of primary intrahepatic T-cell activation in the 'liver tolerance effect'. *Immunology and Cell Biology* **80**(1): 84–92. doi: 10.1046/j.0818-9641.2001.01048.x

Bolin SR, McClurkin AW and Coria MF (1985). Effects of bovine viral diarrhea virus on the percentages and absolute numbers of circulating 3 and T lymphocytes in cattle. *American Journal of Veterinary Research* **46**: 884–886.

Borca MV, Gudmundsdottir I, Fernández-Sainz IJ, Holinka LG and Risatti GR (2008). Patterns of cellular gene expression in swine macrophages infected with highly virulent classical swine fever virus strain Brescia. *Virus Research* **138**(1–2): 89–96. doi: 10.1016/j.virusres.2008.08.009.

Bowen DG, Zen M, Holz L, Davis T, McCaughan GW and Bertolino P (2004). The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *Journal of Clinical Investigation* **114**(5): 701–712. doi: 10.1172/JCI21593

Boysen P and Storset AK (2009). Bovine natural killer cells. *Veterinary Immunology and Immunopathology* **130**(3–4): 163–177. doi: 10.1016/j.vetimm.2009.02.017.

Boysen P, Olsen I, Berg I, Kulberg S, Johansen GM and Storset AK (2006). Bovine CD2-/NKp46+ cells are fully functional natural killer cells with a high activation status. *BMC Immunology* **7**: 10. doi: 10.1186/1471-2172-7-10.

Boysen P, Gunnes G, Pende D, Valheim M and Storset AK (2008). Natural killer cells in lymph nodes of healthy calves express CD16 and show both cytotoxic and cytokine-producing properties. *Developmental and Comparative Immunology* **32**(7): 773–783. doi: 10.1016/j.dci.2007.11.006.

Brackenbury LS, Carr BV and Charleston B (2003). Aspects of the innate and adaptive immune responses to acute infections with BVDV. *Veterinary Microbiology* **96**(4): 337–344.

Brock KV (2003). The persistence of bovine viral diarrhea virus. *Biologicals* **31**(2): 133–135.

Cantor HM and Dumont AE (1967). Hepatic suppression of sensitization to antigen absorbed into the portal system. *Nature* **215**(5102): 744–745.

Chase CCL (2013). The impact of BVDV infection on adaptive immunity. *Biologicals* **41**(1): 52–60. doi: 10.1016/j.biologicals.2012.09.009

Chase CCL, Elmowalid G and Yousif AAA (2004). The immune response to bovine viral diarrhea virus: a constantly changing picture. *Veterinary Clinics of North America: Food Animal Practice* **20** (1): 95–114. doi: 10.1016/j.cvfa.2003.11.004

Choi C, Hwang K-K and Chae C (2004). Classical swine fever virus induces tumor necrosis factor-alpha and lymphocyte apoptosis. *Archives of Virology* **149**(5): 875–889. doi: 10.1007/s00705-003-0275-6

Darweesh M (2013). Using genetic and pathogenesis studies to develop antiviral and BVDV vaccine targets. PhD Dissertation 2013, South Dakota State University, Brookings, SD.

Decker K (1990). Biologically active products of stimulated liver macrophages (Kupffer cells). *European Journal of Biochemistry* **192**(2): 245–261. doi: 10.1111/j.1432-1033.1990.tb19222.x

https://doi.org/10.1017/S1466252315000110 Published online by Cambridge University Press

Decker K (1998). The response of liver macrophages to inflammatory stimulation. *Keio Journal of Medicine* **47**(1): 1–9.

Dobromylskyj M and Ellis S (2007). Complexity in cattle KIR genes: transcription and genome analysis. *Immunogenetics* **59**(6): 463–472. doi: 10.1007/s00251-007-0215-9

Finlay BB and Hancock REW (2004). Can innate immunity be enhanced to treat microbial infections? *Nature Reviews Microbiology* **2**(6): 497–504. doi: 10.1038/nrmicro908

Endsley JJ, Furrer JL, Endsley MA, McIntosh MA, Maue AC, Waters WR, Lee DR and Estes DM (2004). Characterization of bovine homologues of granulysin and NK-lysin. *Journal of Immunology* (Baltimore, Md.: 1950) **173**(4): 2607–2614.

Endsley JJ, Endsley MA and Estes DM (2006). Bovine natural killer cells acquire cytotoxic/effector activity following activation with IL-12/15 and reduce *Mycobacterium bovis* BCG in infected macrophages. *Journal of Leukocyte Biology* **79**(1): 71–79. doi: 10.1189/jlb.0505239

Gabriel C, Her Z and Ng LFP (2013). Neutrophils: neglected players in viral diseases. *DNA and Cell Biology* **32**(12), 665–675. doi: 10.1089/dna.2013.2211

Gånheim C, Johannisson A, Ohagen P and Persson Waller K (2005). Changes in peripheral blood leucocyte counts and subpopulations after experimental infection with BVDV and/or *Mannheimia haemolytica*. *Journal of Veterinary Medicine B, Infectious Diseases and Veterinary Public Health* **52**(9): 380–385. doi: 10.1111/j.1439-0450.2005.00882.x

Graham EM, Thom ML, Howard CJ, Boysen P, Storset AK, Sopp P and Hope JC (2009). Natural killer cell number and phenotype in bovine peripheral blood is influenced by age. *Veterinary Immunology and Immunopathology* **132**(2–4): 101–108. doi: 10.1016/j.vetimm.2009.05.002

He F, Chen Z, Xu S, Cai M, Wu M, Li H and Chen X (2009). Increased CD4+CD25+Foxp3+ regulatory T cells in tolerance induced by portal venous injection. *Surgery* **145**(6): 663–674. doi: 10.1016/j.surg.2009.01.016

Kärre K, Ljunggren HG, Piontek G and Kiessling R (1986). Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* **319**(6055): 675–678. doi: 10.1038/319675a0

Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Büschenfelde KH and Gerken G (1995). Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. *Journal of Hepatology* **22**(2): 226–229.

Knolle PA and Gerken G (2000). Local control of the immune response in the liver. *Immunological Reviews* **174**: 21–34.

Laskin DL and Pendino KJ (1995). Macrophages and inflammatory mediators in tissue injury. *Annual Review of Pharmacology and Toxicology* **35**: 655–677. doi: 10.1146/annurev.pa.35.040195.003255

Ley K, Laudanna C, Cybulsky MI and Nourshargh S (2007). Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nature Reviews Immunology* **7**(9): 678–689. doi: 10.1038/nri2156

Li F and Tian Z (2013). The liver works as a school to educate regulatory immune cells. *Cellular and Molecular Immunology* **10**(4): 292–302. doi: 10.1038/cmi.2013.7

Liebler-Tenorio EM, Ridpath JF and Neill JD (2002). Distribution of viral antigen and development of lesions after experimental infection with highly virulent bovine viral diarrhea virus type 2 in calves. *American Journal of Veterinary Research* **63**(11): 1575–1584. doi: 10.2460/ajvr.2002.63.1575

Liebler-Tenorio EM, Ridpath JF and Neill JD (2003). Distribution of viral antigen and development of lesions after experimental infection of calves with a BVDV 2 strain of low virulence. *Journal of Veterinary Diagnostic Investigation* **15**(3): 221–232.

Ljunggren HG and Kärre K (1990). In search of the 'missing self': MHC molecules and NK cell recognition. *Immunology Today* **11**(7): 237–44.

Lund H, Boysen P, Dean GA, Davis WC, Park KT and Storset AK (2012). Interleukin-15 activated bovine natural killer cells express CD69 and produce interferon-γ. *Veterinary Immunology and Immunopathology* **150**(1–2): 79–89. doi: 10.1016/j.vetimm.2012.08.011.

Lund H, Boysen P, Hope JC, Sjurseth SK and Storset AK (2013). Natural killer cells in afferent lymph express an activated phenotype and readily produce IFN-γ. *Frontiers in Immunology* **4**: Article 392: 1–8. doi: 10.3389/fimmu.2013.00395.

Menge C, Neufeld B, Hirt W, Schmeer N, Bauerfeind R, Baljer G and Wieler LH (1998). Compensation of preliminary blood phagocyte immaturity in the newborn calf. *Veterinary Immunology and Immunopathology* **62**(4): 309–321.

Molina V, Risalde MA, Sánchez-Cordón PJ, Romero-Palomo F, Pedrera M, Garfia B and Gómez-Villamandos JC (2014). Cell-mediated immune response during experimental acute infection with bovine viral diarrhoea virus: evaluation of blood parameters. *Transboundary and Emerging Diseases* **61**(1): 44–59. doi: 10.1111/tbed.12002.

Morarie S (2012). Unraveling the biology of bovine viral diarrhea virus (BVDV) persistent infections: integrating field and laboratory studies. PhD Dissertation 2012, South Dakota State University, Brookings, SD.

Morarie S, Braun L, Smirnova N, Hansen T and Chase C (2012). Liver tolerance may serve a role in the development of BVDV persistent infection. Abstract W33-9. 31st Annual Meeting of the American Society of Virology, Madison, WI, 21–25 July 2012.

Moretta A, Bottino C, Vitale M, Pende D, Cantoni C, Mingari MC, Biassoni R and Moretta L (2001). Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annual Review of Immunology* **19**: 197–223. doi: 10.1146/annurev.immunol.19.1.197.

Paape MJ, Bannerman DD, Zhao X and Lee J-W (2003). The bovine neutrophil: structure and function in blood and milk. *Veterinary Research* **34**(5): 597–627. doi: 10.1051/vetres:2003024

Palomares RA, Brock KV and Walz PH (2014). Differential expression of pro-inflammatory and anti-inflammatory cytokines during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves. *Veterinary Immunology and Immunopathology* **157**(3–4): 149–154. doi: 10.1016/j.vetimm.2013.12.002.

Peterhans E and Schweizer M (2010). Pestiviruses: how to outmaneuver your hosts. *Veterinary Microbiology* **142**(1–2): 18–25. doi: 10.1016/j.vetmic.2009.09.038.

Peterhans E and Schweizer M (2013). BVDV: a pestivirus inducing tolerance of the innate immune response. *Biologicals*, **41**(1): 39–51. doi: 10.1016/j.biologicals.2012.07.006.

Peterhans E, Jungi TW and Schweizer M (2003). BVDV and innate immunity. *Biologicals* **31**(2): 107–112. doi: 10.1016/S1045-1056(03)00024-1

Rai RM, Loffreda S, Karp CL, Yang SQ, Lin HZ and Diehl AM (1997). Kupffer cell depletion abolishes induction of interleukin-10 and permits sustained overexpression of tumor necrosis factor alpha messenger RNA in the regenerating rat liver. *Hepatology* **25**(4): 889–895. doi: 10.1002/hep.510250417

Rajput MK, Darweesh MF, Park K, Braun LJ, Mwangi W, Young AJ and Chase CC (2014). The effect of bovine viral diarrhea virus (BVDV) strains on bovine monocyte-derived dendritic cells (Mo-DC) phenotype and capacity to produce BVDV. *Virology Journal* **11**(1): 44. doi: 10.1186/1743-422X-11-44

Rebhun WC, French TW, Perdrizet JA, Dubovi EJ, Dill SG and Karcher LF (1989). Thrombocytopenia associated with acute bovine virus diarrhea infection in cattle. *Journal of Veterinary Internal Medicine* **3**(1): 42–46.

Ridpath JF, Bendfeldt S, Neill JD and Liebler-Tenorio E (2006). Lymphocytopathogenic activity *in vitro* correlates with high virulence *in vivo* for BVDV type 2 strains: criteria for a third biotype of BVDV. *Virus Research* **118**(1–2): 62–69. doi: 10.1016/j.virusres.2005.11.014

Risalde MA, Gómez-Villamandos JC, Pedrera M, Molina V, Cerón JJ, Martínez-Subiela S and Sánchez-Cordón PJ (2011). Hepatic immune response in calves during acute subclinical infection with

bovine viral diarrhoea virus type 1. *Veterinary Journal* (London, England: 1997) **190**(2): e110–e116. doi: 10.1016/j.tvjl.2011.03.003

Risalde MA, Molina V, Sánchez-Cordón PJ, Romero-Palomo F, Pedrera M and Gómez-Villamandos JC (2014). Effects of preinfection with bovine viral diarrhea virus on immune cells from the lungs of calves inoculated with bovine herpesvirus 1.1. *Veterinary Pathology*. doi: 10.1177/0300985814551579

Roland CR, Mangino MJ, Duffy BF and Flye MW (1993). Lymphocyte suppression by Kupffer cells prevents portal venous tolerance induction: a study of macrophage function after intravenous gadolinium. *Transplantation* **55**(5): 1151–1158.

Roth JA and Kaeberle ML (1983). Suppression of neutrophil and lymphocyte function induced by a vaccinal strain of bovine viral diarrhea virus with and without the administration of ACTH. *American Journal of Veterinary Research* **44**(12): 2366–2372.

Roth JA, Kaeberle ML and Griffith RW (1981). Effects of bovine viral diarrhea virus infection on bovine polymorphonuclear leukocyte function. *American Journal of Veterinary Research* **42**(2): 244–250.

Sánchez-Cordón PJ, NUNez A, Salguero FJ, Pedrera M, Fernández de Marco M and Gómez-Villamandos JC (2005). Lymphocyte apoptosis and thrombocytopenia in spleen during classical swine fever: role of macrophages and cytokines. *Veterinary Pathology* **42**(4): 477–488. doi: 10.1354/vp.42-4-477

Schultz RD, Dunne HW and Heist CE (1973). Ontogeny of the bovine immune response. *Infection and Immunity*, **7**(6): 981–991.

Shuster DE, Kehrli ME, Ackermann MR and Gilbert RO (1992). Identification and prevalence of a genetic defect that causes leukocyte adhesion deficiency in Holstein cattle. *Proceedings of the National Academy of Sciences of the United States of America* **89**(19): 9225–9229.

Sladek Z and Rysanek D (2006). The role of CD14 during resolution of experimentally induced *Staphylococcus aureus* and *Streptococcus uberis* mastitis. *Comparative Immunology, Microbiology and Infectious Diseases* **29**(4): 243–262. doi: 10.1016/j.cimid.2006.06.006

Smirnova NP, Webb BT, Bielefeldt-Ohmann H, Van Campen H, Antoniazzi AQ, Morarie SE and Hansen TR (2012). Development of fetal and placental innate immune responses during establishment of persistent infection with bovine viral diarrhea virus. *Virus Research* **167**(2): 329–36. doi: 10.1016/j.virusres.2012.05.018

Smirnova NP, Webb BT, McGill JL, Schaut RG, Bielefeldt-Ohmann H, Van Campen H, Sacco RE and Hansen TR (2014). Induction of interferon-gamma and downstream pathways during establishment of fetal persistent infection with bovine viral diarrhea virus. *Virus Research* **183**: 95–106. doi: 10.1016 /j.virusres.2014.02.002

Sohn EJ, Paape MJ, Bannerman DD, Connor EE, Fetterer RH and Peters RR (2007). Shedding of sCD14 by bovine neutrophils following activation with bacterial lipopolysaccharide results in down-regulation of IL-8. *Veterinary Research* **38**(1): 95–108. doi: 10.1051/vetres:2006052

Storset AK, Kulberg S, Berg I, Boysen P, Hope JC and Dissen E (2004). NKp46 defines a subset of bovine leukocytes with natural killer cell characteristics. *European Journal of Immunology* **34**(3): 669–676. doi: 10.1002/eji.200324504

Thakur N, Braun L and Chase CCL (2014). Effect of bovine viral diarrhea virus (BVDV) infection on neutrophil survival and surface marker expression. Abstract 71P. 95th Annual Meeting of the Conference for Research Workers in Animal Diseases, Chicago IL, 7–9 December, 2014.

Tiegs G and Lohse AW (2010). Immune tolerance: what is unique about the liver. *Journal of Autoimmunity* **34**(1): 1–6. Review. PubMed PMID: 19717280.

Triger DR, Cynamon MH and Wright R (1973). Studies on hepatic uptake of antigen: I. Comparison of inferior vena cava and portal vein routes of immunization. *Immunology* **25**(6): 941.

Vivier E, Tomasello E, Baratin M, Walzer T and Ugolini S (2008). Functions of natural killer cells. *Nature Immunology* **9**(5): 503–510. doi: 10.1038/ni1582

Zeldin DC, Eggleston P, Chapman M, Piedimonte G, Renz H and Peden D (2006). How exposures to biologics influence the induction and incidence of asthma. *Environmental Health Perspectives*, 620–626. doi: 10.1289/ehp.8379