1 **$\gamma\delta$ T cells and the Immune Response to Respiratory Syncytial Virus Infection**

2

3 Jodi L. McGill¹ and Randy E. Sacco²

4

5 ¹Department of Diagnostic Medicine and Pathobiology, Kansas State University, 1800
6 Denison Ave., Manhattan, Kansas 66503

7 ²Ruminant Diseases and Immunology Research Unit, National Animal Disease Center,
8 Agricultural Research Service, United States Department of Agriculture, 1920 Dayton
9 Ave., Ames, Iowa 50010

10

11 **Corresponding Author**

12 Jodi L. McGill

13 Telephone: 785-532-4602

14 Fax: 785-532-4039

15 Email: jlmcgill@vet.k-state.edu

16

17 **Keywords:** bovine respiratory syncytial virus, bovine respiratory disease, human

18 respiratory syncytial virus, gamma delta T cells, immune response

19

Abstract

$\gamma\delta$ T cells are a subset of nonconventional T cells that play a critical role in bridging the innate and adaptive arms of the immune system. $\gamma\delta$ T cells are particularly abundant in ruminant species and may constitute up to 60% of the circulating lymphocyte pool in young cattle. The frequency of circulating $\gamma\delta$ T cells is highest in neonatal calves and declines as the animal ages, suggesting these cells may be particularly important in the immune system of the very young. Bovine respiratory syncytial virus (BRSV) is a significant cause of respiratory infection in calves, and is most severe in animals under one year of age. BRSV is also a significant factor in the development of bovine respiratory disease complex (BRDC), the leading cause of morbidity and mortality in feedlot cattle. Human respiratory syncytial virus (RSV) is closely related to BRSV and a leading cause of lower respiratory tract infection in infants and children worldwide. BRSV infection in calves shares striking similarities with RSV infection in human infants. To date, there have been few studies defining the role of $\gamma\delta$ T cells in the immune response to BRSV or RSV infection in animals or humans, respectively. However, emerging evidence suggests that $\gamma\delta$ T cells may play a critical role in the early recognition of infection and in shaping the development of the adaptive immune response through inflammatory chemokine and cytokine production. Further, while it is clear that $\gamma\delta$ T cells accumulate in the lungs during BRSV and RSV infection, their role in protection vs. immunopathology remains unclear. This review will summarize what is currently known about the role of $\gamma\delta$ T cells in the immune response to BRSV and BRDC in cattle, and where appropriate, draw parallels to the role of $\gamma\delta$ T cells in the human response to RSV infection.

Introduction

$\gamma\delta$ T cells are present in all vertebrate species examined so far and are deemed critical for immune surveillance and protection; however, their role in the immune response to infection remains poorly defined. While present in low numbers in species such as mice and humans, $\gamma\delta$ T cells are particularly abundant in ruminants, making cattle an excellent animal model to study the functions of these cells. $\gamma\delta$ T cells can have a significant effect on the nature of the inflammatory milieu at the site of infection, particularly in mucosal sites such as the lung and gastrointestinal tract; yet, their role in the response to acute respiratory infection has only recently been examined. Bovine respiratory syncytial virus (BRSV) causes acute respiratory viral infection in calves; with most severe symptoms observed in animals less than six months of age. BRSV infection is also a factor contributing to the development of bovine respiratory disease (BRD) complex, a multifactorial disease condition that is associated with significant morbidity and mortality in weaned dairy calves and feedlot cattle. Here, we will discuss the recently emerging role for bovine $\gamma\delta$ T cells in the immune response to BRSV and how these results have implications for the development of BRDC. Further, we will review how the $\gamma\delta$ T cell response during BRSV compares to the response in rodents and humans infected with the closely related human respiratory syncytial virus (HRSV).

BRSV and HRSV infection

BRSV is a negative sense single-stranded RNA virus that is a leading cause of severe acute lower respiratory tract infection in calves. BRSV infection is most severe in calves less than six months of age, but infects a majority of animals by 1 year of age. The

worldwide frequency of exposure to BRSV infection in beef and dairy herds reportedly exceeds 50% (Gershwin, 2007) and may be as high as 70-100%, depending upon geographic location and animal density (Ohlson et al., 2013). Following outbreaks of BRD, rates of seroconversion to BRSV have been documented as high as 45% (Brodersen, 2010). In the instance of enzootic calf pneumonia, BRSV is a documented cause in as many as 60% of the cases (Meyer et al., 2008).

BRSV is genetically and antigenically closely related to human respiratory syncytial virus (HRSV), a leading cause of severe lower respiratory tract infection in infants and young children worldwide. HRSV infection accounts for up to 70% of hospitalized bronchiolitis cases in industrialized countries (Hall, 2001; Henrickson et al., 2004), and globally, there are an estimated 33 million new episodes of HRSV-associated disease in children under 5 years of age with more than 100,000 resultant deaths (Nair et al., 2010). Severe RSV infection has been linked with the development and exacerbation of recurrent wheezing and asthma (Silvestri et al., 2004; Stein et al., 1999), and is a predisposing factor to the development of otitis media (Hall et al., 1991; Uhari et al., 1995). BRSV infection in cattle presents the opportunity to study a naturally susceptible host-pathogen interaction that is strikingly similar to RSV infection in humans. The similarities between HRSV infection in human infants and BRSV infection in calves, and a detailed description of the disease pathology and ensuing innate and adaptive immune responses have been the subject of several recent, comprehensive reviews (Bem et al., 2011; Gershwin, 2007; Guzman and Taylor, 2015; Sacco et al., 2012; Sacco et al., 2014).

Innate recognition of BRSV by $\gamma\delta$ T cell subsets

89 $\gamma\delta$ T cells comprise a significant portion of the immune compartment in mucosal
90 tissues such as those lining the lung and upper respiratory tract. Their accumulation in
91 these locations suggests that $\gamma\delta$ T cells likely play a role in the early pathogen detection.
92 Critical for activation of the innate immune system, pattern recognition receptors (PRR)
93 bind conserved molecular signatures termed pathogen associated molecular patterns
94 (PAMPs)(Janeway, 1989). Among PRR, the Toll Like Receptor (TLR) and NOD Like
95 Receptor (NLR) families are particularly important. As bridges between the innate and
96 adaptive immune systems, human, murine and bovine $\gamma\delta$ T cells express a number of
97 PRR, including TLR 2, 3, 4 and 7; and the NLR, Nod1 and Nod2(Jutila et al., 2008;
98 Wesch et al., 2011). $\gamma\delta$ T cells respond to direct stimulation through their PRR in the
99 absence of additional T cell receptor (TCR) signaling, suggesting that they have the
100 capacity for innate recognition of early, acute viral infection.

101 Innate recognition of BRSV and HRSV can be mediated by the endosomal TLR
102 receptors for double-stranded and single-stranded RNA, TLR3 and TLR7,
103 respectively(Klein Klouwenberg et al., 2009). Human $\gamma\delta$ T cells can respond directly to
104 TLR3 and TLR7 ligands; and mitogen or TCR-induced production of IFN γ by human
105 adult and neonatal $\gamma\delta$ T cells is significantly enhanced by co-stimulation through TLR3
106 and 7(Gibbons et al., 2009; Wesch et al., 2011). We recently demonstrated that bovine $\gamma\delta$
107 T cell subsets from neonatal calves also respond directly to stimulation through TLR3
108 and TLR7(McGill et al., 2013). Stimulation of purified $\gamma\delta$ T cell subsets with the TLR3
109 agonist Poly(I:C), or the TLR7 agonist Imiquimod, induced significant production of the
110 chemokines CCL2 and CCL3. This result suggests that bovine $\gamma\delta$ T cells have the
111 capacity for innate recognition of BRSV. Indeed, $\gamma\delta$ T cells from naïve, neonatal calves

do respond to direct *in vitro* infection with BRSV and the response parallels that observed following TLR agonist stimulation(McGill et al., 2013). Interestingly, we observed that while $\gamma\delta$ T cell subsets from naïve calves produce inflammatory chemokines in response to TLR stimulation or *in vitro* BRSV infection, they do not undergo extensive proliferation or produce IFN γ . Instead, we only observed IFN γ production when $\gamma\delta$ T cells were activated through their TCR by plate-bound CD3, or when $\gamma\delta$ T cells were purified from BRSV-infected calves and restimulated *in vitro*. These results suggest an important differential regulation between innate $\gamma\delta$ T cell functions, such as chemokine production, and adaptive $\gamma\delta$ T cell functions such as clonal expansion and cytokine production. Others examining the response of $\gamma\delta$ T cells to the Nod2 agonist muramyl dipeptide(Kerns et al., 2009); the TLR2 agonist, Pam3CSK or TLR4 agonist, LPS have confirmed this result(Hedges et al., 2005). Hedges *et al.* observed that TLR2 or 4-stimulatedbovine $\gamma\delta$ T cells exhibit only minor increases in gene transcription for IFN γ and TNF α , but demonstrate significant increases in expression of the genes encoding for inflammatory chemokines such as CXCL8, CCL3, CCL4 and GM-CSF(Hedges et al., 2005). The authors have termed this response “antigen-independent priming” and proposed that it enables more rapid and efficient responses to secondary signals such as encounter with the appropriate antigen in the context of an antigen-presenting cell(Jutila et al., 2008; Kerns et al., 2009). To date, the role of antigen-independent priming of bovine $\gamma\delta$ T cells during *in vivo*BRSV infection or other disease setting remains unclear; however, these results suggest that $\gamma\delta$ T cells may play an important role in viral recognition and in shaping the inflammatory environment in the lungs during early viral infection.

WC1 and $\gamma\delta$ T cells

Bovine $\gamma\delta$ T cells can be divided into phenotypic and functional subsets based upon their expression of CD8 or of Workshop Cluster 1 (WC1) (Machugh et al., 1997). CD8⁺ $\gamma\delta$ T cells are the predominant subset in the mucosal tissues, and are usually WC1^{neg}, CD2⁺; while CD8^{neg} $\gamma\delta$ T cells predominate in the blood and are WC1⁺CD2^{neg} (Hedges et al., 2003; Machugh et al., 1997; Wijngaard et al., 1994; Wilson et al., 1999). WC1, also known as T19, is a family of transmembrane glycoproteins in the scavenger receptor cysteine rich (SRCR) superfamily. WC1 are encoded by 13 individual genes, and are closely related to CD163 in humans (Baldwin and Telfer, 2015). Extensive work by investigators at the University of Massachusetts has identified a role for WC1 as both a PRR and a costimulatory molecule on bovine $\gamma\delta$ T cells, playing a role in recognition of microbial pathogens and in signaling cells for activation. Our current understanding of the role of WC1 on bovine $\gamma\delta$ T cells has been summarized in a recent, excellent review (Baldwin and Telfer, 2015).

To date, most studies in cattle have focused on total $\gamma\delta$ T cells or on the WC1⁺/CD8^{neg} $\gamma\delta$ T cell subset as a whole. However, the WC1⁺/CD8^{neg} population can be further serologically divided into WC1.1⁺, WC1.2⁺ and WC1.3⁺ subsets. WC1.1 and WC1.2 are predominantly expressed by distinct $\gamma\delta$ T cell populations, while the WC1.3⁺ subset is contained within the WC1.1⁺ cells (Rogers et al., 2006; Wijngaard et al., 1994). WC1.1⁺ $\gamma\delta$ T cells are robust producers of IFN γ and have been shown to respond specifically to vaccination or infection with *Leptospira* (Rogers et al., 2005a; Rogers et al., 2005b; Wang et al., 2011). WC1.2⁺ $\gamma\delta$ T cells respond poorly to mitogen

stimulation(Rogers et al., 2005a; Rogers et al., 2005b), but produce IFN γ in specific response to the rickettsial pathogen *Anaplasma marginale*(Lahmers et al., 2005). WC1.2 $^{+}$ $\gamma\delta$ T cells were also initially reported to play a regulatory role in the naive animal(Hoek et al., 2009; McGill et al., 2013), although a recent study by Guzman *et al.* suggests that all three, WC1 neg /CD8 $^{+}$, WC1.1 $^{+}$ and WC1.2 $^{+}$ $\gamma\delta$ T cell subsets in the bovine may have this capacity(Guzman et al., 2014). Interestingly, we recently demonstrated that both WC1.1 $^{+}$ and WC1.2 $^{+}$ $\gamma\delta$ T cell subsets respond to infection with virulent *Mycobacterium bovis*, although the role of WC1 in this infection setting remains to be determined(McGill et al., 2014).

In the context of BRSV, the role of individual WC1-expressing $\gamma\delta$ T cell subsets remains largely undefined. However, we showed that WC1 neg , WC1.1 $^{+}$ and WC1.2 $^{+}$ $\gamma\delta$ T cell subsets respond differently to stimulation through TLR3 or TLR7, as well as to *in vitro* and *in vivo* infection with BRSV(McGill et al., 2013). Following TLR3 or TLR7 stimulation, WC1.1 $^{+}$ $\gamma\delta$ T cells produce CCL3 and GM-CSF, while WC1.2 $^{+}$ $\gamma\delta$ T cells produce IL-10 and TGF- β . Following *in vitro* BRSV infection of purified $\gamma\delta$ T cells from healthy calves, WC1 neg and WC1.1 $^{+}$ $\gamma\delta$ T cells, but not WC1.2 $^{+}$ cells produce CCL3; however, and all three subsets produce CCL2. IFN γ production was not detectable by any subset isolated from healthy calves. However, by day 10 following *in vivo* BRSV infection, WC1.1 $^{+}$ $\gamma\delta$ T cells isolated from peripheral blood produced IFN γ , while WC1.2 $^{+}$ cells primarily produced IL-10. Together, these results suggest critical differences in the function of $\gamma\delta$ T cell subsets in the response to BRSV infection in the calf; however, the role of WC1 in these responses is unknown.

$\gamma\delta$ T cells respond to *in vivo* RSV infection in mice, men and cattle

Emerging evidence suggests that $\gamma\delta$ T cells play a role *in vivo* during RSV infection in humans, mice and cattle. Children with severe RSV bronchiolitis exhibit altered frequencies of $\gamma\delta$ T cells circulating in peripheral blood(De Weerd et al., 1998). In another study, $\gamma\delta$ T cells isolated from the peripheral blood of infants hospitalized with severe RSV infection are inhibited in their ability to produce IFN γ upon secondary stimulation(Aoyagi et al., 2003). $\gamma\delta$ T cells are recruited to the lungs of mice infected with RSV(Dodd et al., 2009; Huang et al., 2015). Mice depleted of $\gamma\delta$ T cells prior to RSV infection display increased viral titers, but a reduction in lung inflammation and overall disease severity, suggesting that $\gamma\delta$ T cells may participate in viral clearance, but also contribute to immunopathology in the lungs during infection(Dodd et al., 2009).

A recent paper by Huang *et al.* suggests that $\gamma\delta$ T cells in the lungs may play a critical role in the response to RSV through production of IL-17(Huang et al., 2015). $\gamma\delta$ T cells from adult mice produce IL-17, while $\gamma\delta$ T cells from neonatal mice are impaired in this ability, likely through a defect in inflammasome activation. The authors propose this as one mechanism to explain the increased susceptibility of neonatal animals to infection with RSV(Huang et al., 2015). It is important to note that the role of IL-17 during RSV infection remains controversial. In both children and small animal models, IL-17 responses can be attributed to both protection and immunopathology in the lungs(Bystrom et al., 2013). It is likely that there is a critical threshold whereby low levels of IL-17 in the lungs can be beneficial during RSV infection, but more significant production can be damaging to the host.

The suggestion by Huang *et al.* that IL-17 produced by $\gamma\delta$ T cells may be playing a role during RSV infection is particularly intriguing. $\gamma\delta$ T cell derived IL-17 has been reported as a critical immune component in a number of other viral infection models as well, including West Nile virus (Welte *et al.*, 2011), herpes simplex virus (Kim *et al.*, 2012; Suryawanshi *et al.*, 2011) and hepatitis virus (Hou *et al.*, 2013). Mice infected with influenza virus are severely predisposed to secondary bacterial infection with *Streptococcus pneumoniae*. A recent paper by Li *et al.* suggests that one factor contributing to this increased susceptibility is impaired $\gamma\delta$ T cell production of IL-17 in the lungs following viral infection (Li *et al.*, 2012). This report may have serious implications if extrapolated to the context of BRD in calves. BRD complex affects stressed or immunocompromised cattle, whereby a primary viral infection with agents such as BRSV, predisposes the host to secondary, and frequently fatal, bacterial pneumonia (Srikumaran *et al.*, 2007). It is currently unknown if $\gamma\delta$ T cells are expressing IL-17 in the lungs of calves with primary BRSV infection; however, interference with this critical anti-bacterial cytokine pathway could explain one mechanism that leads to increased susceptibility of calves to severe BRD. The expression of IL-17 by bovine $\gamma\delta$ T cells in the lungs during BRSV infection, and during secondary bacterial pneumonia, is a subject of current investigation in our laboratory.

The frequency of $\gamma\delta$ T cells circulating in peripheral blood are not altered following BRSV infection in the calf (McInnes *et al.*, 1999); however, $\gamma\delta$ T cells are recruited to the lungs of infected animals and can be identified in areas of pneumonic lung tissue on day 7 post infection. As seen in Figure 1A, low numbers of $\gamma\delta$ T cells (red) are present in the lungs of uninfected control calves, while significantly increased

numbers can be found in the lungs of calves that have been infected with BRSV for seven days (Figure 1B, red). Depletion of $\gamma\delta$ T cells from gnotobiotic calves prior to BRSV infection reportedly does not affect clinical disease course, tissue pathology or viral titers (Taylor et al., 1995; Thomas et al., 1996), although animals depleted of $\gamma\delta$ T cells do exhibit a significant increase in titers of IgM and IgA in lung washes compared to non-depleted control calves (Taylor et al., 1995). It is important to note, that although a seminal study for defining the immune response to BRSV infection in calves, the results from these experiments must be interpreted with caution. Experimental numbers were low and the calves in the study were treated with the anti-inflammatory drug flunixin meglumine. Additionally, neither control nor T cell-depleted calves exhibited signs of clinical disease following BRSV infection.

We have demonstrated that $\gamma\delta$ T cells produce inflammatory chemokines when exposed to BRSV *in vitro* (McGill et al., 2013); however, their capacity for chemokine production *in vivo* remains unknown. To date, investigation of functional immune responses in bovine tissues, at the site of *in vivo* infection or disease has remained challenging due to difficulties in liberating cells from these locations and a more limited availability of cytokine/chemokine-specific bovine reagents. Therefore, in order to determine the role of $\gamma\delta$ T cells present at the site of BRSV infection, our laboratory has turned to the use of RNAScope, an assay developed by Advanced Cell Diagnostics that enables the detection of mRNA expression in fixed or frozen tissue sections. The method is similar to *in situ* hybridization, but displays superior specificity and resolution compared to previous protocols, and can be used to interrogate expression of up to three target genes. With this approach, we have initiated preliminary studies examining

chemokine production by $\gamma\delta$ T cells at the site of BRSV infection. Interestingly, our initial results suggest that neither $\gamma\delta$ T cells present in uninfected control lungs (Figure 1A) nor those present in BRSV-infected pneumonic lungs (Figure 1B) express significant mRNA for the IFN γ -induced cytokine CXCL10. Further, in contrast to our *in vitro* results (McGill et al., 2013), $\gamma\delta$ T cells in pneumonic lungs do not express mRNA for CCL2 (Figure 1C) or CCL3 (Figure 1D) at this time point following infection. It is important to note that production of inflammatory chemokines such as CCL2 likely occurs at early timepoints post infection, as has been observed in murine models (Culley et al., 2006). By initiating our studies at day 7-post infection, we may have missed a critical window for peak $\gamma\delta$ T cell chemokine production. Our future studies will include a more extensive kinetic analysis of $\gamma\delta$ T cell chemokine production in the lungs of BRSV infected calves.

The impact of $\gamma\delta$ T cells on secondary infections and susceptibility to chronic airway diseases

Primary HRSV and BRSV infection causes significant morbidity and mortality in children and calves, respectively. However, the changes that can occur in the lungs and in the immune system following acute RSV infection can have long-term impact on the health of the host. Primary BRSV infection plays a significant role in BRD, predisposing the host to secondary bacterial pneumonia and the development of exacerbated lung disease. We observe the development of bronchiolitis obliterans in the lungs of calves that are recovering from severe BRSV infection (Sacco et al., 2014). Also known as organizing bronchiolitis, this condition is caused by defective healing of the bronchiolar epithelium characterized by fibrous polyps extending into the airway lumen that result in

a permanent reduction in airflow(Sacco et al., 2014). One report has observed changes in airway resistance in calves for as long as 30 days post BRSV infection(LeBlanc et al., 1991). These changes, in addition to increasing susceptibility to BRD, no doubt contribute to an overall reduction in productivity in the animal.

In children, RSV infection is thought to predispose to asthma and allergic respiratory disease. The immune response to RSV is skewed towards a T helper 2 phenotype, and the production of IL-4, IL-5 and IL-13 is increased in the lungs for several days following RSV infection(Kalina and Gershwin, 2004). In experimental models, including mice and cattle, sensitization to an allergen followed by RSV infection results in severe airway hyperresponsiveness and production of high levels of IgE.

$\gamma\delta$ T cells are associated with allergic inflammation. They are increased in the respiratory mucosa of patients with allergic rhinitis(Pawankar et al., 1995; Pawankar et al., 1996), and in the bronchoalveolar fluids of asthma patients (Krug et al., 2001). In the case of RSV-induced allergic responses, $\gamma\delta$ T cells may play a proinflammatory role in the lungs. Zhang *et al.* recently reported that adoptive transfer of $\gamma\delta$ T cells from ovalbumin-sensitized mice, to mice that were subsequently challenged with ovalbumin and infected with RSV resulted in significantly increased allergic inflammation in the lungs compared to mice that did not receive $\gamma\delta$ T cells, or mice that received $\gamma\delta$ T cells from non-sensitized mice(Zhang et al., 2013). The $\gamma\delta$ T cells transferred from sensitized mice produced IL-4 and IL-10, but not IFN γ , in the lungs during RSV infection. Aoyagi *et al.* demonstrated that $\gamma\delta$ T cells isolated from the peripheral blood of infants with severe RSV infection were significantly impaired in their ability to produce IFN γ upon restimulation, and this defect was apparent as far out as 28 days after infection(Aoyagi et

al., 2003). Collectively, these results suggest an important role for $\gamma\delta$ T cells in the pathogenesis of acute RSV disease, but also indicate a role in predisposing the host to secondary conditions such as BRD or the development of wheezing and allergic asthma after recovery from infection.

Conclusions and Future Directions

$\gamma\delta$ T cells form a critical bridge between the innate and adaptive immune system, and have potential to fulfill a wide range of functions in the response to infection and disease. $\gamma\delta$ T cells are a particularly important component of the immune system of the neonate- the same population at increased risk for severe infection with RSV. Although a role for $\gamma\delta$ T cells is still unclear in the context of RSV infection in cattle or humans, emerging evidence suggests that these cells have the capacity for early, innate viral recognition, and the ability to significantly shape the nature of the inflammatory response in the lungs both during infection and during convalescence. We suggest that future studies should be aimed at developing a more thorough understanding of the role of $\gamma\delta$ T cells *in vivo* in both humans and calves, with the ultimate goal of more effectively harnessing or shaping the $\gamma\delta$ T cell response to promote improved disease outcome and recovery following severe infection in the neonate.

Acknowledgements

The authors wish to acknowledge Dr. Mitchell V. Palmer for his expert pathology support and assistance in developing and optimizing the RNAScope procedure for use on bovine lung samples. The authors wish to thank Theresa Waters for her excellent

technical assistance on all aspects of the project. Research for this project was conducted at a USDA research facility and all funding was provided through internal USDA research dollars.

References

- Aoyagi, M., Shimojo, N., Sekine, K., Nishimuta, T., Kohno, Y., 2003. Respiratory syncytial virus infection suppresses IFN-gamma production of gammadelta T cells. *Clin. Exp. Immunol.* 131, 312-317.
- Baldwin, C.L., Telfer, J.C., 2015. The bovine model for elucidating the role of gammadelta T cells in controlling infectious diseases of importance to cattle and humans. *Mol. Immunol.* 66, 35-47.
- Bem, R.A., Domachowske, J.B., Rosenberg, H.F., 2011. Animal models of human respiratory syncytial virus disease. *Am. J Physiol. Lung Cell. Mol. Physiol.* 301, L148-156.
- Brodersen, B.W., 2010. Bovine respiratory syncytial virus. *Vet. Clin. North Am Food Anim. Pract.* 26, 323-333.
- Bystrom, J., Al-Adhoubi, N., Al-Bogami, M., Jawad, A.S., Mageed, R.A., 2013. Th17 lymphocytes in respiratory syncytial virus infection. *Viruses.* 5, 777-791.
- Culley, F.J., Pennycook, A.M., Tregoning, J.S., Hussell, T., Openshaw, P.J., 2006. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-biased immunopathology. *J Virol.* 80, 4521-4527.
- De Weerd, W., Twilhaar, W.N., Kimpen, J.L., 1998. T cell subset analysis in peripheral blood of children with RSV bronchiolitis. *Scand. J. Infect. Dis.* 30, 77-80.

341 Dodd, J., Riffault, S., Kodituwakku, J.S., Hayday, A.C., Openshaw, P.J., 2009.
 342 Pulmonary V gamma 4+ gamma delta T cells have proinflammatory and antiviral effects
 343 in viral lung disease. *J. Immunol.* 182, 1174-1181.
 344 Gershwin, L.J., 2007. Bovine respiratory syncytial virus infection: immunopathogenic
 345 mechanisms. *Anim. Health Res. Rev.* 8, 207-213.
 346 Gibbons, D.L., Haque, S.F., Silberzahn, T., Hamilton, K., Langford, C., Ellis, P., Carr,
 347 R., Hayday, A.C., 2009. Neonates harbour highly active gammadelta T cells with
 348 selective impairments in preterm infants. *Eur. J. Immunol.* 39, 1794-1806.
 349 Guzman, E., Hope, J., Taylor, G., Smith, A.L., Cubillos-Zapata, C., Charleston, B., 2014.
 350 Bovine gammadelta T cells are a major regulatory T cell subset. *J. Immunol.* 193, 208-
 351 222.
 352 Guzman, E., Taylor, G., 2015. Immunology of bovine respiratory syncytial virus in
 353 calves. *Mol. Immunol.* 66, 48-56.
 354 Hall, C.B., 2001. Respiratory syncytial virus and parainfluenza virus. *N. Engl. J. Med.*
 355 344, 1917-1928.
 356 Hall, C.B., Walsh, E.E., Long, C.E., Schnabel, K.C., 1991. Immunity to and frequency of
 357 reinfection with respiratory syncytial virus. *J. Infect. Dis.* 163, 693-698.
 358 Hedges, J.F., Cockrell, D., Jackiw, L., Meissner, N., Jutila, M.A., 2003. Differential
 359 mRNA expression in circulating gammadelta T lymphocyte subsets defines unique
 360 tissue-specific functions. *J. Leukoc. Biol.* 73, 306-314.
 361 Hedges, J.F., Lubick, K.J., Jutila, M.A., 2005. Gamma delta T cells respond directly to
 362 pathogen-associated molecular patterns. *J. Immunol.* 174, 6045-6053.

363 Henrickson, K.J., Hoover, S., Kehl, K.S., Hua, W., 2004. National disease burden of
 364 respiratory viruses detected in children by polymerase chain reaction. *Pediatr. Infect. Dis.*
 365 *J.* 23, S11-18.

366 Hoek, A., Rutten, V.P., Kool, J., Arkesteijn, G.J., Bouwstra, R.J., Van Rhijn, I., Koets,
 367 A.P., 2009. Subpopulations of bovine WC1(+) gammadelta T cells rather than
 368 CD4(+)CD25(high) Foxp3(+) T cells act as immune regulatory cells ex vivo. *Vet. Res.*
 369 40, 6.

370 Hou, L., Jie, Z., Desai, M., Liang, Y., Soong, L., Wang, T., Sun, J., 2013. Early IL-17
 371 production by intrahepatic T cells is important for adaptive immune responses in viral
 372 hepatitis. *J. Immunol.* 190, 621-629.

373 Huang, H., Saravia, J., You, D., Shaw, A.J., Cormier, S.A., 2015. Impaired gamma delta
 374 T cell-derived IL-17A and inflammasome activation during early respiratory syncytial
 375 virus infection in infants. *Immunol Cell. Biol.* 93, 126-135.

376 Janeway, C.A., Jr., 1989. Approaching the asymptote? Evolution and revolution in
 377 immunology. *Cold Spring Harb. Symp. Quant. Biol.* 54 Pt 1, 1-13.

378 Jutila, M.A., Holderness, J., Graff, J.C., Hedges, J.F., 2008. Antigen-independent
 379 priming: a transitional response of bovine gammadelta T-cells to infection. *Anim. Health*
 380 *Res. Rev.* 9, 47-57.

381 Kalina, W.V., Gershwin, L.J., 2004. Progress in defining the role of RSV in allergy and
 382 asthma: from clinical observations to animal models. *Clin. Dev. Immunol.* 11, 113-119.

383 Kerns, H.M., Jutila, M.A., Hedges, J.F., 2009. The distinct response of gammadelta T
 384 cells to the Nod2 agonist muramyl dipeptide. *Cell. Immunol.* 257, 38-43.

385 Kim, J.O., Cha, H.R., Kim, E.D., Kweon, M.N., 2012. Pathological effect of IL-17A-
 386 producing TCRgammadelta(+) T cells in mouse genital mucosa against HSV-2 infection.
 387 Immunol. Lett. 147, 34-40.

388 Klein Klouwenberg, P., Tan, L., Werkman, W., van Bleek, G.M., Coenjaerts, F., 2009.
 389 The role of Toll-like receptors in regulating the immune response against respiratory
 390 syncytial virus. Crit. Rev. Immunol. 29, 531-550.

391 Krug, N., Erpenbeck, V.J., Balke, K., Petschallies, J., Tschernig, T., Hohlfeld, J.M.,
 392 Fabel, H., 2001. Cytokine profile of bronchoalveolar lavage-derived CD4(+), CD8(+),
 393 and gammadelta T cells in people with asthma after segmental allergen challenge. Am. J.
 394 Resp. Cell Mol. Biol. 25, 125-131.

395 Lahmers, K.K., Norimine, J., Abrahamsen, M.S., Palmer, G.H., Brown, W.C., 2005. The
 396 CD4+ T cell immunodominant Anaplasma marginale major surface protein 2 stimulates
 397 gammadelta T cell clones that express unique T cell receptors. J. Leukoc. Biol. 77, 199-
 398 208.

399 LeBlanc, P.H., Baker, J.C., Gray, P.R., Robinson, N.E., Derksen, F.J., 1991. Effects of
 400 bovine respiratory syncytial virus on airway function in neonatal calves. Am. J. Vet. Res.
 401 52, 1401-1406.

402 Li, W., Moltedo, B., Moran, T.M., 2012. Type I interferon induction during influenza
 403 virus infection increases susceptibility to secondary Streptococcus pneumoniae infection
 404 by negative regulation of gammadelta T cells. J. Virol. 86, 12304-12312.

405 Machugh, N.D., Mburu, J.K., Carol, M.J., Wyatt, C.R., Orden, J.A., Davis, W.C., 1997.
 406 Identification of two distinct subsets of bovine gamma delta T cells with unique cell
 407 surface phenotype and tissue distribution. Immunology. 92, 340-345.

408 McGill, J.L., Nonnecke, B.J., Lippolis, J.D., Reinhardt, T.A., Sacco, R.E., 2013.
 409 Differential chemokine and cytokine production by neonatal bovine gammadelta T-cell
 410 subsets in response to viral toll-like receptor agonists and in vivo respiratory syncytial
 411 virus infection. *Immunology*. 139, 227-244.
 412 McGill, J.L., Sacco, R.E., Baldwin, C.L., Telfer, J.C., Palmer, M.V., Waters, W.R., 2014.
 413 Specific recognition of mycobacterial protein and peptide antigens by gammadelta T cell
 414 subsets following infection with virulent *Mycobacterium bovis*. *J. Immunol.* 192, 2756-
 415 2769.
 416 McInnes, E., Sopp, P., Howard, C.J., Taylor, G., 1999. Phenotypic analysis of local
 417 cellular responses in calves infected with bovine respiratory syncytial virus. *Immunology*.
 418 96, 396-403.
 419 Meyer, G., Deplanche, M., Schelcher, F., 2008. Human and bovine respiratory syncytial
 420 virus vaccine research and development. *Comp. Immunol. Microbiol. Infect. Dis.* 31,
 421 191-225.
 422 Nair, H., Nokes, D.J., Gessner, B.D., Dherani, M., Madhi, S.A., Singleton, R.J., O'Brien,
 423 K.L., Roca, A., Wright, P.F., Bruce, N., Chandran, A., Theodoratou, E., Sutanto, A.,
 424 Sedyaningsih, E.R., Ngama, M., Munywoki, P.K., Kartasmita, C., Simoes, E.A.,
 425 Rudan, I., Weber, M.W., Campbell, H., 2010. Global burden of acute lower respiratory
 426 infections due to respiratory syncytial virus in young children: a systematic review and
 427 meta-analysis. *Lancet*. 375, 1545-1555.
 428 Ohlson, A., Alenius, S., Traven, M., Emanuelson, U., 2013. A longitudinal study of the
 429 dynamics of bovine corona virus and respiratory syncytial virus infections in dairy herds.
 430 *Vet. J.* 197, 395-400.

431 Pawankar, R.U., Okuda, M., Okubo, K., Ra, C., 1995. Lymphocyte subsets of the nasal
 432 mucosa in perennial allergic rhinitis. *Am. J. Resp. Crit. Care Med.* 152, 2049-2058.
 433 Pawankar, R.U., Okuda, M., Suzuki, K., Okumura, K., Ra, C., 1996. Phenotypic and
 434 molecular characteristics of nasal mucosal gamma delta T cells in allergic and infectious
 435 rhinitis. *Am. J. Resp. Crit. Care Med.* 153, 1655-1665.
 436 Rogers, A.N., VanBuren, D.G., Hedblom, E., Tilahun, M.E., Telfer, J.C., Baldwin, C.L.,
 437 2005a. Function of ruminant gammadelta T cells is defined by WC1.1 or WC1.2 isoform
 438 expression. *Vet. Immunol. Immunopathol.* 108, 211-217.
 439 Rogers, A.N., Vanburen, D.G., Hedblom, E.E., Tilahun, M.E., Telfer, J.C., Baldwin,
 440 C.L., 2005b. Gammadelta T cell function varies with the expressed WC1 coreceptor. *J.*
 441 *Immunol.* 174, 3386-3393.
 442 Rogers, A.N., Vanburen, D.G., Zou, B., Lahmers, K.K., Herzig, C.T., Brown, W.C.,
 443 Telfer, J.C., Baldwin, C.L., 2006. Characterization of WC1 co-receptors on functionally
 444 distinct subpopulations of ruminant gamma delta T cells. *Cell. Immunol.* 239, 151-161.
 445 Sacco, R.E., McGill, J.L., Palmer, M.V., Lippolis, J.D., Reinhardt, T.A., Nonnecke, B.J.,
 446 2012. Neonatal calf infection with respiratory syncytial virus: drawing parallels to the
 447 disease in human infants. *Viruses.* 4, 3731-3753.
 448 Sacco, R.E., McGill, J.L., Pillatzki, A.E., Palmer, M.V., Ackermann, M.R., 2014.
 449 Respiratory syncytial virus infection in cattle. *Vet. Pathol.* 51, 427-436.
 450 Silvestri, M., Sabatini, F., Defilippi, A.C., Rossi, G.A., 2004. The wheezy infant --
 451 immunological and molecular considerations. *Paed. Resp. Rev.* 5 Suppl A, S81-87.
 452 Srikumaran, S., Kelling, C.L., Ambagala, A., 2007. Immune evasion by pathogens of
 453 bovine respiratory disease complex. *Anim. Health Res. Rev.* 8, 215-229.

454 Stein, R.T., Sherrill, D., Morgan, W.J., Holberg, C.J., Halonen, M., Taussig, L.M.,
 455 Wright, A.L., Martinez, F.D., 1999. Respiratory syncytial virus in early life and risk of
 456 wheeze and allergy by age 13 years. *Lancet*. 354, 541-545.
 457 Suryawanshi, A., Veiga-Parga, T., Rajasagi, N.K., Reddy, P.B., Sehrawat, S., Sharma, S.,
 458 Rouse, B.T., 2011. Role of IL-17 and Th17 cells in herpes simplex virus-induced corneal
 459 immunopathology. *J. Immunol.* 187, 1919-1930.
 460 Taylor, G., Thomas, L.H., Wyld, S.G., Furze, J., Sopp, P., Howard, C.J., 1995. Role of T-
 461 lymphocyte subsets in recovery from respiratory syncytial virus infection in calves. *J.*
 462 *Virol.* 69, 6658-6664.
 463 Thomas, L.H., Cook, R.S., Howard, C.J., Gaddum, R.M., Taylor, G., 1996. Influence of
 464 selective T-lymphocyte depletion on the lung pathology of gnotobiotic calves and the
 465 distribution of different T-lymphocyte subsets following challenge with bovine
 466 respiratory syncytial virus. *Res. Vet. Sci.* 61, 38-44.
 467 Uhari, M., Hietala, J., Tuokko, H., 1995. Risk of acute otitis media in relation to the viral
 468 etiology of infections in children. *Clin. Infect. Dis.* 20, 521-524.
 469 Wang, F., Herzig, C.T., Chen, C., Hsu, H., Baldwin, C.L., Telfer, J.C., 2011. Scavenger
 470 receptor WC1 contributes to the gammadelta T cell response to *Leptospira*. *Mol.*
 471 *Immunol.* 48, 801-809.
 472 Welte, T., Aronson, J., Gong, B., Rachamalla, A., Mendell, N., Tesh, R., Paessler, S.,
 473 Born, W.K., O'Brien, R.L., Wang, T., 2011. Vgamma4+ T cells regulate host immune
 474 response to West Nile virus infection. *FEMS Immunol. Med. Microbiol.* 63, 183-192.
 475 Wesch, D., Peters, C., Oberg, H.H., Pietschmann, K., Kabelitz, D., 2011. Modulation of
 476 gammadelta T cell responses by TLR ligands. *Cell Mol Life Sci.* 68, 2357-2370.

477 Wijngaard, P.L., MacHugh, N.D., Metzelaar, M.J., Romberg, S., Bensaid, A., Pepin, L.,
478 Davis, W.C., Clevers, H.C., 1994. Members of the novel WC1 gene family are
479 differentially expressed on subsets of bovine CD4-CD8- gamma delta T lymphocytes. J.
480 Immunol. 152, 3476-3482.

481 Wilson, E., Aydintug, M.K., Jutila, M.A., 1999. A circulating bovine gamma delta T cell
482 subset, which is found in large numbers in the spleen, accumulates inefficiently in an
483 artificial site of inflammation: correlation with lack of expression of E-selectin ligands
484 and L-selectin. J. Immunol. 162, 4914-4919.

485 Zhang, L., Liu, J., Wang, E., Wang, B., Zeng, S., Wu, J., Kimura, Y., Liu, B., 2013.
486 Respiratory syncytial virus protects against the subsequent development of ovalbumin-
487 induced allergic responses by inhibiting Th2-type gammadelta T cells. J. Med. Virol. 85,
488 149-156.

489

490

Figure Legends

Figure 1. $\gamma\delta$ T cells are increased in the lungs of calves infected with BRSV, but do not express significant amounts of CXCL10, CCL2 or CCL3, therein. Paraffin-embedded sections of lungs from a control calf (1A) or calves experimentally infected with 10^4 TCID₅₀ BRSV strain 375 were analyzed by RNAScope assay for mRNA expression of the $\gamma\delta$ T cell receptor (red, 1A-D) and CXCL10 (green, A and B), CCL2 (green, C) or CCL3 (green, D). Sections are counterstained with hematoxylin. Results are representative of 2-3 animals per condition.