Review Article

Comparative Gamma Delta T Cell Immunology: A Focus on Mycobacterial Disease in Cattle

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A theme among many pathogenic mycobacterial species affecting both humans and animals is a prolonged asymptomatic or latent period that can last years to decades. The mechanisms that favor progression to active disease are not well understood. Pathogen containment is often associated with an effective cell-mediated or T-helper 1 immune profile. With certain pathogenic mycobacteria, such as Mycobacterium avium subspecies paratuberculosis, a shift to active clinical disease is associated with loss of T-helper 1 immunity and development of an ineffective humoral or T-helper 2 immune response. Recently γδ T cells have been shown to play a role early in mycobacterial infections and have been hypothesized to influence disease outcome. The purpose of this paper is to compare recent advancements in our understanding of γδ T cells in humans, cattle, and mice and to discuss roles of γδ T cells in host response to mycobacterial infection.

1. Introduction

The host immune response to mycobacterial infection is complex, and significant differences exist among a diverse group of mycobacterial pathogens and host species infected. A common theme among many pathogenic mycobacterial species in both humans and animals is a prolonged asymptomatic or latent period that can last years to decades. The mechanisms that favor progression to active disease are not well understood. Pathogen containment is often associated with an effective cell-mediated or T-helper 1 immune profile. With certain pathogenic mycobacteria, such as Mycobacterium avium subspecies paratuberculosis, a shift to active clinical disease is associated with loss of T-helper 1 immunity and development of an ineffective humoral or T-helper 2 immune response. Recently γδ T cells have been shown to play a role early in mycobacterial infections and have been hypothesized to influence disease outcome. The purpose of this paper is to compare recent advancements in our understanding of γδ T cells in humans, cattle, and mice and to discuss roles of γδ T cells in host response to mycobacterial infection.

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2. γδ T Cells

First described in humans in 1986 [2] and in cattle in 1989 [3], the γδ T cell receptor (TCR) has not been well characterized compared to the more widely studied αβ TCR. Since their discovery, the immunobiology of γδ T cells has been most studied in humans and mice, and the data have indicated that these cells have a variety of functions...
during both innate and adaptive immunity. Key differences in γδ T cell biology exist between species, yet recent data have indicated several common themes that apparently span the species barrier including anatomic distribution and functional capacity; these topics are further discussed in the following sections.

3. Specialized Anatomic Distribution and Phenotype

Within lymphoid tissues, γδ T cells are considered to be a minor T lymphocyte population, yet γδ T cells are enriched in many organs including skin and mucosal surfaces. This distribution suggests a role for these cells during immune surveillance and antigen sampling at surfaces constantly confronted with invading pathogens [4, 5]. γδ T cells are also well represented in peripheral blood mononuclear cells. γδ T cells typically represent 1–10% of circulating T lymphocytes in adult humans and mice and approximately 10–25% in adult cattle, though this number can be as high as 40% in young calves [6].

Similar to α and β TCR genes, γ and δ TCR genes have variable (V), joining (J), and constant (C) regions. The δ and β genes also contain diversity (D) gene segments [7]. In humans, γδ T cell subsets are defined by their γ and δ gene segment usage. In humans, γδ T cells from different anatomic sites show preferential V segment usage suggesting that human γδ T cell subsets have distinct functional roles [8]. For example, the two major γδ T cell subsets in humans are Vδ1+ and Vδ2+ cells. Vδ2+ cells predominate in peripheral blood and have been shown to significantly expand during a variety of infectious diseases including mycobacterial diseases [9]. The Vδ1+ subset is less frequent in the blood, but is the majority subset in tissues [10, 11]. Expression of additional molecules (CD2, CD4, CD5, CD6, and CD8) on γδ T cells has also been described in humans, mice, and cattle. There is considerable interspecies variability with respect to these markers suggesting that these molecules are less useful for defining functionally distinct subsets, and there is currently no species-wide γδ T cell-specific marker [12, 13].

The γδ TCR of cattle has been cloned and characterized, but little is known about how preferential gene segment usage correlates with tissue distribution or functionality [14, 15]. Surface expression of the cysteine-rich scavenger receptor molecule workshop cluster 1 (WC1) is most commonly used to distinguish γδ T cell subsets of cattle, and expression of WC1 appears to be limited to ruminant γδ T cells though WC1-like genes have been found in sheep, goats, horses, mice, pigs, and humans [16]. Further subdivision of WC1 expressing cells in cattle has been described (WC1.1, WC1.2, and WC1.3) [17, 18]. As in humans, it is thought that different phenotypes represent functionally distinct γδ T cell subsets that preferentially home to different tissue localizations [13]. Based on WC1 molecule expression, bovine γδ T cells are most frequently divided into two categories: the larger of these subsets has the phenotype WC1−CD2+CD3+ and is found primarily within splenic red pulp and the intestinal tract, while the second subset has the phenotype WC1+CD2−CD3+ and is found predominantly in peripheral blood. Two additional features that fundamentally distinguish αβ from γδ T cells appear to be shared by humans and cattle. First, γδ T cells are not clearly defined by surface expression of the CD4 or CD8 accessory molecules, and thus there is no MHC class I or MHC class II restriction. Second, γδ T cells recognize unconventional antigens such as phosphorylated microbial metabolites or lipid antigens [19].

4. Ligands

Specificity of γδ T cells to mycobacterial antigens in humans has been described [20]. Protein antigens such as mycobacterial heat shock protein [21, 22] and nonprotein [23, 24] antigens including phosphoantigens have been shown to induce strong γδ T cell responses. In humans, the majority of studies have examined reactive patterns of the Vδ2+ subset of γδ T cells. Vδ2+ cells recognize low molecular weight nonpeptide phosphate-containing metabolites produced by a variety of bacterial pathogens including mycobacteria [25]. Variations or other important ligands for Vδ2+ cells include microbial byproducts such as negatively charged alkyl phosphate antigens [26] and positively charged alkyl-lamine antigens [27]. Many of the putative microbial ligands described for human γδ T cells have autologous counterparts or endogenous metabolites of the mevalonate pathway, which are upregulated during periods of cellular stress suggesting that γδ T cells also function during noninfectious processes [28]. Specific ligands for human Vδ1+ cells are less well described. Spada et al. reported that Vδ1+ cells directly recognized CD1c molecules [11], which may be a mechanism of antigen presentation to Vδ1+ cells during M. leprae infection [11, 29].

γδ T cell ligands in cattle are not clearly defined. The majority of studies have examined the reactive patterns of WC1+ γδ T cells likely because of their ease of isolation from peripheral blood. An early study by Rhodes et al. demonstrated responsiveness of bovine peripheral blood γδ T cells from M. bovis infected calves to various mycobacterial protein antigens [30]. Work by Welsh et al. confirmed that WC1+ cells respond to both protein and nonprotein M. bovis antigens, and that response to mycobacterial proteins was dominant [31]. Vesosky et al. showed that WC1+ cells from healthy calves could respond to stimulation with live mycobacteria, mycobacterial cell wall, and mycobacterial culture filtrate proteins [32]. In this study, the phosphoantigen identified as a human γδ T cell ligand (isopentenyl pyrophosphate, IPP) was not recognized by naive bovine γδ T cells [32]. In both humans and cattle, the interactions surrounding γδ T cell activation have largely been considered to be MHC-independent [24] and TCR-dependent, although TCR-independent activation has also been shown [33]. Recent work has also demonstrated that purified human and bovine γδ T cells can be directly activated by pathogen-associated molecular patterns (PAMPs) in the absence of antigen presenting cells [34], which may have significant implications for the innate role of γδ T cells. Though their restriction elements during ligand recognition by bovine γδ T cells remain to be fully characterized, it is
clear that γδ T cells from both naïve and infected individuals have the capacity to respond to mycobacterial antigens.

5. Importance of IL-2

In αβ T cells the initial encounter with specific antigen along with the appropriate costimulatory signals (CD28 of T cell binding B7 of APC) induces the synthesis of IL-2 and increased expression of the α chain of the IL-2 receptor (CD25). Subsequent binding of IL-2 to its high-affinity receptor then triggers progression through the cell cycle, proliferation, and differentiation of naïve T cells [7]. Distinct from their αβ T cell counterparts, γδ T cells produce minimal amounts of IL-2 upon activation, and the proliferative response of human γδ T cells after antigenic stimulation is dependent on CD4+ T cell secretion of IL-2 [35]. Welsh et al. and Smyth et al. in separate studies demonstrated marked upregulation of CD25 on the surface of bovine γδ T cells after encountering M. bovis protein antigens, but there was minimal proliferation without addition of IL-2 [31, 36]. Based on these findings, IL-2 is very likely a required secondary signal for activation of γδ T cells, which ultimately drives them to proliferation after recognition of mycobacterial antigens.

6. Effects on Granuloma Formation, Maintenance

There has been recent interest in the role of γδ T cells in generation and maintenance of granulomas that develop at mycobacterial infection sites. In a murine model of Map infection, the frequency of granuloma formation was significantly decreased in γδ TCR depleted mice indicating a potential role of γδ T cells in the generation of granulomas during mycobacterial infection [37]. In cattle, γδ T cells have also been evaluated for a potential role in granuloma formation. Palmer et al. demonstrated that in M. bovis infected calves, CD4+ T cell numbers in lymph node granulomas remained constant over time. The number of CD8+ T cells and WC1+ cells was high during early-stage granulomas, but diminished as granulomas matured. The authors suggested that loss of these T cell subsets during late stages correlates with failure of the immune system to control infection [38]. In contrast, Wangoo et al. showed that late-stage lymph node granulomas from M. bovis-infected calves had significantly greater numbers of WC1+ T cells compared to early stage lesions, and that the WC1+ cells were spatially distributed at the peripheral zone near the fibrotic capsule [39]. However, in a separate study evaluating spatial distribution of T cell subsets, this group was unable to confirm distinct spatial relationships of the γδ T cells within the granulomatous lesions [40]. Simutis et al. in 2005 identified γδ T cells within poorly organized granulomatous lesions induced by subcutaneous Map injection in a calf model [41]. In 2009, Plattner et al. went on to show in this model that in well-organized (Th-1 polarized granulomas) there was stratification of γδ T cells with respect to WC1+ and WC1− phenotypes. This stratification was lacking in poorly organized granulomas associated with Map infection. The conclusion from this study was that the γδ T cells subsets had unique roles in directing bovine granuloma formation and function during Map infection [42].

7. Immediate Effector Function: Cytotoxicity

Numerous effector functions have been reported for subsets of γδ T cells. Activated human Vδ2+ γδ T cells have broad cytotoxic activity. Oliaro et al. demonstrated that Vδ2+ cells were able to directly lyse Brucella-infected macrophages and reduce intracellular bacterial numbers by the Fas/Fas ligand pathway [43]. Dieli et al. showed that generation of perforin and granzyme by Vδ2+ cells reduced the viability of both extracellular and intracellular M. tuberculosis [44, 45]. Fisch et al. specifically examined both major subsets of human γδ T cells and demonstrated broad in vitro cytotoxicity by Vδ2+ cells, but importantly observed that Vδ1+ cells also exhibited this capacity [46]. In cattle, it is known that cytotoxicity mediated by bovine natural killer (NK) cells reduces intracellular viability of M. bovis [47]; however, the evidence for γδ T cell-mediated cytotoxicity is less clear. Bovine peripheral blood-derived and antigen-stimulated γδ T cells (WC1 phenotype not reported, but most likely WC1+ subset) were unable to mediate nitric oxide production and bacterial killing of Map-infected macrophages [48]. Other data have also suggested that cytotoxicity is a feature of bovine γδ T cells during Map infection [49, 50].

8. Immediate Effector Function: Cytokine Secretion

It has been known for several years that a key mechanism by which T lymphocytes respond to infectious agents and mediate immune functions is secretion of specific cytokines. Upon recognition of their ligands, γδ T cells are able to generate a range of proinflammatory cytokines and antimicrobial peptides [51] and provide an initial barrier until antigen-specific αβ T cells have been expanded. Cytokine production by γδ T cell subsets has been analyzed at the gene and protein levels in humans and cattle. Microarray analysis of stimulated human Vδ2+ cells has shown upregulation of proinflammatory genes such as tumor necrosis factor alpha (TNF-α), IFN-γ, macrophage-colony stimulating factor, IL-17, and IL-21 [52, 53]. However, secretion of some of these proteins by stimulated Vδ2+ cells has not been confirmed. Initial studies in humans showed that peripheral blood-derived γδ T cells rapidly expand and produce IFN-γ in response to nonpeptide phosphate antigens [54]. Wang et al. demonstrated that human Vδ2+ cells generate IFN-γ and TNF-α as early as 2 hours following exposure to the live bacterial product iso-butyramine. An interesting observation in this study was that production of cytokines was cyclic and limited to periods of direct contact with live bacteria, suggesting that γδ T cell activity is focused at the infection site [55]. Vδ2+ production of IFN-γ and TNF-α was also confirmed by Wesch et al. [56]. Vδ2+ cells from human
peripheral blood can be driven towards IL-4 production under specific culture conditions [56]. Depending on the physiologic or pathologic context, subsets of murine γδ T cells have also been shown to produce Th2 cytokines [57]. The production of keratinocyte growth factor or connective tissue growth factor by γδ T cells suggests more specialized tissue repair functions [19]. Microarray data for human-stimulated Vδ1+ cells initially demonstrated upregulation of cytokine genes that are considered important during regulatory functions such as IL-10 and IL-11 [52, 53], and recent work has confirmed the ability of Vδ1+ cells to produce IL-10 as well as transforming growth factor-beta (TGF-β) [58].

In cattle, evidence for cytokine secretion by γδ T cell subsets is less clear. Buza et al. correlated IFN-γ production with changes in circulating γδ T cell populations rather than CD4+ or CD8+ T cells of BCG-vaccinated calves [59]. No effects on disease pathology were observed following depletion of WC1+ γδ T cells from M. bovis-infected calves, though increased antigen-specific IL-4, reduced innate IFN-γ, and reduced IgG2 antibody were observed [60]. WC1+ cells from M. bovis-infected calves proliferated strongly when stimulated with M. bovis extracts but produced significantly less IFN-γ compared to autologous CD4+ T cells [36]. Vesosky et al. demonstrated that while proliferation of bovine γδ T cells from healthy cattle could be induced by a variety of mycobacterial antigens, the requirements for IFN-γ production were more stringent. Specifically, purified WC1+ cells produced significant amounts of IFN-γ in response to a nonprotein component of mycobacterial cell wall antigen only when antigen-presenting cells and exogenous IL-2 were added to the cultures [32]. Rogers et al. have further demonstrated that the function of bovine γδ T cells varies with the expressed form of WC1. In a series of experiments, they showed that WC1.1+ and WC1.2+ cells had different proliferation potentials to various bacterial stimuli and that the WC1.1+ cells were the major producers of IFN-γ [17, 18, 61]. Further, WC1.1+ cells are preferentially recruited to the respiratory tract following intranasal BCG vaccination in calves [62]. In a fetal bovine-severe combined immunodeficient (SCID-bo) xenochimeric mouse model, WC1+ cells did not produce significant IFN-γ, but were shown to be involved in recruitment of other cells to mycobacterial infection sites [63]. These results support the hypothesis that WC1+ cells have a role in directing the Th1 bias of the immune response during mycobacterial infections. In contrast to human γδ T cells, no published studies document production of IL-4 or other Th2-like cytokines from bovine γδ T cells. As is the case for human Vδ1+ cells, little is known regarding cytokine secretion by WC1− γδ T cells of cattle, though recent work has shown that this subset can be experimentally induced to generate significant amounts of IFN-γ [64].

Recently, γδ T cells have been shown to play a role during the Th17 response. Th17 responses are defined by the production of IL-17, and are thought to play a critical role in inflammatory responses, particularly at mucosal surfaces [65]. In mice, production of IL-17 has been demonstrated from naive γδ T cells [66]. It has been proposed that γδ T cells initiate Th17 responses by upregulation of IL-6 and IL-8, which in turn enhances neutrophil chemotaxis during early bacterial infections [67]. Sutton et al. recently demonstrated that murine γδ T cells express the IL-23 receptor and the transcription factor RORγT and produce IL-17, IL-21, and IL-22 in response to IL-1β and IL-23 (all features of Th17 response) and that their cytokine production is independent of γδ TCR ligation [68]. Okamoto Yoshida et al. have recently reported that IL-17 is essential for granuloma formation in mice during mycobacterial infection [69]. IL-17 production by γδ T cells has also recently been confirmed in humans [70], but has yet to be identified in cattle.

9. Specific Effects on Other Cell Types

The ability of γδ T cells to innately produce IFN-γ during mycobacterial infection is particularly interesting in the context of mycobacterial diseases. It has been proposed that early IFN-γ production at the site of infection by γδ T cells could stimulate initial killing of bacteria by macrophages [71]. This could enhance antigen presentation by stimulation of infection site dendritic cells (DCs) to mature and migrate to draining lymph nodes thus initiating adaptive T cell immunity [72]. Moreover, direct influence of γδ T cells on DC function has been recently explored. As the primary antigen presenting cells of the innate immune system, DCs are considered to be primary determinants of the efficacy of the T cell-mediated immune response. It is known that compared to antigen alone, the addition of exogenous IFN-γ enhances the maturation of human DCs in vitro and the potency of the ensuing Th1 immune response [73]. γδ T cell-mediated enhancement of DC maturation has been documented in vitro following activation of Vδ2+ phosphoantigen-specific [74] and Vδ1+ CD1c-restricted [75] human γδ T cell subsets. In these studies, there was increased expression of CD86 on cocultured DCs and enhanced IL-12 production by the DCs, which ultimately resulted in improved priming of downstream T cell responses. Leslie et al. have demonstrated that DCs lacking γδ T cell interaction (DCs matured by microbial stimuli alone) resulted in “exhausted” DC populations unable to induce efficient Th1 polarization [75]. This response was found to be partially mediated by the cytokines TNF-α and IFN-γ in a nonantigen-specific manner [75]. Vδ1+ cells are thus uniquely positioned to induce DC maturation at infection sites due to their tissue (mucosal surfaces) distribution. A reciprocal interaction where fully mature DCs stimulate γδ T cells for sustained innate immune responses has been demonstrated at infection sites and in secondary lymphoid organs [76–78]. DC-γδ T cell interaction has also been shown to be important in the control of mycobacterial infections in mouse models [79].

In contrast to studies with humans, studies of the cellular interactions between bovine γδ T cells and DCs are few. In 1996, Collins et al. demonstrated that neither WC1− nor WC1+ cells from cattle were stimulated to proliferate in response to allogeneic DCs [80]. These results contrast to CD4+ and CD8+ T cells from these calves, which were strongly induced to proliferate by DCs. Price and Hope
recently examined interactions between monocyte-derived DCs and WC1+ cells from *M. bovis*-infected calves in vitro [81]. This study demonstrated that WC1+ cells upregulated surface expression of MHC class II and CD25 (IL-2 receptor) and generated significantly greater amounts of IFN-γ when cocultured with DC. Also, the DCs produced significantly greater amounts of IL-12 when cocultured with WC1+ cells. These results further support the hypothesis that in *M. bovis*-infected cattle, γδ T cells are able to provide the initial IFN-γ burst that is required for full maturation of DCs and that γδ T cell-DC interaction can enhance the activation of MHC class II-restricted αβ T cells.

Additional roles for γδ T cells have been described in a variety of experimental systems. The ability of γδ T cells to directly present antigen to αβ T cells was first demonstrated in cattle [82] and pigs [83]. Collins demonstrated in cattle that B7 molecules were widely expressed on the surface of γδ T cells and that antigen-primed WC1+ cells directly induced significant CD4+ T cell proliferation [82]. Similar results have been demonstrated in human Vδ2+ [84, 85] and murine γδ T cells [86].

### 10. Regulatory Function

Immunoregulatory activity has recently been described for γδ T cells of several species including mice [87] and humans [58, 88]. In the study by Küh et al., the Vδ1+ subset was shown to have strong regulatory functions apparently mediated by their production of IL-10 and TGF-β, yet these γδ T cells lack expression of the classic regulatory T cell marker and transcription factor forkhead box P3 (FoxP3) [58]. Kang et al. were successfully able to induce immunosuppressive function and FoxP3 expression in murine splenic-origin but not human blood-origin γδ T cells [89]. An immunomodulatory role for bovine γδ T cells was first described in cattle infected with *Map* where depletion of γδ T cells was shown to enhance the proliferation of *Map* antigen-stimulated CD4+ T cells [49]. Rhodes et al. confirmed regulatory activity by bovine peripheral blood γδ T cells during *M. bovis* infection when they demonstrated suppression of antigen-specific αβ T cell proliferation and enhanced production of both IFN-γ and TGF-β [30]. Jutila and colleagues have also described an immunoregulatory phenotype within subsets of circulating bovine γδ T cells using serial gene expression analysis [90, 91]. Recently, Hoek et al. identified and characterized regulatory function of sorted bovine γδ T cells [92]. In contrast to humans and mice, bovine CD4+CD25highFoxP3+ cells lacked ex vivo regulatory activity, and these authors described T regulatory cell activity by WC1.1+ and WC1.2+ cells with upregulated transcription of IL-10 but not FoxP3 or TGF-β genes [92].

### 11. Memory Function

In 2002, Shen et al. demonstrated the capability of primate γδ T cells to mount a memory response after microbial infection [93]. Using a macaque tuberculosis model, these authors demonstrated characteristic features of memory γδ T cells, which included prolonged recall response upon reinfection. Interestingly in this study, the expansion of Vδ2+ cells in the peripheral blood was associated with clearance of detectable bacteremia [93]. In 2003 Dieli et al. demonstrated effector memory subsets of CD45RA-CD27-human Vδ2+ T cells present in the circulation and within tissues [94].

### 12. Conclusions

With continued exploration of γδ T cell functions, it is becoming clear that γδ T cells have many roles and can be regulatory or stimulatory during host defense against mycobacterial pathogens [95]. This paper has highlighted key recent findings relevant to the pathogenesis of mycobacterial infections: nonclassical mycobacterial antigens are recognized by γδ T cells; γδ T cells play key roles in infection site immunopathology, which is potentially mediated by γδ T cell production of IL-17; γδ T cells lyse infected cells and act as strong producers of IFN-γ at infection sites; interactions between γδ T cells and DCs leading to mutual activation have potential to influence mycobacterial infection.

Limitations in the study of γδ T cell biology such as difficulty obtaining appropriate tissues exist for many species. The study of human and bovine γδ T cell biology has been largely focused on in vitro evaluation of peripheral blood-derived γδ T cells. Evaluation of the less easily accessed minor γδ T cell subsets is important in the future as these subsets have powerful local and downstream effector functions. There is a need for animal modeling systems that allow not only evaluation of multiple tissue-specific γδ T cell subsets, but also the ability to examine these cells in the context of infection site-specific immunopathology. Cattle display some similarity with humans regarding immunopathology of mycobacterial diseases [96], and this coupled with readily-available γδ T cells makes young calves a strong model choice to study the pathogenesis of mycobacterial disease.

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