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CHAPTER

46

Harnessing γδ T Cells as Natural Immune Modulators

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I. INTRODUCTION

Lymphocytes are important in both innate and adaptive immune responses. Innate lymphocytes represent a heterogeneous group of cells that include cells lacking receptors for antigen, such as the group of innate lymphoid cells, of which natural killer (NK) cells are the prototypical example. Innate lymphocytes expressing antigen receptors include B1 B cells, natural killer T (NKT) cells, and γδ T cells. While innate lymphocytes are relatively rare in circulation and in lymphoid tissues, they are found in mucosal surfaces that represent portals of entry into the body [1]. In this chapter, we will focus on some of our laboratory's work on one of the major antigen-specific subsets of innate lymphocytes: γδ T cells.

γδ T cells have important roles in both innate and adaptive immune responses, wound healing, and tissue homeostasis. There are many outstanding reviews of the biology and function of γδ T cells. A select few relevant to the topic of this chapter are listed in Table 46.1. Briefly, γδ T cells express unique T cell receptors (TCRs) that recognize self and foreign antigens in the absence of the requirement for presentation by major histocompatibility complex (MHC) class I or class II molecules. This feature leads to a broad range of innate responses against pathogens, as well as recognition of stressed or tumor cells. Subsets of γδ T cells are defined by restricted TCR gene usage in addition to expression of various surface molecules and preprogrammed functional responses imprinted prior to their egress from the thymus. γδ T cells also express myriad innate receptors, such as toll-like receptors (TLRs), scavenger receptors, and lectin receptors, such as dectin-1, that can directly sense infectious agents. These receptors, along with cytokine receptors, fine-tune sensing and response of γδ T cells adapting to the tissue microenvironment. TCR stimulation leads to a variety of functional responses, such as cytolysis, cytokine production, regulatory effects, and even phagocytosis and antigen presentation, that depend on the activation of receptors and coreceptors. γδ T cells respond rapidly to external signals, leading to early cytokine responses in a

Mucosal Vaccines
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TABLE 46.1 Recent References/Reviews for Key γδ T Cell Functions

γδ T cell function	Selected review article
General γδ T cell	Hayday, Annu Rev Immunol 2000 [2], Chien, Annu Rev Immunol 2014 [3], Chien, Immunol Rev 2007 [4] Ciofani, Nat Rev Immunol 2010 [5], Holderness, Annu Rev Anim Biosci 2013 [6], Holderness, Crit Rev Immunol 2008 [7], Zarin, PNAS 2014 [8], Ribeiro, Frontiers Immunol 2015 [9]
γδ T cell role in cancer and cytolytic responses	Wu, Cell Mol Immunol 2017 [10], Zou, Oncotarget 2017 [11], Silva-Santos, Nat Rev Immunol 2015 [12], Rei, Cancer Res 2015 [13], Paul, Int J Cancer [14], Ramstead, J Interferon Cytokine Res [15]
γδ T cell innate and adaptive responses	Bonneville, Nat Rev Immunol 2010 [16], Jutila, Anim Health Res Rev 2007 [17], Ribeiro, Front Immunol 2015 [9]
γδ T cells in multiple species	Holderness, Annu Rev Anim Biosci 2013 [6]
Myeloid-cell-like features, including APC	Moser, Trends Immunol 2006 [18], Jutila, Anim Health Res Rev 2007 [17], Holderness, Crit Rev Immunol [7]
TLR expression by γδ T cells	Wesch, Cell Mol Life Sci 2011 [19], Dar, Front Immunol 2014 [20]
IL-17-producing γδ T cells	Chien, Trends Immunol., 2013 [21], Papotto, Nature Immunol. 2017 [22], Corpuz, J. Immunol., 2016 [23], McKenzie, Nature Communications, 2017 [24]
Skin/gut-resident γδ T cells	Hayday, Annu Rev Immunol 2000 [2], Holderness, Crit Rev Immunol 2008 [7], Macleod, Havran, Cell Mol Life Sci 2011 [25], Nielsen, Nat Rev Immunol 2017 [1], Ebert, J Immunol 2006 [26], Sheridan, Immunity 2013 [27]
γδ T cells/ immunotherapy	Burjanadze, Br J Immunol 2007 [28], Lawand, Front Immunol 2017 [29], Mirzaei, Cancer Lett 2016 [30]

variety of disease settings. Furthermore, they are uniquely positioned at virtually all portals of entry into the body where this type of innate immune response is critical. Indeed, γδ T cells, like other innate lymphocytes, are found at all mucosal surfaces and make up a large fraction of the intraepithelial lymphocyte population. They are also recruited to sites of inflammation, tumor growth, or other tissue insults.

II. γδ T CELL SURFACE RECEPTORS

In addition to the γδ TCR, γδ T cells express a variety of non-TCR receptors that affect their function. γδ T cells express the NK C-type lectin-like receptors, such as NKG2D, which recognize cellular stress proteins resulting in cellular activation [31,32]. They also express tumor necrosis factor (TNF) receptor family molecules CD27, CD30, and CD137 [9]. CD27 is a costimulatory receptor to the TCR [33], and CD137 is also expressed on TCR-stimulated tumor-reactive γδ T cells [34]. CD28 (of the Ig superfamily) is also a γδ TCR coreceptor. The aryl hydrocarbon receptor (AhR), generally known for its role in homeostasis for mucosal T cells, is also expressed by mouse γδ T cells that produce innate interleukin 17 (IL-17) [35], as well as the mouse skin γδ T cell subset [36]. γδ T cells also express various cytokine receptors that contribute to their activation (IL-2R, IL-15R, IL-23R, etc.) and fine-tune their functional responses. The expression of pathogen-associated molecular pattern receptors has been detected on γδ T cells. These include another lectin receptor, dectin-1, a receptor for fungal, plant, and bacterial-derived polysaccharides [37,38]; the TLRs [20,39]; CD36 [40]; scavenger receptors [41]; and NOD-like receptors [42]. Though not a focus of this chapter, γδ T cells also express a variety of receptors that downregulate their function. Examples include killer cell immunoglobulin-like receptor and leukocyte immunoglobulin-like receptor, B and T lymphocyte attenuator, and programmed cell death 1 receptor, which are regulatory receptors that suppress the function and/or proliferation of the cells [29].

III. SIMILARITIES OF γδ T CELLS TO MYELOID AND MACROPHAGE CELLS

γδ T cells are an ancient immune cell lineage, found in all jawed vertebrates. Phylogenetic evidence suggests that they are the progenitors of both αβ T cells and B cells [43]. They predate adaptive immunity, so it is not surprising that they retain many innate functions similar to those of monocytes and macrophages. Zebrafish γδ T cells both are phagocytic and can present antigen, in addition to their expression of CD8 [44]. We characterized transcript expression in subsets of bovine γδ T cells [45–47]. The primary outcome of these studies was the recognition of multiple transcripts similar to those found in monocyte and macrophage cells, indicating their innate function. As part of these studies, we detected B-lymphocyte-induced maturation protein 1 (BLIMP-1) transcripts in bovine γδ T cells [46]. BLIMP-1, also known as PRDI-BF1, is a key regulator in the differentiation of hematopoietic cells into myeloid or B cells [48]; therefore, its detection in a T cell subset was notable at the time. We recognized this significance and further confirmed the expression of transcripts in resting bovine γδ T cells and not αβ T cells [46]. More recent findings have further confirmed the innate function of γδ T cells in the appropriate contexts.

Transcript analyses in bovine γδ T cells also suggested expression of transcripts encoding solute carrier 11A1 (SLC11A1, also denoted natural resistance-associated macrophage protein 1, or NRAMP-1) in these cells [45]. SLC11A1 is a divalent metal transporter that is thought to be expressed only in myeloid and macrophage cells; it is important in effective responses against intracellular bacterial infections [49–51]. SLC11A1 enhances signaling and activation in macrophages [52]. We defined protein expression and a similar function in activation in bovine and human γδ T cells and NK cells. Expression of SLC11A1 was strongly correlated to the activation and, in particular, the expression of interferon gamma (IFNγ) in these cells [53]. Thus SLC11A1 is an additional monocyte/macrophage protein that is also expressed in γδ T cells with functional relevance.

Another similarity to myeloid cells is the ability of γδ T cells in a number of species to process and present antigen. Effective antigen presentation is required for the initiation of adaptive immunity and is studied primarily in conventional antigen-presenting cells (APCs), such as dendritic cells (DCs), activated macrophages, and B cells. γδ T cells express an array of surface receptors, such as scavenger receptors, CD11b, and CD16, that facilitate uptake of particulate antigens [54,55]. Subsets also express MHC class II and necessary coreceptors for effective antigen presentation to $CD4^+$ T cells [56–58]. Antigen uptake and presentation to $CD4^+$ T cells were first shown for bovine γδ T cells [57]. It was also shown that MHC class II expression and antigen presentation is enhanced in bovine $WC1^+$ γδ T cells during viral infection [59]. Similar functions were described for porcine, human, and mouse γδ T cells [60–62]. γδ T cells in contact with bacteria can transition from cytokine-producing cells to phagocytic APCs, demonstrating their functional plasticity [63]. The phagocytic capacity of γδ T cells is augmented by opsonization [54,63]. Combined, these studies show that subsets of γδ T cells in various species can be induced to present antigens via MHC class II. Clearly, γδ T cells have a unique role in innate immunity that is similar in some respects to that of monocytes and macrophages, and is further involved in the subsequent initiation of antigen-dependent acquired immunity.

IV. γδ T CELL-MEDIATED CYTOTOXICITY

Ligation of receptors expressed on the γδ T cell can lead to potent cytolytic responses

against stressed, infected, and malignant cells [64–67], though γδ T cells can be permissive to growth of some tumors [15]. Ligation of TCR, in combination with other receptors such as NKG2D and cytokine receptors such as the IL-23 receptor, enhances and directs cytotoxic responses along with cytokine production [68,69]. Cytotoxicity is a function of γδ T cells that is conserved across species [70–75]. For example, granzyme B, perforin, and FasL are expressed in $WC1^+$ γδ T cells from bovine peripheral blood mononuclear cells (PBMCs), with FasL expression increasing upon activation of these cells [76,77]. Perforin expression is also found in bison γδ T cells [78]. Perforin and granzyme, FasL-Fas, and the TNF-related apoptosis-inducing ligand pathway are also features of human γδ T cells [79,80]. The cytotoxic activity of γδ T cells likely plays an important role in multiple species for optimal immune responses by these cells to a subset of malignant and infected cells.

V. γδ T CELL CYTOKINE PRODUCTION

Another important functional response of γδ T cells is their regulation of the tissue environment through cytokine generation. These cytokines include those that drive inflammatory responses and contribute to downstream adaptive immune responses as well as cytokines that affect epithelial cell health and tissue homeostasis. Although the number of cytokines produced by γδ T cells is large, a few, such as IL-17, IFNγ, and the tissue cytokines keratinocyte growth factor (KGF) and insulin-like growth factor (IGF), are of particular importance in the function of subsets of these cells. IL-17 and IFNγ are potent activators of cells of the myeloid lineage and contribute to downstream inflammatory responses. In mice, γδ T cells are a major source of innate IL-17 early in response to infection [81,82]. Two populations of γδ T cells contribute to the IL-17 response. One is referred to as "natural" IL-17-producing cells, which acquire effector function prior to egress from the thymus [83]. These cells are found in mucosal tissues and are thought to be early responders to infectious insult. Another population is referred to as "induced," and these cells rapidly acquire effector function after egress from the thymus and in response to antigen and cytokine in the periphery [84]. Some reports suggest that although human and large animal γδ T cells produce IL-17 (induced phenotype), they may not be a major early source of this cytokine in these species [10,85]. Although they are clearly protective in most instances and are thought to be important to the early innate immune response, dysregulation of IL-17 production leading to excessive IL-17 can also be pathogenic [86]. KGF and IGF are also produced by tissue γδ T cells and are important in maintaining epithelial cell health and effective wound repair responses [87–90]. Though defined as important in tissue homeostasis, these responses are also important for host defense, since health of the epithelial cell barrier contributes to protection against various pathogens and the creation of a homeostatic environment for commensal microbiota.

VI. ROLE OF γδ T CELLS IN INFECTIOUS DISEASES

γδ T cells have been shown to respond to and participate in host defense responses in a variety of infectious diseases, including viral, bacterial, and parasite-induced disease, many at the mucosal surface [2,91]. Recently, γδ T cells have been found to be important for protection against emerging viruses such as Chikungunya and West Nile virus [92,93]. In HIV infection, the peripheral subset of human γδ T (Vδ2) cells is severely depleted and does not completely recover, even in patients who have had successful antiretroviral treatment.

This deficit may increase the likelihood for secondary infections and could be a critical target for new immunotherapies for HIV patients [94].

γδ T cells are clearly important in antibacterial immunity as a source of early IFNγ and IL-17 [77,82,95,96]. As human γδ T cells are preprogrammed for recognition of bacterial phosphoantigens, they are particularly important in protection from *Mycobacterium* and *Legionella* infections [97,98]. Human γδ T cells expand during *Salmonella enterica* serovar Typhimurium (ST) infection of the intestinal mucosa [99] and are a source of early IFNγ [100,101]. Bovine γδ T cells also respond to oral ST infection [102]. γδ T cells play a critical role in protection against infection with *Brucella* sp., which are facultative intracellular bacteria [103]. This appears to be primarily through production of IFNγ, and was found in mice, cattle, and sheep [103,104]. However, our results showed no contribution of mouse γδ T cells to infection with another emerging intracellular pathogen, *Coxiella burnetii* (unpublished results). Following mucosal infection but not peripheral infection, mouse γδ T cells were also found to have a role in downstream memory immune responses to *Listeria* infection [27]. Thus, γδ T cells play an important role in response against many different bacterial infections. This suggests that their specific stimulation may contribute to protection and may potentially replace or at least reduce the need for antibiotics and could be considered as a new target for future vaccine development.

γδ T cells also play protective roles in parasite infections. They respond to and are protective following initial infection with the malaria *Plasmodium falciparum*, owing to recognition of phosphoantigens produced by the parasite. However, upon subsequent infection, the numbers of γδ T cells drop, similar to the situation with long-term HIV infection. Nonetheless, higher numbers of functional Vδ2 T cells are correlated with greater protection from reinfection with *Plasmodium* and also increased symptoms upon infection, as they are sources of IFNγ and TNF-α [105]. Similarly, the first instance of bovine IL-17-producing cells was demonstrated and protects against a related parasite [106]. Indeed, in most instances of protection from pathogens, γδ T cells are similarly protective in humans and other animals [6]. Common features across species provide a rationale for the use of various animal models to test the role and importance of γδ T cells in disease settings of relevance to humans, which will lead to the creation of strategic platforms for γδ T cell-targeted vaccine development.

VII. THERAPEUTIC POTENTIAL FOR MANIPULATION OF γδ T CELLS

γδ T cells are characterized by a unique and specific tissue location, rapid response to external signals and insults, and the existence of preprogrammed and induced effector subsets. Combined with the ability to expand these cells *in vitro* and their critical roles in a variety of infectious and cancerous disease settings, γδ T cells have been the target for new immunotherapeutics [11,28–30,34,91]. In humans, both TCR and TLR agonists have been studied for their effects on enhancing γδ T cell function. Prenyl phosphates and bisphosphonates that directly or indirectly drive expansion and cytokine production in a major subset of circulating γδ T cells have been pursued for treatment of certain tumors and infections [29]. Two approaches have been used. In the first approach, γδ T cells are expanded to large numbers *in vitro* and then adoptively transferred to patients. In the second approach, these agonists are given directly to the patient, inducing responses *in vivo*. The *in vivo* responses of γδ T cells to these agonists are impressive, leading to significant expansion in tissues, such as the lung and production of immune cytokines [107]. Of note, though originally pursued for cancer treatments, the potential application of

phosphoantigen stimulation of γδ T cells in infectious disease was recently demonstrated in *Mycobacterium tuberculosis* infection in primates [108]. The application of these therapeutic approaches to stimulate γδ T cells is limited to humans and nonhuman primates, since γδ T cell responses to the prenyl phosphates are restricted to primate cells. Other therapeutic approaches to increase γδ T cell activity have focused on other receptors, such as TLRs and scavenger receptors [19,20].

Our recent endeavor has been to expand the number of materials that enhance the activity of γδ T cells in multiple species. This was achieved by screening various natural product libraries and other sources of natural products, including nutritional supplements. They were assessed for their capacity to upregulate IL-2 receptor expression on primary γδ T cells, thereby enhancing responses to IL-2 in the absence of antigen [7,17,109–112]. Follow-up functional assays examined their cell type specificity, induced cytokine responses, and benefit in various infectious disease models [110,112]. Two classes of plant products—polyphenols and polysaccharides—and one example of a microbial product that stimulate these cells, which came from these studies, are summarized below.

VIII. PLANT POLYPHENOLS FOR THE ACTIVATION OF γδ T CELLS

A class of plant polyphenol called oligomeric procyanidins (OPCs) produced by apples, grapes, and some other plants was determined to be a potent priming agent for γδ T cells. Several studies suggest that ingestion of plant and berry compounds containing polyphenols expand human γδ T cells *in vivo* [113–115]. Our study showed that OPCs from apple peel prime human, mouse, and bovine γδ T cells, and NK cells in some instances [109], for enhanced responses to secondary signals provided by cytokines and antigens. Other groups also found that OPCs expand mouse γδ T cells *in vivo* [116] and stimulate goat γδ T cells [117]. OPC-mediated γδ T cell responses increase the expression of activation markers, but the cells do not actively proliferate in the absence of a secondary signal, such as cytokine or TCR engagement [109]. OPC treatment also induces production of a restricted number of cytokines, many of which act on myeloid cells, such as colony-stimulating factors (CSFs) and chemokines such as IL-8, and various tissue growth factors [109]. One of the consequences of OPC treatment of bovine and human γδ T cells is a significant extension of the stability of CSF and chemokine transcripts [118]. The ability to extend the functional lifetime of these transcripts enables γδ T cells to more rapidly and robustly produce certain cytokines in response to secondary signals. Importantly, OPCs show bioactivity when ingested and are safe over a range of doses in all species tested [7,116,119]. Such supplements increase γδ T cells in the periphery or in tissues [119,120]. Following oral delivery of very large doses of the OPCs in mice, a significant reduction of inflammation was seen in dextran sulfate sodium (DSS)-induced colitis [121]. The anti-inflammatory effects are independent of γδ T cells and require αβ T cells. Interestingly, in the absence of αβ T cells, a Rag-protein-dependent population of cells, likely γδ T cells, is responsible for a robust but noninflammatory cytokine response in OPC treated mice in the DSS model [121]. Consistent with this observation, OPC ingestion in some mice was shown to induce increased levels of G-CSF in circulation without obvious deleterious inflammation (unpublished results). Induced G-CSF is normally considered a proinflammatory response, but it can also contribute to protective immune support in certain instances. Clearly, we have much to learn about the myriad effects of ingestion of OPCs on γδ T cells and other immune cells (e.g., αβ T cells) *in vivo*. We expect that these potent plant

chemicals (e.g., OPC) and their derived products may be a safe novel immunotherapeutic and immunomodulator in some settings.

A. Plant Polysaccharides as γδ T Cell-Targeted Immunomodulator

Our study has also identified unique polysaccharides from various plants that are potent agonists for γδ T cells and other cells of the immune system. The first source of polysaccharide agonist was *Funtumia elastica* bark (Yamoa). Yamoa polysaccharides activate γδ T as well other immune cells, such as monocytes, and, when given *in vivo*, enhance protection from infection [110]. Optimal activation or priming of γδ T cells by these polysaccharides requires monocytes or macrophages in a mixed *in vitro* culture. Following our initial characterization of the Yamoa polysaccharides, similar activity was defined in extracts from other plants, including tansy (unpublished), juniper (unpublished), and, most recently, açai [111,122,123]. Many of the polysaccharide preparations being tested, except for those generated from açai, were positive in the limulus amebocyte lysate assay for lipopolysaccharides [124]. Açai polysaccharide responses are conserved in γδ T cells across species, including humans, cattle, and mice [111]. Monocytes and macrophages are also activated by the polysaccharides and are required for optimal responses by the γδ T cell. Instillation of açai polysaccharides into the lungs of mice induces dose-dependent IL-12 production, accumulation of myeloid cells, and activation of local DCs and macrophages [111]. It was subsequently shown that prophylactic or therapeutic nasal administration of açai polysaccharides significantly enhances host innate defense responses against the intracellular bacterial pathogens *Francisella tularensis* and *Burkholderia pseudomallei* [125]. Protection could also be achieved following oral delivery, although responses were more variable. Mechanism of action studies showed that açai polysaccharides enhance IFNγ expression by γδ T cells and NK cells following *F. tularensis* and *B. pseudomallei* infections. Inhibition of IFNγ blocked the protective effect of the polysaccharides [125]. Thus, the stimulation of γδ T cells, as well as other innate immune cells, by açai or similar plant agonists and subsequent type 1 T helper cell-associated responses, could have therapeutic applications in bacterial infections.

Since açai is a commonly ingested dietary supplement and has shown therapeutic benefit following oral delivery [125], we examined the effects of these agonists in two additional intestinal models. Dysbiosis is a condition usually induced by antibiotic use in which the normal flora is disrupted. This state can lead to increased susceptibility to infection and colitis [126]. Mice with dysbiosis were treated with açai polysaccharides to assess whether these polysaccharides could aid in recovery from this susceptible state. When cytokine expression in mesenteric lymph nodes (MLNs) and spleen cells were measured, the feeding of açai polysaccharides induced expression of IL-12 in supernatant fluids from cultured MLN and spleen cells from the treated mice. IL-12 was also detected in the serum of the mice [127]. Expression of IFNγ was also increased in spleen cells from açai polysaccharide-fed mice, similar to the previous finding using nasal administration [125]. No adverse effects were noted in the açai-treated mice. In a model of chemically induced colitis, mice that were fed açai had a reduced deleterious inflammatory response in the gut [127]. Considering that there are no adverse effects following açai ingestion, this polysaccharide could represent a safe and novel approach to stimulating γδ T cells and other innate cells, potentially to promote their innate protective and homeostatic functions at the mucosal surface.

Our next study aimed to examine potential receptors involved in the sensing and responses to the açai polysaccharides by immune cells. Some of the responses were lost in mice lacking functional TLR4 or the innate adaptor protein MyD88. However, neutrophils were still recruited into the peritoneum of these mice following intraperitoneal injection of açai [111]. The role of the β-glucan receptor dectin-1 was particularly investigated, since IL-12 is produced by immune cells following ingestion of β-glucans [128]. Our result demonstrated that açai polysaccharides contain appropriate linkages for recognition by dectin-1 using an inhibition ELISA against β-glucan [127]. Furthermore, açai polysaccharides specifically block binding of anti-dectin-1 antibodies to immune cells in a flow cytometry based assay. Thus, açai polysaccharides bind to multiple innate immune cell receptors, contributing to unique effects of innate and likely downstream adaptive immune responses. Açai polysaccharides can be considered as a new mucosal immunomodulator molecule for the regulation of antigen-specific immune response and inflammation.

B. Microbial Products for the Regulation of γδ T Cells

Activation-based screening assays resulted in the detection of robust agonist activity for γδ T cells in multiple microbial extracts (unpublished results). One such agonist was determined to be amphotericin B (AmB), produced by *Streptomyces nodosus*. AmB is a commonly used antifungal drug that has previously been shown to stimulate innate immune cells [129–131]. AmB induces expression of cytokines in macrophages, mediated by TLR recognition [132–134]. AmB treatment of bovine PBMCs leads to increased expression of IL-2R selectively on γδ T cells, activation of bovine monocytes and NK cells, and enhanced IFNγ from NK cells [112]. Addition of IL-2 to these cultures induces a robust, antigen-independent proliferation of the treated γδ T cells [112]. The agonist activity of AmB is not restricted to cattle, in that similar effects are seen on expression of activation markers and proliferation of γδ T cells in humans and mice as well [112]. Thus the response is highly conserved. In a separate study, AmB was shown to increase IFNγ production in mouse lung cells following *in vitro* infection and costimulation by avirulent *C. burnetii* bacteria [127]. AmB also enhances antibody responses against ovalbumin when used as an immunizing adjuvant [127]. Thus AmB has potential both to enhance innate and acquired responses to infection and to function as a vaccine adjuvant.

Since bovine γδ T cells and NK cells respond to AmB at very low, nontoxic doses, our next experiment aimed to test it in an *in vivo* model of infectious enterocolitis. Calves were given one intravenous injection of approximately 0.029–0.031 mg/kg AmB or saline 24 hours prior to ST infection by the oral route. AmB-treated calves had lower fevers, had overall reduced morbidity, and shed less bacteria into the environment in comparison to control calves [112]. Thus AmB protected from disease severity and reduced the level of shed bacteria. The result suggested that AmB could be used as a potent immunomodulatory molecule to enhance disease resistance against ST in calves.

Our efforts are continuing to assess the immune protective effects of AmB on very young calves, which are highly prone to infection. When bovine calves are less than a week-old, they have a variable colostrum status, and they experience a broad spectrum of natural scouring and respiratory maladies in their first week to 3 months of life. These symptoms are typically caused by rotavirus, coronavirus, *Cryptosporidium*, or a combination of virus and parasite infections. Regardless of the cause, the calves are treated with a hydration therapy. If signs of a secondary bacterial infection become

apparent, antibiotics are administered. The calves were likely preexposed to a variety of pathogens; this would explain the early disease that occurs when they are housed indoors in clean facilities. With years of data on these occurrences of natural illness in our facilities, our study was directed to test whether early minimal treatments with AmB could potentially be used as a broad-spectrum prophylactic immunomodulator. A dose of 0.25 mg/kg injected intravenously as previously described [112] was used in the study. This is approximately 10-fold less than the doses given to patients for antifungal treatment and was determined to be nontoxic in calves. There were two treated groups ($n = 12$ per group). One group received a single injection of AmB on the day of arrival at our facility (AmB x1). A second group received this initial dose on the day of their arrival and a second dose after 10 days (AmB x2). Thus, for the first 10 days, there were 24 calves treated with one dose of AmB. Health condition was assessed by evaluating each animal's subjective appearance and attitude, appetite, temperature, pulse and respirations, fecal consistency, and treatments on a scale of 0–5. Health condition was assessed for all calves twice daily and was compared to calves acquired in the same 3 months in a 5-year span before and after this experiment that did not receive any treatment. In a given period, the study tallied the number of days the calves had perfect health scores (scores of 0). The calves that received one injection of AmB had improved health assessments in their first 10 days in comparison to calves that received no treatment (Fig. 46.1A). The period was then extended to the first 30 days. In this case, the untreated calves were compared to the AmB x1 and AmB x2 groups. Whereas one dose of AmB appeared to benefit in the short term (in the first 10 days), the AmB x1 treatment had no lasting effect. In contrast, calves treated with AmB x2 had a longer-lasting positive benefit (Fig. 46.1B). These data suggest that minimal early doses of an innate immune stimulant could benefit the health of livestock for extended periods. This is especially important for cattle that are subject to repeated infections early in life. It also provides proof of principle that broad-spectrum

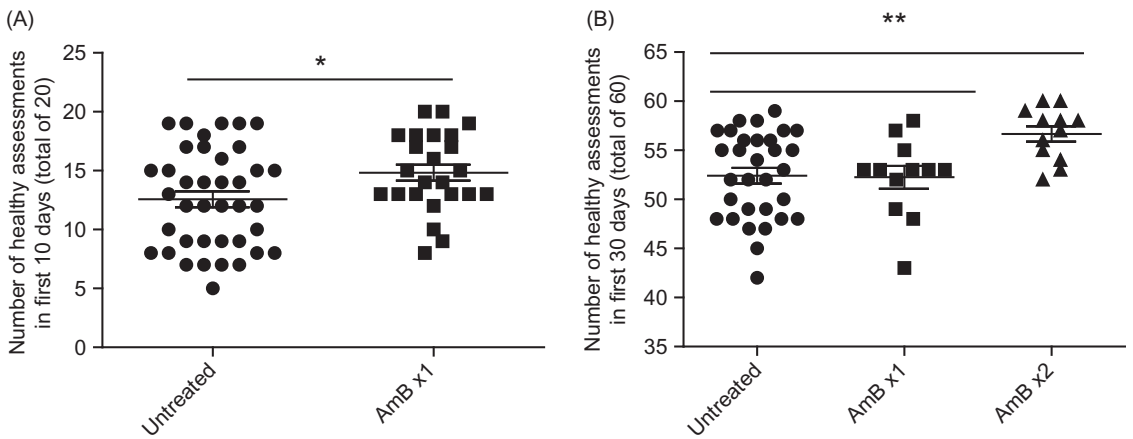

FIGURE 46.1 Early prophylactic treatment of calves with AmB improves their health. Calves were assessed twice daily, and the perfect health assessments in the first 10 days (A) at our facility were tallied for calves that were treated once with AmB (AmB x1), or untreated. (B) Numbers of perfect health assessments in the first 30 days for calves treated with AmB once, on the day of their arrival (AmB x1), or twice, on their first and 10th days in the facility (AmB x2). Statistical analysis was by Student's *t*-test. * P value $< .05$; ** P value $< .01$.

protection against an array of mucosal pathogens can likely be achieved through prophylactic use of an innate immune stimulant.

IX. CONCLUDING REMARKS

Because of their position in the body and their capacity for varied, appropriate responses depending on the environmental signals, γδ T cells are an optimal target for novel immunotherapeutic and vaccine development. Some TCR and TLR agonists that can stimulate γδ T cells have already been used extensively for new cancer treatments. Ample data suggest that the cells might also be specifically stimulated to protect from infectious and inflammatory disease. Considering the growing concerns about the use and overuse of antibiotics, it is critical that such novel approaches to counter infectious agents be pursued.

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ABBREVIATIONS

AmB	amphotericin B
APC	antigen presenting cell
CSF	colony-stimulating factor
IFNγ	interferon gamma
IGF	insulin-like growth factor
KGF	keratinocyte growth factor
MHC	major histocompatibility complex
OPC	oligomeric procyanidin
PBMC	peripheral blood mononuclear cells
ST	*Salmonella enterica* serovar Typhimurium
TCR	T cell receptor
TLR	toll-like receptor
TNF	tumor necrosis factor

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