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CD4⁺ T Cell Responses to Pathogens in Cattle

Anmol Kandel, Magdalena Masello and Zhengguo Xiao

Abstract

Helper CD4⁺ T cells are essential in shaping effective antibody response and cytotoxic T cell response against pathogen invasion. There are two subtypes of pathogen-specific helper T cells in mice and humans; type 1 (Th1) and type 2 (Th2), with Th1 producing interferon-gamma (IFN γ) and Th2 producing interleukin-4 (IL-4). While effective Th1 controls intracellular pathogens like viruses, efficient Th2 controls extracellular pathogens like most parasites. However, the most predominant CD4⁺ T cell subtype in cattle is Th0, which produces both IFN γ and IL-4, and only exists in small amounts in mice and humans. Moreover, in many bovine infections, both IFN γ and IL-4 were detected in the blood and both antigen-specific IgG2 (Th1 associated bovine antibody) and antigen-specific IgG1 (Th2 associated bovine antibody) were upregulated in the serum, suggesting bovine CD4⁺ T cell responses may vary from those in mice and humans. How bovine CD4⁺ T cell differentiation differs from that in mice and humans and how some critical bovine pathogens regulate immunity to establish chronic infections are largely unknown. This chapter summarizes current literature and identifies the knowledge gaps to provide insights into future research in the field.

Keywords: bovine, CD4⁺ T cell differentiation, antigen-specific clones, Th0 responses, pathogens, chronic infections

1. Introduction

CD4⁺ T cells, also called helper T cells, are important regulators of adaptive immune responses, which are antigen-specific and critical in protecting animals from pathogen infections. The control of intracellular pathogens, such as viruses, primarily depends on antigen-specific CD8⁺ T cell response, whereas antibodies (produced by B cells) or humoral immune responses are mostly responsible for the control of extracellular pathogens such as most bacteria and parasites. CD4⁺ T cells are the lynchpin in shaping both CD8⁺ T cell and antibody responses [1, 2].

Common lymphoid progenitor cells migrate from the bone marrow into the thymus for further development and maturation into T cells. Inside the thymus, these progenitor cells proliferate into a large pool of T cells, with each expressing a unique T cell receptor (TCR) through a genetic recombination. After TCR recombination, T cells must go through two selection processes, and only a fraction of them pass through these selections and become either CD4⁺ or CD8⁺ T cells [3]. Surviving CD4⁺ T cells then exit the thymus as naïve CD4⁺ T cells but without the ability to help CD8⁺ T cells and B cells. To become fully functional, naïve CD4⁺ T cells need to become activated and differentiated into specialized effector subtypes; helper

type 1 (Th1) to facilitate CD8⁺ T cell responses, and helper type 2 (Th2) to facilitate antibody responses [4]. Naïve CD4⁺ T cells constantly survey secondary lymphoid tissues to detect pathogens through their antigen-specific TCRs [5]. As opposed to antibodies, which bind directly to pathogens or their derivatives, TCRs can only recognize short chains of amino acids (derived from pathogens) that are presented by major histocompatibility-II (MHC-II) expressed on antigen presenting cells (APCs) [2]. This recognition process provides the 1st signal required to activate naïve CD4⁺ T cells. Along with the 1st signal, APCs also offer co-stimulation as the 2nd signal and cytokine signaling, as the 3rd signal, to the naïve CD4⁺ T cell. Combined, these three signals coordinate CD4⁺ T cell differentiation into distinct effector subtypes with different helper functions [2].

Studies in humans and mice have identified numerous helper subtypes, including: Th1, Th2, Th3, Th9, Th17, Treg, and Tr1 [2, 6]. Among these, Th1 and Th2 are considered to play major roles in defending the host from pathogen invasion [7–9]. Th1 cells help CD8⁺ T cells to gain killing functions, which leads to apoptosis of infected cells and induces Interferon gamma (IFN γ) mediated immunity [10–13]. On the other hand, Th2 cells help B cells differentiate into plasma cells, which produce pathogen-specific antibodies [14]. Antibodies or humoral immunity contribute to the control of extracellular pathogens by mechanisms like neutralizing toxins, preventing bacterial attachment to the host cell, and stimulating basophil and mast cells to release toxic chemicals that induce the expulsion of large gastrointestinal parasites [15, 16]. Although antibodies are mostly responsible for controlling extracellular pathogens, they can also play important roles in cell-mediated killing of intracellular pathogens [17]. For instance, during intracellular infections in mice, Th1 cells help B cells become plasma cells that secrete antigen-specific immunoglobulin subtype G2a (IgG2a), which in turn can help killing infected cells through antibody dependent cytotoxicity (ADCC) [18, 19]. In short, Th1 is responsible for control of intracellular pathogens mostly through shaping CD8⁺ T cell responses and Th2 is for control of extracellular pathogens through antibody responses. In addition, antibodies can be involved in both Th1 and Th2 responses, but with unique subtypes, such as IgG2 for Th1, and IgG1 for Th2 in cattle. This will be discussed further in Section 2.

There are many similarities in the immune system across species. Therefore, knowledge generated from the research in mice and humans has been extensively applicable to study immune responses in cattle [20–23]. In the past several decades, however, unique features have been discovered in the bovine immune system that are not shared with that of mice and humans, such as high prevalence of circulating $\gamma\delta$ T cells [24], production of IL-10 by $\gamma\delta$ T cells [25], regulation of CD4⁺ T cell activation by neutrophils [26], which are able to secrete IL-10, and high prevalence of hybrid helper T cells (*i.e.*, co-express both Th1 and Th2 cytokines), which is relatively low in humans and mice [22, 27, 28].

Cattle industry suffers billions of dollar's losses annually due to infections, and many of the commercially available vaccines for cattle are not fully effective [29–32]. Understanding the mechanisms underlying bovine CD4⁺ T cell differentiation, which seems to be partially different from that of mice and humans, is critical to identify novel strategies to achieve more effective immunity after vaccinations, such as through generating strong Th1 responses against intracellular pathogens and Th2 responses against extracellular pathogens. In this chapter, we will summarize the current knowledge and key findings on bovine CD4⁺ T cell responses, highlight the existing knowledge gaps, and provide some insights on future directions.

2. CD4+ T cells regulate adaptive immunity

Naïve CD4+ T cells exit the thymus and search for pathogen-derived antigens presented by APCs in secondary lymphoid tissues (*e.g.*, lymph nodes and the spleen). During infections, pathogens break through barriers (Physical, chemical etc) of the host to establish infection in the local tissues [33]. As a result, the immune system in the host initiates an inflammatory response through recruitment of immune cells such as neutrophils to the site of infection, which secretes inflammatory cytokines and chemokines [34, 35]. These chemokines provide signals for further recruitment of APCs to the site of infection. APCs constantly search for invading pathogens through recognizing pathogen associated molecular patterns (PAMPS) on pathogens by their pattern recognition receptors (PRRs) [36]. For example, Toll-like receptor-4 (TLR-4) on APCs can recognize the lipopolysaccharide (LPS) present on the cell membranes of gram-negative bacteria [37]. After recognition, APCs engulf the pathogen, break it down into small peptides, and finally present the peptides to CD4+ T cells in the secondary lymphoid tissue. Recognition of this peptide–MHC-II complex by the TCRs on the naïve CD4+ T cells provides the 1st activation signal, as shown in **Figure 1** [41]. At the same time, co-stimulatory molecules on the CD4+ T cell surface (*e.g.*, CD28) recognize their corresponding ligands on the APC surface (*e.g.*, CD80 or CD86), which provides the 2nd activation signal [42]. The final and 3rd signal, which occurs simultaneously with antigen stimulation and co-stimulation, is provided by cytokines such as Interleukin-12 (IL-12) or Interleukin-4 (IL-4) that not only enhance the activation process, but also drive CD4+ T cell differentiation into a specific subtype (*e.g.*, Th1 or Th2) [2, 43]. Therefore, APCs can provide all 3 signals to naïve CD4+ T cells, which facilitates their activation and differentiation (**Figure 1**). Pathogens can regulate host helper T cell response through targeting any of the three signals directly or indirectly, which will be discussed in Section 5. Recently, we have reported that bovine CD4+ T cells respond to three signals in a way similar to that in humans and mice [44]. Furthermore, IL-12 and neutrophils can work on bovine CD4+ T cells synergistically to enhance their production of IFN γ [44].

Figure 1.
 Three-signal model for CD4 + T cell activation: The 1st signal is provided when TCRs recognize the peptide–MHC-II complex presented by APC; the 2nd signal is initiated when CD28 on CD4+ T cells interacts with CD80/86 on APCs, and the 3rd signal is triggered by cytokines released from the APCs and other cells. CD28/CD80/CD86 interaction is used as an example. This figure was adapted from previous reviews [38–40].

2.1 Th1 cells coordinate CD8+ T cell response to intracellular pathogens

During the infection, the host responds to the intracellular pathogens by inducing cytokines such as IFN γ and IL-12 from APCs like macrophages and dendritic cells (DCs), which further leads to the polarization of CD4+ T cells into a Th1 sub-type. IFN γ and IL-12 enhance the expression of transcription factor T-bet, which directs Th1 differentiation in the activated naïve CD4+ T cells (**Figure 2a**) [51, 52]. More specifically, when bound to their receptors on naïve CD4+ T cells, these cytokines induce the activation of transcription factor STAT-1 or STAT-4 respectively, which in turn causes T-bet upregulation [53]. Subsequently, T-bet induces histone modification and binds to the promoter region of Th1-specific cytokine genes, which leads to enhanced expression of IFN γ [51, 52]. In addition, T-bet also inhibits Th2 differentiation by repressing the transcription of Th2 specific genes, such as GATA-3, which is the transcription factor responsible for IL-4 expression [51, 54]. Thus, IFN γ and IL-12 induce Th1 differentiation, which leads to IFN γ production and suppression of Th2 differentiation.

One key functions of differentiated Th1 cells is to facilitate the activation of CD8+ T cells by “conditioning” dendritic cells; a process that induces dendritic cell (DC) maturation by modifying their cytoskeletal structure, upregulating co-stimulatory molecules, and by enhancing their migration to secondary lymphoid tissues [55–57]. Once conditioned, these DCs can induce CD8+ T cell activation as shown in **Figure 2(b)**. Although these two processes, conditioning of DCs and activation of CD8+ T cells, might occur simultaneously, some researchers argue that this process may occur in two sequential steps: conditioning DC first, followed by CD8+ T cell activation [56, 58, 59]. Activated CD8+ T cell secretes cytotoxicity-related proteins such as perforin and granzyme-B. While perforin forms pores at the cell membrane, granzyme enters through these pores and cause apoptosis of the infected cell [60]. Additionally, antigen-specific CD8+ T cells can kill infected cells through caspase mediated pathway, when Fas molecules expressed on the infected cells interact with Fas Ligand expressed on the antigen-specific CD8+ T cells [61].

IFN γ is a critical cytokine performing multiple functions to assist Th1 response against intracellular pathogens in mice, humans and cattle [62]. Although many types of immune cells can produce IFN γ including NK cells, DCs, macrophages and B cells, it is the signature cytokine of Th1 subtype [27]. Th1 produced IFN γ plays a critical role in regulating the Th1 response. IFN γ can recruit immune cells to the site of infection and promote anti-microbial activities of neutrophils and macrophages by inducing oxidative burst and production of reactive oxygen species (ROS) [62–65].

Figure 2. Th1 help to the activation of CD8+ T cell. A) IFN γ and IL-12 bind to their corresponding receptors on naïve CD4+ T cells during activation, which leads to T-bet expression and Th1 differentiation. This figure was adapted from previous reviews [45–47]. B) Once differentiated, Th1 effector cell conditions dendritic cell, which in turn activates CD8+ T cell. This figure was adapted from previous reviews [48–50].

IFN γ is directly involved in blocking viral replication, as well as enhancing the cytotoxic activity of CD8+ T cells [66, 67]. Moreover, IFN γ can enhance the number, mobility, and cytotoxicity of CD8+ T cells [67, 68].

During infection caused by intracellular pathogens, Th1 produced IFN γ can induce IgG subtype switching in activated B cells. However, this subtype switching may differ among the species. For example, it induces production of IgG2a in mice and IgG2 in cattle but IgG1 and IgG3 in humans (Table 1) [18, 69, 73]. These IgG subtypes induced by IFN γ can facilitate multiple mechanisms such as ADCC to kill intracellular pathogens, such as *Coxiella burnetii*, *Listeria monocytogenes*, and *Toxoplasma gondii* in mice [19, 82].

2.2 Differentiated Th2 cells coordinate humoral response against extracellular pathogens

During infections caused by extracellular pathogens, innate immune cells such as basophils, eosinophils, and innate lymphoid cells (ILCs) produce and secrete IL-4 [83, 84]. Together with 1st and 2nd signals, IL-4 signaling on naïve CD4+ T cell upregulates GATA-3 (GATA binding protein-3), a critical transcription factor for Th2 differentiation [85, 86]. GATA-3 knockout mice mounted impaired Th2 responses [87, 88]. When IL-4 binds to its corresponding receptor on the surface of naïve CD4+ T cells, it activates STAT-6, which turns on pathways leading to GATA-3 expression (Figure 3a) [93, 94]. Consecutively, GATA-3 promotes Th2 differentiation by inducing histone acetylation and enhancing transcription of the IL4 gene [83, 95]. In addition, GATA-3 is capable of suppressing Th1 differentiation by downregulating transcription and expression of molecules such as the IL-12 receptor β 2, IFN γ , STAT-4, and possibly T-bet [96].

Once differentiated, Th2 cells are capable of activating B cells to produce antibodies that defend the host against extracellular pathogens [97, 98]. During B cell activation, Th2 cells recognize peptide–MHC-II complexes expressed on B cells [99, 100] and provide co-stimulation via CD40L, which are both necessary for B cell activation [101] (Figure 3b). Importantly, IL-4 signaling induces isotype and subtype switching of B cells towards IgE and IgG1 production, which are key antibodies for controlling extracellular pathogens in mice and cattle [102].

Although antibodies can assist CD8+ T cell responses during intracellular infections, they play a major role in controlling infections caused by extracellular pathogens [13, 103, 104]. Antibodies can prevent the attachment of extracellular

Figure 3.
Th2 help to the activation of B cell. A) IL-4 binds to its receptor on naïve CD4+ T cells during activation, which induces GATA-3 activation and Th2 differentiation. This figure was adapted from previous reviews [46, 89, 90]. B) Once differentiated, Th2 cells secrete IL-4 and provide antigen stimulation and co-stimulation to a B cell. This figure was adapted from previous reviews [91, 92].

bacteria to the host cell, facilitate phagocytic killing, and neutralize toxins [13, 105–108]. In addition, different antibody isotypes and subtypes can have different functions. For instance, IgE can bind to both low and high-affinity receptors (FcεRI and FcεRII) on mast cells and basophils, which results in the degranulation and release of chemicals (*e.g.*, histamine, leukotrienes) that either kill parasites directly, or induce hyper-contraction of intestinal smooth muscle to promote their expulsion [109–112].

In addition to IL-4, other cytokines such as IL-5, IL-9 and IL-13 are also involved in the control of extracellular pathogens. For example, IL-9 promotes production of IgE and proliferation as well as maturation of mast cells, which rapidly infiltrate the site of infection [113, 114]. Similarly, IL-5 induces differentiation, maturation, and infiltration of eosinophils to the site of infection [114]. Infiltrated mast cells and eosinophils, when cross-linked by antigen-specific IgE, degranulate (*i.e.*, release histamine and leukotrienes) to kill or expel gastrointestinal parasites. IL-13 on the other hand, plays a significant role in the expulsion of parasites by inducing regeneration of the intestinal epithelium and contraction of smooth muscle cells in the intestine [98, 115]. Nevertheless, there are multiple cytokines involved in the differentiation of Th2 responses, but IL-4 is considered the most critical one.

2.3 Th1/Th2 cytokines induce immunoglobulin class switching during infection

Antibodies produced by activated B cells during infection are classified into five different classes (*i.e.*, IgM, IgG, IgA, IgD and IgE) based on their structure [116]. Among them, IgG is the most abundant in serum, and it has four different subtypes, namely: IgG1, IgG2, IgG3 and IgG4 [116]. Each antibody has two structural segments (heavy and light chains) and two functional segments (F_{ab} and F_c portions). While association of heavy chain with the light chain at the F_{ab} portion forms antigen-binding sites, only the constant portion of the heavy chain constitutes the F_c segment that regulates the effector function of the antibody. During infection, activated B cells undergo isotype or subtype switching, a process that involves switching of F_c segment but not of the F_{ab} segment. Briefly, DNA in B cells contains multiple heavy chain constant genes (or C_H genes) that encode various types of Fc segments [117]. During infections, Th1 and Th2 cytokines provide signals to the activated B cells to select a specific C_H gene for the heavy chain, thus producing a specific isotype or subtype of immunoglobulins with the same antigen specificity [118]. For example, IFNγ can induce subtype switching to IgG2a to enhance the killing of infected cells in mice; similarly, IL-4 can induce switching to IgG1 to promote humoral immunity (**Table 1**) [70–72, 119]. Historically, characterizing serum IgG subtypes was a common practice to define the immune response in clinically ill cattle; the greater concentration of serum IgG2 typically indicated a Th1 response, whereas greater IgG1 indicated a Th2 response. Interestingly, the Th1 induced IgG subtypes may vary among the mice, humans and cattle species as shown in **Table 1**.

Species	Th1 immunity	Th2 immunity	References
Mice	IgG2a	IgG1	[69–72]
Humans	IgG1 and IgG3	IgG4	[73–79]
Cattle	IgG2	IgG1	[18, 80, 81]

Table 1.
Th1- and Th2-associated IgG subtypes in mice, humans, and cattle.

2.4 Cytokines and transcription factors mediate Th1/Th2 cross-regulation

In humans and mice, multiple lines of evidence support that Th1 differentiation inhibits Th2 differentiation, and vice versa [120, 121]. For example, *in vitro* experiments reveal that IFN γ inhibits Th2 differentiation whereas IL-4 suppresses Th1 differentiation [122–124]. In addition, studies using knockout mice and retroviral-transduced CD4⁺ T cells demonstrate that T-bet blocks Th2 differentiation by inhibiting the transcription of genes associated with Th2 cytokine production [54, 125]. Similarly, GATA-3 prevents Th1 differentiation by suppressing the transcription of genes associated with Th1 cytokines, and interfering with Th1-promoting transcription factors [126, 127]. Collectively, these findings confirm that Th1 and Th2 transcription factors and cytokines cross-regulate each other, ensuring that CD4⁺ T cells differentiate into either Th1 or Th2 cells. In cattle, however, most of the differentiated clones represent a “hybrid” that co-expresses both IFN γ and IL-4 in the same cell (explained in detail in Section 3) [22, 128]. While it is clear in mice and humans that T-bet and GATA-3 are the transcription factors that regulate expression of IFN γ and IL-4 respectively, at this moment, it is unclear if this is equally true for cattle. In addition, we do not know if the co-production of both Th1 and Th2 cytokines in the hybrid bovine clones corresponds to the co-expression of both transcriptional factors. Therefore, further research is needed to understand the underpinning regulatory mechanism of hybrid clone differentiation in cattle.

2.5 Distinct Th1 and Th2 are the most dominant antigen-specific clones in mice and humans

In mice and humans, Mosmann et al. and Romagnani et al. stimulated single CD4⁺ T cells *in vitro* and established antigen-specific CD4⁺ T cell clones, which they classified mostly into Th1 and Th2 subtypes. Although, in both mice and humans, clear-cut Th1 or Th2 were the dominant clones, a small percentage of hybrid clones (named “Th0” clones), that co-produced Th1 and Th2 cytokines (IFN γ and IL-4), were also observed [27, 28]. Subsequently, follow-up research verified the existence of these hybrid clones, which were only a small fraction of the total clones (*i.e.*, only 9.6% clones were Th0) [124, 129–135]. Therefore, at this moment, the consensus in the fields of murine and human immunology is that Th1 and Th2 are the major effector cells that orchestrate immune responses against intracellular and extracellular pathogens, respectively, and that Th0 are short-lived “intermediate” cells [131, 136, 137].

2.6 Th0 is the most dominant antigen-specific clone in cattle

Just a few years after the discovery of the Th1/Th2 subtypes in humans and mice, Brown et al. successfully investigated bovine Th1/Th2 response through the establishment and analysis of antigen-specific CD4⁺ T cell clones. Peripheral blood mononuclear cells (PBMC) were purified from cattle challenged by experimental pathogens: either intracellular pathogens (*Babesia bovis*, *Babesia bigemina*) or extracellular pathogens (*Fasciola hepatica*) [22]. These purified PBMCs (that contained pathogen-specific CD4⁺ T cells), were stimulated with antigens derived from the same pathogen used for the challenge, to generate pathogen-specific CD4⁺ T cell clones, which were then analyzed and classified based on the detection of Th1/Th2 cytokine mRNA. The authors reported that, regardless of the type of pathogen used in the challenge, most bovine clones were Th0 that co-expressed IFN γ and IL-4 (*e.g.*, more than 60% *Babesia* species -specific and more than 90%

Fasciola hepatica-specific clones were Th0) [22]. These observations indicated that bovine Th1/Th2 responses might be at least partially different from the typical murine and human Th1/Th2 responses, as the frequency of bovine Th0 clones was significantly higher than that of murine and humans. Later, when researchers used the Th0 clones specific to an antigen of *Babesia bigemina* to stimulate B cells *in vitro*, both, Th1-related IgG2 and Th2-related IgG1 were detected in the supernatant culture, suggesting that Th0 is capable of performing functions of both Th1 and Th2 cells [138].

3. Many critical bovine pathogens induce Th0 responses

In cattle, mixed Th1/Th2 cytokines (both IFN γ and IL-4) have been detected in cultured PBMCs, or Draining Lymph Nodes (DLNs), or local tissues in large number of diseases. Most researchers commonly refer to this as the bovine Th0 response, which may include clones of all three types (Th1, Th2, and Th0) [128, 139–141]. It is important to note that while Th0 clones can produce both IFN γ and IL-4, Th1 and Th2 clones can only produce a single cytokine, either IFN γ or IL-4 (**Figure 4**). Therefore, a mixed population of Th1, Th2, and Th0 cells possibly contributes to the induction of Th0 responses in most of the bovine diseases as explained in Section 4.

Figure 4. Helper T cell responses to infections in cattle. Pathogen infections in cattle may induce three types of CD4 $^{+}$ T cell responses: Th1, Th2 and Th0. Th1 responses are characterized by Th1 clones that produce IFN γ , Th2 responses include Th2 clones that produce IL-4, and Th0 response could induce mixed populations of clones: Th1, Th2 and Th0. Th0 clones co-express both IFN γ and IL-4.

4. Advancement of technology facilitates the progress in bovine immunology

Technology is a critical factor that drives the advancement of science, and bovine immunology is not an exception, particularly regarding bovine CD4 $^{+}$ T cell research. In the late 80s, the study of bovine Th1/Th2 responses depended heavily on the measurement of cytokines in the supernatant of cultured CD4 $^{+}$ T cells through simple biological assays such as ELISA, or detection of IgG subtypes in the serum of infected animals through ELISA or immunoblotting techniques. In this context, upregulation of supernatant IFN γ and serum IgG2 would represent a Th1 response, upregulation of IL-4 and detection of serum IgG1 would indicate a Th2 response [18, 80], and detection of both cytokines and both IgG subtypes (IgG1 and

IgG2) would represent a Th0 response [142]. In the late 90s, advancements in molecular biology enabled scientists to measure cytokines at the transcriptional level (mRNA). Thus, reverse transcription polymerase chain reaction (RT-PCR) was commonly used to detect the presence of mRNA of Th1/Th2 cytokines in PBMCs, DLNs, and tissues of infected cattle [143–145]. In the next decade, the advent of quantitative PCR (qPCR) improved the detection of Th1/Th2 transcripts from a qualitative to a quantitative level [146]. Later, with the invention and use of flow cytometry, scientists were able to measure protein production of Th1/Th2 cytokines on a population level [147]. More recently, some very exciting technological advancements have been developed, such as single-cell RNA sequencing, proteomics, metabolomics, confocal microscopy, which are considered excellent tools for a deeper understanding of immune mechanisms [148–152]. Therefore, the advancement of bovine immunology research is closely associated with the development of novel technology in science, especially in the context of understanding Th1/Th2 responses in cattle.

4.1 Most intracellular pathogens induce either a Th1 or Th0 response in cattle

During pathogen invasion, the host mounts a CD4⁺ T cell response that may or may not be effective enough to clear the infection. In humans, ineffective CD4⁺ T cell responses are associated with increased pathogenesis and progression towards chronic infections [153]. Cattle mostly launch either Th1 or Th0 responses against intracellular pathogens [154–157]. However, some bovine pathogens are able to establish chronic infections, which is possibly associated with ineffective CD4⁺ T cell responses [128, 158].

As observed in mice and humans, bovine Th1 responses are considered to be protective against diseases caused by intracellular pathogens such as *Theileria annulata*, and *Anaplasma marginale* [154, 155]. Indeed, researchers in the late 80s found that transferring serum from an immune animal into animals infected with theileriosis was not effective at controlling infection [159]. Several groups later discovered that CD8⁺ T cell responses but not humoral responses were effective at controlling disease, since antigen-specific CD8⁺ T cells from recovered animals demonstrated effective cytotoxicity to the autologous infected cells *in vitro* [160–162]. Further research revealed that *in vitro* activation of T cells with *Theileria*-infected macrophages predominantly induced IFN γ expression [163]. Similar to theileriosis, Th1 responses were also protective against *Anaplasma marginale* [155, 164]. In both infected and vaccinated animals, circulatory IFN γ levels were higher relative to their healthy counterparts [155, 164]. Similarly, IgG2 was increased in cattle infected with *Anaplasma marginale* [165]. Collectively, in both theileriosis and anaplasmosis, hosts seem to induce effective Th1 responses.

Bovine pathogens such as *Mycobacterium tuberculosis* and *Mycobacterium paratuberculosis* can shift a Th1-dominant response towards a Th0- or a Th2-dominant response as the infection progressed [128, 158]. In bovine tuberculosis, high levels of circulatory IFN γ are detected at the early stage of disease that could inhibit Mycobacterial growth, suggesting that the host most likely mounts an early Th1 response [166–168]. However, in the chronic tuberculosis increased serum IgG1 (a Th2 associated antibody) is detected in the serum [128]. In line with these observations, in mice and humans, IFN γ expression was upregulated during the early phases of tuberculosis, however, at the chronic phase IL-4 expression was enhanced [169–172]. Collectively, these results suggest that *Mycobacterium tuberculosis* can shift an IFN γ (Th1) dominant response towards an IL-4 (Th0 or Th2) dominant response at the later stages of disease. Interestingly, the frequency of antigen-specific Th0 clones was higher in animals showing severe lung pathology

than in animals having less severe lesions [128]. Therefore, the authors speculated that Th0 clones may play an important role in skewing the immune response from Th1 (IFN γ) response towards Th0 or Th2 (IL-4) response during the progression of infection (**Figure 4**) [128]. As in *Mycobacterium tuberculosis* infections, the immune responses to *Mycobacterium paratuberculosis* switches from Th1 response to Th2 response while the disease progresses from subclinical to clinical stage [158]. In *Mycobacterium paratuberculosis* infections, cattle show high levels of IFN γ in the supernatant of cultured PBMCs and high levels of IFN γ mRNA in the intestinal ileal tissues, suggesting an induction of Th1 response against this pathogen [173, 174]. Importantly, cattle clinically infected with *Mycobacterium paratuberculosis* had significantly lower expression of IFN γ in ileal and caecal lymph nodes compared to cattle at sub-clinical stage of infection [175]. This finding supports the notion that the suppression of the Th1 response at the sub-clinical stage of the disease might have contributed to the progression of disease into the clinical stage. Furthermore, increased antigen-specific IgG1 was detected in animals infected with *Mycobacterium paratuberculosis* at the clinical stage, suggesting a Th2 response [176, 177]. Together, these findings suggest that the shift of an early-induced Th1-dominant response towards a Th0 or Th2-dominant response is associated with disease progression in both bovine tuberculosis and bovine paratuberculosis.

During the early phases of Respiratory syncytial virus (RSV) infection in humans and mice, the host launches a Th1/Th2 mixed response (*i.e.*, both IFN γ and IL-4), which then shifts towards a Th2 response (*i.e.*, increased circulatory IL-4) during chronic infection [178–180]. Consistently, cattle infected with Bovine respiratory syncytial virus (BRSV) seem to mount a Th0 response, which turns into a Th2 response during chronic infection [143, 181]. In the past, reports suggested that both IFN γ and IL-4 were detected in the peripheral blood, lymph sample and pulmonary tissues of BRSV infected animals at the early stage, indicating the induction of a Th0 response [144, 181, 182]. Similarly, both IgG1 and IgG2 were detected in the serum, although they peaked at different times during infection [182]. Conversely, IgE and IgG1 levels increased as the infection progressed towards the chronic stage, suggesting a gradual shift from a Th0 towards a Th2 response [143, 181–183]. Collectively, these studies indicate that these pathogens can switch the early-induced Th0 response towards a Th2 response during chronic infection.

The efficacy of Th0 responses in controlling infections caused by bovine intracellular pathogens is unclear. While Th0 responses seem ineffective against some bovine diseases such as tuberculosis, they can be protective against bovine babesiosis and non-cytopathic Bovine viral diarrhea virus (ncp- BVDV) infection [156, 157, 184]. In Babesiosis, both CD8 $^{+}$ T cell responses and humoral responses appear critical to clear infection. For instance, increased numbers of antigen-specific CD8 $^{+}$ T cells were detected in the peripheral blood of vaccinated animals [156]. Similarly, transferring serum from an immune animal containing both IgG1 and IgG2 can clear infection of sick animals [184]. In this regard, *in vitro* experiments have demonstrated that the majority of Babesia-specific clones are Th0, which are able to stimulate B cells to produce both IgG1 and IgG2 [22, 138, 184]. Furthermore, IgG1 and IgG2 antibodies were found effective to prevent invasion of bovine erythrocytes by *Babesia bovis* merozoite *in vitro* [185]. Collectively, these findings suggest that Th0 responses promote both the cytotoxic activity of CD8 $^{+}$ T cells, and neutralizing activities of IgG subtypes [156].

Cattle might launch different immune responses against different biotypes of the same intracellular pathogen [145, 186, 187]. For instance, while Th0 response was induced against the non-cytopathic (ncp) biotype of Bovine viral diarrhea virus (BVDV), Th1 response was induced during infection caused by the cytopathic biotype (cp) [188]. In experiments with T cells isolated from the ncp-BVDV

Disease	Detected cytokines	Serum antibodies	References
Theileriosis	IFN γ (RT-PCR)	—	[154, 163]
Anaplasmosis	IFN γ (ELISA)	IlgG2 /IlgG1 + IlgG2	[155, 189]
Babesiosis	IFN γ + IL-4 (RT-PCR)	—	[22, 138, 156]
Respiratory syndrome	IFN γ +IL-4(flow cytometry)	IgE	[181, 182]
Bovine viral diarrhea	IFN γ / IFN γ + IL-4 (q-RT-PCR)	IgG2/ IgG1 + IgG2	[145, 186, 187]
Tuberculosis	IFN γ to IL-4 shift (PCR)	IgG2 to IgG1 shift	[190]
Paratuberculosis	IFN γ to IL-4 shift (ELISA+ RT-PCR)	IgG2 to IgG1 shift	[158, 191, 192]

Table 2.
Characterization of helper T cell responses in diseases induced by bovine intracellular pathogens. Th1/Th2 cytokines were detected in cultured PBMCs and DLNs; IgG subtype was tested in the serum.

infected cattle, IL-4 protein in the supernatant of CD4+ T cell culture and IFN γ protein in CD8+ T cell culture were detected, suggesting possible induction of Th0 response [157]. More recently, Palomares et al. analyzed cytokine expression in tracheo-bronchial lymph nodes and found that both IFN γ and IL-4 were detected in ncp-BVDV-infected cattle, but IL-12 mRNA was only detected in cp-BVDV-infected cattle [145]. Additionally, while only IgG2 was detected in the serum of cp-BVDV-infected cattle, both IgG1 and IgG2 were detected in ncp-BVDV infected cattle after day 35 of infection [187]. These results collectively reveal that ncp-BVDV induces a Th0 response whereas cp-BVDV induces a Th1 response in infected cattle.

Thus, available literature supports the notion that cattle launch either Th1 or Th0 responses against most infectious diseases caused by intracellular pathogens (**Table 2**). Moreover, although further research is required to confirm these findings, the shift from an early Th1 or Th0 response towards a Th2 response is associated with progression of disease towards chronic condition.

4.2 Most extracellular pathogens induce either a Th2 or Th0 response in cattle

In mice and humans, Th2 responses are typically effective in controlling extracellular pathogens. In this regard, Th2 cytokines can induce processes such as IgG subtype switching and migration of mast and eosinophils to the site of infection that are critical for defending the host against extracellular bacteria and parasites [98]. In cattle, most of extracellular parasites induce either Th2 or Th0 responses [193–195]. However, some pathogens are capable of suppressing Th2 response, which is associated with the establishment of chronic infections [196].

Generally, Th2 responses are effective in controlling gastrointestinal nematodes such as *Cooperia oncophora* [197, 198]. Infected animals had increased level of antigen-specific IgG1 (Th2 associated antibody) in the serum [199]. Consistently, a high titer of pathogen specific IgG1 was associated with a better immune response [200]. Similarly, increased numbers of peripheral eosinophils (a Th2 response feature) was associated with increased expulsion of cooperial larvae [200]. Importantly, cytokine analysis of the intestinal tissue of disease resistant cattle demonstrated high expression level of IL-4 and IL-13 mRNA compared to those susceptible animals [201, 202]. These results offer compelling evidence that Th2 response is critical to control infection caused by some extracellular pathogens such as *Cooperia oncophora*.

Interestingly, some extracellular parasites such as *Dictyocaulus viviparus* (lung worm) are capable of shifting the initial Th2 or Th0 response into an ineffective Th1 response to establish chronic infections [203, 204]. At the early stage, both IL-4 and IFN γ were detected in the lungs and DLNs after day 15 of lung worm infection, indicating an initial Th0 response [205]. However, subsequent research only detected increased IL-4 mRNA for a short period of time in the Broncho-alveolar lavage fluid (BALF) of infected cattle, suggesting a possible Th2 response [206]. In line with this finding, high level of total IgE (antigen-specific plus non-specific) in the serum and BALF was associated with the clearance of lungworm [203]. Furthermore, in the chronically infected animals the detection of Th1 associated antibody (*i.e.*, IgG2) in the serum, was associated with increased lungworm larval excretion [204]. These data indicate that bovine lungworm might shift the early-induced Th0 or Th2 response towards a Th1 dominant response to establish chronic infection.

In cattle *Fasciola hepatica* (liver fluke) can modulate the early-induced Th1 or Th0 response into an ineffective Th2 response at the later phases of the disease [207, 208]. Of note, although an initial Th1 response was observed in the peripheral blood, a Th0 response was also observed inside the hepatic lymph node, as indicated by the detection of both IFN γ and IL-4 [209–212]. Collectively, these experiments suggest that cattle might launch either a Th1 or a Th0 response at the early stages of liver fluke infection. However, at later stages, the response is shifted to a Th2 response as indicated by the significantly increased expression of IL-4 mRNA (x6) and significantly reduced expression of IFN γ mRNA (x6) in the hepatic tissue of infected animals, which is consistent with several other reports [140, 213, 214]. In line with these observations, peripheral blood lymphocytes obtained from chronically infected animals failed to induce IFN γ secretion when co-cultured with adult fluke antigen *in vitro* [209]. Importantly, chronically infected cattle typically show high levels of antigen-specific IgG1 in the serum [140]. Altogether, these findings suggest that *Fasciola hepatica* might switch a Th1 or a Th0 dominant response to a Th2 dominant response at the chronic stage of disease.

Ostertagia ostertagi (OO), an economically important abomasal nematode, typically induces Th0 response [215]. Bovine OO usually causes chronic infection and requires long-term repetitive exposure (at least 2 years) to develop effective immunity [216]. Both pathogen-specific IgG subtypes (IgG1 and IgG2) were detected in OO-infected cattle, with higher serum IgG1 titer than IgG2 [217]. Similarly, mRNAs of both IL-4 and IFN γ were upregulated in the abomasal lymph nodes of experimentally infected cattle from day 11 to day 28 after infection, suggesting the induction of a Th0 responses [215]. In contrast to this observation, subsequent research demonstrated induction of Th2 response in the abomasal lymph nodes of OO infected cattle [218]. The differences observed between these two experiments might be explained, at least in part, by the differences in time points for cytokine detection and in the number of L3 larvae used for experimental infection. More specifically, while Canals et al. measured cytokine expression from day 11 to day 28 post infection and used 200,000 L3 larvae for experimental infection, Claerebout (2005) measured cytokine expression after 8 weeks post primary infection and only used 25,000 L3 larvae [215, 219]. Recently, Mihi et al. experimentally infected cattle with 200,000 L3 larvae and tested the gene expression of Th1/Th2 cytokines at different time points; interestingly, the authors observed a positive association between upregulation of both IFN γ and IL-4 (in mucosa) with migration of adult (L5) worms out of gastric gland towards abomasal mucosa [146]. These observations suggest that *Ostertagia ostertagi* may modulate the bovine immune response by inducing a Th0 response, which is ineffective in controlling OO and leads to the establishment of chronic infections.

Disease	Detected cytokines	Serum antibodies	References
Cooperiosis	IL-4 (q-PCR)	IgG1	[201, 202, 227]
Lung worm infection	IL-4 /IL-4+ IFN γ (RT-PCR)	IgG1, /IgG1 + IgG2	[228, 229]
Trichomoniasis	—	IgG1 + IgG2	[221, 222, 230]
Fasciolosis	IL-4 (ELISA+ qPCR)	IgG1	[211, 231, 232]
Ostertagiasis	IL-4 + IFN γ (qPCR/RT-PCR)	IgG1 + IgG2	[146, 215, 218]

Table 3.
Characterization of helper T cell responses in diseases caused by bovine extracellular pathogens. Th1/Th2 cytokines were detected in cultured PBMCs and DLNs; IgG subtype was tested in the serum.

Immune response against extracellular pathogens may vary at the systemic and local levels, such as in bovine trichomoniasis, where Th0 response is induced in the serum, and Th2 response in the mucosal secretion [220, 221]. More specifically, *Trichomonas foetus* upregulates both IgG1 and IgG2 in the serum but only IgG1 in local secretions from cervix, vagina, and uterus [220, 221]. Furthermore, animals immunized with specific antigen of *Trichomonas foetus* showed resistance to the experimental challenge, which was associated with the upregulation of both antigen-specific IgG1 and IgG2 in the serum [222, 223]. *Trichomonas foetus* seems to induce a Th0 response in the circulation, but a Th2 response in the mucosa. In addition, the systemic Th0 response may be protective against *Trichomonas foetus* rechallange.

Generally, Th2 response is effective in controlling extracellular bacteria [224]. For instance, Th2 response controls *Clostridium difficile* infection in humans and *Streptococcus suis* infection in pigs [224, 225]. In cattle, only few reports are available on CD4+ T cell response to extracellular bacteria such as *E. coli*. At this moment, the common understanding is both CD8+ T cell and antibodies seem to be critical to generate protective immunity (consistent with humans) in *E. coli* 0157:H7 infection [193, 194, 226].

Collectively, the results obtained from multiple experiments indicates that extracellular pathogens typically trigger Th2 or Th0 responses in cattle as shown in **Table 3**, and some extracellular pathogens modulate initial Th2 or Th0 responses to ineffective Th1 responses that are associated with the development of chronic infection.

4.3 Pathogens regulate the availability and the strength of three critical signals to suppress effective CD4+ T cell responses

Whenever a pathogen invades and starts multiplying, the host mounts a coordinated attack in order to clear the infection. To counteract the host attacks, some pathogens can interfere with helper T cell responses to establish chronic infections. This can be achieved through unique strategies that impair the availability or strength of the signals required for the activation and differentiation of CD4+ T cells (**Figure 1**). For example, pathogens such as *Salmonella*, and *Mycobacterium tuberculosis* can downregulate MHC-II expression in APC, which diminishes the strength of the 1st signal (antigen stimulation) [233, 234]. In addition, pathogens can reduce the expression of co-stimulatory molecules (2nd signal) and change the type of APCs (e.g. dendritic cell vs. macrophage), which

can collectively impair all of the three signals required for the activation and differentiation of T cells (**Figure 1**) [235, 236].

Bovine pathogens escape from effective CD4⁺ T cell responses in a very similar way to those of mice and humans. They can regulate the availability, type, and strength of three signals. Some pathogens such as Bovine herpes virus type-1 (BHV-1), *Bovine papilloma virus* (BPV), and *Mycobacterium paratuberculosis* can undermine the strength of antigen stimulation (1st signal) by downregulating MHC-I expression, which is actively involved in antigen presentation to CD8⁺ T cells [237–239]. Similarly, some pathogens can disrupt the host T cell response through inhibiting the co-stimulatory signals [211, 240, 241]. Co-stimulatory molecules expressed on the surface of CD4⁺ T cells (as shown in **Figure 1**) are of two types: one provides activating signals, and the other provides inhibiting signals [242]. Pathogens such as, *Bovine leukemia virus*, *Anaplasma marginale*, and *Fasciola hepatica* can upregulate the expression of inhibitory molecules like program cell death protein-1 (PD-1), which severely impairs the T cell response when these inhibitory molecules bind to their ligands on the surface of APCs [211, 240, 241]. Additionally, pathogens such as *Ostertagia ostertagi* and *Myctobacterium paratuberculosis* can induce immune-regulatory cytokines that can inhibit the activation, differentiation, and expansion of effector CD4⁺ T cell subtypes [26, 243, 244]. More specifically, *Ostertagia ostertagi* may stimulate neutrophils to produce IL-10, which can suppress bovine CD4⁺ T cell activation [26]. Furthermore, pathogens like *Fasciola hepatica* can reduce the number of APCs by apoptosis, which curtails the availability of all activating signals [211]. Moreover, pathogens such as *Anaplasma marginale*, *Bovine herpes virus – 1* and *Bovine viral diarrhea virus* can directly cause apoptosis of antigen-specific CD4⁺ T cell and starkly compromise the ability of the host to co-ordinate effective CD8⁺ T and antibody responses [241, 245–247]. In short, bovine pathogens regulate the CD4⁺ T cell responses by reducing the availability and strength of the three activating signals by changing the type and number of APCs, or by interfering with co-stimulation and cytokine production.

4.4 Pathogens regulate the CD4⁺ T cell differentiation process to establish chronic infections in cattle

In addition to regulating activation signals, during the course of infection, pathogens can also regulate CD4⁺ T cell differentiation to evade the effective immune response mounted by the host. As already explained, intracellular pathogens can shift effective Th1 response to an ineffective Th2 response; similarly, extracellular pathogens can shift an effective Th2 response to an ineffective Th1 response, in order to promote the chronic infection in the host. For example, *S. japonicum* in mice can shift a Th2 response to an ineffective Th1 response by triggering apoptosis of Th2 cells via granzyme B signal pathway [248]. Similarly, some authors suggested that in chronic diseases such as bovine tuberculosis, immune complexes circulating in the blood might interfere specifically with Th1 response thus leading to a relatively increased Th2 response [249]. In cattle, intracellular pathogens including *Mycobacterium tuberculosis*, *Mycobacterium paratuberculosis* and *Bovine respiratory syncytial virus* (BRSV) shift the immune responses from a Th1 or a Th0 to an ineffective Th2 response, to establish chronic infections [128, 158]. In the same manner, extracellular pathogens such as *Dictyocaulus viviparus* modulate the immune response from a Th0 or a Th2 response to an ineffective Th1 response and establish the chronic infection [203, 204]. In summary, a fraction of bovine pathogens can skew the CD4⁺ T cell polarization to an ineffective subtype that cannot control their infection, which leads to the establishment of chronic infections.

5. Conclusion and future directions

After receiving three stimulation signals from APCs, naïve CD4+ T cells differentiate into effector subtypes such as Th1, Th2, and Th0 cells. While clear-cut Th1 and Th2 are the common subtypes detected in mice and humans, hybrid Th0 is common in cattle infected by both intracellular and extracellular pathogens. In fact, Th0 responses induced in many bovine diseases might consist of a mixed population of Th1, Th2, and Th0 subtypes. Thus, despite similarities in general, bovine CD4+ T cell responses seem to be partially different from the Th1/Th2 responses classically defined in mice and humans. Therefore, understanding the mechanisms of bovine CD4+ T cell differentiation and its regulation by pathogens may facilitate the development of more effective vaccines and designing immune intervention strategies against important chronic bovine infectious diseases.

Acknowledgements

We gratefully acknowledge the Grant 2016-67015-24948 (to Z.X.) and Grant 2019-67015-29831 (to Z.X.), the Jorgensen Foundation (to Z.X.), and MAES program in University of Maryland (to Z.X.). The figures were created with BioRender.com.

Conflict of interest

The authors state no conflict of interests.

Author details

Anmol Kandel, Magdalena Masello and Zhengguo Xiao*
Department of Animal and Avian Sciences, University of Maryland,
College Park, Maryland, USA

*Address all correspondence to: xiao028@umd.edu

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