Chapter

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 (IFNγ) 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 IFNγ and IL-4, and only exists in small amounts in mice and humans. Moreover, in many bovine infections, both IFNγ 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 (IFNγ) 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 γδ T cells [24], production of IL-10 by γδ 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

Naive 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 IFNγ [44].

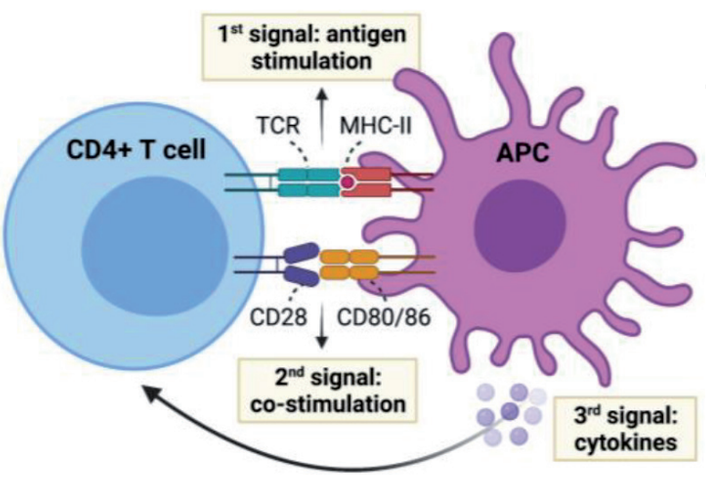

Figure 1.
Three-signal model for CD4 + T cell activation: The 1st signal is provided when TCRs recognize the peptide–MHC-II complex presented by APC; the 2nd signal is initiated when CD28 on CD4+ T cells interacts with CD80/86 on APCs, and the 3rd signal is triggered by cytokines released from the APCs 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 IFNγ and IL-12 from APCs like macrophages and dendritic cells (DCs), which further leads to the polarization of CD4+ T cells into a Th1 subtype. IFNγ 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 IFNγ [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, IFNγ and IL-12 induce Th1 differentiation, which leads to IFNγ 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].

IFNγ 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 IFNγ including NK cells, DCs, macrophages and B cells, it is the signature cytokine of Th1 subtype [27]. Th1 produced IFNγ plays a critical role in regulating the Th1 response. IFNγ 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].

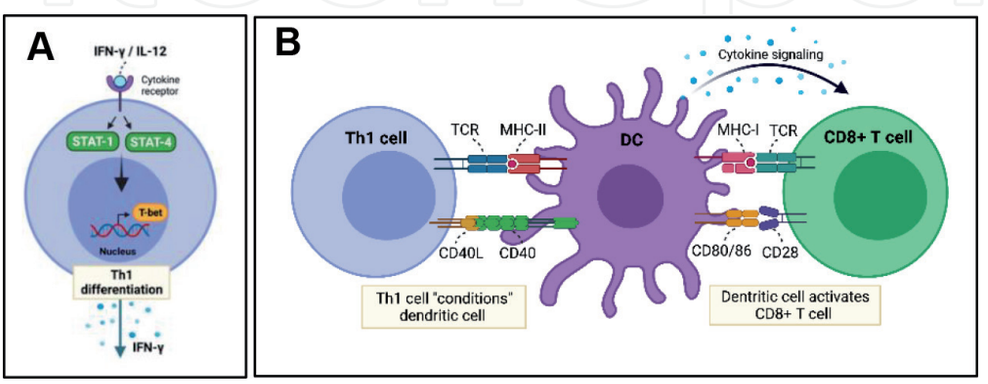

Figure 2.
Th1 help to the activation of CD8+ T cell. A) IFNγ and IL-12 bind to their corresponding receptors on naïve CD4+ T cells during activation, which leads to T-bet expression and Th1 differentiation. This figure was adapted from previous reviews [45–47]. B) Once differentiated, Th1 effector cell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

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

During infection caused by intracellular pathogens, Th1 produced IFNγ 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 IFNγ 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 naive 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 *β2,* IFNγ, 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

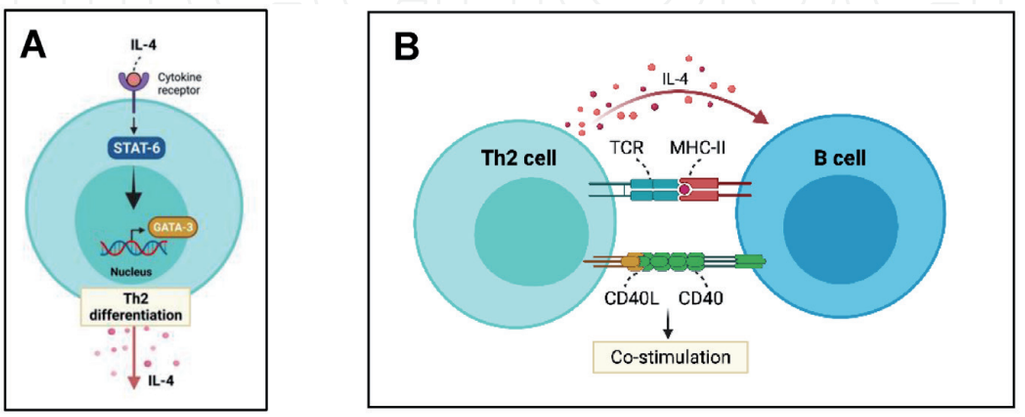

Figure 3.
Th2 help to the activation of B cell. *A) IL-4 binds to its receptor on naïve CD4+ T cells during activation, which induces GATA-3 activation and Th2 differentiation. This figure was adapted from previous reviews [46, 89, 90]. B) Once differentiated, Th2 cells secrete IL-4 and provide antigen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

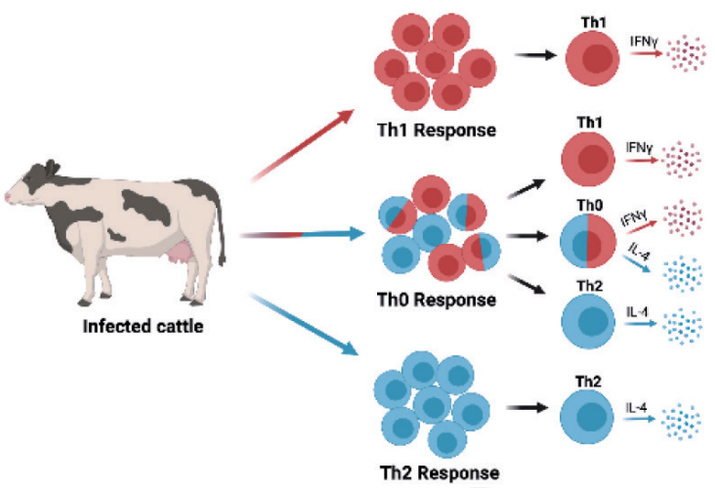

Figure 4.
Helper T cell responses to infections in cattle. Pathogen infections in cattle may induce three types of CD4+ T cell responses: Th1, Th2 and Th0. Th1 responses are characterized by Th1 clones that produce IFNγ, Th2 responses include Th2 clones that produce IL-4, and Th0 response could induce mixed populations of clones: Th1, Th2 and Th0. Th0 clones co-express both IFNγ 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 IFNγ 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 IFNγ expression [163]. Similar to theileriosis, Th1 responses were also protective against *Anaplasma marginale* [155, 164]. In both infected and vaccinated animals, circulatory IFNγ 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 IFNγ 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, IFNγ 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 IFNγ (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 (IFNγ) 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 IFNγ in the supernatant of cultured PBMCs and high levels of IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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	IFNγ (RT-PCR)	—	[154, 163]
Anaplasmosis	IFNγ (ELISA)	IIgG2 /IgG1 + IIgG2	[155, 189]
Babesiosis	IFNγ + IL-4 (RT-PCR)	—	[22, 138, 156]
Respiratory syndrome	IFNγ+IL-4(flow cytometry)	IgE	[181, 182]
Bovine viral diarrhea	IFN γ / IFNγ + IL-4 (q-RT-PCR)	IgG2/ IgG1 + IgG2	[145, 186, 187]
Tuberculosis	IFNγ to IL-4 shift (PCR)	IgG2 to IgG1 shift	[190]
Paratuberculosis	IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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 IFNγ 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+ IFNγ (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 + IFNγ (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

References

[1] Mosmann TR, Fowell DJ. The Th1/Th2 paradigm in infections. Immunology of infectious diseases. 2001:161-174.

[2] Zhu J, Paul WE. Peripheral CD4+ T-cell differentiation regulated by networks of cytokines and transcription factors. Immunological reviews. 2010;238(1):247-262.

[3] Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annual review of immunology. 2003;21(1):139-176.

[4] Infante-Duarte C, Kamradt T, editors. Th1/Th2 balance in infection. Springer seminars in immunopathology; 1999: Springer.

[5] Tomura M, Itoh K, Kanagawa O. Naive CD4+ T lymphocytes circulate through lymphoid organs to interact with endogenous antigens and upregulate their function. The Journal of Immunology. 2010;184(9):4646-4653.

[6] Saravia J, Chapman NM, Chi H. Helper T cell differentiation. Cellular & molecular immunology. 2019;16(7):634-643.

[7] Spellberg B, Edwards JE, Jr. Type 1/Type 2 immunity in infectious diseases. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2001;32(1):76-102.

[8] Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology. 2008;123(3):326-338.

[9] Rivera-Hernandez T, Rhyme MS, Cork AJ, Jones S, Segui-Perez C, Brunner L, et al. Vaccine-induced Th1-type response protects against invasive group A Streptococcus infection in the absence of opsonizing antibodies. Mbio. 2020;11(2).

[10] Casciotti L, Ely KH, Williams ME, Khan IA. CD8+-T-cell immunity against Toxoplasma gondii can be induced but not maintained in mice lacking conventional CD4+ T cells. Infection and immunity. 2002;70(2):434-443.

[11] Jordan KA, Hunter CA. Regulation of CD8+ T cell responses to infection with parasitic protozoa. Experimental parasitology. 2010;126(3):318-325.

[12] Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4+ T cells in immunity to viruses. Nature Reviews Immunology. 2012;12(2):136-148.

[13] Jones K, Fischetti V. The importance of the location of antibody binding on the M6 protein for opsonization and phagocytosis of group A M6 streptococci. The Journal of experimental medicine. 1988;167(3):1114-1123.

[14] Punt J, Stranford SA, Jones PP, Owen JA. Kuby immunology. Eighth edition. ed. New York: Macmillan Education; 2019.

[15] Blackwell NM, Else KJ. B cells and antibodies are required for resistance to the parasitic gastrointestinal nematode Trichuris muris. Infection and immunity. 2001;69(6):3860-3868.

[16] Liu Q, Kreider T, Bowdridge S, Liu Z, Song Y, Gaydo AG, et al. B cells have distinct roles in host protection against different nematode parasites. Journal of immunology (Baltimore, Md : 1950). 2010;184(9):5213-23.

[17] Igietseme JU, Eko FO, He Q, Black CM. Antibody regulation of T-cell immunity: implications for vaccine strategies against intracellular pathogens. Expert review of vaccines. 2004;3(1):23-34.

[18] Estes DM, Closser NM, Allen GK. IFN-gamma stimulates IgG2 production from bovine B cells costimulated with anti-mu and mitogen. Cell Immunol. 1994;154(1):287-295.

[19] Casadevall A. Antibody-mediated protection against intracellular pathogens. Trends in microbiology. 1998;6(3):102-107.

[20] Agnew D, Corbeil L, Munson L, Byrne BA, BonDurant R. A pregnant mouse model for bovine Tritrichomonas foetus infection. Veterinary pathology. 2008;45(6):849-864.

[21] Notebaert S, Meyer E. Mouse models to study the pathogenesis and control of bovine mastitis. A review. Veterinary quarterly. 2006;28(1):2-13.

[22] Brown W, Rice-Ficht AC, Estes DM. Bovine type 1 and type 2 responses. Veterinary immunology and immunopathology. 1998;63(1-2):45-55.

[23] Claerebout E, Vercruysse J. The immune response and the evaluation of acquired immunity against gastrointestinal nematodes in cattle: a review. Parasitology. 2000;120(7):25-42.

[24] Mackay CR, Hein WR. A large proportion of bovine T cells express the gamma delta T cell receptor and show a distinct tissue distribution and surface phenotype. Int Immunol. 1989;1(5):540-545.

[25] Guzman E, Hope J, Taylor G, Smith AL, Cubillos-Zapata C, Charleston B. Bovine γδ T cells are a major regulatory T cell subset. The Journal of Immunology. 2014;193(1):208-222.

[26] Li L, Si H, Wu S-W, Mendez JO, Zarlenga D, Tuo W, et al. Characterization of IL-10-producing neutrophils in cattle infected with Ostertagia ostertagi. Scientific reports. 2019;9(1):1-14.

[27] Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. The Journal of immunology. 1986;136(7):2348-2357.

[28] Romagnani S. Human TH1 and TH2 subsets: regulation of differentiation and role in protection and immunopathology. International archives of allergy and immunology. 1992;98(4):279-285.

[29] Jores J, Baldwin C, Blanchard A, Browning GF, Colston A, Gerdts V, et al. Contagious Bovine and Caprine Pleuropneumonia: a research community's recommendations for the development of better vaccines. NPJ vaccines. 2020;5(1):1-9.

[30] Gomes F, Henriques M. Control of Bovine Mastitis: Old and Recent Therapeutic Approaches. Current microbiology. 2016;72(4):377-382.

[31] Marugan-Hernandez V. Neospora caninum and bovine neosporosis: current vaccine research. Journal of comparative pathology. 2017;157(2-3):193-200.

[32] Vlasova AN, Saif LJ. Bovine Immunology: Implications for Dairy Cattle. Frontiers in Immunology. 2021;12.

[33] Runte F, Renner IV P, Hoppe M. Kuby immunology. 2019.

[34] Surmi BK, Hasty AH. The role of chemokines in recruitment of immune cells to the artery wall and adipose tissue. Vascular pharmacology. 2010;52(1-2):27-36.

[35] Gasteiger G, D'osualdo A, Schubert DA, Weber A, Bruscia EM, Hartl D. Cellular innate immunity: an old game with new players. Journal of innate immunity. 2017;9(2):111-125.

[36] Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. International reviews of immunology. 2011;30(1):16-34.

[37] Janeway Jr CA, Medzhitov R. Innate immune recognition. Annual review of immunology. 2002;20(1):197-216.

[38] Lee H-G, Cho M-Z, Choi J-M. Bystander CD4+ T cells: crossroads between innate and adaptive immunity. Experimental & Molecular Medicine. 2020;52(8):1255-1263.

[39] Alvarez-Dominguez C, Calderón-Gonzalez R, Terán-Navarro H, Salcines-Cuevas D, Garcia-Castaño A, Freire J, et al. Dendritic cell therapy in melanoma. Annals of translational medicine. 2017;5(19).

[40] Eggermont LJ, Paulis LE, Tel J, Figdor CG. Towards efficient cancer immunotherapy: advances in developing artificial antigen-presenting cells. Trends in biotechnology. 2014;32(9):456-465.

[41] Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell. 1994;76(2):263-274.

[42] Allison JP. CD28-B7 interactions in T-cell activation. Current opinion in immunology. 1994;6(3):414-419.

[43] Kambayashi T, Laufer TM. Atypical MHC class II-expressing antigen-presenting cells: can anything replace a dendritic cell? Nature Reviews Immunology. 2014;14(11):719-730.

[44] Xiao Z, Kandel A, Li L. Synergistic Activation of Bovine CD4+ T Cells by Neutrophils and IL-12. Pathogens. 2021;10(6):694.

[45] Pritchard GH, Kedl RM, Hunter CA. The evolving role of T-bet in resistance to infection. Nature Reviews Immunology. 2019;19(6):398-410.

[46] O'Garra A. T-cell differentiation: Commitment factors for T helper cells. Current Biology. 2000;10(13): R492-R4R4.

[47] Biedermann T, Röcken M, Carballido JM, editors. TH1 and TH2 Lymphocyte development and regulation of TH Cell–mediated immune responses of the skin. Journal of Investigative Dermatology Symposium Proceedings; 2004: Elsevier.

[48] Thaiss CA, Semmling V, Franken L, Wagner H, Kurts C. Chemokines: a new dendritic cell signal for T cell activation. Frontiers in immunology. 2011;2:31.

[49] Sarawar SR, Shen J, Dias P. Insights into CD8 T Cell Activation and Exhaustion from a Mouse Gammaherpesvirus Model. Viral immunology. 2020;33(3):215-224.

[50] Borst J, Ahrends T, Bąbała N, Melief CJ, Kastenmüller W. CD4+ T cell help in cancer immunology and immunotherapy. Nature Reviews Immunology. 2018;18(10):635-647.

[51] Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell. 2000;100(6):655-669.

[52] Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-γ production in CD4 and CD8 T cells. Science. 2002;295(5553):338-342.

[53] Afkarian M, Sedy JR, Yang J, Jacobson NG, Cereb N, Yang SY, et al. T-bet is a STAT1-induced regulator of IL-12R expression in naive CD4+ T cells. Nature immunology. 2002;3(6):549-557.

[54] Zhu J, Jankovic D, Oler AJ, Wei G, Sharma S, Hu G, et al. The transcription factor T-bet is induced by multiple pathways and prevents an endogenous Th2 cell program during Th1 cell responses. Immunity. 2012;37(4): 660-673.

[55] Nobile C, Lind M, Miro F, Chemin K, Tourret M, Occhipinti G, et al. Cognate CD4+ T-cell–dendritic cell interactions induce migration of immature dendritic cells through dissolution of their podosomes. Blood, The Journal of the American Society of Hematology. 2008;111(7):3579-3590.

[56] Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature. 1998;393(6684):474-478.

[57] Le Bon A, Durand V, Kamphuis E, Thompson C, Bulfone-Paus S, Rossmann C, et al. Direct stimulation of T cells by type I IFN enhances the CD8+ T cell response during cross-priming. The Journal of Immunology. 2006;176(8):4682-4689.

[58] Le Bon A, Etchart N, Rossmann C, Ashton M, Hou S, Gewert D, et al. Cross-priming of CD8+ T cells stimulated by virus-induced type I interferon. Nature immunology. 2003;4(10):1009-1015.

[59] Bevan MJ. Helping the CD8(+) T-cell response. Nature reviews Immunology. 2004;4(8):595-602.

[60] Trapani JA. Target cell apoptosis induced by cytotoxic T cells and natural killer cells involves synergy between the pore-forming protein, perforin, and the serine protease, granzyme B. Australian and New Zealand journal of medicine. 1995;25(6):793-799.

[61] Harty JT, Bevan MJ. Responses of CD8+ T cells to intracellular bacteria. Current opinion in immunology. 1999;11(1):89-93.

[62] Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases. Biomolecular Concepts. 2018;9(1): 64-79.

[63] Kang S, Brown HM, Hwang S. Direct antiviral mechanisms of interferon-gamma. Immune network. 2018;18(5).

[64] Casbon AJ, Long ME, Dunn KW, Allen LAH, Dinauer MC. Effects of IFN-γ on intracellular trafficking and activity of macrophage NADPH oxidase flavocytochrome b558. Journal of leukocyte biology. 2012;92(4):869-882.

[65] Johnson HM. Gamma interferon: from antimicrobial activity to immune regulation. Frontiers in immunology. 2015;5:667.

[66] Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev. 2001;14(4):778-809, table of contents.

[67] Whitmire JK, Tan JT, Whitton JL. Interferon-γ acts directly on CD8+ T cells to increase their abundance during virus infection. The Journal of experimental medicine. 2005;201(7):1053-1059.

[68] Bhat P, Leggatt G, Waterhouse N, Frazer IH. Interferon-γ derived from cytotoxic lymphocytes directly enhances their motility and cytotoxicity. Cell death & disease. 2017;8(6):e2836-e.

[69] Zakroff SG, Beck L, Platzer EG, Spiegelberg HL. The IgE and IgG subclass responses of mice to four helminth parasites. Cellular immunology. 1989;119(1):193-201.

[70] Firacative C, Gressler AE, Schubert K, Schulze B, Müller U, Brombacher F, et al. Identification of T helper (Th)1- and Th2-associated antigens of Cryptococcus neoformans in a murine model of pulmonary infection. Sci Rep. 2018;8(1):2681.

[71] Uzonna JE, Bretscher PA. Anti-IL-4 antibody therapy causes regression of chronic lesions caused by medium-dose Leishmania major infection in BALB/c mice. Eur J Immunol. 2001;31(11): 3175-3184.

[72] Stevens TL, Bossie A, Sanders VM, Fernandez-Botran R, Coffman RL, Mosmann TR, et al. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. Nature. 1988;334(6179):255-258.

[73] Kawano Y, Noma T. Role of interleukin-2 and interferon-γ in inducing production of IgG subclasses in lymphocytes of human newborns. Immunology. 1996;88(1):40-48.

[74] Gauchat J-F, Henchoz S, Mazzei G, Aubry J-P, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. Nature. 1993;365(6444):340-343.

[75] King C, Nutman T. IgE and IgG subclass regulation by IL-4 and IFN-gamma in human helminth infections. Assessment by B cell precursor frequencies. The Journal of Immunology. 1993;151(1):458-465.

[76] Punnonen J, Aversa G, Cocks BG, McKenzie A, Menon S, Zurawski G, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proceedings of the National Academy of Sciences. 1993;90(8):3730-3734.

[77] Lundgren M, Persson U, Larsson P, Magnusson C, Smith CE, Hammarström L, et al. Interleukin 4 induces synthesis of IgE and IgG4 in human B cells. European journal of immunology. 1989;19(7):1311-1315.

[78] Hjelholt A, Christiansen G, Sørensen US, Birkelund S. IgG subclass profiles in normal human sera of antibodies specific to five kinds of microbial antigens. Pathogens and disease. 2013;67(3):206-213.

[79] Wongkham C, Tantrawatpan C, Intapan PM, Maleewong W, Wongkham S, Nakashima K. Evaluation of immunoglobulin G subclass antibodies against recombinant Fasciola gigantica cathepsin L1 in an enzyme-linked immunosorbent assay for serodiagnosis of human fasciolosis. Clinical and diagnostic laboratory immunology. 2005;12(10):1152-1156.

[80] Heriazon A, Hamilton K, Huffman J, Wilkie BN, Sears W, Quinton M, et al. Immunoglobulin isotypes of lactating Holstein cows classified as high, average, and low type-1 or-2 immune responders. Veterinary immunology and immunopathology. 2011;144(3-4): 259-269.

[81] Estes DM, Brown WC. Type 1 and type 2 responses in regulation of Ig isotype expression in cattle. Veterinary immunology and immunopathology. 2002;90(1-2):1-10.

[82] Shannon JG, Cockrell DC, Takahashi K, Stahl GL, Heinzen RA. Antibody-mediated immunity to the obligate intracellular bacterial pathogen Coxiella burnetii is Fc receptor-and complement-independent. BMC immunology. 2009;10(1):1-8.

[83] Zheng W-p, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89(4):587-596.

[84] Zhu J, Yamane H, Cote-Sierra J, Guo L, Paul WE. GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. Cell research. 2006;16(1):3-10.

[85] Walker JA, McKenzie ANJ. TH2 cell development and function. Nature Reviews Immunology. 2018;18(2): 121-133.

[86] Zhu J. Transcriptional regulation of Th2 cell differentiation. Immunol Cell Biol. 2010;88(3):244-249.

[87] Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in TH 1-TH 2 responses. Nature immunology. 2004;5(11):1157-1165.

[88] Pai S-Y, Truitt ML, Ho I-C. GATA-3 deficiency abrogates the development and maintenance of T helper type 2 cells. Proceedings of the National Academy of Sciences. 2004;101(7): 1993-1998.

[89] Sahoo A, Wali S, Nurieva R. T helper 2 and T follicular helper cells: regulation and function of interleukin-4. Cytokine & growth factor reviews. 2016;30:29-37.

[90] Wang P, Zhang Q, Tan L, Xu Y, Xie X, Zhao Y. The regulatory effects of mTOR complexes in the differentiation and function of CD4+ T cell subsets. Journal of immunology research. 2020;2020.

[91] Cabrera CM, Urra JM. Food allergy and the oral immunotherapy approach. Archivum immunologiae et therapiae experimentalis. 2015;63(1):31-39.

[92] Chapter 5 - B Cell Development, Activation and Effector Functions. In: Mak TW, Saunders ME, Jett BD, editors. Primer to the Immune Response (Second Edition). Boston: Academic Cell; 2014. p. 111-142.

[93] Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for the development of Th2 cells. Immunity. 1996;4(3):313-319.

[94] Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S-i, et al. Essential role of Stat6 in IL-4 signalling. Nature. 1996;380(6575):627-630.

[95] Lee HJ, Takemoto N, Kurata H, Kamogawa Y, Miyatake S, O'Garra A, et al. GATA-3 induces T helper cell type 2 (Th2) cytokine expression and chromatin remodeling in committed Th1 cells. The Journal of experimental medicine. 2000;192(1):105-116.

[96] Yagi R, Zhu J, Paul WE. An updated view on transcription factor GATA3-mediated regulation of Th1 and Th2 cell differentiation. International immunology. 2011;23(7):415-420.

[97] Soliman C, Pier GB, Ramsland PA. Antibody recognition of bacterial surfaces and extracellular polysaccharides. Current opinion in structural biology. 2020;62:48-55.

[98] Cortés A, Muñoz-Antoli C, Esteban JG, Toledo R. Th2 and Th1 responses: clear and hidden sides of immunity against intestinal helminths. Trends in parasitology. 2017;33(9):678-693.

[99] Crotty S. A brief history of T cell help to B cells. Nature reviews Immunology. 2015;15(3):185-189.

[100] Elsner RA, Hastey CJ, Baumgarth N. CD4+ T cells promote antibody production but not sustained affinity maturation during Borrelia burgdorferi infection. Infection and immunity. 2015;83(1):48-56.

[101] Klaus SJ, Pinchuk LM, Ochs HD, Law CL, Fanslow WC, Armitage RJ, et al. Costimulation through CD28 enhances T cell-dependent B cell activation via CD40-CD40L interaction. Journal of immunology (Baltimore, Md : 1950). 1994;152(12):5643-52.

[102] Stavnezer J. Immunoglobulin class switching. Current opinion in immunology. 1996;8(2):199-205.

[103] Pulendran B, Kumar P, Cutler CW, Mohamadzadeh M, Van Dyke T, Banchereau J. Lipopolysaccharides from distinct pathogens induce different classes of immune responses in vivo. The Journal of Immunology. 2001;167(9):5067-5076.

[104] Aboshady HM, Stear M, Johansson A, Jonas E, Bambou J-C. immunoglobulins as Biomarkers for Gastrointestinal nematodes Resistance in Small Ruminants: A systematic review. Scientific reports. 2020;10(1):1-14.

[105] Yousefi M, Tahmasebi F, Younesi V, Razavi A, Khoshnoodi J, Bayat AA, et al. Characterization of neutralizing monoclonal antibodies directed against tetanus toxin fragment C. Journal of immunotoxicology. 2014;11(1):28-34.

[106] Johnson S. Antibody responses to clostridial infection in humans. Clinical infectious diseases. 1997:S173-S1S7.

[107] Wenzel EV, Bosnak M, Tierney R, Schubert M, Brown J, Dübel S, et al. Human antibodies neutralizing diphtheria toxin in vitro and in vivo. Scientific reports. 2020;10(1):1-21.

[108] van Kessel KP, Bestebroer J, van Strijp JA. Neutrophil-Mediated Phagocytosis of Staphylococcus aureus. Front Immunol. 2014;5:467.

[109] Xiang Z, Block M, Löfman C, Nilsson G. IgE-mediated mast cell degranulation and recovery monitored by time-lapse photography. Journal of allergy and clinical immunology. 2001;108(1):116-121.

[110] Segal DM, Taurog JD, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. Proceedings of the National Academy of Sciences. 1977;74(7):2993-2997.

[111] Dvorak AM, Massey W, Warner J, Kissell S, Kagey-Sobotka A, Lichtenstein LM. IgE-mediated anaphylactic degranulation of isolated human skin mast cells. Blood. 1991;77(3):569-578.

[112] Thangam EB, Jemima EA, Singh H, Baig MS, Khan M, Mathias CB, et al. The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets. Frontiers in immunology. 2018;9:1873.

[113] Goswami R, Kaplan MH. A brief history of IL-9. The Journal of Immunology. 2011;186(6):3283-3288.

[114] Takatsu K, Nakajima H. IL-5 and eosinophilia. Current opinion in immunology. 2008 Jun 1;20(3):288-94.

[115] Zhao A, McDermott J, Urban JF, Gause W, Madden KB, Yeung KA, et al. Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. The Journal of Immunology. 2003;171(2):948-954.

[116] Schroeder Jr HW, Cavacini L. Structure and function of immunoglobulins. Journal of Allergy and Clinical Immunology. 2010;125(2):S41-S52.

[117] Stavnezer J, Radcliffe G, Lin Y-C, Nietupski J, Berggren L, Sitia R, et al. Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. Proceedings of the National Academy of Sciences. 1988;85(20):7704-7708.

[118] Renner IV P, Hoppe M. Kuby immunology. 2019.

[119] Bossie A, Vitetta ES. IFN-γ enhances secretion of IgG2a from IgG2a-committed LPS-stimulated murine B cells: implications for the role of IFN-γ in class switching. Cellular immunology. 1991;135(1):95-104.

[120] Rengarajan J, Szabo SJ, Glimcher LH. Transcriptional regulation of Th1/Th2 polarization. Immunology today. 2000;21(10):479-483.

[121] Fishman MA, Perelson AS. Th1/Th2 cross regulation. Journal of theoretical biology. 1994;170(1):25-56.

[122] Wurtz O, Bajénoff M, Guerder S. IL-4-mediated inhibition of IFN-γ production by CD4+ T cells proceeds by several developmentally regulated mechanisms. International immunology. 2004;16(3):501-508.

[123] Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. The Journal of Immunology. 1988;140(12):4245-4252.

[124] Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni M, Rugiu FS, et al. Reciprocal regulatory effects of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. The Journal of Immunology. 1992;148(7):2142-2147.

[125] Usui T, Nishikomori R, Kitani A, Strober W. GATA-3 suppresses Th1 development by downregulation of Stat4 and not through effects on IL-12Rβ2 chain or T-bet. Immunity. 2003;18(3):415-428.

[126] Ferber IA, Lee H-J, Zonin F, Heath V, Mui A, Arai N, et al. GATA-3 significantly downregulates IFN-γ production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. Clinical immunology. 1999;91(2):134-144.

[127] Yagi R, Junttila IS, Wei G, Urban Jr JF, Zhao K, Paul WE, et al. The transcription factor GATA3 actively represses RUNX3 protein-regulated production of interferon-γ. Immunity. 2010;32(4):507-517.

[128] Welsh MD, Cunningham RT, Corbett DM, Girvin RM, McNair J, Skuce RA, et al. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology. 2005;114(1):101-111.

[129] Couissinier-Paris P, Dessein AJ. Schistosoma-specific helper T cell clones from subjects resistant to infection by Schistosoma mansoni are Th0/2. Eur J Immunol. 1995;25(8):2295-2302.

[130] Della Bella C, Benagiano M, De Gennaro M, Gomez-Morales M, Ludovisi A, D'Elios S, et al. T-cell clones in human trichinellosis: Evidence for a mixed Th1/Th2 response. Parasite immunology. 2017;39(3):e12412.

[131] Firestein GS, Roeder WD, Laxer JA, Townsend KS, Weaver CT, Hom JT, et al. A new murine CD4+ T cell subset with an unrestricted cytokine profile. Journal of immunology (Baltimore, Md : 1950). 1989;143(2):518-25.

[132] Kelso A, Troutt AB, Maraskovsky E, Gough NM, Morris L, Pech MH, et al. Heterogeneity in lymphokine profiles of CD4+ and CD8+ T cells and clones activated in vivo and in vitro. Immunological reviews. 1991;123:85-114.

[133] Openshaw P, Murphy EE, Hosken NA, Maino V, Davis K, Murphy K, et al. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. J Exp Med. 1995;182(5):1357-1367.

[134] Paliard X, de Waal Malefijt R, Yssel H, Blanchard D, Chretien I, Abrams J, et al. Simultaneous production of IL-2, IL-4, and IFN-gamma by activated human CD4+ and CD8+ T cell clones. The Journal of Immunology. 1988;141(3):849-855.

[135] Miner KT, Croft M. Generation, persistence, and modulation of Th0 effector cells: role of autocrine IL-4 and IFN-γ. The Journal of Immunology. 1998;160(11):5280-5287.

[136] Vella A, Pearce E. CD4+ Th2 response induced by Schistosoma mansoni eggs develops rapidly, through an early, transient, Th0-like stage. The Journal of Immunology. 1992;148(7):2283-2290.

[137] Infante-Duarte C, Kamradt T. Th1/Th2 balance in infection. Springer seminars in immunopathology. 1999;21(3):317-338.

[138] Brown WC, McElwain TF, Palmer GH, Chantler SE, Estes DM. Bovine CD4(+) T-lymphocyte clones specific for rhoptry-associated protein 1 of Babesia bigemina stimulate enhanced immunoglobulin G1 (IgG1) and IgG2 synthesis. Infection and immunity. 1999;67(1):155-164.

[139] Brown WC, Zhu D, Shkap V, McGuire TC, Blouin EF, Kocan KM, et al. The repertoire of Anaplasma marginale antigens recognized by CD4+ T-lymphocyte clones from protectively immunized cattle is diverse and includes major surface protein 2 (MSP-2) and MSP-3. Infection and immunity. 1998;66(11):5414-5422.

[140] Clery D, Torgerson P, Mulcahy G. Immune responses of chronically infected adult cattle to Fasciola hepatica. Veterinary parasitology. 1996;62(1-2):71-82.

[141] Rhodes SG, Graham SP. Is 'timing'important for cytokine polarization? TRENDS in Immunology. 2002;23(5):246-249.

[142] Kimman TG, Westenbrink F, Schreuder BE, Straver PJ. Local and systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. Journal of clinical microbiology. 1987;25(6):1097-1106.

[143] Gershwin LJ. Immunology of bovine respiratory syncytial virus infection of cattle. Comparative immunology, microbiology and infectious diseases. 2012;35(3):253-257.

[144] Gershwin LJ, Anderson ML, Wang C, Berghaus LJ, Kenny TP, Gunther RA. Assessment of IgE response and cytokine gene expression in pulmonary efferent lymph collected after ovalbumin inhalation during experimental infection of calves with bovine respiratory syncytial virus. American journal of veterinary research. 2011;72(1):134-145.

[145] Palomares RA, Brock KV, Walz PH. Differential expression of pro-inflammatory and anti-inflammatory cytokines during experimental infection with low or high virulence bovine viral diarrhea virus in beef calves. Veterinary immunology and immunopathology. 2014;157(3-4):149-154.

[146] Mihi B, Van Meulder F, Vancoppernolle S, Rinaldi M, Chiers K, Van Den Broeck W, et al. Analysis of the mucosal immune responses induced by single and trickle infections with the bovine abomasal nematode O stertagia ostertagi. Parasite immunology. 2014;36(4):150-156.

[147] Weynants V, Walravens K, Didembourg C, Flanagan P, Godfroid J, Letesson J-J. Quantitative assessment by flow cytometry of T-lymphocytes producing antigen-specific γ-interferon in Brucella immune cattle. Veterinary immunology and immunopathology. 1998;66(3-4):309-320.

[148] Herderschee J, Fenwick C, Pantaleo G, Roger T, Calandra T. Emerging single-cell technologies in immunology. Journal of leukocyte biology. 2015;98(1):23-32.

[149] Estévez O, Anibarro L, Garet E, Martínez A, Pena A, Barcia L, et al. Multi-parameter flow cytometry immunophenotyping distinguishes different stages of tuberculosis infection. Journal of Infection. 2020;81(1):57-71.

[150] Xu G, Qi F, Li H, Yang Q, Wang H, Wang X, et al. The differential immune responses to COVID-19 in peripheral and lung revealed by single-cell RNA sequencing. Cell discovery. 2020;6(1):1-14.

[151] Alegre M-L, Shiels H, Thompson CB, Gajewski TF. Expression and function of CTLA-4 in Th1 and Th2 cells. The Journal of Immunology. 1998;161(7):3347-3356.

[152] van den Ham HJ, Binai NA, Zaaraoui-Boutahar F, Heck AJ, Andeweg AC. Proteomic Profiling of Mouse Helper T Cell Differentiation. Proteomics. 2019;19(7):1800045.

[153] Becker Y. Respiratory syncytial virus (RSV) evades the human adaptive immune system by skewing the Th1/Th2 cytokine balance toward increased levels of Th2 cytokines and IgE, markers of allergy—a review. Virus genes. 2006;33(2):235-252.

[154] Seitzer U, Ahmed J. Tropical theileriosis: cytotoxic T lymphocyte response to vaccination. Vaccine. 2008;26:G24-GG8.

[155] Nazifi S, Razavi S, Kaviani F, Rakhshandehroo E. Acute phase response in cattle infected with Anaplasma marginale. Veterinary microbiology. 2012;155(2-4):267-271.

[156] Rauf U, Suleman M, Abid A, Jamil H, Menghwar H, Durrani AZ, et al. Humoral and Cell-Mediated Immune Response Validation in Calves after a Live Attenuated Vaccine of Babesia bigemina. Pathogens. 2020;9(11):936.

[157] Rhodes SG, Cocksedge JM, Collins RA, Morrison WI. Differential cytokine responses of CD4+ and CD8+ T cells in response to bovine viral diarrhoea virus in cattle. Journal of General Virology. 1999;80(7): 1673-1679.

[158] Stabel J. Transitions in immune responses to Mycobacterium paratuberculosis. Veterinary microbiology. 2000;77(3-4):465-473.

[159] Samad M, Dhar S, Gautam O. Effect of humoral antibodies on Theileria annulata infection of cattle. Haryana Agricultural University Journal of Research (India). 1984.

[160] Preston P, Brown C, Spooner R. Cell-mediated cytotoxicity in Theileria annulata infection of cattle with evidence for BoLA restriction. Clinical and experimental immunology. 1983;53(1):88.

[161] Ahmed J, Rothert M, Steuber S, Schein E. In vitro proliferative and cytotoxic responses of PBL from Theileria annulata-immune cattle. Journal of Veterinary Medicine, Series B. 1989;36(1-10):584-592.

[162] MacHugh N, Burrells A, Morrison W. Demonstration of strain-specific CD8 T cell responses to Theileria annulata. Parasite immunology. 2008;30(8):385-393.

[163] Campbell JDM, Brown D, Nichani A, Howie S, Spooner R, Glass E. A non-protective T helper 1 response against the intra-macrophage protozoan Theileria annulata. Clinical & Experimental Immunology. 1997;108(3):463-470.

[164] Barigye R, GARCÍA-ORTIZ M, Rojas Ramirez E, Rodriguez S. Identification of IgG2-Specific Antigens in Mexican Anaplasma marginale Strains. Annals of the New York Academy of Sciences. 2004;1026(1):84-94.

[165] Brown WC, Shkap V, Zhu D, McGuire TC, Tuo W, McElwain TF, et al. CD4+ T-lymphocyte and immunoglobulin G2 responses in calves immunized with Anaplasma marginale outer membranes and protected against

homologous challenge. Infection and immunity. 1998;66(11):5406.

[166] Pollock J, McNair J, Welsh M, Girvin R, Kennedy H, Mackie D, et al. Immune responses in bovine tuberculosis. Tuberculosis. 2001;81(1-2):103-107.

[167] Pollock J, Pollock D, Campbell D, Girvin R, Crockard A, Neill S, et al. Dynamic changes in circulating and antigen-responsive T-cell subpopulations post-Mycobacterium bovis infection in cattle. Immunology. 1996;87(2):236-241.

[168] Widdison S, Schreuder L, Villarreal-Ramos B, Howard C, Watson M, Coffey T. Cytokine expression profiles of bovine lymph nodes: effects of Mycobacterium bovis infection and bacille Calmette–Guérin vaccination. Clinical & Experimental Immunology. 2006;144(2):281-289.

[169] van Crevel R, Karyadi E, Preyers F, Leenders M, Kullberg B-J, Nelwan RH, et al. Increased production of interleukin 4 by CD4+ and CD8+ T cells from patients with tuberculosis is related to the presence of pulmonary cavities. The Journal of infectious diseases. 2000;181(3):1194-1197.

[170] Heitmann L, Abad Dar M, Schreiber T, Erdmann H, Behrends J, Mckenzie AN, et al. The IL-13/IL-4R α axis is involved in tuberculosis-associated pathology. The Journal of pathology. 2014;234(3):338-350.

[171] Rook GA, Hernandez-Pando R, Dheda K, Seah GT. IL-4 in tuberculosis: implications for vaccine design. Trends in immunology. 2004;25(9):483-488.

[172] Buccheri S, Reljic R, Caccamo N, Ivanyi J, Singh M, Salerno A, et al. IL-4 depletion enhances host resistance and passive IgA protection against tuberculosis infection in BALB/c mice. European journal of immunology. 2007;37(3):729-737.

[173] Dargatz DA, Byrum BA, Barber LK, Sweeney RW, Whitlock RH, Shulaw WP, et al. Evaluation of a commercial ELISA for diagnosis of paratuberculosis in cattle. Journal of the American Veterinary Medical Association. 2001;218(7):1163-1166.

[174] Billman-Jacobe H, Carrigan M, Cockram F, Corner LA, Gill IJ, Hill JF, et al. A comparison of the interferon gamma assay with the absorbed ELISA for the diagnosis of Johne's disease in cattle. Australian veterinary journal. 1992;69(2):25-28.

[175] Sweeney R, Jones D, Habecker P, Scott P. Interferon-gamma and interleukin 4 gene expression in cows infected with Mycobacterium paratuberculosis. American journal of veterinary research. 1998;59(7):842-847.

[176] Coussens PM. Mycobacterium paratuberculosis and the bovine immune system. Animal Health Research Reviews. 2001;2(2):141.

[177] Mundo SL, Fontanals AM, García M, Durrieu M, Álvarez E, Gentilini ER, et al. Bovine IgG1 antibodies against Mycobacterium avium subsp. paratuberculosis protein p34-cx improve association of bacteria and macrophages. Veterinary research. 2008;39(1):1-12.

[178] Hoebee B, Rietveld E, Bont L, Oosten Mv, Hodemaekers HM, Nagelkerke NJ, et al. Association of severe respiratory syncytial virus bronchiolitis with interleukin-4 and interleukin-4 receptor α polymorphisms. The Journal of infectious diseases. 2003;187(1):2-11.

[179] Fu Y, He J, Zheng X, Wu Q, Zhang M, Wang X, et al. Intranasal immunization with a replication-deficient adenoviral vector expressing the fusion glycoprotein of respiratory syncytial virus elicits protective immunity in BALB/c mice. Biochemical

and biophysical research communications. 2009;381(4): 528-532.

[180] Tripp RA, Moore D, Barskey IV A, Jones L, Moscatiello C, Keyserling H, et al. Peripheral Blood Mononuclear Cells from Infants Hospitalized Owing to Respiratory Syncytial Virus Infection Express T Helper-1 and T Helper-2 Cytokines and CC Chemokine Messenger RNA. Journal of Infectious Diseases. 2002;185(10):1388-1394.

[181] Antonis AF, Claassen EA, Hensen EJ, De Groot RJ, de Groot-Mijnes JD, Schrijver RS, et al. Kinetics of antiviral CD8 T cell responses during primary and post-vaccination secondary bovine respiratory syncytial virus infection. Vaccine. 2006;24(10):1551-1561.

[182] Kimman T, Westenbrink F, Schreuder B, Straver P. Local and systemic antibody response to bovine respiratory syncytial virus infection and reinfection in calves with and without maternal antibodies. Journal of clinical microbiology. 1987;25(6):1097.

[183] Guzman E, Taylor G. Immunology of bovine respiratory syncytial virus in calves. Molecular immunology. 2015;66(1):48-56.

[184] Mahoney D. Studies on the protection of cattle against Babesia bovis infection. Parasite lives: papers on parasites, their hosts and their associations to honour JFA Sprent/ edited by Mary Cremin, Colin Dobson and Douglas E Moorehouse. 1986.

[185] Wilkowsky SE, Farber M, Echaide I, De Echaide ST, Zamorano PI, Dominguez M, et al. Babesia bovis merozoite surface protein-2c (MSA-2c) contains highly immunogenic, conserved B-cell epitopes that elicit neutralization-sensitive antibodies in cattle. Molecular and biochemical parasitology. 2003;127(2):133-141.

[186] Molina V, Risalde M, Sánchez-Cordón P, Romero-Palomo F, Pedrera M, Garfia B, et al. Cell-Mediated Immune Response During Experimental Acute Infection with Bovine Viral Diarrhoea Virus: Evaluation of Blood Parameters. Transboundary and emerging diseases. 2014;61(1):44-59.

[187] Rajput MK, Darweesh MF, Braun LJ, Mansour SM, Chase CC. Comparative humoral immune response against cytopathic or non-cytopathic bovine viral diarrhea virus infection. Research in veterinary science. 2020;129:109-116.

[188] Fulton RW, Saliki J, Confer A, Burge LJ, d'Offay J, Helman R, et al. Bovine viral diarrhea virus cytopathic and noncytopathic biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle. Journal of Veterinary Diagnostic Investigation. 2000;12(1):33-38.

[189] Palmer G, Rurangirwa F, Kocan K, Brown W. Molecular basis for vaccine development against the ehrlichial pathogen Anaplasma marginale. Parasitology Today. 1999;15(7):281-286.

[190] Welsh MD, Cunningham RT, Corbett DM, Girvin RM, McNair J, Skuce RA, et al. Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. Immunology. 2005;114(1):101-111.

[191] Koo HC, Park YH, Hamilton MJ, Barrington GM, Davies CJ, Kim JB, et al. Analysis of the immune response to Mycobacterium avium subsp. paratuberculosis in experimentally infected calves. Infection and immunity. 2004;72(12):6870-6883.

[192] Allen AJ, Park K-T, Barrington GM, Lahmers KK, Abdellrazeq GS, Rihan HM, et al. Experimental infection

of a bovine model with human isolates of Mycobacterium avium subsp. paratuberculosis. Veterinary immunology and immunopathology. 2011;141(3-4):258-266.

[193] Mehrzad J, Janssen D, Duchateau L, Burvenich C. Increase in Escherichia coli inoculum dose accelerates CD8+ T-cell trafficking in the primiparous bovine mammary gland. Journal of dairy science. 2008;91(1):193-201.

[194] Corbishley A, Ahmad NI, Hughes K, Hutchings MR, McAteer SP, Connelley TK, et al. Strain-dependent cellular immune responses in cattle following Escherichia coli O157: H7 colonization. Infection and immunity. 2014;82(12):5117-5131.

[195] Steinbach G, Dinjus U, Gottschaldt J, Kreutzer B, Staak C. Course of infection and humoral immune reaction in calves infected orally with different salmonella serovars. Journal of Veterinary Medicine, Series B. 1993;40(1-10):515-521.

[196] Ing R, Su Z, Scott ME, Koski KG. Suppressed T helper 2 immunity and prolonged survival of a nematode parasite in protein-malnourished mice. Proceedings of the National Academy of Sciences. 2000;97(13):7078-7083.

[197] González-Hernández A, Borloo J, Peelaers I, Casaert S, Leclercq G, Claerebout E, et al. Comparative analysis of the immune responses induced by native versus recombinant versions of the ASP-based vaccine against the bovine intestinal parasite Cooperia oncophora. International journal for parasitology. 2018;48(1):41-49.

[198] Van Meulder F, Ratman D, Van Coppernolle S, Borloo J, Li R, Chiers K, et al. Analysis of the protective immune response following intramuscular vaccination of calves against the intestinal parasite Cooperia oncophora. International journal for parasitology. 2015;45(9-10):637-646.

[199] Parmentier H, Ploeger H, Nieuwland M, Souren P, Van Pinxteren L, Rietveld F, et al. Low molecular weight Cooperia oncophora antigens: characterization and humoral immune responses in calves mono-infected with 100 000 infective larvae. Veterinary parasitology. 1995;59(3-4):219-230.

[200] Kanobana K, Koets A, Bakker N, Ploeger H, Vervelde L. T-cell mediated immune responses in calves primary-infected or re-infected with Cooperia oncophora: similar effector cells but different timing. International journal for parasitology. 2003;33(13): 1503-1514.

[201] Bricarello P, Zaros L, Coutinho L, Rocha R, Silva M, Kooyman F, et al. Immunological responses and cytokine gene expression analysis to Cooperia punctata infections in resistant and susceptible Nelore cattle. Veterinary parasitology. 2008;155(1-2):95-103.

[202] Li RW, Gasbarre LC. A temporal shift in regulatory networks and pathways in the bovine small intestine during Cooperia oncophora infection. International journal for parasitology. 2009;39(7):813-824.

[203] Kooyman FNJ, Yatsuda AP, Ploeger H, Eysker M. Serum immunoglobulin E response in calves infected with the lungworm Dictyocaulus viviparus and its correlation with protection. Parasite immunology. 2002;24(1):47-56.

[204] Scott CA, McKeand JB, Devaney E. A longitudinal study of local and peripheral isotype/subclass antibodies in Dictyocaulus viviparus-infected calves. Veterinary immunology and immunopathology. 1996;53(3-4):235-247.

[205] Johnson D, Sales J, Matthews J. Local cytokine responses in Dictyocaulus viviparus infection. Veterinary parasitology. 2005;128(3-4):309-318.

[206] Holmgren S, Hagberg Gustavsson M, Lundén A, Wattrang E. Cytokine m RNA expression in bronchoalveolar lavage cells during D ictyocaulus viviparus infection in calves. Parasite immunology. 2014;36(2):78-86.

[207] McCole D, Doherty M, Baird A, Davies W, McGill K, Torgerson P. T cell subset involvement in immune responses to Fasciola hepatica infection in cattle. Parasite immunology. 1999;21(1):1-8.

[208] Mulcahy G, O'connor F, Clery D, Hogan S, Dowd A, Andrews S, et al. Immune responses of cattle to experimental anti-Fasciola hepaticavaccines. Research in veterinary science. 1999;67(1):27-33.

[209] Clery D, Mulcahy G. Lymphocyte and cytokine responses of young cattle during primary infection with Fasciola hepatica. Research in veterinary science. 1998;65(2):169-171.

[210] Oldham G, WILLIAMS L. Cell mediated immunity to liver fluke antigens during experimental Fasciola hepatica infection of cattle. Parasite immunology. 1985;7(5):503-516.

[211] Garcia-Campos A, Correia CN, Naranjo-Lucena A, Garza-Cuartero L, Farries G, Browne JA, et al. Fasciola hepatica infection in cattle: analyzing responses of peripheral blood mononuclear cells (PBMC) using a transcriptomics approach. Frontiers in immunology. 2019;10:2081.

[212] Hoyle DV, Taylor DW. The immune response of regional lymph nodes during the early stages of Fasciola hepatica infection in cattle. Parasite Immunol. 2003;25(4):221-229.

[213] Mendes EA, de Oliveira Mendes TA, dos Santos SL, Menezes-Souza D, Bartholomeu DC, Martins IVF, et al. Expression of IL-4, IL-10 and IFN-γ in the liver tissue of cattle that are naturally infected with Fasciola hepatica. Veterinary parasitology. 2013;195(1-2):177-182.

[214] Graham-Brown J, Hartley C, Clough H, Kadioglu A, Baylis M, Williams DJ. Dairy heifers naturally exposed to *Fasciola hepatica* develop a type 2 immune response and concomitant suppression of leukocyte proliferation. Infection and immunity. 2018;86(1).

[215] Canals A, Zarlenga DS, Almeria S, Gasbarre LC. Cytokine profile induced by a primary infection with Ostertagia ostertagi in cattle. Vet Immunol Immunopathol. 1997;58(1):63-75.

[216] Michel J, Lancaster M, Hong C. Ostertagia ostertagi: protective immunity in calves: the development in calves of a protective immunity to infection with Ostertagia ostertagi. Experimental parasitology. 1973;33(1):179-186.

[217] Hilderson H, De Graaf D, Vercruysse J, Bergren P. Characterisation of Ostertagia ostertagi antigens by the different bovine immunoglobulin isotypes. Research in veterinary science. 1993;55(2):203-208.

[218] Claerebout E, Vercauteren I, Geldhof P, Olbrechts A, Zarlenga D, Goddeeris B, et al. Cytokine responses in immunized and non-immunized calves after Ostertagia ostertagi infection. Parasite immunology. 2005;27(9):325-331.

[219] Claerebout E, Vercauteren I, Geldhof P, Olbrechts A, Zarlenga DS, Goddeeris BM, et al. Cytokine responses in immunized and non-immunized calves after Ostertagia ostertagi infection. Parasite Immunol. 2005;27(9):325-331.

[220] Soto P, Parma A. The immune response in cattle infected with Tritrichomonas foetus. Veterinary parasitology. 1989;33(3-4):343-348.

[221] Skirrow S, BonDurant R. Immunoglobulin isotype of specific antibodies in reproductive tract secretions and sera in Tritrichomonas foetus-infected heifers. American journal of veterinary research. 1990;51(4):645-653.

[222] Voyich JM, Ansotegui R, Swenson C, Bailey J, Burgess DE. Antibody responses of cattle immunized with the Tf190 adhesin of Tritrichomonas foetus. Clinical and diagnostic laboratory immunology. 2001;8(6):1120-1125.

[223] BonDurant R, Corbeil R, Corbeil L. Immunization of virgin cows with surface antigen TF1. 17 of Tritrichomonas foetus. Infection and immunity. 1993;61(4):1385.

[224] Gómez-Gascón L, Cardoso-Toset F, Tarradas C, Gómez-Laguna J, Maldonado A, Nielsen J, et al. Characterization of the immune response and evaluation of the protective capacity of rSsnA against Streptococcus suis infection in pigs. Comp Immunol Microbiol Infect Dis. 2016;47:52-59.

[225] Donlan A, Petri Jr WA. The inflammasome and type-2 immunity in Clostridium difficile infection. Clinics in colon and rectal surgery. 2020;33(02):067-072.

[226] Hoffman MA, Menge C, Casey TA, Laegreid W, Bosworth BT, Dean-Nystrom EA. Bovine immune response to Shiga-toxigenic Escherichia coli O157: H7. Clinical and Vaccine Immunology. 2006;13(12):1322-1327.

[227] Kanobana K, Ploeger H, Vervelde L. Immune expulsion of the trichostrongylid Cooperia oncophora is associated with increased eosinophilia and mucosal IgA. International journal for parasitology. 2002;32(11):1389-1398.

[228] Hagberg M, Wattrang E, Niskanen R, Tråvén M, Höglund J, Lundén A. Mononuclear cell subsets in bronchoalveolar lavage fluid during Dictyocaulus viviparus infection of calves: a potential role for γ/δ TCR-expressing cells in airway immune responses? Parasite immunology. 2005;27(5):151-161.

[229] Kooyman FN, de Vries E, Ploeger HW, van Putten JP. Antibodies elicited by the bovine lungworm, Dictyocaulus viviparus, cross-react with platelet-activating factor. Infection and immunity. 2007;75(9):4456-4462.

[230] Gault R, Kvasnicka W, Hanks D, Hanks M, Hall M. Specific antibodies in serum and vaginal mucus of heifers inoculated with a vaccine containing Tritrichomonas foetus. American journal of veterinary research. 1995;56(4):454-459.

[231] Bossaert K, Farnir F, Leclipteux T, Protz M, Lonneux J-F, Losson B. Humoral immune response in calves to single-dose, trickle and challenge infections with Fasciola hepatica. Veterinary parasitology. 2000;87(2-3):103-123.

[232] Hughes D, Hanna R, Symonds H. Fasciola hepatica: IgG and IgA levels in the serum and bile of infected cattle. Experimental Parasitology. 1981;52(2):271-279.

[233] Mitchell EK, Mastroeni P, Kelly AP, Trowsdale J. Inhibition of cell surface MHC class II expression by Salmonella. European journal of immunology. 2004;34(9):2559-2567.

[234] Harding CV, Boom WH. Regulation of antigen presentation by Mycobacterium tuberculosis: a role for Toll-like receptors. Nature Reviews Microbiology. 2010;8(4):296-307.

[235] Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, et al. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell. 1995;80(5):707-718.

[236] Sallusto F, Lanzavecchia A. The instructive role of dendritic cells on T-cell responses. Arthritis Research & Therapy. 2002;4(3):1-6.

[237] Nataraj C, Eidmann S, Hariharan MJ, Sur JH, Perry GA, Srikumaran S. Bovine herpesvirus 1 downregulates the expression of bovine MHC class I molecules. Viral immunology. 1997;10(1):21-34.

[238] Araibi EH, Marchetti B, Ashrafi GH, Campo MS. Downregulation of major histocompatibility complex class I in bovine papillomas. The Journal of general virology. 2004;85(Pt 10):2809-2814.

[239] Weiss DJ, Evanson OA, McClenahan DJ, Abrahamsen MS, Walcheck BK. Regulation of Expression of Major Histocompatibility Antigens by Bovine Macrophages Infected withMycobacterium avium subsp. paratuberculosis orMycobacterium avium subsp. avium. Infection and immunity. 2001;69(2):1002-1008.

[240] Okagawa T, Konnai S, Deringer JR, Ueti MW, Scoles GA, Murata S, et al. Cooperation of PD-1 and LAG-3 contributes to T-cell exhaustion in Anaplasma marginale-infected cattle. Infection and immunity. 2016;84(10):2779-2790.

[241] Okagawa T, Konnai S, Nishimori A, Maekawa N, Goto S, Ikebuchi R, et al. Cooperation of PD-1 and LAG-3 in the exhaustion of CD4+ and CD8+ T cells during bovine leukemia virus infection. Veterinary research. 2018;49(1):1-12.

[242] Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nature Reviews Immunology. 2013;13(4):227-242.

[243] de Almeida DE, Colvin CJ, Coussens PM. Antigen-specific regulatory T cells in bovine paratuberculosis. Veterinary immunology and immunopathology. 2008;125(3-4):234-245.

[244] Sheridan MP, Browne JA, Doyle MB, Fitzsimons T, McGill K, Gormley E. IL-10 suppression of IFN-γ responses in tuberculin-stimulated whole blood from Mycobacterium bovis infected cattle. Vet Immunol Immunopathol. 2017;189:36-42.

[245] Eskra L, Splitter GA. Bovine herpesvirus-1 infects activated CD4+ lymphocytes. Journal of general Virology. 1997;78(9):2159-2166.

[246] Chase C, Elmowalid G, Yousif A. The immune response to bovine viral diarrhea virus: a constantly changing picture. The Veterinary clinics of North America Food animal practice. 2004;20(1):95-114.

[247] Han S, Norimine J, Palmer GH, Mwangi W, Lahmers KK, Brown WC. Rapid deletion of antigen-specific CD4+ T cells following infection represents a strategy of immune evasion and persistence for Anaplasma marginale. The Journal of Immunology. 2008;181(11):7759-7769.

[248] Xu X, Wen X, Chi Y, He L, Zhou S, Wang X, et al. Activation-induced T helper cell death contributes to Th1/Th2 polarization following murine Schistosoma japonicum infection. Journal of Biomedicine and Biotechnology. 2010;2010.

[249] Berger S, Ballo H, Stutte H. Distinct antigen-induced cytokine pattern upon stimulation with antibody-complexed antigen consistent with a Th1→ Th2-shift. Research in virology. 1996;147(2-3):103-108.