ISSN 1466-2523

Animal Health Research Reviews 7(1/2); 61–70
DOI: 10.1017/S1466252307001168

# Host responses to *Mycobacterium avium* subsp. *paratuberculosis*: a complex arsenal

J. R. Stabel

*USDA-ARS-NADC, Bacterial Diseases of Livestock Research Unit, 2300 Dayton Rd., Ames, IA 50010, USA*

**Received 25 July 2006; Accepted 28 September 2006**

## Abstract

The immune system is not always successful in recognizing and destroying pathogens it may encounter. Host immunity to mycobacteria is characterized by a very complex series of events, designed to clear the infection. The first line of defense is uptake and processing of the pathogen by macrophages, followed by the initiation of cell-mediated immunity. The secretion of pro-inflammatory cytokines such as IFN-γ is credited with containment of mycobacterial infections. Yet it is clear that activated T-cells may contain but fail to clear the infection in some hosts. Further, it is recognized that if infection progresses to a more clinical state, the production of pro-inflammatory cytokines is suppressed and expression of anti-inflammatory cytokines is increased. It is unclear what defines a host that can successfully contain the infection versus one that succumbs to severe immunopathologic disease. This review will address some of the key elements in host immunity to mycobacterial pathogens, with an emphasis on *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*), in an attempt to understand the dialogue between immune cells and their mediators during infection and what causes this discourse to go awry.

**Keywords:** *Mycobacterium avium* subsp. *paratuberculosis*, Johne's disease, immunity

## Introduction

The agent of the disorder known as paratuberculosis was identified in 1895 by Johne and Frothingham as an acid-fast staining microorganism in granulomatous lesions in the intestines of affected cattle, indicating some type of *Mycobacterium* (Johne and Frothingham, 1895). The organism was fully characterized several years later and named *Mycobacterium paratuberculosis* (*Mycobacterium avium* subsp. *paratuberculosis*). Paratuberculosis is widely distributed both nationally and internationally in domestic ruminants such as cattle, sheep, and goats, as well as wildlife, such as deer, antelope, and bison. Accurate figures on the true prevalence of paratuberculosis in the US are not available although a study conducted in 1996 by the National Animal Health Monitoring System yielded estimates that 22% of US dairy herds were infected (Wells *et al.*, 1998). At the time of that study, it was estimated that annual losses in the US from paratuberculosis in dairy herds would exceed $200 million. Economic losses due to paratuberculosis result from early culling or death from clinical disease, reduced reproductive efficiency and feed efficiency, decreased milk production, and management changes incurred to control the spread of the disease (Ott *et al.*, 1999). The rising incidence of paratuberculosis in the past 10 years suggests that this figure sorely underestimates the current financial burden of this disease.

The primary route of transmission of infection is through ingestion of fecal matter, milk or colostrum containing *M. paratuberculosis* microorganisms, although in utero transmission can occur as well. It is generally thought that neonates are the most susceptible to infection due to their undeveloped immune system and the degree of exposure to *M. paratuberculosis* if their dams are clinically infected (Larsen *et al.*, 1975). However, it has also been demonstrated that adult animals can become infected after exposure to large doses of the bacteria (Rankin, 1962). After infection,

E-mail: jstabel@nadc.ars.usda.gov

animals can remain asymptomatic for long periods of time, up to 2–5 years post-infection. During this subclinical phase of infection, cattle will shed minimal amounts of *M. paratuberculosis* in their feces yet can still be a detriment to the herd due to stealthy contamination of the environment, possibly resulting in further spread of infection throughout the herd. During the clinical phase of infection, fecal shedding of the microorganism is quite high and can exceed $10^{10}$ cfu $g^{-1}$ of feces (Chiodini *et al.*, 1984). The terminal clinical stage of disease is characterized by chronic diarrhea, rapid weight loss, diffuse edema, decreased milk production, and infertility.

Understanding host immunity to *M. paratuberculosis* infection is tantamount to controlling the spread of this disease as it is central to the development of better diagnostic tests, characterization of protective immunogens for use as vaccine candidates, and the identification of potential therapeutic agents. A reciprocal relationship has been documented between the host T-cell responses and the extent of disease during mycobacterial infections (Orme, 1993; Koets *et al.*, 2002; Welsh *et al.*, 2005). A temporal shift in Th1 to Th2-dominated immune responses has been demonstrated in both experimentally and naturally infected sheep and cattle with paratuberculosis (Thorel *et al.*, 1992; Stabel, 2000). As with other mycobacterial diseases, Th1-mediated immunity appears to be essential to control infection yet many aspects of immunity contribute to the regulation of the infection within the host.

## Innate immunity

The study of host immunity to mycobacterial diseases presents a tremendous challenge due to the finely honed evasive maneuvers of the bacteria and the seemingly paradoxical immune responses induced during the course of infection. Similar to other mycobacteria, *M. paratuberculosis*, the causative agent of Johne's disease (paratuberculosis), is an intracellular pathogen that can evade the aggressive defense mechanisms invoked upon phagocytosis by macrophages. It is well documented that mycobacteria can survive and replicate within macrophages by inhibiting the maturation of the phagolysosome. Numerous studies have described mechanisms credited for this inhibition including reduced activity of a macrophage enzyme, sphingosine kinase (Kusner, 2005); secretion of a lipid phosphatase that inhibits phosphatidylinositol 3-phosphate production thereby disallowing the acquisition of lysosomal constituents by the phagosome (Vergne *et al.*, 2005); and the interaction of mycobacterial mannose-capped lipoarabinomannan (ManLAM) with mannose receptors on the macrophage resulting in limited phagolysosome fusion (Kang *et al.*, 2005).

The aforementioned mechanisms have not been delineated for *M. paratuberculosis* but are likely involved in reduced macrophage killing. However, characteristics indicative of the early phagosome, such as reduced acidification, have been observed upon phagocytosis of live *M. paratuberculosis* by a mouse macrophage cell line, suggesting an inhibition of phagosome maturation (Cheville *et al.*, 2001; Kuehnel *et al.*, 2001). Further validation of the inhibition of phagosome maturation was demonstrated by increased levels of an early marker [transferrin receptor (TFR)] and decreased levels of a late maturation marker [lysosome-associated membrane protein one (Lamp-1)] on phagosomes containing live *M. paratuberculosis* relative to those containing killed *M. paratuberculosis*, *Mycobacterium smegmatis*, and zymosan A (Hostetter *et al.*, 2003). The addition of interferon-γ (IFN-γ) and LPS to macrophage cultures increased acidification and maturation of phagosomes containing *M. paratuberculosis* with a concomitant decrease in the survival of *M. paratuberculosis* within the activated macrophages (Hostetter *et al.*, 2002). These results indicate that phagosome maturation is directly correlated with activation of the macrophage (Hostetter *et al.*, 2002).

Acquisition of LAMP-1 and cathepsin D are involved in the accumulation of proton–ATPase complexes, key components of acidification of the phagosome, following phagocytosis of other mycobacteria (Sturgill-Koszycki *et al.*, 1996). Gene expression analysis of bovine macrophages infected with *M. paratuberculosis* for 18 h demonstrated a decrease in ATPase expression that correlated with a lack of phagosome acidification (Weiss *et al.*, 2004). Acidification of the phagosome–lysosome is associated with the production of bactericidal products such as nitric oxide, superoxide, and lysosomal hydrolases and, consequently, has a high correlation with the control of intracellular replication of pathogens.

In addition to effects on phagosomal maturation, mycobacterial ManLAMs have been suggested as potential virulence factors. ManLAMs purified from *Mycobacterium bovis* BCG and *M. tuberculosis* inhibited production of IL-12 upon incubation with human monocyte-derived dendritic cells (DCs) (Nigou *et al.*, 2001), and may also potentiate secretion of TGF-β by monocytes (Chatterjee *et al.*, 1992; Dahl *et al.*, 1996). In a more recent study, candidate virulence factors for *M. paratuberculosis* were identified after screening of over 1100 transposon mutants (Shin *et al.*, 2006). Upon evaluation of a selected group of candidate genes in mice, disruption of genes gcpE, pstA, kdpC, papA2, umaA1, and fabG2_2, resulted in decreased tissue colonization and lesion formation, suggesting roles in virulence. Many of these genes are involved in the synthesis of cell wall components and, as such, act as the first defense for the bacterium against the macrophage. Further work is necessary to unravel the mechanisms of bacterial resistance, leading to an understanding of host–pathogen interactions.

In addition to activation of macrophages via adaptive immune responses such as IFN-γ secretion, toll-like

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press

receptors (TLR) have also been demonstrated to modulate antibacterial and inflammatory immune responses of antigen-presenting cells (APCs) (Nguyen and Pieters, 2005). The interaction of TLRs and DCs is often the first step in the initiation of adaptive immune responses, followed by interaction of co-stimulatory molecules, CD28, CTLA-4, CD80, CD86, and then, finally, signaling by cytokines or chemokines. TLRs help the macrophage/DC process information about the pathogen it has encountered and then provide this information to the cell so the appropriate immune response can take place. Mechanisms of action by which TLRs can modulate the host immune response are regulation of the transcription of various response genes such as IL-12, nitric oxide synthase (iNOS), B7 and lysosomal peptides (Sieling and Modlin, 2001). Thus far, eleven TLRs have been identified, however, only TLR2 (in association with TLR1/TLR6) and TLR4 are recognized by mycobacterial components, with the majority of mycobacterial antigens signaling through TLR2 (Krutzik and Modlin, 2004; Quesniaux *et al.*, 2004). Other receptors that are unrelated to TLRs such as CD14, CR3 (complement receptor), DC-SIGN (DC-specific adhesion molecule-3) have also been associated with APC–T-cell interactions that stimulate cellular activation in mycobacterial infections (Quesniaux *et al.*, 2004).

## Th1/Th2 helper cells and cytokine secretion

Although macrophages, DCs, and other APCs are the first line of defense to intracellular pathogens such as mycobacteria, once the infection takes place the host response is orchestrated primarily by T-cells. Th1 cells are key components of the type-1 pathway (cellular immunity) which is involved in defense against intracellular and viral pathogens. Th2 cells are responsible for type-2 responses (humoral immunity) which promote strong antibody responses to extracellular pathogens. T helper cell differentiation occurs upon stimulation of naive T-cells by APCs. Factors that influence the polarization to either Th1 or Th2 helper cells include the dose of the antigen, the nature of the antigen, and the dynamic of the cell-to-cell contact of T-cells with APCs (Rogers and Croft, 1999). Differentiation of a naive T-cell to either a Th1 or Th2 subtype appears to be permanent although it has been hypothesized that 'switching' from one subtype to another may occur *in vivo*. One theory suggests that Th1/Th2 differentiation may occur further upstream in the host response and, in fact, may be influenced by polarization of APCs upon initial contact with an antigen.

Each of these pathways results in distinct patterns of cytokine secretion. Th1-mediated responses are characterized by the secretion of IFN-γ, IL-2, and TNF-β and are regulated by IL-12, whereas Th2-type regulators of immunity include IL-4, IL-5, IL-6, and IL-10 (Kidd, 2003). Cellular cross-talk and the induction of host responses to infection require the secretion of cytokines. Cytokines mediate host immunity by either activating cells to clear a pathogen or by modulating responses to control pathogenesis. In the case of *M. paratuberculosis* infection and that of other mycobacterial pathogens, a very simplistic viewpoint describing dominant Th1-mediated responses in early subclinical disease and Th2-driven responses in clinical disease has been petitioned for many years. Yet as technological advances have allowed more detailed study of host immunity, particularly in domestic livestock and wildlife, it has become clear the immune responses to *M. paratuberculosis* and other mycobacterial pathogens are highly variable and complex, resulting in overlap between the two pathways at various time points during infection (Ritacco *et al.*, 1991; Dlugovitzky *et al.*, 1997; Jiao *et al.*, 2003; Kidd, 2003).

A strong Th1 bias dominated by IFN-γ secretion has been observed upon initial exposure to mycobacterial pathogens and, as the key Th1 effector cytokine, IFN-γ plays a crucial role in Th1 differentiation. IFN-γ contributes to the production of reactive oxygen and nitrogen intermediates by macrophages (Sato *et al.*, 1998), promotes T-cell activation and DC maturation (Shankar *et al.*, 2003) and up-regulates expression of MHC class I and II genes (Fruh and Yang, 1999; Delvig *et al.*, 2002). IFN-γ induces the secretion of IL-12 by APCs, resulting in Th1 induction via a paracrine pathway but also acts directly to augment Th1 polarization via an autocrine mechanism that does not involve IL-12 (Teixeira *et al.*, 2005). The broad base of actions noted above suggests a critical role for IFN-γ in the control of infection. Although secretion of other Th1-driven cytokines has been studied, the enhanced expression and secretion of IFN-γ in the early stages of infection suggest that this cytokine is critical for controlling mycobacterial infections. Levels of IFN-γ in the serum were directly associated with the degree of pulmonary involvement of *M. tuberculosis*, as patients with advanced tuberculosis had significantly reduced IFN-γ compared to patients categorized with mild or moderate disease (Dlugovitzky *et al.*, 1997).

Two recent studies utilizing mouse models of tuberculosis demonstrated that either infection with *M. tuberculosis* or vaccination with *M. bovis* bacillus Calmette Guerin (BCG) result in a dominance in IFN-γ gene expression or the expression of genes regulated by IFN-γ (Mollenkopf *et al.*, 2006; Rodgers *et al.*, 2006). A singular role for IFN-γ in controlling mycobacterial infection was demonstrated after infection of IFN-γ knockout mice (gko) with *M. tuberculosis* (Flynn *et al.*, 1993). Colonization of spleen, liver, and lung was significantly higher for gko mice compared to wild-type littermates by 15 days post-infection, and mean survival time for mice was reduced as well (15 versus >60 days). Interestingly, the level of infection decreased in gko mice that were pre-treated with exogenous IFN-γ prior to infection. Several studies have reported higher expression of IFN-γ in subclinically infected animals (Sweeney *et al.*, 1998; Khalifeh and Stabel, 2004) and this correlates

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press

with higher levels of IFN-γ secretion by PBMC isolated from animals in the early stages of *M. paratuberculosis* infection, whether it be natural or experimental infection (Stabel, 2000; Waters *et al*., 2003). These studies provide key evidence of an essential role for IFN-γ in modulating the immune response to mycobacterial infection.

Due to its rapid and robust appearance after infection, the measurement of *in vitro* IFN-γ has successfully been used as a diagnostic tool for detection of *M. paratuberculosis* in the early stage of disease as well as other mycobacterial infections such as *M. tuberculosis* in humans and *M. bovis* in cattle and wildlife (Gwodz *et al*., 2000; Stabel and Whitlock, 2001; Huda *et al*., 2003). However, strong IFN-γ responses may also be detrimental to the host if the proper balance of Th1/Th2 cytokines is not achieved. The inflammatory effects of IFN-γ result in the up-regulation of adhesion molecules such as I-CAM on endothelial cells, reduced tight junction expression, and increased vascular permeability as noted in murine models of colitis (Oshima *et al*., 2001). Overproduction of IFN-γ and other Th1 cytokines at the site of inflammation may lead to severe tissue damage.

The release of other inflammatory cytokines such as TNF-α, IL-1, and IL-6 has been observed upon stimulation of bovine monocytes and RAW 264.7 cells with *M. paratuberculosis* lipoarabinomannan or muramyl dipeptide antigens (Adams and Czuprynski, 1994), yet, significant differences in TNF-α and IL-6 gene expression were not observed between healthy cows and cows infected with *M. paratuberculosis* (Adams *et al*., 1996). More recently, a pro-inflammatory pattern of gene expression was observed in peripheral blood mononuclear cells isolated from cattle with subclinical and clinical paratuberculosis (Coussens *et al*., 2004). An extensive analysis of cytokine gene expression revealed up-regulation of pro-inflammatory genes such as IFN-γ, TNF-α, IL-1α, IL-6, and IL-8, with a similar but not redundant pattern of expression apparent when assessed in ileal tissues as compared to peripheral cells (Coussens *et al*., 2004). Secretion of these pro-inflammatory cytokines triggers a sequence of events that potentially contribute to the formation of lesions. Although pro-inflammatory cytokines are critical for recruitment of immune cells to fight infection, protracted activation may result in damage and lesion formation (Clarke, 1997). IFN-γ has also been demonstrated to play a role in the inhibition of Th2 polarization, resulting in reduced production of IL-4 and IL-5 and reduced proliferation of Th2 cells (Teixeira *et al*., 2005). Modulation of Th2 responses has also been credited to IL-12, however, IL-12 expression/secretion does not appear to be induced by *M. paratuberculosis* infection (Coussens *et al*., 2004; Berger and Griffin, 2006).

Th2 regulatory cytokines are responsible for activation and proliferation of B-cells and subsequent secretion of immunoglobulins. Due to the intracellular nature of mycobacterial pathogens, antibody-mediated immunity is not considered a significant factor in the control of infection yet Th2 mediators may play a more critical role in the constraint of Th1-mediated responses. IL-10 is immunosuppressive to IFN-γ, resulting in decreased production through inhibition of the IFN-γ-induced gene, IP-10 (Ito *et al*., 1999). Inhibition of IP-10 results in the disruption of the tyrosine phosphorylation of STAT1, a key transcription factor involved in IFN-γ production (Ito *et al*., 1999). Expression of the transcription factor, T-β, is also critical for IFN-γ production by CD4+Th1 cells (Szabo *et al*., 2000). A recent study utilized T-β−/− mice to demonstrate the selective up-regulation of IL-10 after infection with *M. tuberculosis* (Sullivan *et al*., 2005). T-β deficiency did not affect the recruitment or activation of T-cells in the lungs of mice receiving aerosol preparations of *M. tuberculosis* but did result in impaired IFN-γ production by CD4+ T-cells after antigen stimulation.

IL-4 has been shown to play a specific role in the inhibition of IFN-γ production by CD4+ Th1 cells (Peleman *et al*., 1989). It has also been demonstrated that IL-4 does not interfere with binding of IFN-γ to its receptor but does result in the inhibition of transcriptional activation by IP-10 (Larner *et al*., 1993). Although independent inhibitory effects of IL-4 and IL-10 are evident, the interplay between these Th2 cytokines was demonstrated in lymph node cells harvested from mice immunized with picryl chloride (TNP) and cultured with IL-2 and IL-4. Upon re-exposure to antigen, lymph node cells secreted high levels of IL-4 and IL-10 but an abrogation of IFN-γ production occurred. The inhibitory effects of IL-4 were reversed and IFN-γ production re-instated upon addition of a monoclonal antibody to IL-10, indicating that IL-10 is involved in IL-4-mediated regulation of IFN-γ. Similarly, the addition of a monoclonal antibody to IL-10 increased IFN-γ secretion by PBMC from patients in various stages of tuberculosis, particularly those that were skin test (PPD) positive (Gong *et al*., 1996). More recently, neutralization of IL-10 activity in johnin purified protein derivative-stimulated whole blood increased *in vitro* IFN-γ production 23-fold in cattle categorized in the subclinical stage of *M. paratuberculosis* infection (Buza *et al*., 2004). These studies suggest that Th2 cytokines, IL-4 and IL-10, play key role in the regulation of IFN-γ production and attenuate the immunopathology caused by this pro-inflammatory cytokine.

The shift in Th1/Th2 dominance in early and late stages of mycobacterial infection has been well documented but recent evidence would suggest an overlap of these pathways may occur during a transitional period of infection. The kinetics of the T-cell response to two major MalE epitopes in mice chronically infected with a recombinant BCG.MalE construct reflected a strong initial IFN-γ response followed by a shift to a mixed IFN-γ/IL-4 response (Jiao *et al*., 2003). Broadening the scope

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press

to include two additional MalE epitopes demonstrated a T-cell response dominated by IL-4 by 16 weeks post-infection. A similar pattern of responsiveness was noted after PPD stimulation of splenocytes from mice immunized with wild-type BCG, indicating that this pattern of cytokine secretion was not antigen specific (Jiao *et al.*, 2003). The polarization of Th1/Th2 immunity was evaluated during a 6–9 month period in calves after intranasal inoculation with *M. bovis* (Welsh *et al.*, 2005). T-cell clones were established from CD4+ cells isolated from calves at 17 weeks post-infection for the evaluation of cytokine induction and compared to the extent of host pathology at the termination of the study. Animals with extensive pathology in multiple tissues had a greater frequency of Th0 (IFN-γ/IL-4++) clones, whereas animals with less disseminated pathology demonstrated a dominance of Th1 clones. Increased pathology was correlated with IL-10 but not IL-4 expression in PBMC after stimulation with PPD-bovis.

Due to the protracted escalation of infection in paratuberculosis it has been difficult to develop an infection model that would appropriately mimic the progression from subclinical to clinical disease. This has severely hindered our abilities to conduct longitudinal experiments such as described above. However, comprehensive studies have been conducted to compare host immune responses during the subclinical and clinical stages of *M. paratuberculosis* to identify potential mechanisms that might account for the shift in Th1 to Th2 dominance. Higher gene expression of IFN-γ was demonstrated in the ileum and cecal lymph nodes of asymptomatic cows compared to cows demonstrating clinical signs of disease (Sweeney *et al.*, 1998). IL-4 gene expression tended to be higher for clinically affected cows compared to subclinical and control uninfected cows but this effect was not statistically significant. Similarly, up-regulation of IFN-γ expression was noted in the ileum, mesenteric lymph node, and ileocecal valve of cows with subclinical *M. paratuberculosis* infection, whereas IL-10 gene expression was higher in tissues that exhibited extensive pathology with multifocal lesions and high numbers of bacteria obtained from cows that had progressed to the clinical stage of disease (Khalifeh and Stabel, 2004a). Correlation of cytokine gene expression directly with pathologic lesions in draining ileal lymph nodes demonstrated higher levels of IL-4, IL-10, and IL-2 in *M. paratuberculosis*-infected cattle that were multibacillary (lepromatous) when compared to those that were paucibacillary (tuberculoid) (Tanaka *et al.*, 2005). Although expression of IFN-γ and IL-12 did not differ among infection groups, IL-18 expression was down-regulated in the multibacillary group, an interesting observation since IL-18 is a major factor in the induction of IFN-γ. Although a pattern of Th1-mediated immunity in early infection segueing to Th2-regulated immune responses in later infection is recognizable, it is also clear that these responses are not distinct from one another and typically traverse over both pathways during various points of infection.

## T regulatory cells and cytokine secretion

T regulatory cells are a class of T-cells that negatively affect the immune response, or have suppressive functions. The characteristic phenotype of Tregs is CD4+CD25+ and induction of these cells is favored in a Th2 environment as opposed to a Th1 environment. CD4+CD25+ naive Tregs are produced in the thymus and are often associated with autoimmune disorders and self-tolerance (Beissert *et al.*, 2006). However, another class of Tregs has been described that are induced upon exposure to antigen, resulting in differentiation of CD4+CD25− cells to adaptive Tregs. These Tregs are referred to as either Tr1 or Th3 cells, produce immunosuppressive cytokines, IL-10 and TGF-β, and are involved in controlling immune responses during infection. The immunosuppressive actions of IL-10 on IFN-γ have already been discussed, but TGF-β also acts in a regulatory role through inhibition of T-cell activation and proliferation with an associated down-regulation of IFN-γ production (Ludviksson *et al.*, 2000).

Tregs can traffic to affected tissues to control Th1/Th2 immunity, a factor that is critical to the prevention of immunopathology associated with overproduction of pro-inflammatory cytokines such as IFN-γ. A recent study demonstrated local immune hyporesponsiveness in the ileum of cows naturally infected with *M. paratuberculosis* (Weiss *et al.*, 2004). Infected cows had higher numbers of CD4+CD25+ cells (21% versus 6%) and lower numbers of CD2+CD62L+ cells (7% versus 28%) as compared to noninfected controls. In addition, ileal lymphocytes isolated from infected cows failed to proliferate in response to *M. paratuberculosis* whole-cell sonicate and responded poorly to T and B-cell mitogens. Although concomitant measurement of cytokines was not performed in this study, these results strongly suggest that the population of Treg cells in infected animals may be inducing localized secretion of IL-10 and TGF-β, resulting in immune suppression and contributing to the escalation of disease. Further, patients with active TB had increased numbers of CD4+CD25+ T-cells and 2.2-fold higher FoxP3 (forkhead box P3) expression concomitant with increased secretion of IL-10 and TGF-β compared to noninfected control patients (Guyot-Revol *et al.*, 2006). A further 2.3-fold increase in FoxP3 expression was observed in patients with extrapulmonary TB compared to those with pulmonary TB. FoxP3 is a transcription factor that has been associated with activation of CD25+ Tregs in humans. Although a recent report suggests that FoxP3 expression may be also be induced in activated CD25-PBMC and CD8+ cells, FoxP3 appears to be more specific as a marker for Tregs than other cell-surface molecules such as CD25, CD45RB, CTLA-4, and GITR

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press

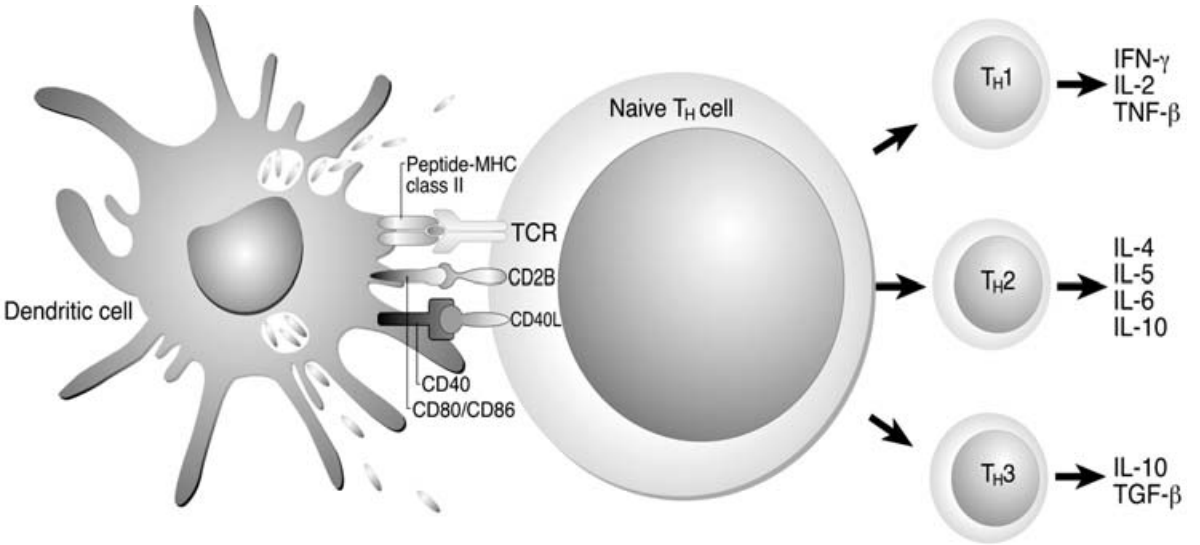


**Fig. 1.** Interaction between co-stimulatory molecules on the DC and the naive Th cell, resulting in differentiation into Th1, Th2, or Th3 cells after exposure to the antigen (adapted from Kapsenberg, 2003).

which are also associated with activated effector or memory T-cells (Hori *et al.*, 2003; Morgan *et al.*, 2005). Other studies have also demonstrated a key role for TGF-β in the induction of FoxP3, a key component in the conversion of naive CD4+CD25−T-cells to the CD4+CD25+ T suppressor cell type associated with Tregs (Yamagiwa *et al.*, 2001; Chen *et al.*, 2003).

Increased secretion and/or expression of TGF-β are associated with advanced fulminate tuberculosis as well as the end-stage of diseases caused by other mycobacteria including *Mycobacterium leprae*, *M. bovis*, and *M. avium* subsp. *paratuberculosis* (Goulart *et al.*, 2000; Khalifeh and Stabel, 2004a; Chung *et al.*, 2005; Fiorenza *et al.*, 2005; Wangoo *et al.*, 2005). The up-regulation of TGF-β is a protective correlate invoked to protect the host by inhibiting production of Th1 and Th2 cytokines and the resulting inflammatory damage in a hyper-responsive state. Information on the role of TGF-β in host immunity to paratuberculosis is very limited to date, however, it was recently demonstrated that cattle in the clinical end-stage of infection had significantly higher expression of IL-10 and TGF-β in ileal tissues and associated lymph nodes as compared to healthy control cows or cows in the early stage of disease (Khalifeh and Stabel, 2004a). *In vitro* study demonstrated increased secretion of IL-10 and TGF-β by PBMC from naturally infected cows after culture with live *M. paratuberculosis* for 6 days (Khalifeh and Stabel, 2004b). A highly significant reduction in IFN-γ production was observed with the addition of exogenous recombinant human TGF- and IL-10, with and without the presence of live *M. paratuberculosis* and regardless of infection status of the cows. Interestingly, an increase in IL-10 secretion after the addition of exogenous rTGF-β to cell culture was observed. Similarly, exogenous rIL-10 and rTGF-β suppressed the production of PPD-induced IFN-γ in PBMC from PPD skin test-positive individuals (Otheino *et al.*, 1999). Synergistic effects of TGF-β and IL-10 were observed with a greater reduction in IFN-γ production noted for cultures containing both cytokines as compared to cultures containing only TGF-β or IL-10. The least suppression was noted with the addition of IL-10 alone, indicating that TGF-β mediates suppression of IFN-γ both directly and indirectly through IL-10.

## DCs and co-stimulatory molecules

Development of Tregs is dependent upon Th cell interactions with mature DCs and, in reciprocity, Treg cytokines, IL-10 and TGF-β, exercise control through inhibition of DC maturation and down-regulation of co-stimulatory molecules such as B7 and CD40 (Cederbom *et al.*, 2000; Misra *et al.*, 2004). Co-stimulatory molecules B7.1 (CD80), B7.2 (CD86), and CD40 are present on DCs, whereas their counterparts, CD28, CTLA-4 (CD152), and CD40L are present on resting and activated T-cells. They play key roles in APC–T-cell interactions, initiating the activation of T-cells and culminating in the production of IFN-γ. A schematic representing the interactions between DCs and T-cells and their associated co-stimulatory molecules is presented in Fig. 1. Exposure to mycobacterial antigens has been shown to up-regulate expression of MHC I and II, and co-stimulatory molecules such as CD80, CD86, and CD40 on APCs (Latchumanan *et al.*, 2002; Florido *et al.*, 2004). CD80 and CD86 were up-regulated on a DC line after incubation with live *M. tuberculosis* for 18 h (Tascon *et al.*, 2000). In addition, increased expression of TNF-α, IL-6, IL-1α, and IL-12 was noted in the DC, indicating that up-regulation of the co-stimulatory molecules was correlated with protective immunity. Depending upon the presence of factors such as cytokines, chemokines, heat shock proteins,

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press

eicosanoids, and extracellular matrix components, DCs can bias the polarization of T-cells into Th1, Th2, or T regulatory subsets (Kapsenberg, 2003). In the event that Treg cells are able to generate cell-to-cell contact with DCs through CD80, CD86, and CTLA-4, they can negatively impact T-cell activation (Mills, 2004).

Exposure to *M. tuberculosis* antigens has been shown to induce differentiation and maturation of DC (Latchumanan *et al.*, 2005). Following re-exposure to *M. tuberculosis* cell extract, DC expression of CD86 was up-regulated at least 10-fold along with concomitant increases in IL-10 and TGF-β secretion, resulting in decreased secretion of IFN-γ. After blocking CD86, IL-10 and TGF-β with antibodies, IFN-γ production was restored indicating that re-exposure to antigen could effectively shift the T-cell response from a pro-inflammatory response to a suppressor response (Latchumanan *et al.*, 2005). The induction of IL-1 secretion by DC upon exposure to mycobacteria may also play a role in determining which pathway will be stimulated. Mononuclear cells exposed to mycobacteria, including *M. paratuberculosis*, respond with a robust secretion of IL-1 (Stabel, 2000; Greenwell-Wild *et al.*, 2002; Cagatay *et al.*, 2005), yet its influence on innate versus adaptive immunity is not fully understood. A recent study evaluated the effects of IL-1-driven maturation of monocytes on the ability of resulting DC to activate T-cell responses to *M. bovis* BCG (Makino *et al.*, 2006). Inhibition of T-cell responses was dependent upon the concentration of IL-1α used to pretreat the monocytes with declining levels of IFN-γ and IL-12p70 observed. Although a mechanism for the IL-1α inhibition was not elucidated, the experimental evidence precluded a role for IL-10 as a contributor to the impaired T-cell responses.

IL-10-mediated suppression of Th1 responses as observed in the latter stages of mycobacterial infection has been further associated with the down-regulation of co-stimulatory molecules on APC and associated T-cells. This was demonstrated after priming of PBMC from patients with pulmonary tuberculosis with heat-killed *M. tuberculosis* resulted in decreased expression of CTLA-4 (Gong *et al.*, 1996). The addition of anti-IL-10 to cell cultures resulted in increased IFN-γ production through stimulation of IL-12, and up-regulation of CTLA-4 expression on T-cells (Gong *et al.*, 1996). Similarly, reduced expression of CD86 and CD40 on monocytes from tuberculin positive donors was observed after pretreatment of cells with IL-10 and following stimulation with irradiated *M. tuberculosis* (de la Barrera *et al.*, 2004). Neutralization of IL-10 activity reversed these effects, resulting in the up-regulation of these co-stimulatory molecules as well as inducing CD8+ lytic activity. Although proliferation and activation of CD4+ and γδ T-cells after stimulation with *M. tuberculosis* were inhibited by IL-10 and TGF-β, the cytokines did not demonstrate either additive or synergistic behavior and appeared to utilize different mechanisms of inhibition (Rojas *et al.*, 1999). In this study, down-regulation of co-stimulatory molecules CD40, CD80, and CD86 was observed on monocytes incubated with *M. tuberculosis* and either IL-10 or TGF-β, with more significant effects contributed by IL-10.

TGF-β was further demonstrated to reduce CD40 expression on APC and inhibit production of IL-12 due to direct effects on IL-12 p40 gene transcription and transcript stability (Takeuchi *et al.*, 1998). The ligation of CD40 on APCs with CD40L on T-cells provides a stimulus for T-cell-mediated immunity against intracellular pathogens such as mycobacteria (Yamauchi *et al.*, 2000; Demangel *et al.*, 2001). Although studies have suggested key roles for both co-stimulatory molecules in mycobacterial infections other reports have suggested that CD40L plays little part in mediating host responses (Larkin *et al.*, 2002; Lazarevic *et al.*, 2003). Currently, there are no reports in the literature providing information on the expression of co-stimulatory molecules on APCs and T-cells during *M. paratuberculosis* infection. Although it is likely that the pattern of expression of these molecules is similar in other mycobacterial infections, this area of research needs to be addressed before one can draw any conclusions on the involvement of such molecules in the regulation of paratuberculosis.

## Summary

The host immune response to mycobacterial pathogens is a complex series of coordinated events, leading in the best case scenario to clearance of the pathogen but most likely to adequate control of infection. The loss of control observed in some hosts may be due to genetic factors increasing the risk of susceptibility or may be provoked by exogenous stressors such as parturition, malnutrition, or secondary viral or bacterial infections. Some of the perturbations in the host immune response to mycobacterial infections that contribute to the loss of control and subsequent immunopathology of advanced disease have been discussed here yet it is clear that these are only pieces of the puzzle. Once a broader understanding of the factors that invoke either protective or evasive immunity is obtained, it will be possible to design intervention strategies to prevent infection.

## References

Adams J and Czuprynski CJ (1994). Mycobacterial cell wall components induce the production of TNF-α, IL-1, and IL-6 by bovine monocytes and the murine macrophage cell line RAW 264.7. *Microbial Pathogenesis* **16**: 401–411.

Adams JL, Collins MT and Czuprynski CJ (1996). Polymerase chain reaction analysis of TNF-α and IL-6 mRNA levels in whole blood from cattle naturally or experimentally infected with *Mycobacterium paratuberculosis*. *Canadian Journal of Veterinary Research* **60**: 257–262.

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press

Beissert S, Schwarz A and Schwarz T (2006). Regulatory T cells. *The Society for Investigative Dermatology* **126**: 15–24.

Berger ST and Griffin FT (2006). A comparison of ovine monocyte-derived macrophage function following infection with *Mycobacterium avium* ssp. *avium* and *Mycobacterium avium* spp. *paratuberculosis*. *Immunology and Cell Biology* **84**: 349–356.

Buza JJ, Hikono H, Mori Y, Nagata R, Hirayama S, Bari AM, Aodon-geril, Shu Y, Tsuji NM and Momotani E (2004). Neutralization of interleukin-10 significantly enhances gamma-interferon expression in peripheral blood by stimulation with johnin purified protein derivative and by infection with *Mycobacterium avium* subsp. *paratuberculosis*. *Infection and Immunity* **72**: 2425–2428.

Cagatay T, Kiran B, Yurt S, Gulbaran Z, Kosar F and Cagatay P (2005). Levels of tumour necrosis factor-alpha and IL-1alpha in newly diagnosed and multidrug resistant tuberculosis. *Respirology* **10**: 290–294.

Cederbom L, Hall H and Ivars F (2000). CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *European Journal of Immunology* **30**: 1538–1543.

Chatterjee D, Roberts AD, Lowell K, Brennan PJ and Orme IM (1992). Structural basis of capacity of lipoarabinomannan to induce secretion of tumor necrosis factor. *Infection and Immunity* **60**: 1249–1253.

Chen W and Wahl SM (2003). TGF-beta: the missing link in CD4+CD25− regulatory T cell-mediated immunosuppression. *Cytokine Growth Factor Reviews* **14**: 85–89.

Chen W, Jin W, Hardegen N, Lei K, Li L, Marinos N, McGrady G and Wahl SM (2003). Conversion of peripheral CD4+CD25− naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *The Journal of Experimental Medicine* **198**: 1875–1886.

Cheville NF, Hostetter J, Thomsen BV, Simutis F, Vanloubbeeck Y and Steadham E (2001). Intracellular trafficking of *Mycobacterium avium* ssp. *paratuberculosis* in macrophages. *Deutsch Tierarztliche Wochenschrift* **108**: 236–243.

Chung Y, Lee SH, Kim DH and Kang CY (2005). Complementary role of CD4+CD25+ regulatory T cells and TGF-beta in oral tolerance. *Journal of Leukocyte Biology* **77**: 906–913.

Clarke CJ (1997). The pathology and pathogenesis of paratuberculosis in ruminants and other species. *Journal of Comparative Pathology* **116**: 217–261.

Coussens PM, Verman N, Coussens MA, Elftman MD and McNulty AM (2004). Cytokine gene expression in peripheral blood mononuclear cells and tissues of cattle infected with *Mycobacterium avium* subsp. *paratuberculosis*: evidence for an inherent proinflammatory gene expression pattern. *Infection and Immunity* **72**: 1409–1422.

Dahl KE, Shiratsuchi H, Hamilton BD, Ellner JJ and Toosi Z (1996). Selective induction of transforming growth factor B in human monocytes by lipoarabinomannan of *Mycobacterium tuberculosis*. *Infection and Immunity* **64**: 399–405.

Delvig AA, Lee JJ, Chrzanowska-Lightowlers ZM and Robinson JH (2002). TGF-beta1 and IFN-gamma cross-regulate antigen presentation to CD4 T cells by macrophages. *Journal of Leukocyte Biology* **72**: 163–166.

Demangel C, Palendira T, Feng CG, Heath AW, Bean AG and Britton WJ (2001). Stimulation of DCs via CD40 enhances immune responses to *Mycobacterium tuberculosis* infection. *Infection and Immunity* **69**: 2456–2461.

Fiorenza F, Rateni L, Farroni MA, Bogue C and Dlugovitzky DG (2005). TNF-alpha, TGF-beta and NO relationship in sera from tuberculosis (TB) patients of different severity. *Immunology Letters* **98**: 45–48.

Florido M, Goncalves AS, Gomes MS and Appelberg R (2004). CD40 is required for the optimal induction of protective immunity to *Mycobacterium avium*. *Immunology* **111**: 323–327.

Flynn JL, Chan J, Triebold KJ, Dalton DK, Stewart TA and Bloom BR (1993). An essential role for interferon-γ in resistance to *Mycobacterium tuberculosis* infection. *Journal of Experimental Medicine* **178**: 2249–2254.

Fruh K and Yang Y (1999). Antigen presentation by MHC class I and its regulation by interferon gamma. *Current Opinion in Immunology* **11**: 76–81.

Gong J-H, Zhang M, Modlin R, Linsley PS, Iyer D, Lin Y and Barnes PF (1996). Interleukin-10 downregulates *Mycobacterium tuberculosis*-induced Th1 responses and CTLA-4 expression. *Infection and Immunity* **64**: 913–918.

Goulart IM, Mineo JR and Foss NT (2000). Production of transforming growth factor-beta1 (TGF-beta1) by blood monocytes from patients with different clinical forms of leprosy. *Clinical and Experimental Immunology* **122**: 330–334.

Greenwell-Wild T, Vazquez N, Sim D, Schito M, Chatterjee D, Orenstein JM and Wahl SM (2002). *Mycobacterium avium* infection and modulation of human macrophage gene expression. *The Journal of Immunology* **169**: 6286–6297.

Guyot-Revol V, Innes JA, Hackforth S, Hinks T and Lalvani A (2006). Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. *American Journal of Respiratory and Critical Care Medicine* **173**: 803–810.

Gwodz JM, Thompson KG, Murray A, Reichel MP, Manktelow BW and West DM (2000). Comparison of three serological tests and an interferon-gamma assay for the diagnosis of paratuberculosis in experimentally infected sheep. *Australian Veterinary Journal* **78**: 779–783.

Hori S, Nomura T and Sakaguchi S (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**: 1057–1061.

Hostetter JM, Steadham EM, Haynes JS, Bailey TB and Cheville NF (2002). Cytokine effects on maturation of the phagosomes containing *Mycobacterium avium* subspecies *paratuberculosis* in J774 cells. *FEMS Immunology and Medical Microbiology* **34**: 127–134.

Hostetter J, Steadham EM, Haynes J, Bailey T and Cheville NF (2003). Phagosomal maturation and intracellular survival of *Mycobacterium avium* subspecies *paratuberculosis* in J774 cells. *Comparative Immunology, Microbiology, and Infectious Diseases* **26**: 269–283.

Huda A, Jungersen G, Christoffersen AB and Lind P (2003). Diagnosis of bovine paratuberculosis by interferon-gamma (IFN-gamma) test. *Acta Veterinaria Scandanavica* **44**: 281.

Ito S, Ansari P, Sakatsume M, Dickensheets H, Vazquez N, Donnelly RP and Larner AC (1999). Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma-induced genes by suppressing tyrosine phosphorylation of STAT1. *Blood* **93**: 1456–1463.

Jiao X, Lo-Man R, Winter N, Deriaud E, Gicquel B and Leclerc C (2003). The shift of Th1 to Th2 immunodominance associated with the chronicity of *Mycobacterium bovis* bacille Calmette-Guerin infection does not affect the memory response. *The Journal of Immunology* **170**: 1392–1398.

Johne HA and Frothingham J (1895). Ein eigenthuemlicher fall von tuberculose beim rind. *Deutsche Zeitschrift für Thiermedizin und vergleichende Pathologie* **21**: 438–454.

Kapsenberg ML (2003). Dendritic-cell control of pathogen-driven T-cell polarization. *Nature Reviews Immunology* **3**: 984–993.

Kang PB, Azad AK, Torrelles JB, Kaufman TM, Beharka A, TIbesar E, DesJardin LE and Schlesinger LS (2005). The

human macrophage mannose receptor directs *Mycobacterium tuberculosis* lipoarabinomannan-mediated phagosome biogenesis. *Journal of Experimental Medicine* **202**: 987–999.

Khalifeh MS and Stabel JR (2004a). Up-regulation of transforming growth factor-beta and interleukin-10 in cows with clinical Johne's disease. *Veterinary Immunology and Immunopathology* **99**: 39–46.

Khalifeh MS and Stabel JR (2004b). Effects of gamma interferon, interleukin-10, and transforming growth factor beta on the survival of *Mycobacterium avium* subsp. *paratuberculosis* in monocyte-derived macrophages from naturally infected cattle. *Infection and Immunity* **72**: 1974–1982.

Krutzik SR and Modlin RL (2004). The role of toll-like receptors in combating mycobacteria. *Seminars in Immunology* **16**: 35–41.

Kuehnel MP, Goethe R, Habermann A, Mueller E, Rohde M, Griffiths G and Valentin-Weigand P (2001). Characterization of the intracellular survival of *Mycobacterium avium* ssp. *paratuberculosis*: phagosomal pH and fusogenicity in J774 macrophages compared with other mycobacteria. *Cellular Microbiology* **3**: 551–566.

Kusner DJ (2005). Mechanisms of mycobacterial persistence in tuberculosis. *Clinical Immunology* **114**: 239–247.

Larsen AB, Merkal RS and Cutlip RC (1975). Age of cattle as related to resistance to infection with *Mycobacteirum paratuberculosis*. *American Journal of Veterinary Research* **36**: 255–257.

Latchumanan VK, Singh B, Sharma P and Natarajan K (2002). *Mycobacterium tuberculosis* antigens induce the differentiation of dendritic cells from bone marrow. *The Journal of Immunology* **169**: 6856–6864.

Latchumanan VK, Balkhi MY, Sinha A, Singh B, Sharma P and Natarajan K (2005). Regulation of immune responses to *Mycobacterium tuberculosis* secretory antigens by dendritic cells. *Tuberculosis* **85**: 377–383.

Ludviksson BR, Seegers D, Resnick AS and Strober W (2000). The effect of TGF-beta1 on immune responses of naive versus memory CD4+ Th1/Th2 T cells. *European Journal of Immunology* **30**: 2101–2111.

Makino M, Maeda Y, Mukai T and Kaufmann SH (2006). Impaired maturation and function of dendritic cells by mycobacteria through IL-1beta. *European Journal of Immunology* **36**: 1443–1456.

Mills KHG (2004). Regulatory T cells: friend or foe in immunity to infection? *Nature Reviews Immunology* **4**: 841–855.

Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD and Kaveri SV (2004). Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. *The Journal of Immunology* **172**: 4676–4680.

Mollenkopf H, Hahnke K and Kaufmann SHE (2006). Transcriptional responses in mouse lungs induced by vaccination with *Mycobacterium bovis* BCG and infection with *Mycobacterium tuberculosis*. *Microbes and Infection* **8**: 136–144.

Morgan ME, van Bilsen J, Bakker AM, Heemskerk B, Schilham MW, Hartgers FC, Elferink BG, van der Zanden L, de Vries R, Huizinga T, Ottehhoff T and Toes R (2005). Expression of FOXP3 mRNA is not confined to CD4+CD25+ T regulatory cells in humans. *Human Immunology* **66**: 13–20.

Nigou J, Zelle-Rieser C, Gilleron M, Thurnher M and Puzo G (2001). Mannosylated lipoarabinomannans inhibit IL-12 production by human dendritic cells: evidence for a negative signal delivered through the mannose receptor. *The Journal of Immunology* **166**: 7477–7485.

Nguyen L and Pieters J (2005). The Trojan horse: survival tactics of pathogenic mycobacteria in macrophages. *Trends in Cell Biology* **15**: 269–276.

Otheino C, Hirsch CS, Hamilton BD, Wilkinson K, Ellner JJ and Toosi Z (1999). Interaction of *Mycobacterium tuberculosis*-induced transforming growth factor beta 1 and interleukin-10. *Infection and Immunity* **67**: 5730–5735.

Ott SL, Wells SJ and Wagner BA (1999). Herd-level economic losses associated with Johne's disease on US dairy operations. *Preventative Veterinary Medicine* **40**: 179–192.

Rankin JD (1962). The experimental infection of cattle with Mycobacterium johnei. IV. Adult cattle maintained in an infectious environment. *The Journal of Comparative Pathology* **72**: 113–117.

Ritacco V, Lopez B, De Kantor IN, Barrera L, Errico F and Nader A (1991). Reciprocal cellular and humoral immune responses in bovine tuberculosis. *Research in Veterinary Science* **50**: 365–367.

Rodgers A, Whitmore KM and Walker KB (2006). Potential correlates of BCG induced protection against tuberculosis detected in a mouse aerosol model using gene expression profiling. *Tuberculosis* **86**: 255–262.

Rogers PR and Croft M (1999). Peptide dose, affinity, and time of differentiation can contribute to the Th1/Th2 cytokine balance. *The Journal of Immunology* **163**: 1205–1213.

Rojas RE, Balaji KN, Subramanian A and Boom WH (1999). Regulation of human CD4+ αβ T-cell-receptor-positive ($TCR^+$) and γδ $TCR^+$ T-Cell Responses to *Mycobacterium tuberculosis* by interleukin-10 and transforming growth factor-β. *Infection and Immunity* **67**: 6461–6472.

Sato K, Akaki T and Tomioka H (1998). Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha). *Clinical and Experimental Immunology* **112**: 63–68.

Seiling PA and Modlin RL (2001). Activation of toll-like receptors by microbial lipoproteins. *Scandanavian Journal of Infectious Diseases* **33**: 97–100.

Shankar G, Pestano LA and Bosch ML (2003). Interferon-g added during Bacillus Calmette-Guerin induced dendritic cell maturation stimulates potent Th1 immune responses. *Journal of Translational Medicine* **1**: 1–12.

Shin SJ, Wu C, Steinberg H and Talaat AM (2006). Identification of novel virulence determinants in *Mycobacterium paratuberculosis* by screening a library of insertional mutants. *Infection and Immunity* **74**: 3825–3833.

Stabel JR (2000). Cytokine secretion by peripheral blood mononuclear cells from cows infected with *Mycobacterium paratuberculosis*. *American Journal of Veterinary Research* **61**: 754–760.

Stabel JR and Whitlock RH (2001). An evaluation of a modified interferon-gamma assay for the detection of paratuberculosis in dairy herds. *Veterinary Immunology and Immunopathology* **79**: 69–81.

Sturgill-Koszycki S, Schaible UE and Russell DG (1996). *Mycobacterium*-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. *The EMBO Journal* **15**: 6960–6968.

Sullivan BM, Jobe O, Lazarevic V, Vasquez K, Bronson R, Glimcher LH and Kramnik I (2005). Increased susceptibility of mice lacking T-bet to infection with *Mycobacterium tuberculosis* correlates with increased IL-10 and decreased IFN-γ production. *The Journal of Immunology* **175**: 4593–4602.

Sweeney RW, Jones DE, Habecker P and Scott P (1998) Interferon-gamma and interleukin-4 gene expression in cows infected with *Mycobacterium paratuberculosis*. *American Journal of Veterinary Research* **59**: 842–847.

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

Tanaka S, Sato M, Onitsuka T, Kamata H and Yokomizo Y (2005). Inflammatory cytokine gene expression in different types of granulomatous lesions during asymptomatic stages of bovine paratuberculosis. *Veterinary Pathology* **42**: 579–588.

Tascon RE, Soares CS, Ragno S, Stavropoulos E, Hirst EMA and Colston MJ (2000). *Mycobacterium tuberculosis*-activated dendritic cells induce protective immunity in mice. *Immunology* **99**: 473–480.

Teixeira LK, Fonseca BP, Vieira-de-Abreu A, Barboza BA, Robbs BK, Bozza PT and Viola JP (2005). IFN-gamma production by CD8+ T cells depends on NFAT1 transcription factor and regulates Th differentiation. *The Journal of Immunology* **175**: 5931–5939.

Vergne I, Chua J, Lee HH, Lucas M, Belisle J and Deretic V (2005). Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 4033–4038.

Wangoo A, Johnson L, Gough J, Ackbar R, Inglut S, Hicks D, Spencer Y, Hewinson G and Vordermeier M (2005). Advanced granulomatous lesions in *Mycobacterium bovis*-infected cattle are associated with increased expression of type I procollagen, gammadelta (WC1+) T cells and CD 68+ cells. *Journal of Comparative Pathology* **133**: 223–234.

Waters WR, Miller JM, Palmer MV, Stabel JR, Jones DE, Koisteinen KA, Steadham EM, Hamilton MJ, Davis WC and Bannantine JP (2003). Early induction of humoral and cellular immune responses during experimental *Mycobacterium avium* subsp. *paratuberculosis* infection of calves. *Infection and Immunity* **71**: 5130–5138.

Weiss DJ, Evanson OA, Deng M and Abrahamsen MS (2004). Gene expression and antimicrobial activity of bovine macrophages in response to *Mycobacterium avium* subsp. *paratuberculosis*. *Veterinary Pathology* **41**: 326–337.

Wells S, Ott SL and Seitzinger AH (1998). Key health issues for dairy cattle – new and old. *Journal of Dairy Science* **81**: 3029–3035.

Welsh MD, Cunningham RT, Corbett DM, Girvin RM, McNair J, Skuce RA, Bryson DG and Pollock JM (2005). Influence of pathological progression on the balance between cellular and humoral immune responses in bovine tuberculosis. *Immunology* **114**: 101–111.

Yamagiwa S, Gray JD, Hashimoto S and Horwitz DA (2001). A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. *The Journal of Immunology* **166**: 7282–7289.

Yamauchi PS, Bleharski JR, Uyemura K, Kim J, Sieling PA, Miller PA, Brightbill H, Schlienger K, Rea TH and Modlin RL (2000). A role for CD40–CD40 ligand interactions in the generation of type 1 cytokine responses in human leprosy. *The Journal of Immunology* **165**: 1506–1512.

https://doi.org/10.1017/S1466252307001168 Published online by Cambridge University Press