



Review

An insight review on immunopathogenesis of bovine and human mycobacteria infections

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Mycobacterium is one of the first infectious agents to spring to mind in connection with chronic or persistent infections. The causative organism of bovine tuberculosis is *Mycobacterium bovis* (*M. bovis*), a member of the *Mycobacterium tuberculosis* complex (MTBC), which includes *Mycobacterium tuberculosis* (*M. tuberculosis*), *M. bovis*, *Mycobacterium africanum* (*M. africanum*), *Mycobacterium microti* (*M. microti*), *Mycobacterium canetti* (*M. canetti*), *Mycobacterium caprae* (*M. caprae*) and *Mycobacterium pinnipedii* (*M. pinnipedii*), and many of the species and subspecies of MTBC show specific host association. Immunity against mycobacteria is multifactorial and it is believed that the host innate immunity provides initial resistance to mycobacteria before the adaptive cell-mediated immunity fully develops. There are still many unsolved problems associated with the pathogenesis and immune response to tuberculosis. Therefore multi-disciplinary approach to develop more complete understanding of the pathogenic strategies is mandatory. Special consideration to bovine tuberculosis might help scientists to devise proper mechanisms to prevent human tuberculosis as they are closely related.

Key words: Granuloma, immune evasion, immunity, mycobacteria, pathogenesis.

INTRODUCTION

Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide. Currently, a third of the world's population is infected with *Mycobacterium tuberculosis*, the causative agent of TB, and annually there are 10 million new cases of clinical TB and approximately 2 million deaths. TB kills more individuals each year than any other bacterial pathogen, and alarmingly, current control practices have not been able to significantly reduce the incidence over the past 15 years (World Health Organization (WHO), 2010). The global incidence rate of TB per capita fell at a rate of 2.2% between 2010 and 2011 (WHO, 2012), with Sub-Saharan Africa displaying

the highest annual risk of infection, probably catalyzed by the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) pandemic (Corbett et al., 2003).

The causative organism of bovine tuberculosis is *Mycobacterium bovis*, a member of the *M. tuberculosis* complex (MTBC), which includes *M. tuberculosis*, *M. bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canetti*, *Mycobacterium caprae* and *Mycobacterium pinnipedii*, and many of the species and subspecies of MTBC show specific host association (Smith et al., 2006). The most notable member of the

complex is *M. tuberculosis*, the most important bacterial pathogen of human. In contrast to *M. tuberculosis* which is largely host restricted to humans, *M. bovis* is primarily maintained in bovine, in particular, domesticated cattle, although the pathogen can frequently be recovered from other mammals, including humans (Smith et al., 2006).

In developing countries, the conditions for *M. bovis* transmission to humans not only exist unchanged, but the human population has a greater vulnerability due to poverty, HIV and reduced access to health care (Ayele et al., 2004). Bovine TB has been reduced/eliminated from domestic cattle in many developed countries by the application of a test-and-cull policy that moves (Amanfu, 2006; Thoen et al., 2006). In Africa, although bovine TB is known to be common in both cattle and wildlife, control policies have not been enforced in many countries due to cost implications, lack of capacity, and infrastructure limitations (Amanfu, 2006; Renwick et al., 2007).

Pathogenesis of human and bovine tuberculosis occurs in a similar way, beginning with bacterial entry to host lungs by inhalation and bacteria phagocytosis by alveolar macrophages. Establishment of a chronic infection status is accomplished due to mycobacterial virulence factors that allow it to enter and survive within the host phagocytic cells. It is well known that macrophages play an important role in tuberculosis pathogenesis, being the first defense line, the niche for the bacteria and the main control mechanism (Uziel et al., 2011). When disease develops, the associated granulomatous pathological changes are seen mainly in the lower and upper respiratory tract and because of this pattern, it is considered that infection most often follows aerosol exposure to *M. bovis* (Neill et al., 2001). Modeling of bovine tuberculosis believed to help a lot in the production of effective vaccine for human (Van Rhijn et al., 2008). Therefore this review highlights on the immunopathology of bovine and human mycobacteria infections.

NATURE OF MYCOBACTERIA

According to the latest list of bacterial names with standing in nomenclature, there are more than 100 recognized species in the genus *Mycobacterium* (Euze'by, 2004). A number of species of mycobacteria are important pathogens of animals or humans. Human tuberculosis is chiefly associated with infection with the species *M. tuberculosis*, although *M. africanum* is also important in some regions. Bovine tuberculosis is caused by intracellular infection with the acid-fast bacterium, *M. bovis* (Pollock et al., 2005). In cattle, exposure to this organism can result in a chronic disease that jeopardizes animal welfare and productivity, and in some countries leads to significant economic losses (Pollock and Neill, 2002). *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*

and *M. pinnipedii* together with *M. microti* (associated with infection of rodents) form a very closely related phylogenetic group and may be referred to collectively as the *M. tuberculosis* complex (MTBC). Human infection with members of the MTBC produces an indistinguishable clinical picture and the individual species cannot be distinguished from each other based on microscopic examination of stained tissues or other clinical specimens (Annon, 2003).

Virulence factors

Early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are potent IFN- γ inducing antigens of tuberculous mycobacteria. These two proteins are co-secreted and form a tight 1:1 complex upon export (Renshaw et al., 2002). Genes for ESAT-6 and CFP-10 are absent in many environmental, non-tuberculous mycobacteria as well as in the TB vaccine strain, *M. bovis* bacille calmette guerin (BCG). Use of ESAT-6 and/or CFP-10 as antigens in IFN- γ -based TB assays enhances specificity when compared to use of *M. bovis* purified protein derivative (PPD) (Buddle et al., 2003). ESAT-6 has also been used to discriminate between cattle naturally infected with *M. bovis* and cattle sensitized/infected with environmental, non-tuberculous strains of mycobacterial or vaccinated for paratuberculosis.

Route of infection and lesion distribution

With some exceptions, it is agreed that cattle become infected with *M. bovis* by either oral or respiratory routes. The oral route is likely most important in calves nursing tuberculous cows. In the late 1990's, surveys of tuberculous cattle in Great Britain (Phillips et al., 2003) revealed that 67% of tuberculous lesions were within the lungs and pulmonary lymph nodes (tracheobronchial and mediastinal). Although many studies demonstrate a tendency toward lesion development in pulmonary lymph nodes rather than in lungs, meticulous examination often reveals lesions in the lungs; most < 1 cm in diameter.

IMMUNE RESPONSES TO MYCOBACTERIAL INFECTION

Immunity against mycobacteria is multifactorial and dependent on the balance between an inflammatory response that allows the host to develop a granuloma, which contains the microorganism and an anti-inflammatory response that restricts the extent of the granuloma and allows contact of effectors T-cells with the infected cells resulting in the killing of the infecting pathogen

(Villarreal-Ramos et al., 2003).

Innate immunity

It is believed that the host innate immunity provides the initial resistance to infections with intracellular pathogens before the adaptive type 1 cell-mediated immunity fully develops. The major cellular components involved in innate immunity include phagocytes, macrophages, neutrophils, dendritic cells (DCs), natural killer (NK) cells, $\gamma\delta$ T cells, and soluble mediators released by these cells serve as a linker to cell-mediated immunity (Wolf et al., 2008). Macrophages and DCs have been suggested as important in inducing the immune response, though they are likely to have different roles in immunity for killing or T cell stimulation, respectively (Hope et al., 2004).

Macrophages

Classical activation of macrophages is well known since 1964 and demonstrated that *M. bovis* BCG or *Listeria monocytogenes* infection in a mouse model increased macrophage microbicide activity in a stimulus dependent manner, but not antigen specific (Uziel et al., 2011). Alveolar macrophages resident within the lung are considered to be the main cellular host for mycobacteria *in vivo* and the major role of these cells is the rapid killing of the invading organism. This is due to the release of toxic reactive oxygen and nitrogen intermediates or killing by lysosomal enzymes following fusion with the bacterial phagosome. The type of receptor that is engaged by the bacteria can influence the response generated within the macrophage (Liebana et al., 200).

The receptor molecules that have been implicated in the uptake of mycobacteria include mannose receptors that bind mannosylated molecules on the bacterial surface, Fc receptors binding opsonised cells and complement receptors. The use of complement receptor three (CR3) by Mycobacterial species may be advantageous for the bacterium, as triggering this receptor does not induce the release of potentially cytotoxic reactive oxygen intermediates (Liebana et al., 2000). Binding to the mannose receptor has also been suggested as a possible safe route of entry for mycobacteria that facilitates their intracellular survival. Following uptake into the phagosome, phago-lysosome fusion occurs followed by the destruction of bacteria and the processing and presentation of bacterial antigens to T cells in the context of MHC molecules.

The stimulation of T cells in this way activates the adaptive arm of the immune response and induces IFN- γ release and CD8⁺ T cell cytolytic capacity that can further enhance the anti-microbial defence system (Lopez et al.,

2003). Effects on cytokine synthesis and expression of molecules on the cell surface of macrophages have been reported in some studies in the interaction of mycobacteria and antigen presenting cells. Macrophages infected with *M. tuberculosis* preferentially secrete pro-inflammatory cytokines including TNF- α , IL-1 and IL-6 (Giacomini et al., 2001; Hickman et al., 2002). Infected macrophages are known also to secrete chemokines including IL-8, RANTES and MCP-1 which would aid the recruitment of lymphocytes to the lung and granuloma formation, thus leading to containment of the mycobacteria (Peters and Ernst, 2003).

In addition *M. tuberculosis* infected macrophages secrete IL-10, rather than IL-12, which could act to suppress Th1 responses (Giacomini et al., 2001; Hickman et al., 2002). IL-10 may also inhibit export of MHC class II molecules to the cell surface, which would, in turn, down-regulate T cell responses. Reduced MHC class II expression in *M. tuberculosis* infected macrophages has been reported and proposed to be linked to stimulation of TLR2 by mycobacterial lipopeptides (Noss et al., 2001). A reduced ability of these cells to signal T lymphocyte activation combined with recruitment of cells to form granulomas may help mycobacterial persistence within the host. However it is known that stimulation of macrophages by other components of the immune response, such as IFN- γ or TNF- α released by T cells, can enhance macrophage microbicidal activity (Giacomini et al., 2001; Hickman et al., 2002).

Dendritic cells

Dendritic cells (DCs) are a system of cells that are specialized for the presentation of antigen to T cells. They are the most potent of the antigen presenting cells and are central to the initiation of immune responses in naive animals. They originate in the bone marrow but recent investigations suggest that they may be derived from either myeloid or lymphoid precursors (Tizard, 2004).

DCs are a trace population in most tissues but notably form networks underlying major body surfaces such as skin, trachea and intestine, where their function is the uptake of antigens, and after migration to the draining lymphnodes, the presentation of processed antigen. A number of properties have been established that are critical to the function of DC as the ultimate antigen-presenting cell population. These include the ability to effectively take up antigen by a number of routes, which may include endocytosis by clathrin-coated pits, macropinocytosis or phagocytosis depending on the maturation stage of the cell. The interaction of DC and mycobacteria augments their expression of surface molecules that are involved in the interaction with T cells,

notably MHC II and the costimulatory molecules CD40 and CD80 (Hope et al., 2004; Tizard, 2004). Taken together this suggests that infected DCs have an augmented capacity to stimulate mycobacteria reactive T cells. Also of importance for the interaction with T cells and modulation of immune responses by DC is the altered cytokine profile that is observed following mycobacterial infection of these cells (Hope et al., 2004).

Infection of DC with either *M. tuberculosis* or BCG is associated with increased expression of IL-12, TNF- α , IL-1 and IL-6 (Giacomini et al., 2001). These cytokines play major roles in protective anti-mycobacterial immune responses. As noted, IL-12 secreted by DC can potentiate IFN- γ and TNF- α secretion by T cells and this in turn may serve to enhance the anti-microbial activity of macrophages to destroy invading bacilli (Hickman et al., 2002). In addition to the production of proinflammatory cytokines, mycobacterial infection of DC is also associated with the secretion of IL-10, which may inhibit the cellular response to mycobacteria through the down-regulation of IL-12 secretion (Giacomini et al., 2001; Hickman et al., 2002). This may serve to limit the extent of DC and macrophage activation and thus regulate the potentially damaging immune response that occurs in tissues *in vivo*.

Like non-activated macrophages, DCs are reported to provide an environment within which mycobacteria can survive and replicate (Tailleux et al., 2003a). DCs may therefore be a reservoir for Mycobacteria *in vivo*, particularly within lymph nodes to which they have migrated following the initial response to mycobacterial infection (Tailleux et al., 2003b). Thus, survival and/or replication of mycobacteria within the DC is likely to induce T cell activation but may also eventually lead to granuloma formation and persistence of infection. This would potentially contribute to disease pathogenesis. In contrast, uptake by macrophages may lead to lower T cell based immune responses and a failure to control infection. Alternatively, the mycobacteria may be killed leading to disease resolution. Thus, it may be advantageous in terms of immunity to have an extended range of cells that are permissive for infection, each with differing functions that should allow more effective clearance of the invading pathogen (Kaufmann and Schaible, 2003).

Natural killer (NK) cells

NK cells are a type of cytotoxic lymphocyte that is a major component of the innate immune system. These cells have been implicated in early immune responses to a variety of intracellular pathogens, including mycobacteria, through their capacity to rapidly produce IFN- γ and other immunoregulatory cytokines (Brill et al., 2001). NK cells are hypothesized to be important in the

initiation and regulation of various immune responses and it has been shown that NK cells induce a granulomatous response to a glycolipid fraction of *M. tuberculosis* cell wall (Taniguchi et al., 2003).

Neutrophils

Polymorphonuclear cells, principally neutrophils, are the first phagocytes to arrive from circulation and attempt to eliminate invading pathogens via oxygen-dependent and oxygen-independent mechanisms. The former mechanism results from the generation of reactive oxygen species, whereas the latter mechanism reflects the capacity of neutrophils to degranulate and release preformed oxidants and proteolytic enzymes from granules (Lacy and Eitzen, 2008). Neutrophils have been implicated in the control of mycobacterial infections (Martinaeu et al., 2007), but the mechanisms by which they exert direct protective functions are not completely resolved (Kisich et al., 2002). Mycobacteria-infected macrophages acquired the contents of neutrophil granules and their antimicrobial molecules by the uptake of apoptotic neutrophil debris, which was trafficked to endosomes and co-localized with intracellular bacteria (Tan et al., 2006). Neutrophils may play an important role in the transition from innate to adaptive immune responses by producing critical cytokines and chemokines (Sawant and McMurry, 2007).

$\gamma\delta$ T cells

Human T cells expressing $\gamma\delta$ TCR represent a unique lymphocyte population with an unusual tissue distribution and antigen recognition pathway. Conditions that lead to responses of $\gamma\delta$ T cells are not fully understood and current concepts of $\gamma\delta$ T cells as first line of defense or bridge between innate and adaptive responses are also still vague (Holtmeier and Kabelitz, 2005). Murine studies have indicated that the induction of $\gamma\delta$ T cells in the immune response against TB precedes that of conventional CD4⁺ and CD8⁺ cells, hence plays an important role in modulating the effectors' response against tuberculosis. Intranasal infection of mice with BCG resulted in an early accumulation of $\gamma\delta$ T cells in the lungs, and the peak of $\gamma\delta$ T cells expansion at 7 days post infection preceded the 30 day peak of $\alpha\beta$ T cells (Dieli et al., 2003), suggesting that $\gamma\delta$ T cells in the lungs might help to control mycobacterial infection before the onset of adaptive immunity. Studies using $\gamma\delta$ TCR knockout mice indicate that $\gamma\delta$ T cells may be involved in the regulation of granuloma formation, which is critical for the control of mycobacteria (Ehlers et al., 2001).

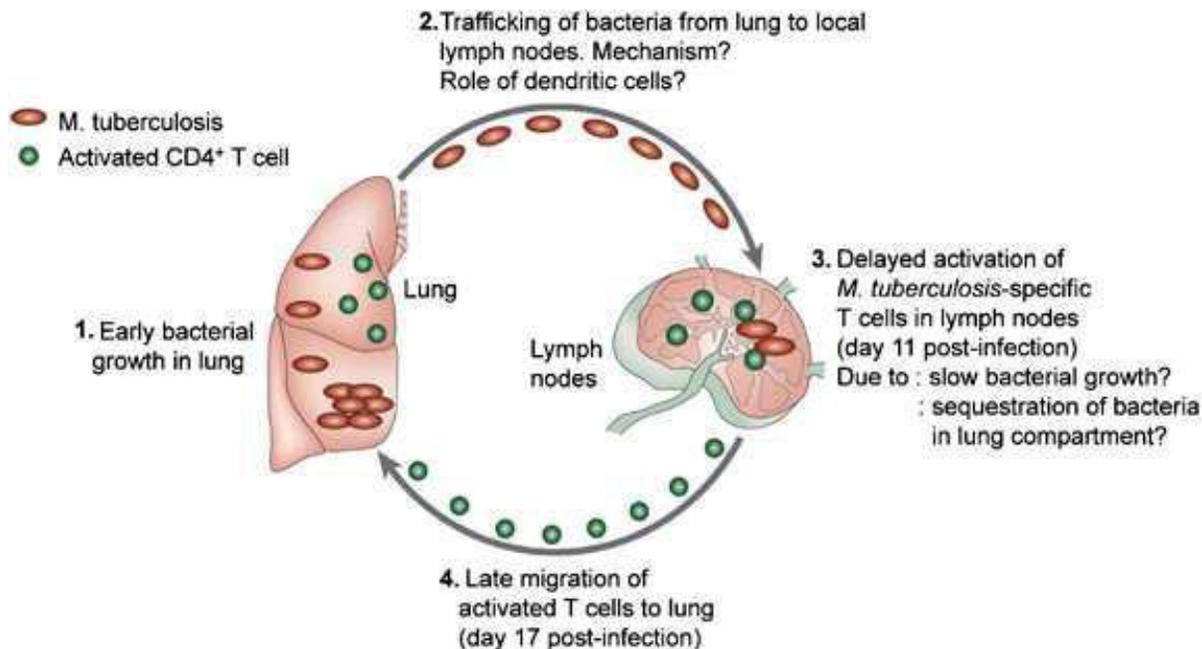


Figure 1. Overview of the events that influence the initiation of the adaptive immune response after infection with *M. tuberculosis*.

Source: Triccas and Davenport (2008).

Adaptive immunity

In cattle, both humoral and cell-mediated responses can be induced following *M. bovis* infection. Several studies have shown that protective immunity to TB is dependent on the adaptive TH1 immune responses (Ngai et al., 2007). It is mediated by macrophages, DCs, T cells and their interactions, which depends on the interplay of cytokines produced by these cells (Berrington and Hawn, 2007). The adaptive immune response is initiated when mycobacteria infected DCs mature and migrate to local lymph nodes (LN), where recognition by T cells takes place (Flynn, 2004). The hallmark of chronic infections such as TB is the significant delay between infection and the induction of the adaptive immune response, which allows early growth of the pathogen and the establishment of persistent infection. Recently, it was demonstrated that activation of *M. tuberculosis*-specific CD4⁺ T cells is dependent on trafficking of bacteria from the lung to local LN, and that delayed dissemination from the lung to sites of antigen presentation accounts for the lag in the initiation of adaptive immunity (Triccas and Davenport, 2008; Wolf et al., 2008) (Figure 1). While the precise mechanisms for this delay are unclear, it has been suggested that low levels of antigen in early infection may help evade immune recognition and that some threshold level of antigen is required to stimulate

the T-cell response (Russell et al., 2007). On the other hand, late migration of activated T cells to the lung was suggested to contribute to the delay in the onset of adaptive immunity (Wolf et al., 2008).

Cell mediated immunity (CMI)

Numerous studies performed in humans and various species of animal have demonstrated the central role of cell mediated immunity in the resolution of mycobacterial infections. The key players in anti-mycobacterial immune responses are T lymphocytes and antigen presenting cells. Both CD4⁺ and CD8⁺ T cells are implicated in the response; these cells produce IFN- γ and display cytolytic activity against mycobacteria infected cells. Responses mediated by $\gamma\delta$ T cells are also involved in the response to mycobacteria. Central to the induction of immune responses to invading pathogens are the antigen presenting cells (Ngai et al., 2007). Robust delayed type hypersensitivity (DTH) and IFN- γ responses are elicited upon experimental and natural infection with *M. bovis*. The bias of the immune response to *M. bovis* is a T helper type-1 response as evidenced by IFN- γ , IL-12, and TNF- α production to pathogen-associated antigen (Flynn, 2004).

Immunohistological examination of early granulomatous

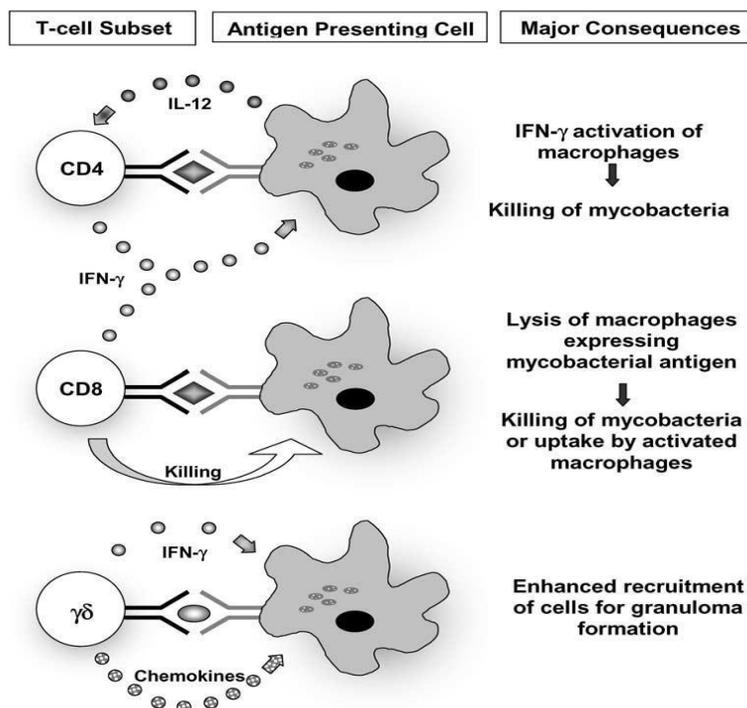


Figure 2. T-cell sub-sets which have a role in protection against tuberculosis.

Source: Buddle et al. (2002).

lesions induced by experimental *M. bovis* infection of cattle has shown T-cells to be among the first cells involved in the reaction (Cassidy et al., 2001). This has pointed to the importance of cell mediated immune responses in bovine tuberculosis, a concept supported by both field and experimental studies which recognize a complex spectrum of immune activity with early domination by T-cell driven responses. These observations have led to a recent expansion of studies to dissect the early CMI response in bovine tuberculosis (Pollock et al., 2001).

All of the main T-cell subsets ($\gamma\delta$ T-cells, $CD4^+$ and $CD8^+$ $\alpha\beta$ T-cells) have been shown to be involved in the anti-mycobacterial immune response in cattle (Figure 2) (Buddle et al., 2002). Study of the dynamics of lymphocyte subsets in the circulation of cattle infected experimentally with *M. bovis* has revealed a sequential involvement of $\gamma\delta$ then $CD4^+$ and later in the infection a more prominent involvement of $CD8^+$ T-cells. T-helper type-1 (TH1) type of immune response, was characterised by production of IFN- γ , which is deemed to be essential for the activation of macrophage microbicidal pathways. In *M. bovis* infected cattle, $CD4^+$ T-cells appear to be the most dominant cell population producing IFN- γ leading to the activation of macrophage anti-mycobacterial

capabilities, with $CD8^+$ T-cells having a greater involvement in the lysis of infected cells (Lie'bana et al., 2000). Effective immune responses are believed to primarily rely on CMI or TH1 responses that involve macrophages, dendritic cells and an adaptive T cell response. These responses are controlled by cytokines released from antigen-specific T cells with the pivotal cytokine of this response being IFN- γ . Evidence indicating that IFN- γ plays a significant role primarily comes from studies using IFN- γ knockout mice. Mice deficient in IFN- γ production quickly succumb to infection. Other pro-inflammatory cytokines, such as IL-12 and TNF- α likely play a role in the TH1 response and granuloma formation (Flynn and Chan, 2001). The culmination of the CMI response is granuloma formation in which the bacteria are walled off, presumably to prevent their spread (Ulrichs and Kaufmann, 2006) (Figure 2).

The precise contributions of the TH1 response to immunity and pathology have not been delineated. T-Helper type-2 (TH2) responses induced by *M. bovis* infection are thought to inhibit type-1 T cell responses and thus contribute to pathology (Tylers et al., 2007). These responses are characterized by the production of cytokines such as IL-4, IL-5 and IL-10. It has been suggested that during the course of infection, cattle convert

convert from a predominant TH1 response early after infection to a TH2 like response and that this conversion correlates with increased pathology (Welsh et al., 2005).

Humoral immunity

Since the organism is an intracellular pathogen, the serum components are thought not to get access to the pathogen and hence, may not play any protective role. However, this view has been challenged by recent studies, showing that the humoral immune response has shown effectiveness against other intracellular pathogens (Glatman-Freedman, 2006), suggesting that it may contribute to protective immunity to tuberculosis. Studies has shown that monoclonal antibodies against surface antigens of *M. tuberculosis* give rise to protective immunity in mice and prolong their survival after infection with lethal doses of *M. tuberculosis* or *M. bovis* through a more organized and compact granuloma formation where the bacilli were contained (Chambers et al., 2004).

It is also known that mycobacteria specific antibodies can both influence mycobacterial dissemination and modulate potentially detrimental inflammatory tissue responses (Maglione et al., 2007). Generally, antibodies seem to have an opsonizing role and thereby improve phagocytosis by macrophages or the cytotoxic actions of killer lymphocytes. The ability of human antibodies induced by *M. bovis* BCG vaccination has been studied recently and internalization of BCG by phagocytic cells was significantly enhanced in post-vaccination serum samples. Furthermore, the inhibition effects of neutrophils and macrophages on mycobacterial growth were significantly enhanced by BCG-induced antibodies. BCG-induced antibodies were shown to significantly enhance the cell-mediated immune response with an increased proliferation and IFN- γ production in mycobacterium specific CD4⁺ and CD8⁺ T cells. Mycobacterium specific antibodies seem capable of enhancing both innate and cell mediated immune responses to mycobacteria (De Valliere et al., 2005). It is increasingly recognized that B cells can exert an influence on T cells (Lund et al., 2006) and are an important constituent of granuloma architecture (Tsai et al., 2006). It is important to note that intracellular pathogens can also be found in the extracellular space during their life cycle, either before entering the host cells or after cell death, and then can easily be reached by antibodies, preventing their dissemination (Hiwa et al., 2007).

IMMUNE EVASIVE MECHANISMS

M. tuberculosis invades and replicates in macrophages, cells of the host innate defense system designed to

eliminate pathogenic microorganisms, through a variety of immune evasion strategies. The use of non-activating complement receptors (CR) to enter into macrophages may be advantageous for the bacterium, since engagement of these receptors does not induce the release of cytotoxic reactive oxygen intermediates (ROI) (Lie bana et al., 2000). The ability of pathogenic mycobacteria to adapt to the hostile environment of macrophages has been instrumental in its success as a pathogen. Mycobacteria interfere with host trafficking pathways by modulating events in the endosomal/phagosomal maturation pathway to create a protective niche (Houben et al., 2006). The mycobacteria containing phagosome, while connected to the endocytic pathway, does not fuse with lysosomes or mature into phagolysosomes (Nguyen and Pieters, 2005). By blocking its delivery to lysosomes, the mycobacterium is able to avoid the acidic proteases of lysosomes; avoid exposure to the bactericidal mechanisms within lysosomes; prevent degradation and hence processing and presentation of mycobacterial antigens to the immune system (Pieters, 2001).

Another mechanism by which mycobacteria could interfere with phagolysosomal fusion is by retention of an important host protein called tryptophan aspartate containing coat protein (TACO), also known as coronin 1 on the phagosome, thereby behaving as self antigens. TACO represents a component of the phagosome coat, and retention of TACO prevents phagosomes from fusing with lysosomes, thereby contributing to the long-term survival of bacilli within the phagosome (Nguyen and Pieters, 2005). The recognition of infected macrophages by CD4⁺ T cells depends on constitutively expressed major histocompatibility complex (MHC) class II on professional antigen presenting cells (APCs), level of which is upregulated upon activation with IFN- γ . One mechanism by which *M. tuberculosis* avoids elimination by the immune system after infection is through the inhibition of MHC II expression or antigen processing or presentation by macrophages (Fulton et al., 2004). Inhibition of MHC II expression or antigen processing does not require viable bacilli and can be achieved by exposure to bacterial lysate (Noss et al., 2000).

PATHOGENESIS

The pathogenesis of bovine tuberculosis is not as well understood as the pathogenesis of tuberculosis in humans. Advances in the field of human tuberculosis have been made using various small animal models of *M. tuberculosis* infection (Mitchell and Waters, 2006). The host response to the tubercle bacillus is complex and broad, involving all aspects of the immune system (Flynn and Chan, 2001). The organism has evolved to avoid

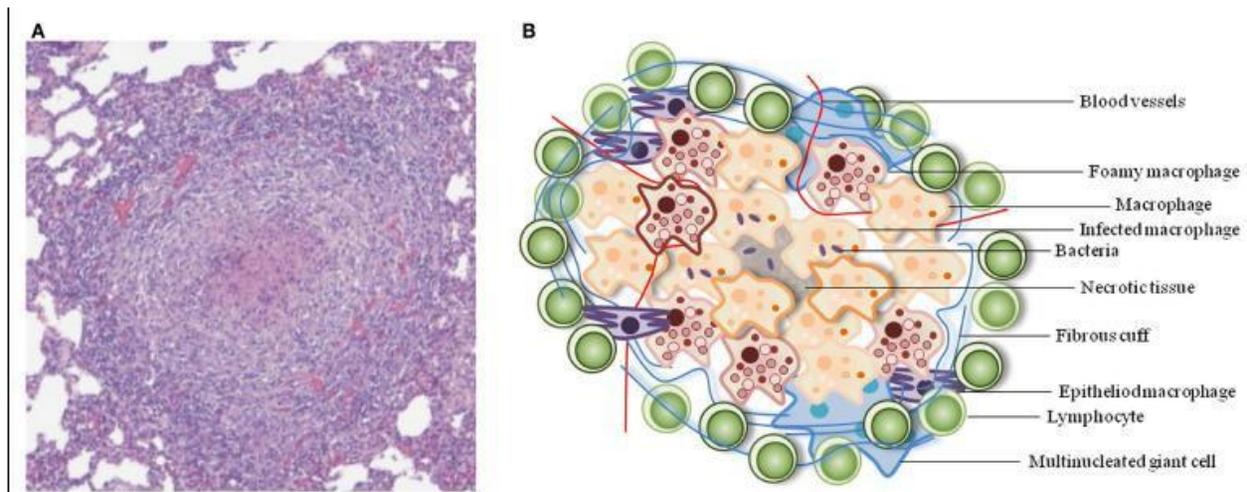


Figure 3. Typical architecture of a TB granuloma. (A) Representative granuloma with central necrosis from mini pig lung tissue. Histological samples were formalin-fixed cut and stained with hematoxylin-eosin. (B) Schematic of the cellular constituents of a TB granuloma.

Source: Guirado and Schlesinger (2013).

immune clearance and induce chronic lesions ensuring transmission by infectious aerosol droplets (North and Jung, 2004). Paradoxically, lesions (that is, granulomas) are elicited as a mechanism to limit spread of the bacillus, thereby preventing early demise of the host (Mitchell and Waters, 2006). Although granulomas limit the spread of the pathogen, they contribute to tissue damage. The balance between controlling bacterial spread and tissue damage may represent the most significant biological challenge to the host immune response (Tyler et al., 2007). Inflammatory response induced by persistent presence of the mycobacterium in the tissue is characterized by granuloma, a distinctive pattern of chronic inflammatory reaction. Granulomatous formations, that surround infected cells and caseous necrosis, are an evidence of cellular response against mycobacteria infection (Tonya et al., 2005) (Figure 3).

Granuloma is multi-cellular structure where there is predominate macrophages, multinucleated giant cells, lymphocytes and necrosis. These macrophages respond to bacterial infections by the process of phagocytosis; the engulfment of bacteria (Algood et al., 2005). Activated macrophages become efficient at phagocytosis and bacterial killing due to the presence of T cells and cytokines. The granuloma serves three major purposes; it is a barrier to dissemination of bacteria throughout the lungs and other organs, a local environment in which immune cells can interact to kill bacteria, and a focus of inflammatory cells that prevent inflammation from occurring throughout the lungs (Roach et al., 2001).

Phagocytes are attracted to sites of infection via the release of chemokines and cytokines (for example, IFN- γ) by a variety of cell types. Infected macrophages

release various chemokines (for example, IL-8, MIP-2, IP-10, and MCP-1) that attract macrophages, neutrophils and T cells to sites of infection (Mitchell and waters, 2006). Additionally, macrophages produce cytokines (for example, IL-12, TNF- α) that both up- and down-regulate adaptive immunity (Flynn and Chan, 2001; Tonya et al., 2005). The immunopathology of granuloma formation is complex and appears to develop in distinct stages of advancement. Bovine models of *M. bovis* infection have shown that lesions, induced following experimental infection, are generally indistinguishable from natural cases of infection (Neill et al., 2001). In an experimental study, a deer was infected with virulent *M. bovis* via intratracheal inoculation. Gross lesions were seen in the upper and lower respiratory tract and associated lymph nodes at 6 to 23 weeks post infection.

Histologically, there are different types of granulomas. Initially, epithelioid cells may be surrounded by an acellular necrotic region, with a ring of Band T cells. The granulomas can displace parenchymal tissue and may necrotize, caseate, and/or calcify. Caseous granulomas might turn calcified during chronic or latent infection. Other types of granulomas may not have a necrotic area and are composed primarily of macrophages and a few lymphocytes. Host-pathogen interactions in the granuloma over the course of infection lead to adaptive changes of the tubercle bacilli, phenotypes of the host immune cells, and levels of the immune mediators they produce. These features allow for the formation of a wide spectrum of granuloma structures even within a single human host, therefore implying the presence of several unique micro environments for *M. tuberculosis* as well as for the immune response (Algood et al., 2005).

Generally, granulomatous lesions were small pale yellow in colour with a caseous core. Microscopically, lesions were observed with macrophage, giant cells (containing acid-fast bacilli) and neutrophilic debris in evidence. As lesion development progressed, there was more extensive necrosis, consisting of intact and degenerate neutrophils, macrophages and lymphocytes. Some mineralization and fibrosis was also seen (Pollock et al., 2006). Previous studies classified granulomatous lesions in lymph nodes from calves experimentally infected with *M. bovis* into four developmental stages (Linda et al., 2006).

1. Stage I (initial): Irregular, unencapsulated cluster of epithelioid macrophages, with interspersed lymphocytes and few admixed neutrophils. Langhans' giant cells may be present. No necrosis is present.
2. Stage II (solid): Partially or completely thinly encapsulated granuloma composed primarily of epithelioid macrophages. Haemorrhage is often noted along with infiltration of lymphocytes, neutrophils and often Langhans' giant cells. Minimal necrosis may be present, and is generally composed of necrotic inflammatory cells.
3. Stage III (minimal necrosis): Fully encapsulated granuloma with central necrosis which is caseous and mineralized. Epithelioid macrophages surround necrosis admixed with Langhans' giant cells. A peripheral zone of macrophages mixed with clusters of lymphocytes and scattered neutrophils extends to the fibrous capsule.
4. Stage IV (necrosis and mineralization): Thickly encapsulated, large and irregular, multicentric granuloma with prominent caseous necrosis and extensive islands of mineralization comprising the greatest area of the lesion. Epithelioid macrophages and multinucleated giant cells surround the necrosis, with clusters of lymphocytes distributed more densely near the peripheral fibrotic capsule.

The granuloma is an elaborated aggregate of immune cells found in non-infectious as well as infectious diseases. It is a hallmark of tuberculosis (TB). Predominantly thought as a host-driven strategy to constrain the bacilli and prevent dissemination, recent discoveries indicate that the granuloma can also be modulated into an efficient tool to promote microbial pathogenesis (Guirado and Schlesinger, 2013). It would be unreasonable to call the TB granuloma an unsuccessful host defense, as it successfully contains the infectious focus in more than 90% of cases. The 10% of individuals that progress toward TB disease suffer from a disbalanced inflammatory reaction, be it due to too little innate or adaptive immunity or due to unrestrained hypersensitivity reactions (Ehlers and Schaible, 2013).

Trehalose-6, 6 dimycolate (TDM), the mycobacterial cord factor is the most abundant cell wall lipid of virulent

mycobacteria, is sufficient to cause granuloma formation, and has long been known to be a major virulence factor (Lang, 2013).

According to Shaler et al. (2013) the true nature of the granuloma still remains to be defined, it is now clearly evident that the granuloma is not just a host-mediated entity of segregation and rather, it is a dynamic battlefield bearing the scars left both by the pathogen and the host immune response. The same authors stated that it may have been originally destined to restrain bacterial dissemination; *M. tuberculosis* efficiently hijacks the granuloma to provoke the generation of an immunologically sheltered niche to reside within and persist until the situation is favorable to bacterial transmission.

CONCLUSION

The immune response to bovine tuberculosis is apparently multifaceted and there are still many unsolved problems associated with the pathogenesis and immune response to tuberculosis. There is considerable benefit in drawing from knowledge of the immunopathological mechanisms elucidated in studies of tuberculosis in humans and in laboratory animal models. These can provide new insights into many of the fundamental mechanisms shared with the bovine disease. The variation of immunopathological responses in a variety of susceptible hosts makes the understanding challenging. Although our knowledge is still limited it is already clear that mycobacterium employs novel pathogenic strategies for both replication and persistence *in vivo*.

It is already clear that a more complete understanding of the pathogenic strategies of this highly successful intracellular organism will elucidate novel feature of host immune response. A broad, multi-disciplinary approach in comparative pathology, immunology and molecular biology will be required for the understanding of comprehensive pathogenesis of the disease. Clear contribution of both the humoral and cell mediated immunity in protection against mycobacterium need to be elucidated. The future research on identification and detail understanding of how host cell regulate mycobacterial infection will be of exquisite importance to develop biomarkers and therapeutics. Rapid advancement and explosion of research nourishes our hope for a giant leap in better diagnosis and treatment of mycobacterial infections in the future.

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