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Mycobacterium avium Subspecies *paratuberculosis* Infection and Immunopathological Changes in Animals: A Review

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ABSTRACT

Mycobacterium avium subspecies *paratuberculosis* (MAP) is a chronic granulomatous intestinal disease and causative agent of Johne's disease (JD) in domestic and wild ruminants. This study explores the intricate dynamics of the host immune response in MAP infection and its implications for disease progression and control. Our investigation revealed the pivotal role of interleukin-10 (IL-10) in mediating the communication between the host's innate and adaptive immunological responses. IL-10 inhibits interferon-gamma (IFN-γ) secretion, altering the balance between Th1 and Th2 responses. In the early stages, a robust Th1 response marked by increased IFN-γ production is observed, but as the disease progresses, a shift towards a humoral Th2 response occurs. Furthermore, we found that IL-10 exerts suppressive effects on macrophage (Mφ) anti-mycobacterial action, leading to increased intracellular survival of mycobacteria. IL-10 also hampers Mφ antimicrobial activity by suppressing the production of essential cytokines such as IL-12 and tumor necrosis factor alpha (TNF-α), crucial for activating natural killer (NK) cells and inducing differentiation of CD4+ T lymphocytes into Th1 effector cells. Importantly, our study sheds light on the intricate interplay between past infections or concurrent active co-infections and their influence on the immune response to unrelated pathogens in the context of paratuberculosis. This review underscores the multifaceted role of IL-10 in the immunopathological manifestation of MAP infection and host immunomodulation. These findings provide valuable insights for both animal and human research in the fight against MAP infection, emphasizing the importance of balancing immune responses and considering the influence of concurrent infections in disease progression.

Key words: Animals, Bacille Calmette-Guerin, Immune response, Johne's disease, *Mycobacterium avium* subspecies *paratuberculosis*, Pathogenesis.

Mycobacterium avium subspecies *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a chronic granulomatous intestinal illness that affects cattle, primates and small/wild ruminants (Garvey, 2018). The disease poses a significant economic burden due to its negative impact on livestock farming and animal welfare (Chaubey *et al*., 2017). MAP infection is known to cause paratuberculosis (pTB) in a wide range of animals (Chaubey *et al*., 2016). With its slow and insidious spread, pTB exhibits a long incubation period, with initial asymptomatic signs typically manifesting in young calves after approximately a year (Karcher *et al*., 2008). The global prevalence of pTB has been a cause of concern, as it is documented in specific regions and continues to spread rapidly within the livestock industry, leading to substantial financial losses (Roller *et al*., 2020). Diagnosis of MAP infection is very tough and challenging because infection producing non-specific symptoms and it comes under observation after long incubation period of the exposure, very long or variable sub-clinical stage of disease in individual infected animals and intermittent or continuous shedding of the MAP bacilli in their milk and faeces (Sweeney *et al*., 2011; Chaubey *et al*., 2016; 2017). Chaubey *et al*. (2019) compared different screening test where they reported histopathological analysis and immunomodulation in host due to MAP infection and also showed pathological test having higher sensitivity than other screening tests. Sharma *et al*. (2022) explained that changes in microbiome profile of the host may modulate the host immune responses. The complex interplay between MAP and the

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host's immune responses has been a subject of extensive research, shedding light on the immunopathological changes that occur during the course of the infection (Kravitz *et al*., 2021).

In this comprehensive review, we aim to investigate into the diverse aspects of MAP infection and its association with immunopathological alterations in various animal species. By findings from multiple studies, we seek to provide a deeper understanding of the mechanisms underlying the establishment and progression of pTB. Additionally, we will explore the implications of MAP infection on animal health, agriculture and the economy, with a focus on the global impact of the disease. Through this exploration, we hope to contribute to the growing body of knowledge surrounding MAP infection and its consequences, which could potentially pave the way for improved diagnostics, prevention strategies and therapeutic interventions. As the understanding of pTB continues to evolve, addressing this significant health concern will be crucial for safeguarding animal populations and promoting sustainable livestock practices.

Phases of pTB infection

The disease progresses through three clinical stages. In stage I (subclinical with undetectable excretion), there is appreciable shedding of bacteria. There are no clinical indications during the silent infection period, however animals periodically shed MAP in their faeces (Cocito *et al*., 1994). This is followed by a subclinical excretory phase (stage II), during which the concentration of mycobacteria in the intestinal mucosa and lumen progressively increases. The terminal phase (stage III, clinical and excretory) is characterized by an intractable chronic diarrhoea and the symptoms of a generalized infectious process (Whitlock and Buergelt, 1996). The majority of the animals die in the clinical phase caused by severe dehydration and emaciation due to disease.

Host-pathogen responses in pTB

The immune response to pTB starts with a strong T-helper1 (Th1) response that is marked by increased interferon-gamma (IFN-γ) production (Stabel and Whitlock, 2001). With the advancement of the disease, the immunological response shifts from a cell-mediated Th1-type response to a body's immune Th2-type response (Leite *et al*., 2015). Strong Th1 immunological responses targeted against the causal pathogen, MAP is thought to be required to keep bacterial reproduction under control (Begg *et al*., 2018). Macrophages (Mφ) then take up MAP and engage with gut-associated lymphoid tissue (GALT), causing a variety of local alterations. Therefore, the result of MAP infection is believed to be determined by the local immune responses induced by lymphocytes in various layers of the gut (Munjal *et al*., 2005). Th1 response, which is defined by the generation of IFN-γ controls the early host response to MAP infections (Park *et al*., 2018).

Furthermore, in pTB bovine model, as shown in Fig 1 about the overview of immune responses, the innate immune response in natural and challenged MAP infected animals declining with progression of the disease (Begg *et al*., 2011). Pathogenic mycobacteria communicate through toll like receptor 2 (TLR2) to avoid Mφ antigen presentation and antimicrobial responses, according to several investigations (Banaiee *et al*., 2006). By interacting with TLR2 on bovine mononuclear phagocytes, MAP activates mitogen-activated protein kinase-p38 (MAPK-p38), resulting in enormous levels of interleukins-10 (IL-10) synthesis and a small level of IL-12 synthesis. For the elimination of microbial infection, successful phago-lysosomal maturation is a critical innate immunological response. During mycobacterial infection, MAPK-p38 plays a key role in signalling pathways (Khalifeh and Stabel, 2004) and it's linked to blocking phagosome-lysosome fusion and maturation. IL-10 can be a powerful anti-inflammatory

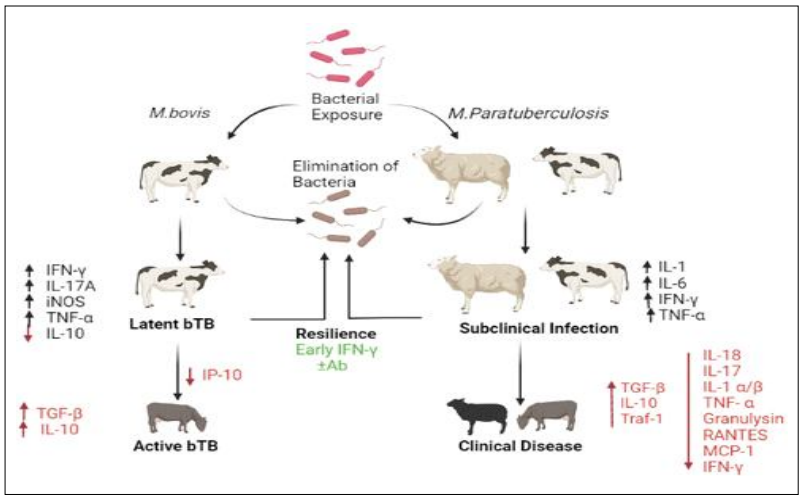

Fig 1: Overview of immune response in MAP infection.

cytokine that prevents phagosome formation and apoptosis. We also discuss techniques for targeting IL-10, MAPK and signal transducer and activators of transcription 3 (STAT3) in various intracellular pathogen contamination (Hussain *et al*., 2016). MAP infection alters miRNA expression, which is important for fine-tuning the host antibody response and could be used as biomarkers and diagnostic tools for MAP infection (Malvisi *et al*., 2016). MAP infection has a higher susceptibility rate than other diseases because pTB can spread horizontally and vertically in the animals (Nielsen *et al*., 2013). MAP infection has been also reported as chronic inflammatory bowel disease in human and present in the blood, intestine and breast milk (Naser *et al*., 2004). At the time of infection of subclinical phases, Mφ form granulomas in the gut lymphoid tissue due to the insistence of MAP. Faecal shedding is known to take place with the down regulation of cell-mediated immunological response and over expression of the humoral immune response however, in a few cases sick animals pointed enhanced faecal shedding along when cellular immunity is activated (Ganusov *et al*., 2015).

Pathophysiological connection between Mφ and other immune cells in MAP infection

In certain situations, the production of IL-1 can reach excessive levels, raising the chances that few clinical JD symptoms because of IL-1 toxicity and that increased IL-1 production at infection sites coincides with increased expression of tumor necrosis factor receptor-associated protein 1 (raf-1) in invading Mφ. The pathophysiology of MAP infection is thus consistent with increased IL-1 expression, especially because lesions associated with JD often feature a large number of Mφ (Aho *et al*., 2003). IFN-γ and IL-6 transcript expression is similarly increased in intestinal tissues when MAP infection is active (Coussens *et al*., 2004). Cluster of differentiation 40 (CD40) signalling is impaired on Th1 cells, which is a key Mφ target for CD40 ligand (CD40L) to keep a Th1 immunological response. Mononuclear phagocytic cells (MPC) provide an intracellular environment for MAP survival and proliferation. Pathogen recognition receptors (PRRs) on Mφ are important in the absorption of mycobacteria. One of the immunological evasion strategies: MAP can impede the formation and acidification of the phagocytic vacuole within Mφ, preventing bacteria from being exposed to bactericidal lysosomal enzymes and oxygen-obtaining radicals (Sohal *et al*., 2008). As a result, MAP can proliferate within Mφ phagosomes while utilising resources of host cell. MAP is thought to cause apoptosis and infect nearby Mφ, drift into the adjacent lymphatic organ, allowing bacteria to spread to lymph nodes, including the mesenteric lymph nodes (Allen *et al*., 2001). MAP can block Mφ apoptosis, lowering the immunological response and permitting intracellular reproduction, based at the level of strain's infection virulence (Kabara and Coussens, 2012).

Infected mice's spleens had an increased number of cytokines and MAP-reactive T cells and their serum had modestly raised levels of IFN-γ (Abdissa *et al*., 2020). The pathological alterations found in groups leporine and bovine were clearly demarcated, non-encapsulated granuloma comprising epithelioid cells, Mφ and the infrequent giant cell. Although no acid-fast bacteria (AFB) was seen in some granulomata, a varied no. of AFB had been detected in particular granulomata's cells. But lesions have been found in the jejunum of an organism (B3), with granuloma in both the jejunal Peyer's patch (JPP) and the overlaying lamina propria, as well as a limited variety of AFB in the JPP granulomata (Beard *et al*., 2001).

The use of macroscopic alterations in the mesenteric lymph nodes and small intestine as a prescription in the post-mortem diagnosis of JD illness in goats have been observed. Macroscopical alterations were frequently absent or non-specific. Caseous and/or calcified foci in infected animals' mesenteric lymph nodes were frequently detected, whereas reported intestinal alterations were surprisingly rare. However, substantial epithelioid cell infiltrations and numerous acid-fast bacilli were frequent in portions of macroscopically unaltered gut. Infected animals had productive inflammation with tubercle development in lymph nodes on a random basis (Fodstad and Gunnarsson, 1979).

Immunological biomarkers and their role in MAP infection

Antibodies (Ab)

In spite of the fact that the significance of the production of antibodies in immunity of host to intracellular mycobacterial infections is unknown, certain Abs detected in the serum, can play a role in preservative immunity (Achkar and Prados-Rosales, 2018). Although less widely used in Bovine tuberculosis (bTB), ELISA conjugated with serum and milk antibody is common diagnostics for pTB. Commercially available pTB test methods have the maximum diagnostic performance in the later phases of the disease, when animals are infected, but lower sensitivity in the initial stages of infection (Nielsen and Toft, 2008). In an experiment in sheep, animals designated as disease resistant has a greater response of antibody than those in which the infection developed (De Silva *et al*., 2018). This opens up few latest possibilities for serological testing to be utilized to detect hardy animals during the initial phases of sickness. In a study, a indigenously developed ELISA was employed to screen serum samples from cattle, evaluating the antibody response to MAP infection and the results indicated a 27.22% prevalence of MAP infection in the cattle population in the Madhya Pradesh region (Audarya *et al*., 2022).

Multiplex bead-based assays exhibit a dependable capability for detecting MAP infection, even in its initial stages, when conventional commercial ELISA tests often fail to detect antibody responses in animals. In terms of improving early diagnosis in both pTB and bTB, a variety of

antigens have been investigated (Li *et al*., 2017). The most common subtype employed in mycobacterial antibody ELISA is immunoglobulin G (IgG); although, targeting alternative isoforms may be more revealing. IgA, the most common subtype seen in mucosal secretion, has also holds promise in identifying resilience, since it has been linked to protective responses in mycobacterial infection in mice (Tjarnlund *et al*., 2006). During the course of pTB disease, MAP-specific faecal IgA immunoglobulin can be detected; however this is temporary and seems to be related to ambient MAP infection (Begg *et al*., 2015).

Cytokines, IFN-γ, chemokines and ILs in MAP

Assessing the IFN-γ response directly at the infection site could offer a more pertinent parameter for gaining a deeper understanding of the cytokine's involvement in fecal shedding. Unfortunately, implementing such a diagnostic test is not feasible (De Silva *et al*., 2018). Sheep has a larger initial IFN-γ response when they are immature, this cytokine has the potential to be used as a biomarker of resistance. Other cytokines and chemokines that are differentially regulated between infected and uninfected individuals and between active and hidden infection, have been reported and these are likely relevant to other mycobacterial diseases. In research on human mycobacterial infections, activated T cells and associated cytokines, such as IL-2, IL-3, IL-6, IL-7, IL-8, IL-9 and IL-10 were found to vary considerably even between infected and normal groups (Anbarasu *et al*., 2013). Hence, further investigation is imperative, especially concerning pathogen-specific reactions, to ascertain whether cytokine levels can serve as a reliable means of determining and differentiating in clinical contexts (Table 1).

As the clinical condition worsens in both sheep and goats, the immune response is reduced, most likely due to overexpression of immune-suppressive cytokines (Lybeck *et al*., 2009). The release of IFN-γ from Th-1 in goats is induced by neutralisation of IL-10 *in vitro* with monoclonal antibodies supporting IL-10's inhibitory impact. IFN-γ assays results in the context of pTB can offer valuable insights into the extent of exposure within a herd, serving as a robust indicator of herd-level exposure. Existing diagnostic tests in cattle use IFN-γ as a marker of MAP exposure; however, due to its low specificity, it is not a suitable marker of infection (Plain *et al*., 2012). An elevation in IL-10 was detected in sheep, four months following experimental infection with MAP, implying that infected sheep as alternative develop Th1 immune response over Th2 immunological response in initial phase of the disease. As a result, the significance of IL-10 in the response to MAP is debatable and the basis for the disparity in reaction between the two types has unexplained. Overall total leucocyte counts and lymphocytes have been shown to be significantly diminished in natural cases of MAP infection in buffaloes (Sharma *et al*., 2020).

MAP targets the host's lymphoid tissues linked with the mucosa. The micro fold cells (M cells) of neonatal calves, goat kids and mice's ileal Peyer's patches are one way that MAP attacks the organism (Bermudez *et al*., 2010). Fig 2 depicts the infection of MAP in calves and its subsequent host interactions. M cells play a pivotal role as antigen-presenting cells and have been demonstrated to internalize and transport various pathogenic microorganisms, such as *Mycobacterium* species, without undergoing degradation. Bacteria can remain and delivered whole to the fundamentally immune-competent, sub-epithelial lymphoid organ due to lacking of lysosomes and hydrolytic enzymes by M-cells (Sigurardóttir *et al*., 2004). On its cell wall, MAP produces fibronectin attachment protein (FAP), allowing it to be opsonized by fibronectin and afterwards uptaken by M cells, that have numerous β1 integrin receptors at its luminal side. During MAP absorption, M cells activate defensins, which are AMP to guard the host. A transitory rise in β-defensin is observed in the first four hours after exploratory MAP infection of cattle. Enterocytes in lambs and mice have also been proven to occupy MAP (Bermudez *et al*., 2010). IL-12 plays a pivotal role in fostering the development of Th1 cells that produce IFN-γ when naive CD4+ T cells are activated. Furthermore, in the context of infection caused by intracellular parasites, IL-12 produced by macrophages stimulates NK cells to generate IFN-γ, thereby contributing to the protective response during the acute phase of infection.

Clusters of differentiation (CD) markers, Mφ and Th cells

MAP is relocates the mucosal epithelium after initial invasion by M cells, then penetrates and continue in sub-epithelial Mφ, triggering a cellular immunological response centred at the CD4+ Th1 cell mileu. Complement receptors, immunoglobulin receptors and scavenger receptors all play a role in Mφ mediated MAP uptake (Whittington *et al*., 2012). MAP is delivered to CD4+ Th-1 cells by Mφ at the time of primary infection. IFN-γ is secreted by CD4+ T-cells, which inhibits bacterial proliferation. Throughout the subclinical stage of JD, the amount of IFN-γ rises in the ileal and cecal lymph nodes of cattle. MAP is thought to infiltrate and inactivate Mφ by interfering with their potential to respond to normal T-cell signalling, according to some studies. T-cells have compelled to acknowledge to extracellular indicator *via* the CD154-CD40 pathway during MAP infection, favouring unsuitable Th2-like pursuit, as well as IL-10 production and unable to start phagosome acidification, which should fight infection (Sommer *et al*., 2009). The progression of JD from subclinical to clinical forms has linked to a movement in immunological response from Th1 to Th2, which results in the development of a significant cellular response as well as a simultaneous antibody and IFN-γ response (Begg *et al*., 2011). Lambs infected with MAP's C strain developed a combined cellular and humoral response, but animals affected with S strain only developed a weak innate immunity (Fernández *et al*., 2014). Sheep's immunological response to MAP infection has influenced by the strain, which influences the clinical condition they suffer. Antigen mediated humoral immune response of Th1

Table 1: Cytokine, chemokine and interleukin responses were observed in MAP infection.

Disease	Medium	Samples	Markers	Responses	Reference
MAP infection/ pTB	Sheep, goats, cattle	Bovine intestinal tissue	TNF-α, IL-18, IL-1α, 1β,	(IL-1β and TNFα ↑), IL-1α No change, IL18 ↓	Smeed *et al.*, 2007
		Plasma and MDMs	IFN-γ, osteopontin, tumor necrosis factor-α, IL-1β, IL-6, IL-23, transforming growth factor-β	↑	Dudemaine *et al.*, 2014
		THP-1	IFN-γ, Leukemia inhibitory factor (LIF), IL-22, IL-13, IL-17	↓	Berry *et al.*, 2018
		Bovine PBMC/intestine	TNF-α, IFN-γ, Transforming growth factor-β (TGF-β), IL-17A and IL-10	↑	Palmer *et al.*, 2016b
		Spleen and liver	IFN-γ, TNF-α, IL-4, IL-10	(IFN-γ, TNF-α and IL-4 ↑), IL-10 ↓	Thacker *et al.*, 2007
		Caprine PBMC	IFN-γ, TNF-α, IL4, IL10	↑	Witchell *et al.*, 2010
	Buffaloes, Cattle	Spleen or lung	IFN-γ, IP-10, IL-17a granzyme B and A	↑	Aranday-Cortes *et al.*, 2012
		Multinucleated giant cells	IL-4, IL-6, IFN-γ	(IL-4, IL-6 ↑), IFN-γ ↓	Widdison *et al.*, 2006
		Tissue	IL-17A, IFN-γ, TGF-β, IL10, IL-22,TNF-α,	↑	Palmer *et al.*, 2016a
		Lymph node	IFNγ, IL-12p40, IL-1β1, TGFβ, IL-10	↑	Hodgkinson *et al.*, 2012

cells and CD4+/CD8+ ratio >1 has been seen during paucibacillary enteritis (Reddacliff *et al*., 2004). Sheep with the paucibacillary degenerative disease have elevated amounts of IFN-γ, whereas those with the multibacillary structure have a strong humoral response to MAP and a weak cell-mediated immunological response to MAP, as well as a lower ratio of CD4+ and CD8+ cells. IFN-γ production is lower in this stage of the disease (Smeed *et al*., 2007).

TH1- Mφ interaction

A cytotoxic and proinflammatory Th1-like response is required to sway MAP infections. Maintaining Th1 response and Mφ activation requires interaction between CD40 receptors and CD154 (CD40L) on Mφ and activated T cells respectively (Sommer *et al*., 2009). Although MAP is mostly restricted to Mφ, infection control requires a proinflammatory and cytotoxic Th1 response marked by proinflammatory cytokines like IL-12 and IFN-γ, as well as the generation of nitric oxide (NO). An adequate Th1 response emerges soon after MAP exposure (Coussens, 2001). The proinflammatory Th1-like response is shifted during the subclinical stage of MAP infection and a Th2-like response, marked by antibody formation, takes over (Stabel, 2000).

Th1/TH17axis

In the initial stages of mycobacterial infection, Th17-derived cytokines play a critical role. IL-26 and IL-17F are linked to host defence against intracellular microorganisms (Coulter *et al*., 2017). IL-26 causes immune cells to be primed and infections to be killed directly by forming membrane pores. Furthermore, through modulating the Th1 response and neutrophil recruitment, IL-17 provides protection against intracellular infections (Umemura *et al*., 2007). IL-17, IL-23 and IL-26 are Th17-derived cytokines that improve granuloma integrity by modulating neutrophil recruitment *via* CXCR3 signalling. In sick animals, peripheral and intestinal CMI responses had been lower than in subclinical animals. The destruction of CD4+ T cells and leads to a steady decline in T cells appeared to be linked to disease progression. Deregulation of Mφ function mediated by MAP is the underlying problem that could be linked to CMI depression (Tooker *et al*., 2002). Activation of gene and signalling along genes encoding TNF-α, IL-1, 12 and γ, can result in disruption in the immunological response that controls disease. Mφ that active, containing MAP travel to lymph nodes and available an antigen, which stimulates natural T cells to produce IFN-γ and cytokines like IL-6, IL-1 and IL-2 inducing the Th1 response (Coussens, 2001). Activated Th1 cells cause the immunological response that cell-mediated by producing IL-2, TNF and IFN-γ during the subclinical phages. Tim3 galectin 9 interaction have also been shown to regulate antiviral (Machala *et al*., 2019; Sharma *et al*., 2011) and anti-bacterial (Sada-Ovalle *et al*., 2012) immune responses.

Toll like receptors (TLRs)

TLRs recognise PAMPS originating from diverse microorganisms and emulate in innate immunological system. TLRs trigger the activation of the transcription factors NF-κB and IFN-regulatory factors (IRFs), which control the consequence of innate immunological responses, by attracting certain adaptor molecules (Kawasaki and Kawai, 2014). Innate immune cells including dendritic cells (DCs) and Mφ, as well as non-immune cells like fibroblast cells and epithelial cells, express TLRs. TLR2 identifies a broad range of pathogen-associated molecular patterns (PAMPs), as well as lipoproteins, lipotechoic acids, peptidoglycans, zymosan, mannan and tGPI-mucin, in combination with TLR1 or TLR6 (Kawai and Akira, 2010). For the early identification of microorganisms, the innate immunological system uses germline-encoded pattern recognition receptors (PRRs).

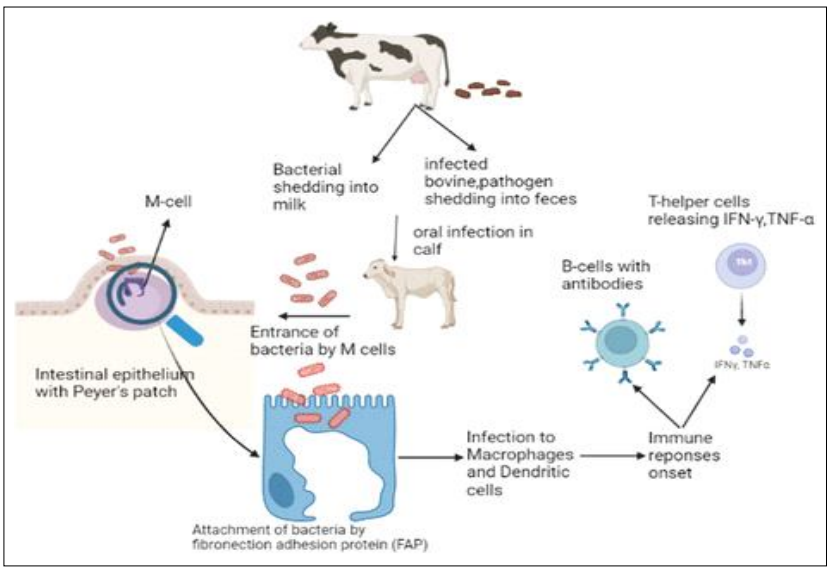

Fig 2: Infection of MAP in calf and the mode of entrance into intestine.

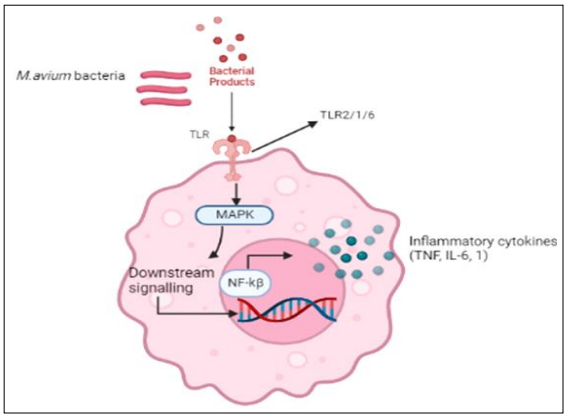

Fig 3: TLR response in Infection of MAP.

PAMPs and own generated molecules formed from injured cells, known as damage-associated molecular patterns (DAMPs), are recognized by PRRs. By generating inflammatory cytokines, such as type I IFN and other mediators, PRRs activate downstream signalling pathways which contribute in the development of innate immunological responses. Antigen-specific adaptive immunological responses are primed and orchestrated by these pathways, which not only generate immediate host defensive responses like inflammation. These reactions are required for both the clearance of invading microorganisms and the subsequent training of antigen-specific adaptive immune responses (Kawasaki and Kawai, 2014).

Changes in TLR gene expression in sheep at the time of experimental infection with MAP have been observed in both *in vitro* and *in vivo* experiments (Plain *et al*., 2010). TLR2 regulates the specific immune system in the initial phases of JD and influences development of disease in selected tissues and in sheep PBMCs (Fiorentina *et al*., 2021). TLR2 mRNA expression rises in the multibacillary form of JD, owing to a stronger inflammatory response in regions at which the majority of microbes are located (Nalubamba *et al*., 2008). TLR4 expression is higher in animals suffering from the multibacillary type of the disease. In both paucibacillary and multibacillary stages of disease, expression of TLR1 and TLR 6, particularly TLR6 in sheep, increases in target organs. TLR6 and TLR2 expression levels rise, allowing the TLR2/TLR6 heterodimer to form in response to MAP (Plain *et al*., 2010). TLR9 expression is likewise up in the PBMC of calves who have been experimentally infected with MAP (Arsenault *et al*., 2013). On the other hand, no alterations have been observed in TLR9 over expression in PBMCs of sheep and cattle spontaneously diseased with MAP (Thirunavuk karasu *et al*., 2013). TLR activation in Mφ and dendritic cells, especially in response to mycobacterial infection, stimulates intracellular signalling pathways, which result in the generation of proinflammatory cytokines (TNF-α, IL-6, IL-1) *via* NF-κB pathway (Fig 3). MAP infection in sheep and goats has been studied using these expression markers (Purdie *et al*., 2012).

Micro-RNA (miRNA)

The role of miRNAs and their influence on Mφ activation during MAP pathogenesis is still unknown. MiRNAs have been identified as a significant arbitrator of the host immunological response to infection in recent research, primarily by modulating proteins that are intricate in both innate and adaptive immunological pathways (Drury *et al*., 2017). According to certain studies, both *in vivo* and *in vitro*, Micro-RNA-27a-3p (miRNA-27a-3p) modification is down regulated during MAP infection. miRNAs are short non-coding endogenous fragments of RNA with a length of 21-25 nucleotides that control gene expression by binding to the 32 -UTR of target mRNA genes (Hoentjen *et al*., 2005). Furthermore, miRNAs play a role in Mφ polarization as well as T and B cell differentiation. The miRNA-27a and miRNA-27b have antimicrobial activity against murine CMV infection in distinct mouse cell lines, according to early research (Buck *et al*., 2010). Farrell unveiled several newly discovered circulating miRNAs present in bovine serum and pinpointed miRNAs whose levels are associated with bovine development, which could be regarded a new technique for pTB control. These miRNAs retain their stability after being stored for a long time and are used as a diagnostic marker (Farrell *et al*., 2015). The role of miRNAs in the regulation of innate immunity in pTB remains unknown (Meng *et al*., 2014).

CONCLUSION

In pTB, the significance of IL-10 is widely recognized as it plays a pivotal role in mediating the communication between the host's innate and adaptive immunological responses. Studies have demonstrated that IL-10 inhibits the secretion of IFN-γ in the peripheral blood of calves and goats infected with MAP. Notably, antigen-presenting cells (APCs) generated from ovine and bovine monocytes secrete IL-10 in response to MAP. While IL-10 serves as a vital immunoregulator, it also exerts suppressive effects on Mφ anti-mycobacterial action, leading to increased intracellular survival of mycobacteria. Moreover, IL-10 inhibits Mφ antimicrobial activity by suppressing the production of other crucial cytokines such as IL-12 and TNF-α. IL-12, an essential pro-inflammatory cytokine, is produced by APCs like monocytes, Mφ and dendritic cells. IL-12 primarily activates natural killer cells (NK cells) and induces differentiation of CD4+ T lymphocytes into Th1 effector cells, which produce IFN-γ. In the context of pTB, IL-10 plays a multifaceted role in the disease process, including guiding Th2-type cell growth in the lungs. Animal models and human investigations have revealed that past infections or concurrent active co-infections can significantly influence the immune response to unrelated pathogens. This study highlights the immunopathological manifestation caused due to MAP infection and host immunomodulation due to MAP infections and also provides valuable insights in animal and human research against MAP infection. Paucibacillary and multibacillary pathologies are

associated with type 1 and type 2 Th responses, respectively. Nevertheless, numerous other cytokines exhibit distinct expression patterns, underscoring their involvement in a intricate network of interactions that contribute to the development of these final pathologies. The notable rise in IL10 expression concurrent with IFN-γ expression following *M. bovis* infection could suggest that the host is attempting to mitigate the pathological damage linked to robust pro-inflammatory immune responses.

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Conflict of interest

All authors declare no conflict of interest.

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