Chapter

The Ambiguous Role of Macrophages in Pulmonary Tuberculosis

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Abstract

Tuberculosis persists among the top 10 causes of death globally; causing 1.7 million deaths and 10 million new infections in 2018. Approximately 1/3 of the global population is infected with *Mycobacterium tuberculosis*; 10% of which are expected to develop active TB at some point in their life. The high burden of tuberculosis in the world is owed to lack of adherence to treatment, diminishment in treatment options and post-infection bacterial metabolic dormancy called latent TB (LTB), along with logistic, financial and political obstacles impeding successful TB control programs globally. Infections with *M. tuberculosis* leave no component of the immune system unengaged, hallmarked with granulomatous pathology as a function of the adaptive immune system. The hallmark of infection is a granulomatous pathological course, with the purpose of containing the difficult-to-kill bacilli, although the nature of the granuloma remains moot. The cells responsible for granuloma formation are professional alveolar macrophages, which seem to have both a beneficial and detrimental role in TB immunopathology. Herein, we discuss relevant immunological intricacies of macrophages in TB, ranging from immunogenetics, receptor-mediated uptake, macrophage-mediated immunopathology and the infamous tuberculosis granuloma.

Keywords: tuberculosis, tuberculosis granuloma, macrophages and tuberculosis, tuberculosis immunopathology, macrophages

1. Introduction

Pulmonary tuberculosis (TB), a severe respiratory infection whose causative agent is *Mycobacterium tuberculosis* (Mtb), persists as one of the top 10 causes of death in the world and has successfully maintained its position as the leading infectious cause of death from a single infectious agent. Approximately one third of the global population (2.3 billion individuals) is considered to be infected with Mtb in a clinically silent manner, although determining the exact number has proven to be quite difficult [1, 2]. Concern regarding this infectious disease is rising due to the emergence of resistant strains that are not adherent to specific geographies [3]. Numerous lineages of Mtb have to date been identified, three of which (lineages 2, 3, and 4) are

considered the 'modern lineages' to which the global TB epidemic has been ascribed [3]. Emergence of these severely pathogenic and geographically unanchored lineages occurred due to the loss of theMmpS6/MmpL6-encoding Mtb-specific deletion 1 region (TbD1) throughout the evolutionary course of Mtb [3].

Pulmonary tuberculosis is divided into asymptomatic infection and active TB disease (ATB) [4]. Asymptomatic (latent) TB (LTB) is a clinically dormant form of infection whose mechanisms of activation and physiological maintenance with respect to host-pathogen dynamics are not fully characterized and although advances in imaging and high-throughput approaches have allowed for findings of tremendously higher resolution, details of the intricate dance between Mtb and the host remain moot [5]. Active tuberculosis is characterized with a wide spectrum of clinical manifestations, hallmarked by a purulent, bloody cough that aerosolizes into infectious droplet nuclei containing the pathogen [6, 7]. Although progress has been made in studying the immunogenetics and antimycobacterial mechanisms of the host, developing a broader comprehension of the matter has stagnated due to the underlying complexity of the relationship between the host and Mtb. Regardless, the innate immune response to inhalation of viable Mtb cells has been well studied and characterized with modest comprehensiveness, with a rigorously preserved hypothesis ascribing the most pathological importance to the TB granuloma [8–11]. Whether inhalation of infectious droplet nuclei will result in active TB disease or LTB, depends on a plethora of genetic and external factors, some of which are better understood than others, and research is currently being directed at understanding these issues [12]. One such factor is the natural degree of resistance humans possess to the development of active TB disease upon being infected [8, 9, 13, 14]. Resistance to TB disease, however, is evidently influenced by numerous factors originating from the host, the pathogen and the environment. Innate resistance to the Mtb, however, is not fully understood from the aspect of human immunogenetics, although significant progress has been made in this field [15]. Furthermore, primary and post-primary tuberculosis—two entities of infection with distinct immunopathologic continua—seem to be studied in a disproportionate manner; primary TB is at the forefront of research, whilst the relevance of secondary TB is substantially overlooked [15]. Available evidence, however, suggests that the subsequent initiation of secondary TB is essential for Mtb survival in the host, which may elicit a need for a broadening of research focus within the domain of TB prophylaxis [2, 16, 17]. Regardless, the innate response to Mtb inhalation has been a topic of intense study in the field of immunology. These molecularly-oriented studies have synthesized a rather large number of vaccine candidates, many of which are currently undergoing clinical trials [17, 18]. In fact, nearly each novel human vaccine candidate primarily functions by amplifying the TH1 innate immune response; an approach owed to the general understanding that an insufficient magnitude of TH1 immunity leads to poor control and subsequent proliferation of Mtb; something based in a rather large body of literature [17, 19–21].

Immunization strategies for tuberculosis currently undergoing clinical trials include killed, whole cell mycobacteria (DAR-901, *Mycobacterium vaccae*, MIP), live, attenuated mycobacteria (MTBVAC, VMP-1002); adjuvanted protein vaccines (M72/AS01E, H56:IC31, ID93 + GLA-SE), and viral-vectored vaccines (Ad5Ag85A, ChAdOx185A/MVA85A, TB/FLU-04 L) [2]. Quite prominent emphasis on the obscurity of substantial progress in regards to research and development of immunization methods for TB, is the fact that, in August 2019, there were 14 vaccine candidates being clinically assessed [19]. Ironically, a recent study on macaques concluded that intravenous (iv) administration of the Bacillus Calmette-Guérin (BCG) vaccine—the

only clinically approved vaccine for tuberculosis—dramatically improved the efficacy of the vaccine in this animal model [22]. Considering the generally limited efficacy and temporary protection of the BCG vaccine when administered intramuscularly, subcutaneously, orally, and intranasally, this new study has placed the 99-year-old immunization method under the spotlight, once again [19].

This chapter comes at a crucial and exciting epoch in the domain of tuberculosis research, primarily facilitated by the rise of extensively-drug-resistant tuberculosis (XDR TB), multiple-drug-resistant tuberculosis (MDR TB) and totally-drug-resistant TB (TDR TB) across the world, and the diminishment of sensical and safe treatment options for all forms of TB [2, 23]. An imperative to expedite current research efforts directed towards development and discovery of more efficient treatment and diagnostic methods, has even been a topic of discussion by the United Nations, although this did not prove to be as fruitful as initially [2, 24]. Recent studies have incorporated other therapeutics into this treatment protocol, however the results on their efficacy appear to be population and circumstance-specific [23, 25–27]. Thus, tuberculosis is currently categorized as a global health 'emergency' by the WHO. It seems a rather sensical approach to focus research and provide comprehensive reviews on the immunopathologic course of TB and the relevant underlying genetic background that influences the outcome of TB infection.

The scope of this chapter includes the variability of the immune response to Mtb infection, concordant to differences in the immunogenetic profiles of the infected hosts across different populations in the context of immunoreceptors expressed on macrophage surfaces. We aim to present the most relevant findings in TB-related immunopathology and the corresponding implications in treatment and patient outcome in the context of macrophage involvement.

1.1 Immunopathological events of primary infection

Five stages of pulmonary tuberculosis (**Figure 1**) have been distinguished by Lurie's 1964 study on rabbits [5, 12, 18, 28, 29]. The importance of this study is seen in its high degree of fidelity with respect to the natural mode of contagion, which cannot be replicated by in vitro conditions [30]. Upon inhalation of even as few as 10 Mtb cells, the first stage is characterized by the rapid action taken by the innate immune defenses; phagocytosis of Mtb cells by resident macrophages (Mϕs) and other antigen-presenting cells (APCs) such as pulmonary dendritic cells (DCs) [31]. Varying immunopathologic continua may be observed for different phagocytic cells lines that phagocytize Mtb once it reaches the lung tissue upon inhalation, as the pathogen is able to employ different tactics to evade the host's immune defenses and interfere with every involved component of the immune system [7, 13, 32]. The evolutionary battle between the human host and Mtb prompted the pathogen to enhance its entry tactics into phagocytes by engaging a specific set of phagocytic receptors and efficiently modulating and interfering with every immunobiological process that plays a role in TB infection, often with staggering success [7]. Ergo, the ability of Mtb to create a survivability niche within the bactericidal environment of phagocytes is considered essential for bacterial survival and intracellular persistence.

Pattern recognition receptors (PRRs) located on the surface of APCs and respiratory epithelial cells that are engaged by Mtb include complement receptors (CRs), immunoglobulin fragment carrying the constant region of the heavy chain (Fc), C-type lectins (CTLs), toll like receptors (TLRs) and the scavenger receptors (SRs) (Image 1) [29]. Despite several receptors displaying partial redundancy in knockout

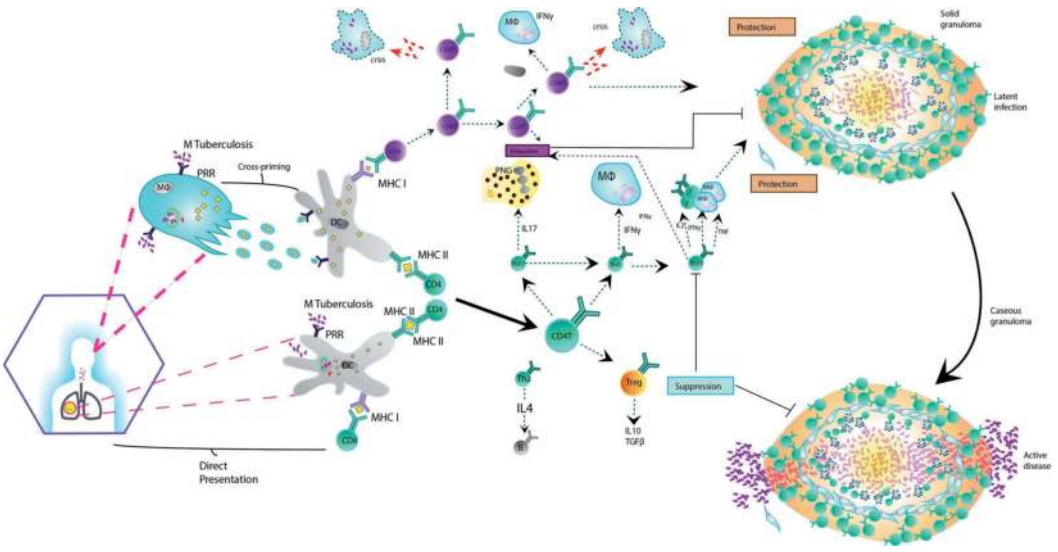

Figure 1.
The immune response to inhalation of Mycobacterium tuberculosis *within the context of Lurie's five stages of pulmonary tuberculosis. Abbreviations: MΦ-macrophage PRR-pattern recognition receptor:* M. tuberculosis—Mycobacterium tuberculosis*; DC—dendritic cell; MHC II—major histocompatibility complex type II; CD4—cluster of differentiation 4; CD8—cluster of differentiation 8; CD8T—cytotoxic T; IFNγ—interferon gamma; PNG—polymorphonuclear granulocytes; IL17— interleukin 17; Th17—T helper cells 17; CD4T—T helper cells; Th2—T helper cell type 2; IL4—the interleukin 4; B—B lymphocyte; Treg—regulatory T cells; L10—Interleukin-10; TGFβ—transforming growth factor beta, IL2—Interleukin-2; TNF—tumor necrosis factor; T—T lymphocyte.*

murine models, engagement of each receptor has pathologically relevant implications in TB pathogenesis. For instance, studies on murine models revealed significant reduction in the number of opsonized mycobacteria actively internalized by Mϕs and other phagocytes in the absence of CR3s [28, 33]. C-type lectins located on Mϕs and DCs are likely favored by Mtb since their engagement opens an opportunity for the pathogen to modulate the intensity of the associated inflammatory responses [34]. Specifically, Mtb has the ability to suppress the MR-TLR2-associated pro-inflammatory response by secreting the early secreted antigenic target protein 6 (ESAT-6), which inhibits the activity of NF-kappa (NF-κB) by downregulating reactive oxygen species (ROS) production [26, 35, 36]. Relevant to this discussion is that ESAT-6 is used in the IGRA due to its potency as a T cell antigen; thus, this secretory mycobacterial protein is an important hallmark of TB infection [37]. Fcγ receptors (FcγR) play a role in regulating the intensity of the host immune response and are concordantly divided into activating and inhibiting types. Toll-like receptors (TLRs) play a cardinal role in priming both pro-inflammatory and anti-inflammatory responses [38]. The cytoplasmic domain of these phylogenetically conserved transmembrane proteins is homologous to the interleukin-1 receptor (IL-1R) signaling domain, which links to IL-1R-associated kinase (IRAK) in order to activate transcription factors that will promote cytokine production. Furthermore, phagocytosis of Mtb does not lead to a pro-inflammatory immune response in the absence of TLRs; particularly relevant is the TLR2, although other TLRs have demonstrated notable roles in immunopathology [7, 32]. With regards to an efficient immune response, TLR recognition of Mtb cell wall lipoproteins will induce the secretion of IL-12—a proinflammatory cytokine that promotes maturation of naïve T cells into Th1 cells—by infected phagocytes [7, 13]. Mutations or genetic polymorphisms will likely, therefore, compromise the ability of

the human host to mount an appropriately controlled inflammatory response to TB [39–46]. Aforementioned avenues for phagocytic receptor engagement make it all the more sensical that Mtb favors entry routes that seldom elicit a pro-inflammatory response, at least in early stages of infection. Pathways activated through the engagement of these receptors allow for immunobiological modulations that comprise the mycobacterial intracellular and intragranular persistence mechanism [37]. Although the intrinsic potency of Mϕs to kill internalized mycobacteria is evidently high through utilization of a barrage of bactericidal tactics, sufficiently virulent Mtb cells manage to evade or block these mechanisms [4, 47]. Thus, the intrinsic Mϕ competence and mycobacterial virulence significantly influence the course of the infection upon phagocytosis. Mycobacterial cells that evade the intrinsic killing mechanisms of phagocytes trigger an immunopathologic continua designated as the second stage, in which logarithmically multiplying Mtb cells inhibit the process of phagosome maturation and disrupt other incoming immune cells [18, 28, 48–55]. Immature phagocytes infected with multiplying mycobacteria attract monocytes and other inflammatory-oriented immune cells in an attempt to swiftly contain the bacilli [56–58]. Studies conducted on murine models and human monocytic cell lines have somewhat elucidated the process by which Mtb blocks the phagosome maturation process, and are further discussed in the following sections.

Approximately 2–3 weeks after engulfing Mtb, infected phagocytes release cytokines in order to recruit antigen-specific T cells (CD4+) to primary tubercle lesions in order to form adaptive immunity [32, 49, 56]. Activated T cells undergo clonal expansion and initiate the killing mechanisms of Mϕs infected with mycobacteria. Within the Mϕ environment, the third stage commences, whereupon logarithmic Mtb growth is halted through various bacteriostatic mechanisms [26, 37]. Infected Mϕs generally go through apoptosis, a consequence of recruiting the tumor necrosis factor alpha (TNF-α), whose inhibition significantly improves Mϕ survivability in the host lungs [37]. Progressive intragranular apoptosis leads to central solid necrosis, which may be followed by liquification of the caseous foci [26]. Survivability of Mtb in dead phagosomes, however, has been continuously reported throughout the years, as necrosis is an outcome favorable for pathogen dissemination and immune evasion [58–60]. Progression of the solid necrotized lesions containing metabolically dormant bacilli into liquified caseous lesions forms ideal conditions for bacterial re-activation and rapid multiplication [5, 7, 13–15, 61–65]. Although immunocompetent patients are evidently able to suppress the development of TB disease, host genetics and pathogen virulence determine whether dormant Mtb bacilli remain contained in the granuloma, or cause the formation of liquified caseous foci [8, 9, 59]. These foci progress to form cavities in which Mtb extracellularly multiplies, causing subsequent bronchial rupture and migration of the bacilli outside of their containment [10].

2. The innate immune response to *M. tuberculosis*: the pivotal role of macrophages

Perhaps the most direct evidence of constitutional human resistance to TB infection and disease may be extrapolated from the 1926 Lübeck disaster, in which 251 neonates were orally administered a large dose of live, virulent *M. tuberculosis* [22]. Clinical or radiological signs of infection were detected in 173 infants that were able to survive the infection, whereas 72 died from TB disease [12, 22]. The remarkable ability of the surviving 173 infants to survive the clinical manifestations of TB clearly

demonstrates the efficacy and undisputable relevance of the innate immune response to Mtb, a large portion of which belongs to macrophages [12]. Studies spanning over recent decades have shown that development of symptomatic TB does not always occur upon initial infection in humans, cattle, mice and rabbits [12]. Further perplexing is the fact that a relevant portion of close contacts with microbiologically confirmed TB cases displays no diagnostically valuable immunopathologic changes (ex. negative tuberculin skin test (TST) and interferon gamma (IFN-γ) Release Assay (IGRA)) [66]. Interestingly, even data on acquired sensitivity to the TST is inconsistent for individuals considered to be at risk of infection; some develop sensitivity sooner than others [12, 18, 22, 28, 67, 68]. The source of this perplexity may very well be located in the host's immune genes and their functionality rather than the sole virulence of the pathogen, although both factors play relevant roles in TB pathogenesis. Disputed among clinicians and researchers is data suggesting that patients immunized with the standard BCG vaccine test positive for tuberculosis, regardless of the presence of an active or latent infection [66, 68, 69]. Conclusively to the TST cut-off research, an increase in TST cut-off criteria up to >15 mm, as opposed to the standard >5 mm cut-off, has been recommended for BCG-immunized patients; optimally throughout a time interval of 15 years after immunization [70]. Evidently, the response to inhalation of Mtb is not uniform in phenotypic manifestation for all individuals. The aforementioned variability is owed to the immunopathology governed by the host's immunogenetic profile, potential presence of comorbidities, pathogen virulence and prominence of numerous environmental factors, along with factors pertaining to receptor-specific internalization of the bacillus by macrophages [71]. Mutations and polymorphisms in genes coding for phagocytic receptor proteins seem to influence the competency of the host's constitutional resistance to progression from primary infection to primary disease or post-primary symptomatic infection [40, 41, 43, 44]. Significant and perhaps disproportional attention has been given T cell mediated immunity in contrast to humoral immunity. Whether humoral immunity poses a significant role in Mtb immunopathology has not been sufficiently elucidated yet, although there is evidence linking B cell immunity and immune competence in murine models [4].

Innate immunity is critical for early anti-mycobacterial responses, it is also important for the progression of infection and long-term control of Mtb by continually priming and educating adaptive immune responses and by regulating inflammation [28]. The system comprises two components, cellular and humoral, the latter including circulating complement proteins, defensins, cytokines and chemokines secreted by innate immune cells [72]. The cellular component which requires our attention comprises of innate immune cells which consist of epithelial cells, endothelial cells (ECs), granulocytes (neutrophils, basophils, eosinophils, mast cells (MCs)), monocytes, macrophages, natural killer (NK) cells, dendritic cells (DCs), invariant NKT cells (iNKT cells), γδT cells, innate immune T cells called mucosal invariant T cells (MAIT) cells and innate lymphoid cells (ILCs). These cells are crucial for the maintenance of the immune homeostasis and regulation of the adaptive immune system; they act as antigen presenting cells (APCs) as well as provide other signaling molecules/factors required in the effective adaptive immune response in response to infection or chronic inflammatory diseases [72].

In the case of TB, the earliest encounter between the host's immune system and Mtb occurs at the interface between resident lung (alveolar) macrophages and the virulent bacterial cells. These cells are often niches for bacterial replication and Mtb utilizes a myriad of strategies that subvert innate immune responses to establish a

chronic infection [4]. During the past several decades, much has been uncovered and mechanisms through which the immune system responds to Mtb are in many ways illuminated, even though much still lingers in the shadows, which will hopefully be cast away over time. The first step is the recognition of mycobacteria as invading pathogens, followed by activation of innate host defense responses, and the subsequent initiation of adaptive immune responses [4]. Knowledge about these processes is crucial for understanding the pathophysiology of tuberculosis and for the development of novel strategies for vaccination and treatment such as immunotherapy.

The initiation process of the innate immune response starts with pattern recognition of microbial structures called pathogen-associated molecular patterns (PAMPs). Recognition of PAMPs is performed by germline-encoded receptors expressed mainly on immune cells termed pattern recognition receptors; in this case, being alveolar macrophages [26].

2.1 Expression levels of cytokines and cytokine receptors influence the extensity of immunopathology

Recruitment of innate immune cells in the early stages of infection is the result of secretion of cytokines and chemokines either by infected phagocytic cells or respiratory lung epithelial cells [56]. Studies have shown that primary lung epithelial cells possess the ability to cross-talk with infected macrophages, which benefits the process of monocyte recruitment. Infected phagocytic cells, inside of which the first stage of Mtb infection commences, may either migrate to the mediastinal lymph nodes in order to prime a T cell response via antigen presentation, or directly prime naïve T cells [73]. In the context of innate immunity, recruitment of polymorphonuclear leukocytes (PMNLs) to the sight of infection seems to be a 'double-edged' sword that either leads to effective control of infection, or damaging inflammation [73]. A mouse study on the recruitment of PMNs by alveolar epithelial cells during early stages of infection, concluded that severely damaging inflammation can occur as a consequence of this process [73]. It was discovered that CXCR2 and CXCL5 significantly contribute to a high influx of PMNLs, which is the mechanism behind the destructive inflammatory response during the initial stages of infection in mice and non-human primates (NHP) [73, 74]. Numerous CXCR2 ligands seem to positively regulate PMNL recruitment in murine models.

With regards to TB in vivo infection, TLR2 recognition of Mtb molecular patterns induces expression of CXCL5 by alveolar epithelial cells, which subsequently recruits PMNLs via CXCR2. Tlr2−/− mutants demonstrated significantly diminished, although not abolished, secretion of CXCL5 in vivo [73, 75]. Considering that alveolar epithelial cells are not the only cells present in the bronchoalveolar space during Mtb infection, and that those cells secrete inflammatory mediators that promote secretion of CXCL5, this finding is not at all surprising. There is an absolute dependency on CXCR5 for recruitment of PMNLs into the bronchoalveolar space during Mtb infection [73]. In contrast, PMNL recruitment induced by CXCL5 was found to account for roughly 60% of PMNLs recruited to the airspaces in vivo [73]. Exacerbated inflammation occurs with an incredibly high degree of dependence to CXCL5 secretion and the dose of Mtb that has infected the host [56, 73]. CXCL5 is not only expressed by alveolar epithelial cells, but rather a variety of other tissue-resident cells, making its role in tissue inflammatory responses well emphasized [76]. Although much work lies ahead in understanding the implications of CXCL5 and CXCR2 in TB immunopathology in the context of innate and acquired immunity, having a better understanding

of these pathways could aid in devising treatment approaches that would allow for avoidance of the devastating inflammatory damage seen in certain subpopulations of TB patients. As for these findings corresponding to human immunopathology in TB, the ENA-78 neutrophil attractant secreted by human pulmonary epithelial cells is very similar to murine CXCL5 [77]. Of course, further studies should be conducted in order to comprehensively understand whether the studies on CXCL5 could be translated to human TB. Unfortunately, this particular aspect of Mtb infection in humans has thus far received modest attention, however current research is indicative of this likely being the case.

2.2 The behavior of macrophages infected with *M. tuberculosis*

The continuous development in the field of immunology has established their role in various immunological and non-immunological processes including embryonic development. Along with acting as phagocytic cells involved in the phagocytosis of pathogens, xenobiotics, these cells also secrete various cytokines, chemokines, and growth factors including TNF-α, TGF-β, platelet-derived growth factor (PDGF), endothelial growth factor (EGF), and vascular endothelial growth factor (VEGF) [72].

Alveolar macrophages encounter Mtb within the first 48 hours of infection, thereby representing the primary replicative niche for the bacillus [77]. Once Mtb is recognized by alveolar macrophages, it is engulfed through surface receptors, which leads to phagocytosis of the bacterium into phagosomes, which typically fuses with lysosomes for pathogen eradication and further consequent acidification of the pathogen-containing phagolysosome. Until recently the mechanism behind the establishment of a chronic infection in mammalian primates remained rather obscure; the lung interstitium, however, was definitely known to be the focal point of the infection based on previous studies [77]. In a 2018 study, performed by Cohen et al., discovered that alveolar macrophages transport Mtb from the alveoli to the interstitial tissue, under the influence of interleukin-1 signaling and the Mtb ESX1 secretion system [77]. Furthermore, localization of infected alveolar macrophages to the lung interstitial tissue leads to virulent Mtb cells being introduced to replication-permissive monocytes [13, 78]. Involvement of IL-1 in this process is not surprising considering that Mtb is a potent stimulator of the inflammasome, which regulates IL-1 production [79]. Contextually to recognition of mycobacterial antigens, one of the most important receptors for mycobacteria is the complement receptor 3, while other receptors such as CR1 and CR4, mannose receptor, surfactant protein A receptor, CD14, Fcγ receptor, scavenger receptors, have also been implicated in phagocytosis and internalization of mycobacteria inside the Mϕs [57].

The mycobacterial surface glycoprotein, mannose-capped lipoarabinomannan (Man-LAM) is recognized by the C-type lectins and the macrophage mannose receptor (MMR). An important role of toll-receptors, mainly the TLR2, has been demonstrated for the attachment of mycobacteria to macrophages [57]. Mtb is capable of inhibiting that process of phagosome maturation, as a result of which acidification of the phagosome is compromised, thus avoiding degradation and antigen processing [57]. Mtb is equipped with a variety of mechanisms that enable such form of survival, the key ones of which are stress-adaptive genes that are expressed in Mtb in order to counter the nitrosative, oxidative, hypoxic, and nutrient-diminished phagosome environment [80]. From an evolutionary point of view, it is clear that Mtb has developed alongside humans and thus adapted for a lifestyle inside the Mϕ, employing many strategies to survive within these cells.

Mtb entry intro Mϕs through different receptors can induce the activation of different pathways that can either inhibit or promote bacterial replication. Mϕ defenses include antimicrobial peptides (AMPs), nitrosative stresses, phagolysosome fusion and autophagy and may operate independently of or subsequent to IFN-γ signaling [7]. The overall interaction of multiple receptors and their engagement with Mtb ligands is a complex and dynamic issue. For instance, TLR-2 recognition of mycobacterial ManLAM activates NF-κB and NOS2 gene transcription that leads to antimycobacterial nitric oxide (NO) production, which is strongly associated with resistance to Mtb, even though evidence for that is stronger in mouse models [72, 81]. It has been shown that the reactive nitrogen intermediates (RNI) in mice are toxic to mycobacteria in vitro and by inhibition of NOs in vitro or in vivo infection can be exacerbated [44]. In relation, mice with disrupted NOS2 alleles display exacerbated disease following Mtb infection [44]. As for humans, in vitro studies using human alveolar macrophages and primary monocytes showed no anti-mycobacterial properties for NO, but specific staining for NOS2 in the bronchoalveolar lavage (BAL) of TB patients revealed upregulation in infected individuals compared to healthy controls. Another interesting fact is that mutations in Gp91phox, encoded by CYBB, a subunit of phagocyte oxidase enzyme complex (NADPH), pivotal for ROS (reactive oxygen species) are significantly correlated with reduced risk of TB [44].

In human macrophages, TLR-mediated recognition of Mtb is reported to synergize with the vitamin D pathway to induce the antimicrobial peptide (AMP), cathelicidin [77]. That process happens through calcitriol, a biologically active vitamin D metabolite, which induces the hCAP-18 gene encoding the pro-form of cathelicidin, following TLR ligation of macrophages. Studies have shown that cathelicidin exerts antimicrobial functions by activating transcription of host autophagy genes Beclin-1 and Atg5 [4]. Besides that, the vitamin D pathway also synergizes with IFN-γ secreted by T-cells to induce IL-15 autocrine signaling to promote autophagy and phagosome maturation in Mtb-infected human macrophages [4, 79].

Autophagy also plays a role in promoting phagosome maturation to enhance bacterial killing and it is integrated into the host response to Mtb infection by synergizing with pathogen sensing, phagosome maturation, and IFN-γ inducible pathways to mediate anti-mycobacterial immunity [4]. Autophagy-related proteins are likely to perform multiple functions and care must be taken when interpreting specific knockouts or knockdowns of individual genes. For instance, myeloid cell-specific ablation of Atg5 compromised control of Mtb in mouse studies. Deletion of autophagy-related genes Ulk1, Ulk2, Atg4B, or p62 compromised the ability to induce autophagy, but were dispensable for control of *M. tuberculosis*. Further analysis of lung sections from Mtb-infected mice showed that Atg5 knockout indicated that it may be involved in regulations of neutrophil responses during infection.

The factor responsible for macrophage activation is IFN-γ produced by CD8+ cytotoxic T (Tc1) cells, CD4+ T helper 1 (Th1) T cells, and natural killer (NK) cells. IFN-γ activation leads to conversion of macrophages to potent phagocytotic cells with increased production of reactive oxygen intermediates and reactive nitrogen intermediates, superoxides and proinflammatory cytokines helping the cells to efficiently kill the intracellular pathogens. This type of IFN-γ-mediated activation induces M1 macrophages [57].

On the other hand, T helper 2 (Th2) type of cytokines, IL-4 and IL-13, induce a response different from the one induced by IFN-γ with distinct set of genes being expressed to form what is known as the alternative activation pathway of macrophages, and the cells are named as alternative activated type 2 or M2 macrophages [13, 72].

Various immune complexes, IL-10, vitamin D3 can also contribute to the activation of M2 macrophages [57]. M2 macrophages generally exhibit a higher phagocytic activity, mannose and galactose receptors, produce higher concentration of ornithine and polyamines due to high arginase pathway, secrete high amount of IL-10 and express higher levels of the IL-1 decoy receptor and IL-1RA. Having all these characteristics, M2 macrophages play a crucial role in anti-parasitic immune response [72].

3. Immunoreceptors expressed on macrophages and their role in the immunopathological course of TB

3.1 *M. tuberculosis* is capable of turning macrophages into non-bactericidal environments in an Immunoreceptor-specific manner

Mannose receptors (CD206) play an important role in TB innate immunity due to their efficacy and specificity as endocytic receptor through engagement of virulence-associated mycobacterial cell wall components that contain mannose, particularly glycoproteins and sulphated and non-sulphated polysaccharides [82]. MR-positive immune cells are able to deliver various antigens to sites where humoral and cellular responses occur, therefore playing a role in bridging innate and acquired immunity [83–86]. This CTL is a Ca^{2+}-dependent type I transmembrane glycoprotein contains an extracellular N terminal, cysteine-rich (CR) domain, a fibronectin II (FNII) domain, eight carbohydrate recognition domains, a cytoplasmic tail and a transmembrane domain. The cytoplasmic tail contains 49 amino acids and there is a tyrosine residue on the 18th position that has been heavily implicated in endocytosis [51]. Much has been uncovered about the signaling pathways of CD206 in recent years, implicating this receptor in functions such as M2 macrophage polarization, antigen presentation, entry trafficking, macrophage-associated tumor biology and receptor targeting for therapeutic purposes [71, 79, 87]. CTL signaling is achieved through various adaptor proteins, of which the FcRγ chain is the most common [51]. Although the implications of this remain in question, heat shock proteins (HSP) in unstimulated cells interact with CD206 [88, 89]. Furthermore, it is not fully understood how this alters the structural configuration of MR upon their activation in vivo, although strides have been made in uncovering the significance of actin remodeling in this process, and its implication in phagolysosome maturation [51]. Phagocytosis is an actin-dependent process, therefore MR-mediated phagocytosis requires receptor clustering, recruitment and engagement of various adaptor proteins and activation of the Rho family of small GTPases in order to facilitate cytoskeletal remodeling [51]. Surface localization of CD206 depends on its ability to interact with FcRγ, which likely occurs at the interface of the positively-charged transmembrane region of the receptor [51]. This is where the value of murine models in understanding receptor-related aspects of TB immunopathology in humans sees its shortcomings: the cytoplasmic tail and TM region of the murine MR is neutrally charged, likely leading to reduced FcRγ tail binding and diminished surface MR exposure [51]. In the contest of human macrophages, recruitment and activation of the Src homology region 2 domain-containing phosphatase 1 (SHP-1) is an MR-dependent event that occurs during Mtb infection; SHP-1 phosphorylates and co-localizes with phagosomes containing Mtb [51]. As a consequence of this, SHP-1 reduces the activity of class III PI3P by interfering with the serine/threonine-protein kinase Vps15 and the phosphatidylinositol 3-kinase (PI3) hVPS34 [29, 87, 90]. Inhibition of SHP-1 has been found to lead to enhanced phagolysosome fusion [34].

Once Mtb is phagocytosed through CD206 engagement, subsequent immunomodulation facilitated by mycobacterial cell wall components may lead to the development of active disease. Mycobacterial ManLAM heavily influences several immunobiological processes throughout the continua of the immune response (**Figure 2**) [91]. ManLAM inhibits the process of phagolysosome fusion, which is considered a key aspect of TB infection with regards to mycobacterial intracellular persistence. It does so by blocking a crucial phosphatidylinositol 3-phosphate (PI3P)-regulated pathway involved in transportation of lysosomal and other crucial components from the trans-Golgi network to immature phagosomes; a process required for phagosome maturation [29, 87, 92, 93]. Two rab5 effector hVPS34 and the early endosomal antigen 1 (EEA1) are components crucial for phagosome maturation [32]. EEA1 binds to the membrane-associated PI3P via its FYVE and PX domains, to which ManLAM may competitively bind and thus preclude phagolysosome fusion. It was discovered that Mtb ManLAM interferes with a pathway involving Ca^{2+}, calmodulin and the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [29, 32, 87, 91, 92, 94]. EEA1 and Syntaxin 6 deliver various lysosomal components from the trans-Golgi network to immature phagosomes, making them crucial for phagosome maturation. However, Mtb uses ManLAM to disrupt recruitment of EEA1 to phagosome membranes by inhibiting the rise in cytosolic Ca^{2+}, thus rendering the Ca^{2+}/Calmodulin pathway, impotent [7]. Considering that hVPS34 interacts with calmodulin in order to generate PI3P, physiological maintenance of PI3P on phagosomes and other intracellular membranes requires calmodulin [91]. In summary, Mtb-associated ManLAM

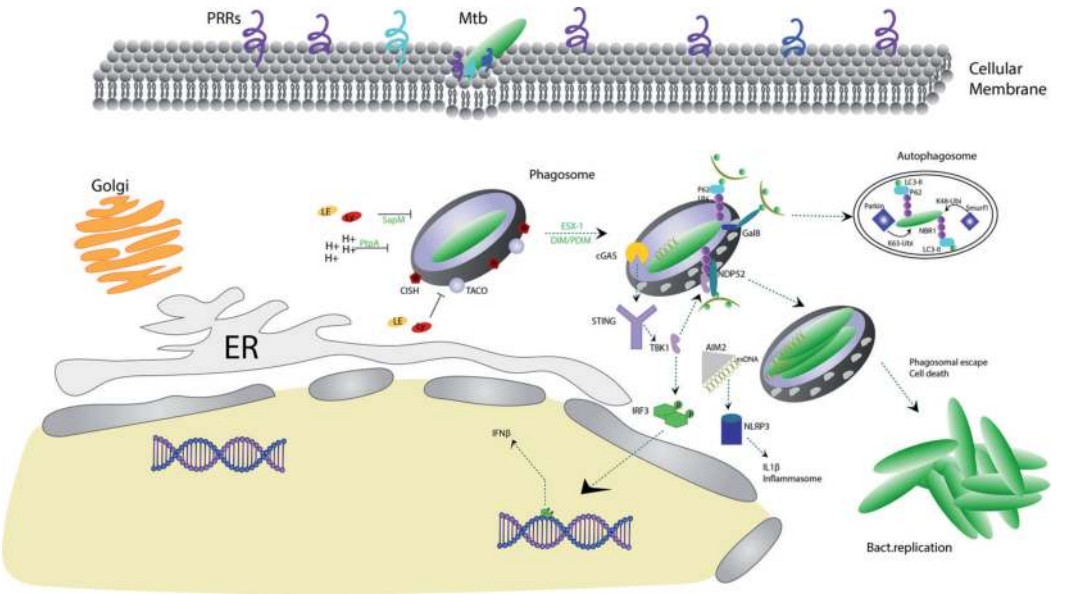

Figure 2.
*The process of successful and unsuccessful phagosome-lysosome fusion during pulmonary tuberculosis. Abbreviations: Golgi—Golgi apparatus, ER—endoplasmic reticulum, PRRs—pattern recognition receptors, Mtb—*Mycobacterium tuberculosis*, PtpA—protein tyrosine phosphatase, SapM—secreted acid phosphatase, CISH—cytokine-inducible SH2-containing protein, TACO—tryptophan-aspartate containing coat protein, IFNβ—interferon beta, IRF3—interferon regulatory factor 3, DIM/PDIM—Phthiocerol dimycocerosate, cGAS—cyclic GMP-AMP synthase, STING—stimulator of interferon genes, TBK1—TANK-binding kinase 1, P62—nucleoporin 62, Ubi—ubiquitin, Gal8—Galectin-8 protein, INDP52—calcium-binding and coiled-coil domain-containing protein 2, AIM2—absent in melanoma 2, NLRP3—NLR family pyrin domain containing 3 inflammasome, IL1β—interleukin 1β, LC3-II—microtubule-associated proteins 1A/1B light chain 3B, parkin—E3 ubiquitin ligase, K63-Ubi—polyubiquitinations K63, K48-Ubi—ubiquitination K48, NBR1—next to BRCA1 gene 1 protein, Smurf1—E3 ubiquitin-protein ligase SMURF1.*

provides a survivability niche within macrophages by blocking the increase in Ca2+ transients, therefore effectively disrupting a Ca^{2+}/Calmodulin associated pathway required for phagosome maturation. Physiological increase in cytosolic Ca^{2+} is heavily influenced by sphingosine kinase (SK), whose signaling pathways is triggered by FcR clustering. Studies suggest that the mechanism by which ManLAM disrupts the increase in cytosolic Ca^{2+} is likely through interference with SK signaling pathways [30].

Cytotoxicity of ManLAM, however, is diminished when dissociated from mycobacterial cells via the activity of the respiratory mucosa [92]. ManLAM debris are incapable of inhibiting the process of phagolysosome fusion, allowing for the attractive assumption that the human respiratory tract evolved in such a way to amplify the immune response to Mtb by creating these highly immunostimulatory debris in the early stages of infection [92]. Despite the evident potency and relevance of this Mtb-associated mechanism, ManLAM-mediated prevention of phagolysosome fusion is but one of the several mechanisms in the arsenal of Mtb, some of which have only been recently discovered [92].

Preferred engagement of the CR and MR-dependent phagocytic pathways was a sensical evolutionary approach by Mtb, since engulfment of microbes via CRs and MRs do not necessarily incite an inflammatory response by the host, and MR-abundant alveolar macrophages act as chaperones that deliver Mtb to replication-permissive cells [26, 30]. The mechanism by which CTLs elicit an anti-inflammatory response is found in the ManLAM-mediated stimulation of a nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ), which in turn triggers such a response. Limited phagosome-lysosome fusion is specific only for monocytes expressing MR, whereupon absence of this receptor on Mϕs and DCs does not lead to the inhibition of phagosome maturation. LAM may be exported from the infected Mϕs and presented to T-cells via the MHC I CD1 molecules [21, 95]. Previous research suggests that Mtb uses LAM to recruit host immune cells whose function it will subsequently modulate in order to facilitate survival.

3.2 Macrophage Toll-like receptors and their implications for tuberculosis

Toll-like receptors are type 1 transmembrane pattern recognition receptors instrumental to animal immunity [96]. They contain an extracellular leucine-rich repeat (LRR) domain—involved in signal transduction and molecular recognition—and intracellular toll/interleukin-1 receptor (TIR) domain, which represents a highly conserved protein-protein module. These receptors are expressed in a wide range of cells, with the most relevant for this discussion being Mϕs. Thus far 10 TLRs have been nominated in humans, with each of them playing cardinal roles in both innate and acquired immunity to Mtb infection. Upon recognition of Mtb molecular patterns via the receptor LRR domain, the Myeloid Differentiation Primary Response 88 (MyD88) is activated, which is utilized by all TLRs lest except for TLR3 (**Figure 3**). A wide spectrum of anti-mycobacterial actions taken by the immune system involve the synergic activity of TLRs and MyD88, leading to the subsequent involvement of IRAK, TNF receptor associated factor 6 (TRAF6), transforming growth factor beta-activated kinase 1 (TAK1) and mitogen-activated protein kinases (MAPK); it should be noted, however, that each TLR alone is capable of initiation a separate immunopathologic continua as means of amplifying the anti-Mtb immune response. Each TLR can bind a specific subset of pathogen-associated molecular patterns (PAMPs), they activate the innate immune system and help with the host protection. TLR2,

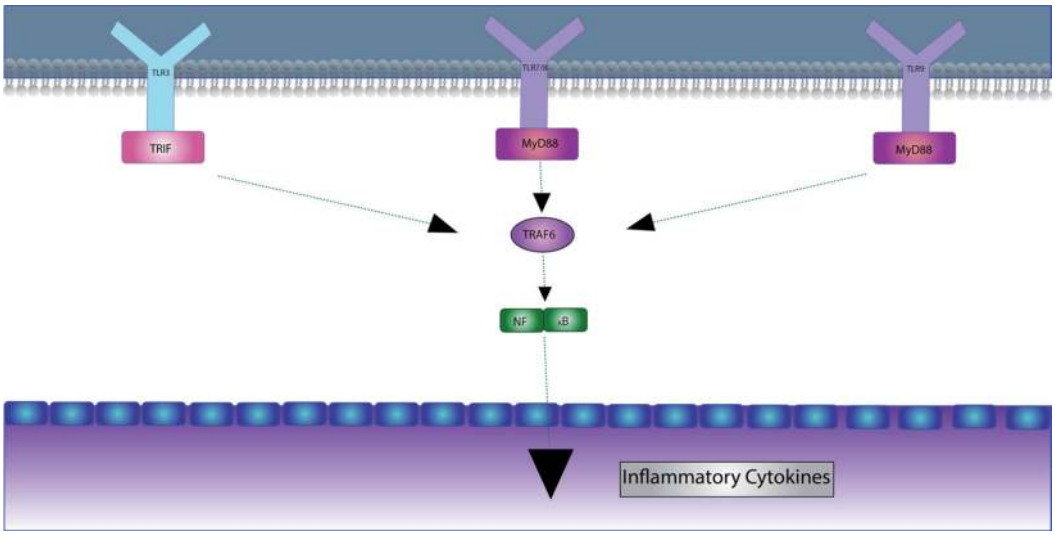

Figure 3.
Schematic representation of toll like (TLR) signaling. NF-κB gets translocated into the nucleus to initiate the transcription of inflammatory cytokine genes. Abbreviations: MyD88—Myeloid differentiation primary response, TRIF—TIR-domain-containing adapter-inducing interferon-β, NF-κB—NF-kappa B.

TLR4, and TLR9 are the most common TLRs that detect Mtb, with TLR2 playing the most important role 5. TLRs recognize Mtb PAMPs, which triggers an intracellular signaling cascade that binds the myeloid differentiation primary response protein 88 (MyD88) to TLRs intracellular domains6. MTB is an acid-fast bacterium because its cell wall is mostly made up of hydrophobic mycolic acids. This is a component of the mycobacterial cell wall that accounts for half of its dry weight. The admission of nutrients is slowed by this thick layer of mycolic acids, causing mycobacteria to proliferate slowly, but it also boosts cellular resistance to lysosomal enzyme destruction. The mycolic acids are typically found in a thick layer on the cell wall's exterior surfaces7. Lipids and polysaccharides make up the mycobacterial wall, which also contains a lot of mycolic acid.

TLR2 and TLR4 are activated by purified cell wall components of mycobacteria [81]. Lipomannan and lipoarabinomannan are two lipoglycans with substantial immunomodulatory properties [30, 38]. TLR2, in conjunction with TLR1, can identify Mtb cell wall lipoprotein antigens, which cause macrophages to produce cytokines. TLR9 is triggered by mycobacterial DNA. The engagement of IL-1 receptor-associated kinases, TNF receptor-associated factor 6, TGF-activated protein kinase 1 and mitogen-activated protein kinase is then enhanced by MyD88 [97]. The transcription factor NF-κB is activated and translocated to the nucleus as a result of this signaling cascade. Multiple pro-inflammatory cytokines, including tumor necrosis factor, interleukin-1 and interleukin-12, are produced as a result [98]. Tumor necrosis factor and interleukin-12 are then secreted, causing nearby natural killer and T cells to produce IFN-y. IFN-y is a key macrophage stimulator and activator of major histocompatibility complex class II molecule production. IFN's importance in the immune system arises from its capacity to directly suppress virus replication, as well as its immunomodulatory properties. IFN-y stimulates macrophages, enhancing antigen presentation and promoting anti-mycobacterial effector mechanisms such as reactive oxygen and nitrogen intermediates, autophagy, phagolysosome fusion and acidification autophagy [99, 100].

For instance, TLR2 has been implicated in regulating T cell trafficking by inducing the production of CCL8, a CD4+ chemokine, and in recruiting regulatory T cells (Treg) to infection foci in order to regulate inflammation. Tampering with the inflammatory response in the context of TLR2 is at least in part owed to the TLR2-induced secretion of CXCL5, considering that PMNL recruitment by this chemokine has the potency to drive destructive inflammation during early stages of infection [81]. Mycobacterial ESAT-6 readily promotes macrophage apoptosis by activating the TLR2/NF-κB [101]. One of the ways that Mtb causes a delay in priming the adaptive immune response is through the activities of mycolic acid and various mycobacterial lipoproteins, which have potency to downregulate MHC II expression and pro-inflammatory responses. Furthermore, TLR2 enhances the expression of vitamin D receptor genes [81, 102–104]. It should be noted that induction of ROS can be achieved through TLR2/dectin-1 cooperation [105]. Induction of a rather wide range of anti-mycobacterial mechanisms is also partly owed to TLR2 [63–65, 106, 107]. TLR2−/− mice have also exhibited increased inflammation, pneumonitis of the interstitial lung tissue and abnormal granuloma morphology, with a very modest increase in bacterial burden [108]. Concordantly to these findings, concluding that TLR2 plays both beneficial and detrimental roles in Mtb infection seems to be rather prudent, as TLR2 signaling is influenced by numerous components of both the immune system and mycobacterial virulence, with notable implications in innate immunity and priming of adaptive immunity.

Functions of TLR4 have been reported to include promotion of CD4 and CD8 T cell recruitment, polarization of T effector cells towards a Th1 cell phenotype and numerous other activities that may be construed as both beneficial and detrimental [81]. Mycobacterial phosphatidyl inositol mannosides can inhibit the production of proinflammatory cytokines and NO by interfering the synergic activity of TLR4 and MyD88. Repressing the host's ability to produce NO is a sensical approach, as low levels of NO have been associated with the activation of dormancy-related genetic programs such as the DosRST regulon [106]. With the recently uncovered detrimental role of alveolar macrophages during early stages of infection, this could be a mechanism used by Mtb to ensure survivability of infected phagocytes in order to be transported to the lung interstitial tissue abundant in replication-permissive monocytes. The Mtb-associated resuscitation-promoting factor B (RpfB) interacts with TLR4 on DCs, activating the synergic signaling of MyD88 and toll/IL-1R homology domain-containing adapter-inducing IFN-β (TRIF), in order to ensure downstream signaling to MAPK and NF-κB [108]. This signaling pathway promotes education of naïve T cells and their subsequent polarization to CD4+ and CD8+, which will secrete IFN-γ and IL-2 [105]. Furthermore, this pathway induces T cell proliferation and polarization in the context of Th1 immunity, further emphasizing the importance of TLR2 signaling in TB immunopathology [105].

Plasmacytoid DCs (pDC), a special subset of DCs, function in close cooperation with TLR9 in such a way as to allow these immune cells to conduct their immune functions, including initiation of the immune response and control of inflammation through the induction of chemokines (**Figure 4**). pDCs are known to play an important role in recruiting NK cells; pDC−/− mice show a drastic reduction in NK cell recruitment upon intraperitoneal injection. Considering that pDCs express the CCR5, CCR2 and the CXCR3 ligand receptors on their surface, this likely translates to diminished binding of chemokines CCL2, CCL3, CCL4, CXCL10 and CXCL9 [109]. Mycobacterial DNA, the unmethylated CpG oligodeoxynucleotide motif in particular, acts as a TLR9 ligand and initiates the signaling pathway that promotes

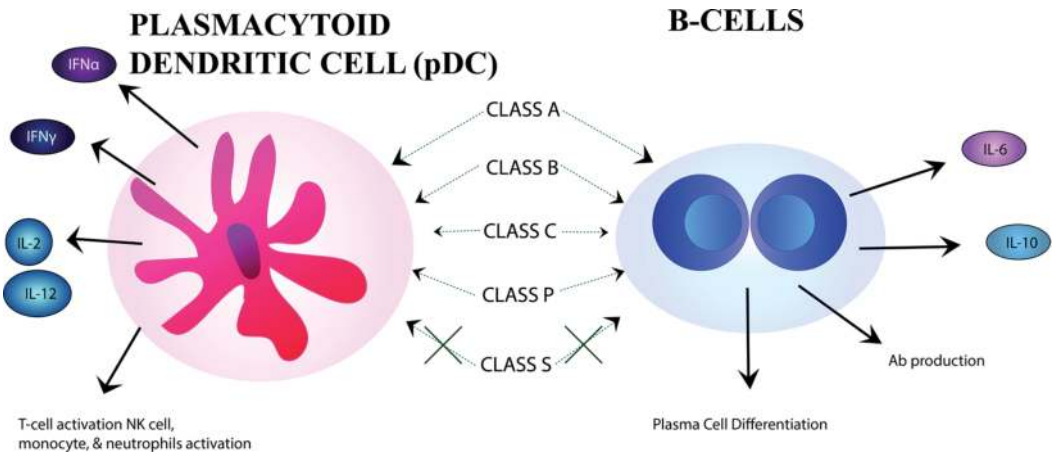

Figure 4.
Plasmacytoid cells in tuberculosis immunopathology. Abbreviations: IFNα/γ—interferon α/γ; IL-2/6/10/12—Interleukin-2/6/10/12, Ab—antibodies, NK cells—natural killer cells.

such pDC activity in Mtb infection [105]. Abrogation of TLR9 in mutant mice leads to higher susceptibility to TB diseases, in contrast to wild-type mice. Interestingly, previous studies on the synergic activity of TLR9 and TLR2 found that TLR9−/− mice demonstrated only mild differences in overall lung histopathology and granuloma formation [105]. However, focal necrosis was seen in TLR9/2−/− mice, a pathological outcome that also occurs for MyD88−/− mice, albeit the lung pathology in MyD88−/− mice is evidently more severe, further emphasizing the importance of MyD88 in TB immunopathology [109]. It should be noted, however, that no relevant difference in susceptibility was discovered in either TLR9−/− or TLR2−/− mice; alterations in granulomatous pathology and TNF-α secretion occur only for high-dose inoculums of Mtb as a consequence of TLR9 deficiency [105]. Considering that pDCs secrete IL-12—a cytokine that induces and controls the production of IFN-γ by CD4+ T cells—the relevance of TLR9-mediated signaling is evidently pivotal in both innate and acquired immunity [110, 111]. It would appear that synergic activity of a minimum of two TLRs is important in the signaling pathways that initiate competent immune responses to TB, although each receptor alone has proven to be relevant in vivo in gene knockout studies conducted on mice. Interestingly, a recent mouse study has concluded that TLR2 and TLR9 signaling is not necessary for vaccine-induced immunity [24, 112]. It should be noted, however, that an immunization strategy based on the combination of enhancement of TLR4, TLR3 and TLR9 signaling and the Mtb antigen Rv2034—a powerful CD4+ cell stimulant—has been successfully explored in mice and guinea pigs [113].

Although certain TLRs are part of signaling pathways relevant to TB immunopathology, they alone are only but a cog in a very complex machinery that comprises the TLR-mediated immune response to Mtb infection. It is attractive to speculate that devising immunotherapies guided towards enhancing TLR signaling due to their pivotal role in priming Th1 immunity through cytokine secretion, could be a fruitful avenue to pursue. Rampant inflammation at the level of innate immunity, influenced by TLR signaling, may also be a target of novel immunotherapies directed at diminishing the violent effects that excess PMNL recruitment has on the lung tissue [33, 105, 108]. However, to infer that enhanced TLR signaling alone would be sufficient in combating tuberculosis, would be rather misinformed. Namely, it was

discovered that, in murine models, XDR TB reduces the expression of TLR2 and TLR4 and consequently the production of cytokines that were otherwise abundantly present in mice infected with DS Mtb [114]. The inhibitory effects of XDR strains reduced the overall lung pathology in such a way that alveolar damage was reduced and granulomatous formations were smaller in size, in contrast with the detrimental immunopathologic events that were caused by DS TB. In light of this particular finding, one may infer that XDR TB was less virulent due to its down regulating effects on TLRs than DS strains, although much work is needed to further understand the implications of these results.

3.3 Implications of specific macrophage TLRs in tuberculosis pathogenesis

TLR2 signaling is triggered by heterodimerization with TLR1 or TLR6, and it follows a well-known signaling pathway. The "bridging adaptor" Mal is recruited by dimerized receptors, which aids in the recruitment of MyD88 and the formation of the myddosome complex, which consists of Mal, MyD88, and IRAK proteins. The nuclear translocation of NFkB and AP1 to commence transcription of cytokine and chemokine genes is triggered by the activation of IRAK4 followed by IRAK1/IRAK2 and activation of TRAF6 and TAK112. Mtb produces a vast number of TLR2 ligands [97]. It's secreted antigen 19 kDa lipoprotein (LpqH) was the first *M. tuberculosis* ligand to be demonstrated to signal through TLR2 [115]. TLR2 receptors sense mycobacterial lipoproteins LprA (Rv1270), LprG (Rv1411c), and PhoS113 [98, 116]. TLR2 is usually thought to play a minor function in Mtb immunity [97]. In Mtb infection, TLR2 signaling promotes three functional responses: protection, evasion, and regulation [97]. These responses are not required for the control of acute Mtb infection, but they may be required in chronic infection [81]. TLR2-activated pro-inflammatory cytokines induce protective mechanisms that keep Mtb control, while immune evasion mechanisms allow Mtb to evade antibacterial effector molecules [115–117]. TLR2 signaling is inhibited by immune regulatory pathways that have been activated by the pathogen, much like in the case of MRs. Mtb persists with low immunopathology and collateral harm to host tissue as a result of the multi-factorial functional response's combinatorial effect. In chronic infection, TLR2 signaling benefits both the host and the bacteria [97]. While TLR2 activation in macrophages is critical for controlling Mtb infection, it may not always be advantageous to host cells because Mtb has developed strategies to exploit TLR2 activation for its own gain. TLR2 has the capacity to engage several, structurally unique ligands and elicit different signals, and is one of the few receptors that can heterodimerize with TLR1 or TLR6, and also connect to additional co-receptors like CD14. One theory is that TLR2's interaction different binding partners helps to diversify ligand recognition [104].

TLR4 is best known for recognizing the Gram-negative bacteria's lipopolysaccharide (LPS) [118]. TLR4 detects lipids in cell walls, glycoproteins, and secretory proteins in Mtb [118]. The LAM precursor LM, as previously mentioned, causes macrophages to produce pro-inflammatory cytokines. Because BMDMs produce TNF and reactive nitrogen intermediates (RNI) in a TRL4-dependent manner in the presence of Ac4LM, the tetra-acylated version of LM (Ac4LM) operates as a particular TLR4 activator [119]. TLR4 is activated by a variety of mycobacterial proteins, including several heat shock proteins, Mtb H37Rv 38-kDA glycoprotein and the Mtb 50S ribosomal protein Rv0652. TLR4 stimulation can initiate the MyD88-independent TIR-domain including adapter-inducing IFN-β pathway [118]. It later on increases the expression of IRF3 to generate IFN-β secretion in this route,

and both IRF-3 and IFN-β have important parts to play in TB pathogenesis [120]. While some Mtb strains only engage TLR2, others trigger TLR4, resulting in distinct cytokine profiles marked by varied IFN-β production and as a result different bacterial pathogenicity ensues [30, 81].

TLR9 has been found in endosomes and phagolysosomes, where it can be activated by mycobacterial DNA upon pathogen uptake [105]. Thus, TLR9 is an important pattern-recognition receptor that could explain the host resistance to Mtb being dependent on MyD88. It identifies CG motifs (CpG) in bacterial DNA that are undermethylated, including Mtb DNA [121]. CpG sites are DNA areas in which cytosine is followed by guanine in a 5′ → 3′ direction linear sequence of bases. TNF-α, is produced with treatment of primary macrophages which can be inhibited with a TLR9 blocker or DNA methylation [121]. Mtb is a powerful stimulator of TLR9-dependent proinflammatory cytokine production by dendritic cells and macrophages, and these cells' in vitro responses to live mycobacteria are also TLR9-dependent [105]. Furthermore, this suggest that TLR9 is involved in the control of mycobacteria-induced Th1 responses in vivo during Mtb infection [105].

4. Macrophages and their role in tuberculosis granuloma formation

Many have questioned the purpose of the granuloma (**Figure 5**) within the body, but some would argue that the formation of the granuloma by the various white blood cells and macrophages in the body is an attempt by the adaptive immune system to contain the cells already infiltrated by Mtb cells [58]. It is begun as an innate immune system response but evolves in complexity as the adaptive immune system takes over [122]. The granuloma will begin forming as a group of macrophages who have been infiltrated and infected by Mtb, which are then surrounded by other macrophages and white blood cells in order to isolate these infected macrophages with their surrounding environment [123]. The granuloma will then be enclosed by a fibrous cuff and the surrounding area will undergo significant angiogenesis, similar to what

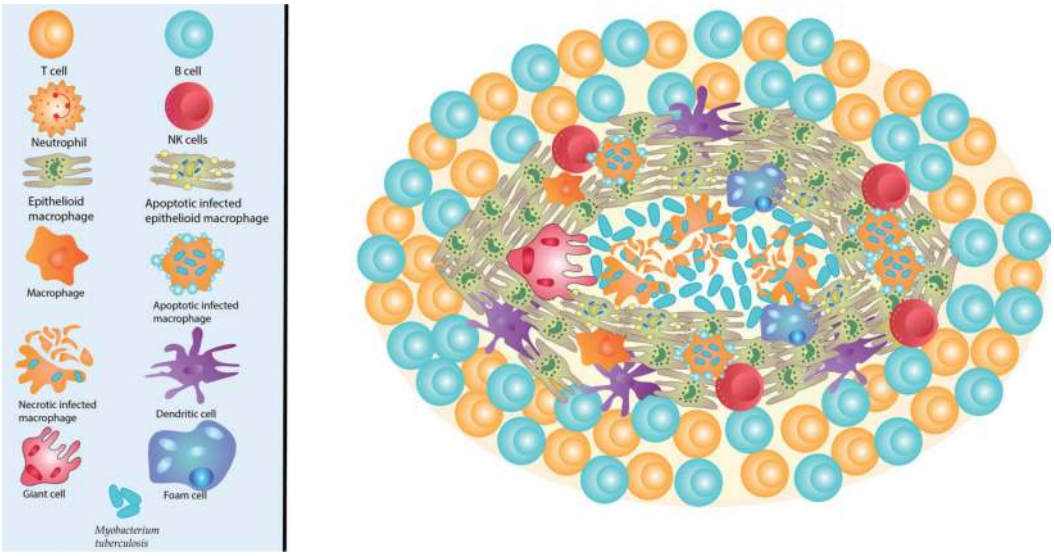

Figure 5.
Graphic illustration of the tuberculosis granuloma.

occurs when a cancerous tumor forms [124]. The macrophages and white blood cells within the granuloma will trigger conditions of hypoxia and lower nutrient availability [122]. Within the granuloma, it is believed that the bacteria do not continue replicating but instead focus their resources on gluconeogenesis to ensure their survival [58].

Of course, this also has a negative effect on the human body's own cells, and can eventually result in a necrotic or caseating granuloma [125]. This is partly due to "foamy" or lipid droplet-containing macrophages, which are formed by increased diffusion of LDL vesicles in the macrophage cells, and whatever is not exported by ATP active transport methods is esterified to convert it into lipid bodies, which give the cell its foamy appearance [123]. These cells are proinflammatory, and the increased presence of them promotes Mtb within the granuloma, enhancing the deteriorating conditions around and inside it and leading to eventual necrosis [55]. Lipid droplets released from the cell in the form of triglycerides can be absorbed by Mtb-infected macrophages, where they are then used by the Mtb bacteria for lipid metabolism [123]. Additionally, superoxide and NO production from the macrophages is severely limited due to the hypoxic conditions, so many times in the case of a granuloma, the best that can be hoped for is an isolated environment containing the bacteria, as bactericidal action by the macrophages becomes more difficult [123]. The most devastating problem presented by the granuloma is Mtb's ability to hijack the process for its own survival. Mtb bacteria have adapted to the formation of the granuloma, and mycobacterial recruitment proteins like ESX-1 release compounds to recruit macrophages to form the granuloma [4]. The reason for this is to provide the bacteria a fresh supply of macrophages for them to infect while simultaneously cutting themselves off from lymphocytes, who have a more pronounced bactericidal action against them [123]. The structure of the granuloma also creates difficulty for penetration by drugs and other therapies, and some have even considered treating the granuloma using similar methods to cancerous tumor treatment [126]. There are various types and stages of the granuloma ranging from primary granulomas to acute caseating granulomas. Caseating granulomas will begin descending into necrosis, releasing the Mtb into the surrounding lung tissue, which can then further infect others by being released in aerosolized droplets [18, 127].

4.1 Macrophages transport living *M. tuberculosis* into replication-permissive interstitial tissue

The bacillus once engulfed by the alveolar macrophage (AM) into the phagolysosome sabotages the lysosomal pathways. By incorporating proton pumps into the phagolysosome membrane, fusion to lysosomes is blocked, as they accommodate the environment suitable for replication and hence polarize into the M2 macrophage phenotype [128, 129]. In later stages, the bacillus may subvert the functional role of lysosome pathways by inducing cell necrosis as a means of dissemination, creating granulomas in the lung interstitium [130, 131]. Granulomas exist in several types wherein diverse macrophages are primary residents, leading to a range of unique microenvironments that are statistically independent of each other in a host body [132]. The outcome of the infection will depend on the phenotype of macrophages present (M1/M2 polarization) altered by the bacillus within the granulomas. The precedent to granuloma establishment—the translocation of the AM from the alveolus to the lung interstitium—depends on several signaling pathways. ESX-1 secretion system is necessary for the bacillus' escape from the AM phagolysosome by

potentiating inflammasome (comprised of NLRP3, ASC and caspase-1) activation within the AM and hence interleukin-1β (IL-1β) release, which increases alveolar permeability once bound to IL-1R on the epithelial barrier [133]. The RD1-dependent inflammasome signaling pathway transfers the living Mtb-infected AM whereas the STING pathway facilitates movement across the epithelial barrier by transferring the bacillus from the AM to a *mycobacterium* growth-permissive monocyte for crossing [134]. This is accomplished primarily by the glycolipids on the cell-surface of bacillus. Specifically, the phthiocerol dimycoceroserate (PDIM) lipid prevents recognition of pathogen-associated molecular patterns (PAMPs) by Toll-like receptors (TLRs) [135]. The phenolic glycolipid (PGL) thereon induces chemokine CCL2 via cytosolic signaling pathway STING to recruit growth-permissive monocytes for bacterial transfer by leveraging their CCR2 receptor [135]. STING activation does not induce CCL2 through type 1 IFNs therefore is independent to type 1 IFN involvement in contrast to the RD1-dependent inflammasome signaling pathway [134]. The CCL2 elicited monocytes then play an inflammatory role in the interstitium- however, the monocytes may switch roles to favor the host by offering antigens to pulmonary lymph nodes for T cell response.

5. Conclusions

Human tuberculosis is a tremendously complex infection, leaving no compartment of the immune system spared. Thus, conclusive studies on this disease from an immunological standpoint are difficult to conduct, due to the heterogenicity present within different populations in the context of host immunogenetics and potential previous exposure to pathogenic and non-pathogenic mycobacteria. Though other innate immune cells eventually come into play, macrophages play a pivotal role in the immune response to infections with *Mycobacterium tuberculosis*, as they represent the first professional line of defense against respiratory infections in general. Indeed, macrophages are equipped with the necessary bactericidal mechanisms to effectively destroy infectious agents, however Mtb has successfully developed a barrage of methods by which they may be circumvented, thereby converting the macrophage from a foe into a safe confinement. Macrophages that have failed to eliminate the bacillus tend to shuttle and localize the pathogen in environments that are rich in replication-permissive cells, thereby perpetuating further bacterial replication and dissemination. Failure of macrophages to clear out the infection is primarily rooted in receptor-mediated uptake of Mtb, where the pathogen favors MR-mediated uptake, as it allows for the bacillus to manipulate the intracellular environment in order to avoid destruction in the phagosome by preventing phagosome-lysosome fusion. However, even in the case where infected macrophages shuttle the pathogen the replication-permissive lung interstitium, the cytokines produced by these infected cells recruit novel APCs, seemingly in order to physically entrap Mtb. This results in the formation of a tuberculosis granuloma, characterized by an infected core, surrounded by non-infected APCs such as macrophages, with the granuloma exterior being further supported by T cells. Though these granulomas seem like a sensical and efficient method of preventing further dissemination of the pathogen, mycobacterial survivability within the hypoxic and noxious granuloma environment is enabled through reprogramming of the metabolic profile of Mtb. This reprogramming enables a state of metabolic dormancy, clinically distinguished by the term latent tuberculosis—an asymptomatic form of TB. When in this state, Mtb is capable of utilizing

the abundant concentration of lipids, among other molecules, present in foamy macrophages in order to facilitate survival. In spite of this, the human immune system is capable of continuously maintaining the structural integrity of the granuloma and ensure that viable but dormant mycobacterial cells are contained. Whether a dormant infection will become activated depends on the competence of the host's immune system. Erosion of the granuloma leads to rapid activation and replication of the bacillus and its dissemination through the airways. Furthermore, eroded granulomas are characterized by extensive caseous necrosis which leaves radiographically observable lung cavities in TB patients. Thus, professional macrophages that are engaged in combating Mtb infection are difficult to categorize in terms of their efficiency at resolving the infection. It appears that every infection with Mtb, in the very least, leads to latent TB, thereby making the conversation of complete elimination of the bacillus from the immune system, rather improbable. Rather, it is more prudent to understand the immune response to Mtb infection as containment rather than infectant elimination, and this is primarily owed to the ambiguous dual role those professional macrophages play in infectious with Mtb, supplemented by factors that include macrophage immunoreceptors, cytokine expression profiles and the overall virulence of the bacillus itself.

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Conflict of interest

None to declare.

Notes/thanks/other declarations

We sincerely hope that, with the rapid expansion and improvement of scientific methods, we will eventually understand *M. tuberculosis* as well as it understands us.

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References

[1] Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Medicine. 2016 Oct 25;**13**(10):e1002152

[2] World Health Organization. Global tuberculosis report 2019. 2019. Recuperado: https://www. who.int/tb/publications/global_report/en. 2020 Apr

[3] Bottai D, Frigui W, Sayes F, di Luca M, Spadoni D, Pawlik A, et al. TbD1 deletion as a driver of the evolutionary success of modern epidemic mycobacterium tuberculosis lineages. Nature Communications. 2020;**11**(1):684. Available from: http://www.nature.com/articles/s41467-020-14508-5

[4] Sia JK, Rengarajan J. Immunology of *Mycobacterium tuberculosis* infections. Microbiology Spectrum. 2019 Jul 12;**7**(4):4-7

[5] Lurie MB. Native and acquired resistance to tuberculosis. The American Journal of Medicine. 1950 Nov 1;**9**(5):591-610

[6] Guerrero Manriquez GG. Immunogenetic and Immunotherapy in Tuberculosis [Internet]. 2019. Available from: www.intechopen.com

[7] Nguyen L, Pieters J. The Trojan horse: Survival tactics of pathogenic mycobacteria in macrophages. Trends in Cell Biology. 2005;**15**:269-276

[8] Abel L, El-Baghdadi J, Bousfiha AA, Casanova JL, Schurr E. Human genetics of tuberculosis: a long and winding road. Philosophical Transactions of the Royal Society B: Biological Sciences. 2014 Jun 19;**369**(1645):20130428

[9] Derek C, Gail C. Novartis Foundation. Genetics and Tuberculosis. Cape Town, South Aftica: Wiley; 1998. p. 269

[10] Pagán AJ, Ramakrishnan L. Immunity and immunopathology in the tuberculous granuloma. Cold Spring Harbor Perspectives in Medicine. 2015 Sep 1;**5**(9):a018499

[11] Kiran D, Podell BK, Chambers M, Basaraba RJ. Host-directed therapy targeting the Mycobacterium tuberculosis granuloma: A review. Seminars in Immunopathology. 2016;**38**:167-183

[12] Cassidy JP, Martineau AR. Innate resistance to tuberculosis in man, cattle and laboratory animal models: Nipping disease in the bud? Journal of Comparative Pathology. 2014;**151**:291-308

[13] Pieters J. Mycobacterium tuberculosis and the macrophage: Maintaining a balance. Cell Host and Microbe. 2008;**3**:399-407

[14] Silva Miranda M, Breiman A, Allain S, Deknuydt F, Altare F. The tuberculous granuloma: an unsuccessful host defence mechanism providing a safety shelter for the bacteria?. Clinical and Developmental Immunology. 2012 Oct;**2012**

[15] Hunter RL, Olsen MR, Jagannath C, Actor JK. Review: Multiple Roles of Cord Factor in the Pathogenesis of Primary, Secondary, and Cavitary Tuberculosis, Including a Revised Description of the Pathology of Secondary Disease [Internet]. 2006. Available from: http://www.granuloma.homestead.com

[16] Behr M, Ellis R, Ernst J, Evans T, Flynn J, Hatherill M, et al.

Developing vaccines to prevent sustained infection with Mycobacterium tuberculosis. Vaccine. 2015 Jun 12;**33**(26):3056-3064

[17] Schrager LK, Harris RC, Vekemans J. Research and development of new tuberculosis vaccines: a review. F1000Research. 2018;7

[18] Hunter R, Actor J. The pathogenesis of post-primary tuberculosis. A game changer for vaccine development. Tuberculosis. 2019;**116**:S114-S117

[19] Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature. 2020;**577**(7788):95-102

[20] Rao M, Valentini D, Poiret T, Dodoo E, Parida S, Zumla A et al. B in TB: B cells as mediators of clinically relevant immune responses in tuberculosis. Clinical Infectious Diseases. 2015 Oct 15;**61**(suppl_3):S225-S235

[21] Steigler P, Verrall AJ, Kirman JR. Beyond memory T cells: mechanisms of protective immunity to tuberculosis infection. Immunology and Cell Biology. 2019 Aug;**97**(7):647-655

[22] Fox GJ, Orlova M, Schurr E. Tuberculosis in newborns: the lessons of the "Lübeck Disaster"(1929-1933). PLoS Pathogens. 2016 Jan 21;**12**(1):e1005271

[23] Migliori GB, Tiberi S, Zumla A, Petersen E, Chakaya JM, Wejse C, et al. MDR/XDR-TB management of patients and contacts: Challenges facing the new decade. The 2020 clinical update by the Global Tuberculosis Network. International Journal of Infectious Diseases. 2020 Mar 1;92:S15-S25

[24] WHO | Technical report on critical concentrations for TB drug susceptibility testing of medicines used in the treatment of drug-resistant TB. WHO. 2018

[25] Shibabaw A, Gelaw B, Gebreyes W, Robinson R, Wang S-H, Tessema B. The burden of pre-extensively and extensively drug-resistant tuberculosis among MDR-TB patients in the Amhara region, Ethiopia. Anupurba S, editor. PLOS ONE. 2020;**15**(2):e0229040. Available from: https://dx.plos.org/10.1371/journal.pone.0229040

[26] van Crevel R, Ottenhoff THM, van der Meer JWM. Innate immunity to mycobacterium tuberculosis. Clinical Microbiology Reviews. 2002;**15**:294-309

[27] Zimri K, Casper R, Hoddinott G, Schaaf HS, Garcia-Prats AJ, Rose PC, et al. A novel approach for eliciting adolescent MDR-TB treatment tolerability: Qualitative data from South Africa. The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union against Tuberculosis and Lung Disease. 2020;**24**(1):43-47

[28] Dheda K, Schwander SK, Zhu B, van Zyl-Smit RN, Zhang Y. The immunology of tuberculosis: From bench to bedside. Respirology. 2010;**15**:433-450

[29] Kang PB, Azad AK, Torrelles JB, Kaufman TM, Beharka A, Tibesar E, et al. The human macrophage mannose receptor directs *Mycobacterium tuberculosis* lipoarabinomannan-mediated phagosome biogenesis. Journal of Experimental Medicine. 2005;**202**(7):987-999

[30] Harding C, v., Boom WH. Regulation of antigen presentation by *Mycobacterium tuberculosis*: A role for Toll-like receptors. Nature Reviews Microbiology. 2010;**8**(4):296. Available from: /pmc/articles/PMC3037727/

[31] Russell DG. The evolutionary pressures that have molded *Mycobacterium tuberculosis* into an infectious adjuvant. Current Opinion in Microbiology. 2013;**16**:78-84

[32] Strohmeier GR, Fenton MJ. Roles of lipoarabinomannan in the pathogenesis of tuberculosis. Microbes and Infection. 1999 Jul 1;**1**(9):709-717

[33] Pathak SK, Basu S, Basu KK, Banerjee A, Pathak S, Bhattacharyya A, et al. Direct extracellular interaction between the early secreted antigen ESAT-6 of *Mycobacterium tuberculosis* and TLR2 inhibits TLR signaling in macrophages. Nature Immunology. 2007;**8**(6):610-618

[34] Aderem A, Underhill DM. Mechanisms of phagocytosis in macrophages. Annual Review of Immunology. 1999 Apr;**17**(1):593-623

[35] Medzhitov R, Janeway CA. Innate immunity: Impact on the adaptive immune response. Current Opinion in Immunology. 1997;**9**(1):4-9

[36] Visintin A, Mazzoni A, Spitzer JH, Wyllie DH, Dower SK, Segal DM. Regulation of Toll-like receptors in human monocytes and dendritic cells. The Journal of Immunology. 2001 Jan 1;**166**(1):249-55

[37] Kleinnijenhuis J, Oosting M, Joosten LA, Netea MG, Van Crevel R. Innate immune recognition of Mycobacterium tuberculosis. Clinical and Developmental Immunology. 2011 Oct;**2011**

[38] Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. Science. 1999;**285**(5428): 732-736

[39] Selvaraj P, Alagarasu K, Harishankar M, Vidyarani M, Nisha Rajeswari D, Narayanan PR. Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis. Cytokine. 2008;**43**(1):26-33

[40] Akahoshi M, Nakashima H, Miyake K, Inoue Y, Shimizu S, Tanaka Y, et al. Influence of interleukin-12 receptor β1 polymorphisms on tuberculosis. Human Genetics [Internet]. 2003;**112**(3):237-243. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12596048

[41] Remus N, El Baghdadi J, Fieschi C, Feinberg J, Quintin T, Chentoufi M, et al. Association of IL12RB1 polymorphisms with pulmonary tuberculosis in adults in Morocco. The Journal of Infectious Diseases [Internet]. 2004;**190**(3):580-587. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/422534

[42] Anand SP, Selvaraj P, Jawahar MS, Adhilakshmi AR, Narayanan PR. Interleukin-12B & interleukin-10 gene polymorphisms in pulmonary tuberculosis. Indian Journal of Medical Research. 2007;**126**(2):135-138

[43] Liu C, He T, Rong Y, Du F, Ma D, Wei Y, et al. Association of mannose-binding lectin polymorphisms with tuberculosis susceptibility among Chinese. Scientific Reports. 2016 Nov 4;**6**(1):1-6

[44] Liu Q, Wang J, Sandford AJ, Wu J, Wang Y, Wu S, et al. Association of CYBB polymorphisms with tuberculosis susceptibility in the Chinese Han population. Infection, Genetics and Evolution. 2015;**33**:169-175

[45] Zhang H, Zhou G, Zhi L, Yang H, Zhai Y, Dong X, et al. Association between mannose-binding

lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. The Journal of Infectious Diseases [Internet]. 2005;**192**(8):1355-1361. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/491479

[46] Ansari A, Hasan Z, Dawood G, Hussain R. Differential combination of cytokine and interferon-γ+ 874 T/A polymorphisms determines disease severity in pulmonary tuberculosis. PLoS One. 2011 Nov 29; **6**(11):e27848

[47] Vergne I, Deretic V. The role of PI3P phosphatases in the regulation of autophagy. FEBS Letters. 2010;**584**:1313-1318

[48] Kaufmann SHE. How can immunology contribute to the control of tuberculosis? Nature Reviews Immunology. 2001;**1**:20-30

[49] Huang Z, Luo Q, Guo Y, Chen J, Xiong G, Peng Y, et al. *Mycobacterium tuberculosis*-induced polarization of human macrophage orchestrates the formation and development of tuberculous granulomas in vitro. PloS One. 2015 Jun 19;**10**(6):e0129744

[50] Patankar YR, Sutiwisesak R, Boyce S, Lai R, Lindestam Arlehamn CS, Sette A, et al. Limited recognition of *Mycobacterium tuberculosis*-infected macrophages by polyclonal CD4 and CD8 T cells from the lungs of infected mice. Mucosal Immunology. 2020;**13**(1):140-148

[51] Rajaram MVS, Arnett E, Azad AK, Guirado E, Ni B, Gerberick AD, et al. *M. tuberculosis*-initiated human mannose receptor Signaling regulates macrophage recognition and vesicle trafficking by FcRγ-chain, Grb2, and SHP-1. Cell Reports. 2017;**21**(1):126-140

[52] Axelsson-Robertson R, Ju JH, Kim HY, Zumla A, Maeurer M. *Mycobacterium tuberculosis*-specific and MHC class I-restricted CD8+ T-cells exhibit a stem cell precursor-like phenotype in patients with active pulmonary tuberculosis. International Journal of Infectious Diseases [Internet]. 2015;**32**:13-22. Available from: http://www.ijidonline.com/article/S1201971214017342/fulltext

[53] De Martino M, Lodi L, Galli L, Chiappini E. Immune response to *Mycobacterium tuberculosis*: a narrative review. Frontiers in Pediatrics. 2019:350

[54] Moreira-Teixeira L, Mayer-Barber K, Sher A, O'Garra A. Type I interferons in tuberculosis: Foe and occasionally friend. Journal of Experimental Medicine. 2018;**215**(5):1273-1285. DOI: 10.1084/jem.20180325

[55] Russell DG, Barry CE, Flynn JL. Tuberculosis: What we don't know can, and does, hurt us. Science. 2010;**328**:852-856. Available from: https://science.sciencemag.org/content/328/5980/852

[56] Monin L, Khader SA. Chemokines in tuberculosis: The good, the bad and the ugly. Seminars in Immunology. 2014;**26**:552-558

[57] Bhat KH, Yaseen I. *Mycobacterium tuberculosis*: macrophage takeover and modulation of innate effector responses. Mycobacterium-Research and Development. 2018 Jun 20

[58] Ehlers S, Schaible UE. The granuloma in tuberculosis: dynamics of a host–pathogen collusion. Frontiers in Immunology. 2013 Jan 7;**3**:411

[59] Simeone R, Bobard A, Lippmann J, Bitter W, Majlessi L, Brosch R, et al. Phagosomal rupture by *Mycobacterium tuberculosis* results in toxicity and host cell death. PLoS Pathogens.

2012;**8**(2):e1002507. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22319448

[60] Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, et al. Incipient and Subclinical Tuberculosis: A Clinical Review of Early Stages and Progression of Infection [Internet]. 2018. Available from: http://cmr.asm.org/

[61] Denholm J. The use of anti-tuberculosis therapy for latent TB infection. Infection and Drug Resistance. 2010;**3**:63. Available from: /pmc/articles/PMC3108738/?report=abstract

[62] Denholm JT, Street AC. Diagnosis and management of latent tuberculosis infection. Medicine Today. 2010;**11**:72-76

[63] Leistikow RL, Morton RA, Bartek IL, Frimpong I, Wagner K, Voskuil MI. The *Mycobacterium tuberculosis* DosR regulon assists in metabolic homeostasis and enables rapid recovery from nonrespiring dormancy. Journal of Bacteriology. 2010;**192**(6):1662-1670

[64] Mehra S, Foreman TW, Didier PJ, Ahsan MH, Hudock TA, Kissee R, et al. The DosR regulon modulates adaptive immunity and is essential for *Mycobacterium tuberculosis* persistence. American Journal of Respiratory and Critical Care Medicine. 2015;**191**(10):1185-1196

[65] Reed MB, Gagneux S, DeRiemer K, Small PM, Barry CE. The W-Beijing lineage of *Mycobacterium tuberculosis* overproduces triglycerides and has the DosR dormancy regulon constitutively upregulated. Journal of Bacteriology. 2007;**189**(7):2583-2589

[66] Diel R. Long-term effect of Bacille Calmette-Guérin vaccination in tuberculin skin testing: A new reality for TB prevention. Chest. 2017;**152**:235-236

[67] Rivas-Santiago CE, Sarkar S, Cantarella P, Osornio-Vargas Á, Quintana-Belmares R, Meng Q, et al. Air pollution particulate matter alters antimycobacterial respiratory epithelium innate immunity. Infection and Immunity. 2015;**83**(6):2507-2517

[68] Wang L, Turner MO, Elwood RK. A Meta-Analysis of the Effect of Bacille Calmette Guérin Vaccination on Tuberculin Skin Test Measurements [Internet]. 2002. Available from: www.thoraxjnl.com

[69] Schito M, Hanna D, Zumla A. Tuberculosis eradication versus control. International Journal of Infectious Diseases. 2017;**56**:10-13

[70] Maglione PJ, Xu J, Casadevall A, Chan J. Fcγ receptors regulate immune activation and susceptibility during *Mycobacterium tuberculosis* infection. The Journal of Immunology. 2008;**180**(5):3329-3338

[71] Martinez-Pomares L. The mannose receptor. Journal of Leukocyte Biology. 2012;**92**(6):1177-1186

[72] Kumar V. Macrophages: The Potent Immunoregulatory Innate Immune Cells. London, UK: IntechOpen; 2019. Available from: www.intechopen.com

[73] Nouailles G, Dorhoi A, Koch M, Zerrahn J, Weiner J, Faé KC, et al. CXCL5-secreting pulmonary epithelial cells drive destructive neutrophilic inflammation in tuberculosis. Journal of Clinical Investigation. 2014;**124**(3):1268-1282

[74] Mehra S, Pahar B, Dutta NK, Conerly CN, Philippi-Falkenstein K, Alvarez X, et al. Transcriptional reprogramming in nonhuman primate (*Rhesus macaque*) tuberculosis

granulomas. PLoS ONE. 2010;**5**(8):e12266. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20824205

[75] Lin Y, Zhang M, Barnes PF. Chemokine production by a human alveolar epithelial cell line in response to *Mycobacterium tuberculosis*. Infection and Immunity. 1998 Mar 1;**66**(3):1121-1126

[76] Rohani MG, Beyer RP, Hacker BM, Dommisch H, Dale BA, Chung WO. Modulation of expression of innate immunity markers CXCL5/ENA-78 and CCL20/MIP3α by protease-activated receptors (PARs) in human gingival epithelial cells. Innate Immunity. 2010 Apr;**16**(2):104-114

[77] Cohen SB, Gern BH, Delahaye JL, Adams KN, Plumlee CR, Winkler JK, et al. Alveolar macrophages provide an early *Mycobacterium tuberculosis* niche and initiate dissemination. Cell Host and Microbe. 2018;**24**(3):439-446.e4

[78] Martinon F, Burns K, Tschopp J. The Inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-β. Molecular Cell. 2002;**10**(2):417-426

[79] Apostolopoulos V, McKenzie IF. Role of the mannose receptor in the immune response. Current Molecular Medicine. 2001 Sep 1;**1**(4):469-474

[80] Queval CJ, Brosch R, Simeone R. The macrophage: A disputed fortress in the battle against *Mycobacterium tuberculosis*. Frontiers in Microbiology. 2017;**8**:2284

[81] Faridgohar M, Nikoueinejad H. New findings of Toll-like receptors involved in *Mycobacterium tuberculosis* infection. Pathogens and Global Health. 2017;**111**:256-264

[82] Schweizer A, Stahl PD, Rohrer J. A di-aromatic motif in the cytosolic tail of the mannose receptor mediates endosomal sorting. Journal of Biological Chemistry [Internet]. 2000;**275**(38):29694-29700. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10896932

[83] Azad AK, Rajaram MV, Schlesinger LS. Exploitation of the macrophage mannose receptor (CD206) in infectious disease diagnostics and therapeutics. Journal of Cytology & Molecular Biology. 2014 Jan 10;**1**(1)

[84] Gazi U, Martinez-Pomares L. Influence of the mannose receptor in host immune responses. Immunobiology. 2009;**214**:554-561. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19162368

[85] Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Frontiers in Bioscience. 2008;**13**:453-461

[86] Stahl PD, Ezekowitz RAB. The mannose receptor is a pattern recognition receptor involved in host defense. Current Opinion in Immunology. 1998;**10**(1):50-55

[87] Kang BK, Schlesinger LS. Characterization of mannose receptor-dependent phagocytosis mediated by Mycobacterium tuberculosis lipoarabinomannan. Infection and immunity. 1998;**66**(6):2769-2777. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9596746

[88] Lowell CA. Src-family and Syk kinases in activating and inhibitory pathways in innate immune cells: Signaling cross talk. Cold Spring Harbor Perspectives in Biology. 2011;**3**:1-16

[89] May RC, Machesky LM. Phagocytosis and the actin cytoskeleton. Journal of Cell Science. 2001;**114**:1061-1077

[90] Bekale RB, Du Plessis SM, Hsu NJ, Sharma JR, Sampson SL, Jacobs M, et al. *Mycobacterium tuberculosis* and interactions with the host immune system: Opportunities for nanoparticle based immunotherapeutics and vaccines. Pharmaceutical Research. 2019 Jan;**36**(1):1-5

[91] Vergne I, Chua J, Deretic V. Tuberculosis toxin blocking phagosome maturation inhibits a novel Ca 2+/ calmodulin-PI3K hVPS34 cascade. Journal of Experimental Medicine. 2003;**198**(4):653-659

[92] Turner J, Torrelles JB. Mannose-capped lipoarabinomannan in Mycobacterium tuberculosis pathogenesis. Pathogens and Disease. 2018 Jun;**76**(4):fty026

[93] Correia-Neves M, Sundling C, Cooper A, Källenius G. Lipoarabinomannan in active and passive protection against tuberculosis. Frontiers in Immunology. 2019:1968

[94] Yuan C, Qu ZL, Tang XL, Liu Q, Luo W, Huang C, et al. *Mycobacterium tuberculosis* mannose-capped lipoarabinomannan induces IL-10-producing B cells and hinders CD4+Th1 immunity. iScience. 2019;**11**:13-30

[95] Cardona P, Cardona PJ. Regulatory T cells in *Mycobacterium tuberculosis* infection. Frontiers in Immunology. 2019:2139

[96] Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science. 2006;**311**(5768):1770-1773

[97] Gopalakrishnan A, Salgame P. Toll-like receptor 2 in host defense against *Mycobacterium tuberculosis*: To be or not to be-that is the question. Current Opinion in Immunology. 2016;**42**:76. Available from: /pmc/articles/ PMC5086274/

[98] Gehring AJ, Dobos KM, Belisle JT, Harding CV, Boom WH. *Mycobacterium tuberculosis* LprG (Rv1411c): A novel TLR-2 ligand that inhibits human macrophage class II MHC antigen processing. Journal of Immunology (Baltimore, Md: 1950). 2004;**173**(4):2660-2668. Available from: https://pubmed.ncbi.nlm.nih.gov/15294983/

[99] Taha RA, Kotsimbos TC, Song YL, Menzies D, Hamid Q. IFN-gamma and IL-12 are increased in active compared with inactive tuberculosis. American journal of Respiratory and Critical Care Medicine. 1997 Mar;**155**(3):1135-1139

[100] Leopold Wager CM, Hole CR, Campuzano A, Castro-Lopez N, Cai H, Caballero Van Dyke MC, et al. IFN-γ immune priming of macrophages in vivo induces prolonged STAT1 binding and protection against Cryptococcus neoformans. PLoS Pathogens. 2018 Oct 10;**14**(10):e1007358

[101] Alemán M. Neutrophil apoptosis in the context of tuberculosis infection. Tuberculosis. 2015;**95**:359-363

[102] Esin S, Counoupas C, Aulicino A, Brancatisano FL, Maisetta G, Bottai D, et al. Interaction of mycobacterium tuberculosis cell wall components with the human natural killer cell receptors NKp44 and toll-like receptor 2. Scandinavian Journal of Immunology. 2013;**77**(6):460-469. Available from: http://www.ncbi.nlm.nih.gov/ pubmed/23578092

[103] Ferwerda G, Girardin SE, Kullberg BJ, le Bourhis L, de Jong DJ, Langenberg DML, et al. NOD2 and toll-like receptors are nonredundant

recognition systems of mycobacterium tuberculosis. PLoS Pathogens. 2005;**1**(3):0279-0285

[104] Medzhitov R. Toll-like receptors and innate immunity. Nature Reviews Immunology. 2001;**1**:135-145

[105] Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to *Mycobacterium tuberculosis*. Journal of Experimental Medicine. 2005;**202**(12):1715-1724

[106] Bartek IL, Rutherford R, Gruppo V, Morton RA, Morris RP, Klein MR, et al. The DosR regulon of *M. tuberculosis* and antibacterial tolerance. Tuberculosis. 2009;**89**(4):310-316

[107] Black GF, Thiel BA, Ota MO, Parida SK, Adegbola R, Boom WH, et al. Immunogenicity of novel DosR regulon-encoded candidate antigens of *Mycobacterium tuberculosis* in three high-burden populations in Africa. Clinical and Vaccine Immunology. 2009;**16**(8):1203-1212

[108] Kim J-S, Kim WS, Choi H-G, Jang B, Lee K, Park J-H, et al. *Mycobacterium tuberculosis* RpfB drives Th1-type T cell immunity via a TLR4-dependent activation of dendritic cells. Journal of Leukocyte Biology. 2013;**94**(4):733-749

[109] Guillerey C, Mouriès J, Polo G, Doyen N, HKW L, Chan S, et al. Pivotal role of plasmacytoid dendritic cells in inflammation and NK-cell responses after TLR9 triggering in mice. Blood. 2012;**120**(1):90-99

[110] Sun Z, Ren W, Xu Y, Zhang H, Li C. Preliminary study on the virulence of XDR-TB: Low virulence owing to less cytokine expression through the TLR 2 and TLR4 pathways in BLAB/C mice. Bio-Medical Materials and Engineering. 2014 Jan 1;24(6):3873-3882

[111] Commandeur S, van den Eeden SJF, Dijkman K, Clark SO, van Meijgaarden KE, Wilson L, et al. The in vivo expressed *Mycobacterium tuberculosis* (IVE-TB) antigen Rv2034 induces CD4+ T-cells that protect against pulmonary infection in HLA-DR transgenic mice and Guinea pigs. Vaccine. 2014;**32**(29):3580-3588. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24837764

[112] Schön T, Juréen P, Giske CG, Chryssanthou E, Sturegård E, Werngren J, et al. Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for *Mycobacterium tuberculosis*. The Journal of Antimicrobial Chemotherapy. 2009;**64**(4):786-793. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19633001

[113] Salie M, Daya M, Lucas LA, Warren RM, van der Spuy GD, van Helden PD, et al. Association of toll-like receptors with susceptibility to tuberculosis suggests sex-specific effects of TLR8 polymorphisms. Infection, Genetics and Evolution. 2015;**34**:221-229

[114] Bukhari M, Aslam MA, Khan A, Iram Q, Akbar A, Naz AG, et al. TLR8 gene polymorphism and association in bacterial load in southern Punjab of Pakistan: An association study with pulmonary tuberculosis. International Journal of Immunogenetics [Internet]. 2015;**42**(1):46-51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25572425

[115] Padhi A, Pattnaik K, Biswas M, Jagadeb M, Behera A, Sonawane A. *Mycobacterium tuberculosis* LprE suppresses TLR2-dependent cathelicidin and autophagy expression to enhance bacterial survival in macrophages.

The Journal of Immunology. 2019;**203**(10):2665-2678. Available from: https://www.jimmunol.org/content/203/10/2665

[116] Pecora ND, Gehring AJ, Canaday DH, Boom WH, Harding CV. *Mycobacterium tuberculosis* LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. Journal of Immunology (Baltimore, Md: 1950). 2006;**177**(1):422-429. Available from: https://pubmed.ncbi.nlm.nih.gov/16785538/

[117] Heldwein KA, Liang MD, Andresen TK, Thomas KE, Marty AM, Cuesta N, et al. TLR2 and TLR4 serve distinct roles in the host immune response against *Mycobacterium bovis* BCG. Journal of Leukocyte Biology. 2003;**74**(2):277-286. Available from: https://pubmed.ncbi.nlm.nih.gov/12885945/

[118] Sepehri Z, Kiani Z, Kohan F, Ghavami S. Toll-like receptor 4 as an immune receptor against *Mycobacterium tuberculosis*: A systematic review. Laboratory Medicine. 2019;**50**(2):117-129. Available from: https://pubmed.ncbi.nlm.nih.gov/30124945/

[119] Stamm CE, Collins AC, Shiloh MU. Sensing of *Mycobacterium tuberculosis* and consequences to both host and bacillus. Immunological Reviews [Internet]. 2015;**264**(1):204. Available from: /pmc/articles/PMC4339209/

[120] Donovan ML, Schultz TE, Duke TJ, Blumenthal A. Type I interferons in the pathogenesis of tuberculosis: Molecular drivers and immunological consequences. Frontiers in Immunology. 2017;**8**(NOV):1633

[121] Kiemer AK, Senaratne RH, Hoppstädter J, Diesel B, Riley LW, Tabeta K, et al. Attenuated activation of macrophage TLR9 by DNA from virulent mycobacteria. Journal of Innate Immunity. 2009;**1**(1):29-45. Available from: https://pubmed.ncbi.nlm.nih.gov/20375564/

[122] Orme IM, Basaraba RJ. The formation of the granuloma in tuberculosis infection. Seminars in Immunology [Internet]. 2014;**26**(6):601-609. Available from: https://pubmed.ncbi.nlm.nih.gov/25453231/

[123] Korb VC, Chuturgoon AA, Moodley D. *Mycobacterium tuberculosis*: manipulator of protective immunity. International journal of Molecular Sciences. 2016 Mar;**17**(3):131

[124] Stockmann C, Schadendorf D, Klose R, Helfrich I. The impact of the immune system on tumor: Angiogenesis and vascular remodeling. Frontiers in Oncology. 2014;**4**(Apr):69

[125] Tezera LB, Bielecka MK, Chancellor A, Reichmann MT, Al Shammari B, Brace P, et al. Dissection of the host-pathogen interaction in human tuberculosis using a bioengineered 3-dimensional model. Elife. 2017 Jan 7;**6**:e21283

[126] Datta M, Via LE, Kamoun WS, Liu C, Chen W, Seano G, et al. Anti-vascular endothelial growth factor treatment normalizes tuberculosis granuloma vasculature and improves small molecule delivery. Proceedings of the National Academy of Sciences of the United States of America [Internet]. 2015;**112**(6):1827-1832. Available from: https://pubmed.ncbi.nlm.nih.gov/25624495/

[127] Jee B. Understanding the early host immune response against *Mycobacterium tuberculosis*. Central-European Journal of Immunology. 2020;**45**(1):99. Available from: /labs/pmc/articles/PMC7226546/

[128] Flynn JL, Chan J. Immunology of tuberculosis. Annual Review of Immunology. 2001;**19**:93-129. Available from: https://pubmed.ncbi.nlm.nih.gov/11244032/

[129] Rohde K, Yates RM, Purdy GE, Russell DG. *Mycobacterium tuberculosis* and the environment within the phagosome. Immunological Reviews. 2007;**219**(1):37-54. Available from: https://pubmed.ncbi.nlm.nih.gov/17850480/

[130] Lee J, Hartman M, Kornfeld H. Macrophage apoptosis in tuberculosis. Yonsei Medical Journal [Internet]. 2009;**50**(1):1-11. Available from: https://pubmed.ncbi.nlm.nih.gov/19259342/

[131] Upadhyay S, Mittal E, Philips JA. Tuberculosis and the art of macrophage manipulation. Pathogens and Disease. 2018;**76**(4):37. Available from: https://academic.oup.com/femspd/article/76/4/fty037/4970761

[132] Flynn JL, Chan J, Lin PL. Macrophages and control of granulomatous inflammation in tuberculosis. Mucosal Immunology [Internet]. 2011;**4**(3):271-278. Available from: https://pubmed.ncbi.nlm.nih.gov/21430653/

[133] Wong KW. The role of ESX-1 in *Mycobacterium tuberculosis* pathogenesis. Microbiology Spectrum. 2017 May 19;**5**(3):5-3

[134] Manzanillo PS, Shiloh MU, Portnoy DA, Cox JS. *Mycobacterium tuberculosis* activates the DNA-dependent cytosolic surveillance pathway within macrophages. Cell Host & Microbe. 2012;**11**(5):469. Available from: /labs/pmc/articles/PMC3662372/

[135] Cambier CJ, O'Leary SM, O'Sullivan MP, Keane J, Ramakrishnan L. Phenolic glycolipid facilitates mycobacterial escape from microbicidal tissue-resident macrophages. Immunity. 2017;**47**(3):552. Available from: /labs/pmc/articles/PMC5610147/