Chapter 2

Mycobacterium tuberculosis: Macrophage Takeover and Modulation of Innate Effector Responses

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Additional information is available at the end of the chapter

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Abstract

Macrophages mediate the first line of defense in the host against various intracellular pathogens. They are armed with several immune-effector mechanisms to detect and combat pathogens. However, intracellular pathogens have developed strategies to overcome the macrophage protective immune responses and colonize inside the macrophages. Tuberculosis (TB), both pulmonary and extrapulmonary, is an infectious disease of global concern caused by *Mycobacterium tuberculosis*. *M. tuberculosis* is a highly successful pathogen and has acquired various strategies to downregulate critical innate-effector immune responses of macrophages such as phagosome-lysosome fusion, antigen presentation, autophagy, and inhibition of reactive oxygen (ROI) and reactive nitrogen (RNI) species to ensure its longer survival inside the macrophages. In addition to these, the bacilli also modulate T cell immune response which can help the bacilli to survive inside the host for a long time. In this chapter, we focus to describe important macrophage innate defense mechanisms and the signaling that can influence T cell adaptive response and the strategies adopted by the bacilli to exploit these signaling cascades to favor its replication and persistence inside the macrophages for establishing a productive infection.

Keywords: *Mycobacterium tuberculosis*, monocytes/macrophages, macrophage effector response and signaling cascades, host responses and *M. tuberculosis* pathogenesis

1. Introduction

Macrophages mediate the first line of defense in the host against various intracellular pathogens [1]. They are armed with several immune-effector mechanisms to detect and combat pathogens [2, 3]. However, intracellular pathogens have developed strategies to overcome the macrophage protective immune responses and colonize inside the macrophages. Tuberculosis (TB), is an

infectious disease caused by a extremely successful pathogen, *Mycobacterium tuberculosis*, as it has evolved numerous clever strategies over time to modulate important macrophage innate-effector immune responses such as phagosome maturation, antigen presentation, inhibition of reactive oxygen (ROI) and reactive nitrogen (RNI) species, and autophagy to ensure its survival inside the macrophages [4–6]. In addition to these, the bacilli also modulate T cell immune response which can help the bacilli to survive inside the host for a long time [7]. In this chapter, we focus to describe important macrophage innate defense mechanisms and the signaling that can influence T cell adaptive response and the strategies adopted by the bacilli to exploit these signaling cascades to favor its replication and persistence inside the macrophages for establishing a productive infection.

2. Monocytes/macrophages

2.1. History and development

Eli Metchnikoff's obsession, the "phagocyte" [phagos-to eat, cyte-cell], is a constituent of Ludwig Aschoff's reticuloendothelial system (RES) [8]; the macrophage plays a key role at almost all the stages of immune response including innate and adaptive immune responses. Macrophages provide the first line of defense against the invading pathogens. In addition to protecting the body against attacks by foreign organisms, macrophages regulate important physiological functions. Their role in homeostasis has been well established. Macrophages clear almost 2×10^{11} erythrocytes per day. This enormous metabolic turnover is crucial for iron homeostasis and to prevent formation of toxic intermediates [9]. Macrophages are equipped with scavenger receptors such as phosphatidylserine receptors, thrombospondin receptor, integrins, and complement receptors to clear the cell debris and rapidly remove the apoptotic cells to help in tissue-remodeling processes. Antigens from the engulfed cells are presented along with the MHC molecules to activate the adaptive immune responses [10]. Thus, macrophage serves as a professional scavenger of the dying cells that not only clears the corpus but also regulates the immune system.

The circulating monocytes that are considered to be the developmental intermediates between bone marrow precursors and tissue macrophages emigrate from the blood vessels and differentiate into tissue macrophages [11]. Macrophages and monocytes originate from hematopoietic stem cell-derived progenitors with myeloid-restricted differentiation potential [1]. The bone marrow progenitors, monocytes, and macrophages collectively were classified into mononuclear phagocytic system, a concept pioneered by van Furth [12]. Monocytes are initially identified by the expression of CD14 molecules and lack of CD16 expression on the surface. These monocytes are termed as "classical monocytes" with $CD14^{++}CD16^{-}$ phenotype and accounting for about 90% of human blood monocytes. However, later studies have proved the expression of CD16 on the surface of some cell populations that were termed as "non-classical monocytes" with $CD14^{+}CD16^{++}$ phenotype [13]. The replenishment of tissue macrophages with the circulating monocytes is well established, but in some instances like in microglial cells of brain, local proliferation of macrophages has been established. Owing to the adaptability and plasticity of macrophages and their responsiveness to different

microenvironments in different tissues such as lung, spleen, liver, gut, and brain, a considerable heterogeneity exists among them [14]. For example, lung alveolar macrophages being constantly exposed to a variety of antigens, express a high level of pattern recognition receptors and scavenger receptors on the surface. In contrast, the macrophages of the gut exhibit high levels of phagocytic and antibacterial activities compared to other macrophages [15].

2.2. Macrophage activation

Activation is defined as the acquisition of competence to execute a complex function [16]. The factor responsible for macrophage activation was found to be the interferon-gamma (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. These macrophages have increased antigen presentation activity, thus they mount an effective immune responses in the host. The IFN-γ-mediated activation is known as "classical activation" and the macrophages are classified as "type 1 or M1 macrophages" [3, 18] (**Figure 1**). IFN-γ stimulation is not enough for the classical activation of macrophages, and may require additional stimulation by TNF-α. As TNF-α is not

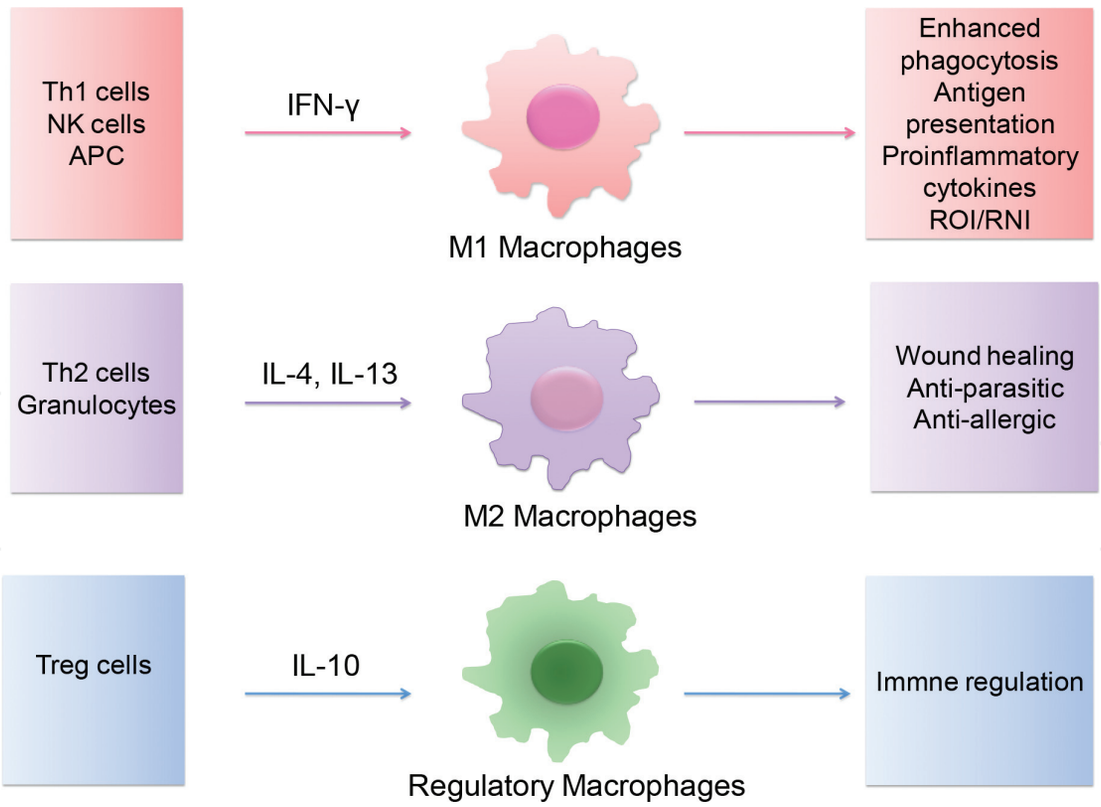

Figure 1. Schematic diagram showing different types of macrophages. Th1 cytokines primarily produced by T cells, natural killer (NK) cells and other antigen presenting cells (APC) result in the development of classically activated (M1) macrophages having microbicidal functions. While Th2 cytokines result in the development of macrophages that predominantly perform tissue repair and anti-inflammatory functions (M2), T reg cells result in the development of regulatory macrophages having immunosuppressive activities.

constitutively present in the environment, specific receptor ligands like lipopolysaccharides (LPS) and various microbial ligands may help in the induction of endogenous expression of TNF-α in macrophages [19, 20].

The T helper 2 (Th2) type of cytokines, IL-4 and IL-13, induce a response distinct from the one induced by IFN-γ with distinct set of genes being expressed and is known as "alternative activation" pathway of macrophages, and the cells are named as "alternative activated type 2 or M2 macrophages" [21]. In addition to T cells and B cells, IL-4 and IL-13 are also produced by various other cells such as mast cells, basophils, eosinophils, NK T cells, and macrophages that are involved in regulation of innate immune responses. Hence, alternative activation can be of both innate and acquired origin. Other than these two cytokines, immune complexes, IL-10, glucocorticoid, or secosteroid (vitamin D3) hormone can also contribute to the activation of M2 macrophages [22–25]. M2 macrophages are characterized by expression of scavenger, mannose [26], and galactose-type receptors, and markers such as dectin-1, arginase 1, Ym1, and FIZZ1 [27]. The M2 macrophages have anti-inflammatory properties and are associated with allergic and anti-parasite responses, and are thought to regulate humoral immunity [27, 28]. The alternatively activated macrophages are found to be recruited to wounds and other sites of tissue injury and are programmed to perform a wound healing function by expressing arginase. These macrophages are termed as "repair macrophages" or "wound healing macrophages" [19, 29, 30]. The M1 and M2 macrophages thus represent two populations of cells with different biological functions [31]. For example, the M1 macrophages, but not the M2 macrophages, produce high levels of reactive oxygen and nitrogen intermediates) and inflammatory cytokines (IL-1β, TNF-α, IL-6), and have low arginase activity, express relatively high levels of CD86, and are efficient APCs. While the M1 cells have an IL-12^{high}, IL-23^{high}, and IL-10^{low} phenotype and play an important role in inducing a dominant Th1 response and provide resistance against intracellular pathogens and tumors [17, 23, 32–34], the various forms of M2 macrophages share an IL-12^{low} and IL-23^{low} phenotype, virtually devoid of the co-stimulatory molecules and fail to mount a strong T cell proliferation [35, 36]. The innate and adaptive immune responses can also lead to the production of the "regulatory macrophages" (M reg) (**Figure 1**). The M reg cells are shown to be very stable in their phenotype and have regulatory activity. These cells are a novel type of suppressor macrophage which induces tolerance during organ transplantation. They have potent T cell suppressive function [37] and inhibit production of the IL-12 cytokine [38].

The activated macrophages exhibit a profound change in their capacities and functions. In addition to other physiological changes, there is a rapid membrane turnover found in case of macrophages even in the resting stage. This membrane flow is enormously increased in the activated state as a result of enhanced phagocytic activity and lysosomal degradation of the ingested material [39, 40]. Phagosomes undergo a series of maturation steps resulting in gradual acidification and increase in the hydrolytic activity. In addition to hydrolases, lethal superoxide generating enzyme activities become prevalent toward the end of phagosome maturation [40]. The NADPH oxidase activity of the enzyme complex leads to formation of H_2O_2 in presence of superoxide dismutase enzyme [41].

3. *M. tuberculosis*: infection and disease

Mycobacteria are rod-shaped bacteria of phylum Actinobacteria mostly found in soil or water. The *M. tuberculosis, M. bovis, M. africanum, M. microti, M. canettii*, *M. caprae*, *M. pinnipedii*, and *M. mungi* all cause TB disease and are classified as *M. tuberculosis* complex [42–44]. The *M. tuberculosis*, a facultative intracellular pathogen, was discovered by Robert Koch in 1882 as a causative agent for TB disease in human, those days commonly known as "consumption" or "white plague" [45]. Different strains of *M. tuberculosis* differ in virulence and in distribution among different human populations. *M. tuberculosis* W-Beijing strain is one of the most pathogenic strains distributed throughout the world [46, 47]. The use of chemotherapeutics against *M. tuberculosis* has resulted in the appearance of drug-resistant strains [48]. Multiple drug-resistant (MDR), extensive drug-resistant (XDR), and total drug-resistant (TDR) strains are becoming increasingly prevalent [49–52]. The *M. tuberculosis* bacteria are highly aerobic, non-sporulating, and non-motile bacteria. They have a high guanine plus cytosine (G + C) content (61–71%) in their genomic DNA, and are characterized by the presence of large hydroxylated branched-chain fatty acids called mycolic acids in their cell envelope [53, 54]. Although they have been classified with other Gram-positive actinomycetes due to their lack of an outer cell membrane, mycobacteria stain weakly with crystal violet and are resistant to decolorization with acid-alcohol solutions after staining with alkaline arylmethane dyes such as carbol fuchsin, hence called acid-fast. *M. tuberculosis* primarily infects not only lungs (pulmonary) but can also colonize other body parts (extra-pulmonary). The symptoms of TB disease include chronic cough, blood with sputum, weight loss, fever and night sweats, cavitation, and fibrosis [55, 56].

TB is a major public health burden. Despite the availability of effective short-course chemotherapy (DOTS) and *M. bovis* bacillus Calmette-Guérin (BCG) vaccine, more than 9 million new cases of *M. tuberculosis* infections are reported every year that accounts to more than 2 billion (one third of world population) being positive for the infection, resulting in 2 million deaths every year and one fifth of all adult deaths in developing countries. Developing countries are the most affected by this pandemic with 30% of the cases being reported from Africa and 55% from Asia. India and China alone are harboring 35% of the cases. With no new drug in use for a while, TB has become increasingly resistant to drugs and multi-, extensive-, and total-drug resistant TB have emerged. Interaction with other infectious diseases like HIV is making it challenging to handle the disease [57]. Other pathological conditions and risk factors associated with TB such as diabetes mellitus, renal diseases, hematological disorders and use of anti-TNF-a drugs has complicated the problem [58]. Socioeconomic factors and variable efficacy of BCG vaccination are also responsible for further aggravating the already complex problem [55, 59].

After infection with *M. tuberculosis*, an individual may not necessarily develop active disease. In case the immune system is competent enough, an individual will either clear the infection or remain latently infected with no clinical signs of disease throughout the life or can have reactivation of TB during weakening of immune system or co-infection with other pathogens like HIV [60, 61]. The molecular factors or environmental conditions that influence the progression of latent phase to active disease are not well understood. During latency, the *M. tuberculosis* bacterium remains inside the infected macrophages in granulomas. These tiny granulomas show no clinical symptoms although they may be visible in chest X-rays and give

positive tuberculin skin test [62, 63]. In 5% of the cases where immune system is weakened, the microscopic primary lesion progresses to a larger primary caseous lesion. The caseation of the primary lesion may lead to hematogenous spread of bacteria causing miliary TB or extrapulmonary TB, where the infection spreads to liver, spleen, and kidneys [63]. In case the bacteria find their way into brain, they may cause tuberculous meningitis [63]. Patients with active pulmonary TB are diagnosed most commonly by sputum smear microscopy where bacteria are directly observed under microscope in the sputum samples of patients or culturing the samples to check for colony forming units and by chest X-ray. Diagnosis of extrapulmonary TB patients is carried out by tissue biopsy, urine culture, cerebrospinal fluid test, CT scan, or MRI. Latent TB has long been diagnosed by tuberculin skin test; however, its specificity has been questioned due to false positive results as a result of infection with other non-tuberculous bacteria or prior vaccination with BCG [64, 65]. Therefore, in recent years, interferon gamma release assays (IGRAs) have been used as an alternative for the diagnosis of both latent TB infection and active TB cases [66].

4. Infection of macrophages with *M. tuberculosis* and host immune responses

4.1. The host-bacilli interplay

M. tuberculosis infection is transmitted via aerosol route. Prolonged and close contacts with infected patients result in the transmission of the pathogen in healthy persons as it is known that survival of the bacterium ranges from one to few hours in the aerosol droplets, which are about 1–2 μm or less size [67, 68]. Once the pathogen enters the respiratory track, it is finally engulfed by the alveolar macrophages of lung through surface receptors. A number of studies reveal that complement receptors and complement-mediated opsonization are majorly involved in the entry of *M. tuberculosis* inside the macrophages. One of the most important receptors for mycobacteria is complement receptor 3 (CR3), while other receptors such as CR1 and CR4, mannose receptor, surfactant protein A receptor, CD14, Fcγ receptor, scavenger receptors, etc., have also been implicated in phagocytosis and internalization of the bacteria inside the macrophages [69–71]. For alveolar dendritic cells (DC), DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) is the main receptor for *M. tuberculosis* [72]. Though complement receptors are found to be important for phagocytosis of both the avirulent and virulent strains of *M. tuberculosis*, the decline of mannose receptors was found to be associated with reduced binding of only the virulent strains [73]. The mycobacterial surface glycoprotein, mannose-capped lipoarabinomannan (Man-LAM) is recognized by the C-type lectins and the macrophage mannose receptor (MMR) [74, 75]. An important role of toll-receptors, mainly the TLR2, has been demonstrated for the attachment of mycobacteria to macrophages [76]. Interestingly, a large number of surface proteins of *M. tuberculosis* interact with the TLR2 receptors [76]. After binding, the bacteria are internalized and engulfed into phagosomes, where they can be killed by several defense mechanisms. Thoma-Uszynski et al., (2001) have shown a role of the TLR2-triggered signaling to induce cytotoxicity against *M. tuberculosis* in alveolar macrophages [77]. Soon after

the first contact of *M. tuberculosis* with alveolar macrophages, generally a robust proinflammatory immune response is induced that confers protection against the bacilli. It is observed that dampening of the proinflammatory signaling can increase *M. tuberculosis* infection burden in mice [78]. Following intracellular infection, adaptive immunity is generated against the invading pathogen via activation of $CD4^+$ T cells and $CD8^+$ T cells. Many times this adaptive immunity fails to provide a sterilizing immunity resulting in longer persistence of the infection and reactivation of *M. tuberculosis* bacteria. The bacilli are found to inhibit the class I, class II, and cross presentation of mycobacterial antigen to T cells, thus avoiding immune recognition by T cells. It has been observed that integrity of bacterial cell wall is important for *M. tuberculosis* in evading adaptive responses. At the site of infection, proinflammatory IFNs and cytokines are secreted, which help in the recruitment of $CD4^+$ T cells, $CD8^+$ T cells, natural killer T cells, and neutrophils [79]. Many a time, the induction of acquired immune response against *M. tuberculosis* is slow and the establishment of infection wins against the induction of full-fledged response [4, 80]. The cell-mediated immune response initiated at the sites of infection is found to be modulated by the bacilli. After establishing the infection, the *M. tuberculosis* antigens move with the help of alveolar dendritic cells to the draining lymph node, which leads to the stimulation of naive $CD4^+$ T cells. The active role of $CD4^+$ T cells in fighting against *M. tuberculosis* infection was proposed in 1974 in mice. In addition, the HIV mediated depletion of $CD4^+$ T cells and a defective macrophage activation in some genetic disorders has been associated with worst TB prognosis [4, 81]. Furthermore, $CD4^{-/-}$ knockout and MHC-$II^{-/-}$ knockout mice have been found to be prone to infection by *M. tuberculosis* [82]. The inhibitory effect of stimulated Th1-type $CD4^+$ T cells is by the production proinflammatory cytokines such as IFN-γ and TNF-α, which inhibit bacillary growth [81]. In comparison, when stimulated in the context of MHC class II, Th2-type $CD4^+$ T cells proliferate and produce anti-inflammatory cytokines such as IL-4, IL-5, and IL-10, which are favorable for the bacilli to establish a productive infection [79, 81]. Many studies indicate that *M. tuberculosis* bacilli suppress the pro-inflammatory cytokines such as IL-12 and IFN-γ and activate production of anti-inflammatory cytokines like IL-10 to skew the anti-mycobacterial immune response from a protective Th1 to a non-protective Th2-type. At the early stages of infection, activation of $CD8^+$ T cells by APC leads to bacterial killing. However, the role of $CD8^+$ T cells at later stages of infection has not been established [82]. The $CD8^+$ T cells can also be activated by the cross presentation of *M. tuberculosis* antigen along with MHC class I molecules. Post infection, $CD8^+$ T cells migrate to the infected tissue and produce IFN-γ. This migration of $CD8^+$ T cells is a characteristic of the granuloma establishment [83]. Higher bacterial load was found in mice deficient in some of the components of class I presentation and $CD8^+$ T cell activation pathways like β_2 microglobulin, transporter associated with antigen processing protein (TAP), and T cell co-receptor CD8α [83].

4.2. Formation of granuloma

M. tuberculosis infection of alveolar macrophages leads to the activation of alveolar dendritic cells which migrate to lymph nodes. In the lymph nodes, $CD4^+$ T cells, $CD8^+$ T cells, and $\gamma\delta$ T cells proliferate in response to activation by the alveolar dendritic cells. At the site of infection, the resulting immune activation leads to a microenvironment of cytokines and chemokines which induces the expression of integrins, selectins, and addressins on the surfaces of

lymphocytes and endothelial cells. This facilitates a mass migration of immune cells resulting in the formation of a focus of immune cells called "tubercle" or granuloma around the primary site of infection. Macrophages and giant nucleated epithelioid cells form layers around the granuloma. While the inner core of granuloma becomes necrotic, the outer surface is covered by fibrous tissue and vasculature is developed. The granuloma is formed by the immune system to contain *M. tuberculosis* to the site of infection and not allowing its spread to other normal tissues. The latent infection may persist and remain dormant for life time without proceeding to diseased condition [79, 84]. At the nascent stage of granuloma, the recruitment of uninfected and susceptible macrophages to the site of infection may lead to their infection due to apoptosis of the existing infected macrophages. This may lead to initial proliferation of the bacteria till equilibrium is attained by containment of bacteria in the granuloma and control of infection due to adaptive immune response. Some of the newly infected macrophages egress from granuloma and nucleate the formation of new granulomas at other uninfected parts of the lungs (**Figure 2**) [85]. It has been noticed that bacterial dissemination and rapid disease progression are related with larger necrotic granulomas [57], however, containment of disease is found to be associated with smaller solid granulomas [86]. Furthermore, *M. tuberculosis* contributes to the formation of granuloma by secreting pathogenic factors

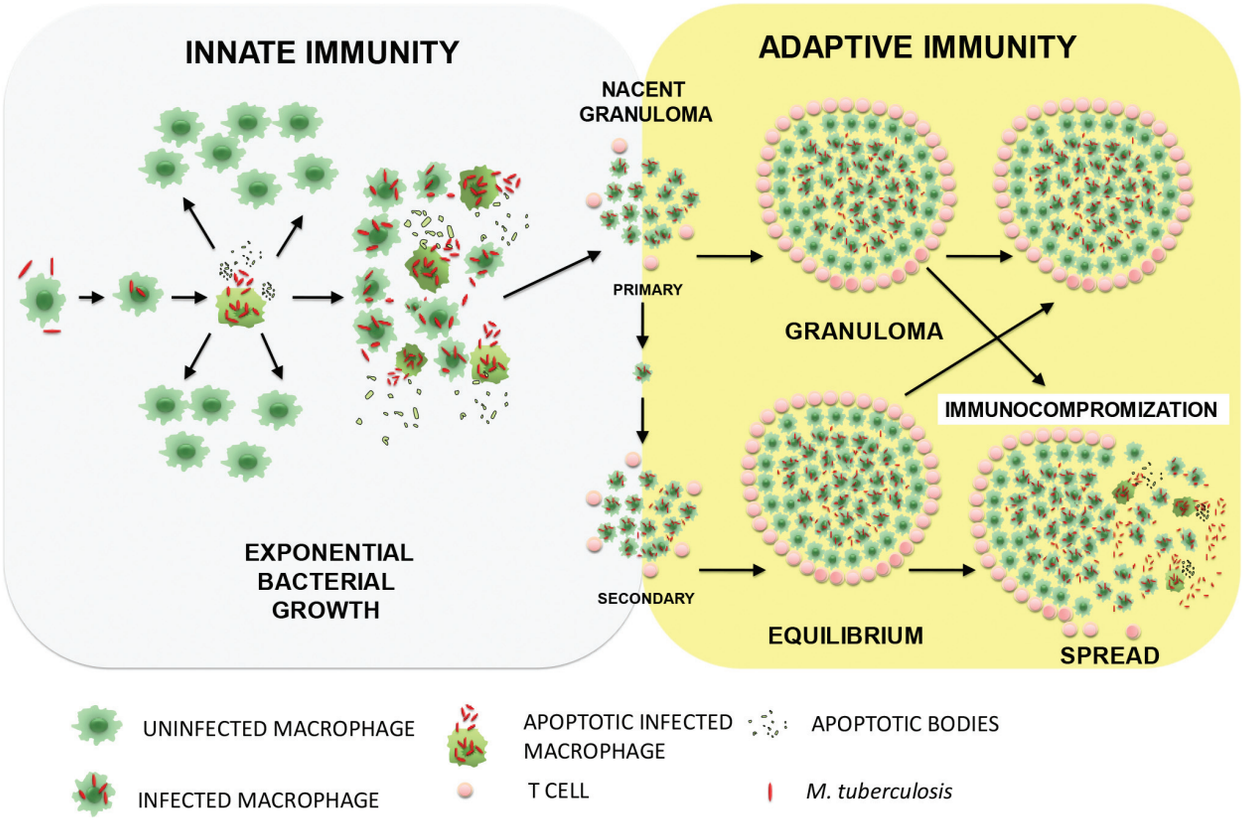

Figure 2. Development of granuloma. Immediately post infection, innate immune response is induced. The immune activation and apoptosis of infected cells lead to a microenvironment of cytokines and chemokines which attracts more uninfected macrophages. At this stage, some newly infected macrophages can migrate to uninfected areas and initiate the formation of new granulomas. This results in initial proliferation of the bacilli. Adaptive immunity although inherently slow in development, checks further spread of infection resulting in an equilibrium state. Immunocompromization of an individual at later stages can lead to caseation of the granuloma and the bacteria are rapidly disseminated.

as RD1 deficient bacteria show attenuated granuloma formation and macrophage migration to the primary site of infection in zebra fish model [85]. Studies in non-human primate, rabbit, and guinea pig models indicate hypoxic inside the environment of granuloma. In combination with nutrient deficiency, hypoxia induces a dormancy program in the bacilli which is characterized by changes in gene expression and alterations in the bacterial metabolism. Hence probably a latent infection is established which may be activated latter during immunocompromization. Thus, after entry in the human lungs, *M. tuberculosis* faces a series of host defense attacks. However, the overall outcome of infection with *M. tuberculosis* depends on the balance between (i) outgrowth and killing of *M. tuberculosis* and (ii) the extent of tissue necrosis, fibrosis, and regeneration.

4.3. Phagosome maturation response

After phagocytosis, *M. tuberculosis* bacteria reside inside the endosomes of the macrophages. Normally, endosome fuses with lysosome to degrade the pathogens, but *M. tuberculosis* bacteria are capable of inhibiting the process of phagosome maturation, as a result of which acidification of phagosome is compromised. The intracellular survival and persistence of the tubercle bacilli rests upon its ability to prevent phagosome-lysosome fusion, thus avoiding degradation, antigen processing, and cidal properties of the phagolysosome. Lipoarabinomannan capped with mannose (Man-LAM), a cell wall component of *M. tuberculosis* and SapM, a phosphatidylinositol 3-phosphate (PI3P) phosphatase secreted by the bacilli, are found to interfere with phosphoinositide metabolism of macrophages by depleting PI3P in phagosome [87, 88]. The latter is used as a docking molecule by peripheral proteins of lysosomes [7]. *M. tuberculosis* also possess protein phosphatases (Ptp A and B) that may interfere with host trafficking process possibly by modulating vacuolar sorting proteins [89].

The transport of *M. tuberculosis* containing vacuoles to lysosomes is mediated by a class of GTPases called Rab GTPases. A phagosome, when it normally matures into the phagolysosome, undergoes a transition between the stages marked by early endocytic Rabs (e.g., Rab5) and late endocytic GTPases (e.g., Rab7). Under normal circumstances, Rab proteins are actively recruited to the vesicles and assembled resulting in fusion of different vesicular compartments. In case of *M. tuberculosis* infection, it was observed that recruitment of Rab7 to the vacuole containing *M. tuberculosis* was inhibited while Rab5 was recruited normally indicating a maturation block between a Rab5 and Rab7 stage in infected macrophages that causes inhibition of phagosome-lysosome fusion. The exchange of Rab5 protein with Rab7 was later named as Rab conversion [90, 91]. Rab5 interacts with early endosomal autoantigen 1 (EEA1), which in turn interacts with phosphatidylinositol 3-phosphate (PI3P). Thus, inhibition of PI3P production by *M. tuberculosis* appears to be also critical for the inhibition of maturation of endolysosomes [91]. A series of Rab proteins are bind to phagosomes to ensure its acidification and recruitment of cathepsin D, while the process is severely inhibited in case of *M. tuberculosis* infection. A soluble eukaryotic-like protein kinase PknG of pathogenic *M. tuberculosis* is shown to be crucial for the prevention of phagosome-lysosome fusion [92]. A recent study has also shown that the *M. tuberculosis* secretory proteins, ESAT-6 and CFP-10 encoded by RD1 region play crucial roles in preventing phagolysosomal fusion [93]. Genetic screens using comprehensive mutant libraries of *M.*

tuberculosis and BCG suggest that additional mycobacterial products directly or indirectly can influence trafficking processes which probably are important for intracellular survival [94, 95]. Coronin1, exclusively recruited to *M. tuberculosis* containing phagosome, is an important host factor that specifically prevents the lysosomal delivery and death of mycobacteria inside macrophage [96]. Coronin1 prevents phagosome-lysosome fusion by regulating Ca^{2+}-dependent signaling processes when macrophages are infected with *M. tuberculosis* [97]. The *Nramp1* (natural-resistance-associated macrophage protein 1) gene involved in macrophage activation and mycobacterial killing [98] becomes part of the phagosome following phagocytosis and displays reduced phagosomal maturation and acidification [99].

A family of IFN-γ-inducible GTPases, also called immunity-related GTPases (IRGs), was found to play a critical role in host innate immunity against intracellular pathogens [100–102]. A member of IRG family, 47 kDa Irgm1 (also called LRG-47) protein (which is strongly inducible by IFN-γ and *M. tuberculosis* infection in mice) is an important anti-*M. tuberculosis* protein [100]. The anti-mycobacterial role of Irgm1 is due to its interaction with phosphatidylinositol-3,4-bisphosphate (PtdIns (3,4)P2) and PtdIns(3,4,5)P3 present on the phagosomal surface harboring *M. tuberculosis*. Irgm1 also increases phosphorylation of lipids by augmenting the PI3K activity. Normally, Irgm1 interacts with a membrane trafficking protein Snapin, which interacts with SNARE to ensure the fusion of phagosomes with lysosomes resulting in the elimination of bacilli. *M. tuberculosis* has developed a way to counter Irgm1 effect by exploiting a natural pathway of Irgm1 inhibition by Rab14 protein that is critical for phagosome-lysosome fusion. It has been shown that the mycobacterial phagosomes recruit and retain Rab14 [103]. Rab 14 is inhibited by unphosphorylated form of AS160 protein. Manipulation of Rab14-pathway by *M. tuberculosis* is mediated by the activation of Akt1 that phosphorylates AS160. Phosphorylated AS160 is unable to inhibit Rab14 hence leaving it free to inhibit Irgm1 recruitment to phagolysosomal compartment resulting in the failure of phagolysosome maturation (**Figure 3**) [103–105]. The fusion of phagosomes with lysosomes has been shown to be dependent on Ca^{2+} ions. Ca^{2+} ions and Ca-binding protein calmodulin are critical for the delivery of lysosomal components to phagosomes using PI3P-dependent pathways. Blockade of Ca^{2+}/calmodulin pathway by *M. tuberculosis* is also found to be one of the ways to block phagosomal maturation [70].

4.4. Autophagy

Autophagy (also called xenophagy) is an evolutionary conserved basic homeostatic mechanism of a cell to digest intracellular organelles and large protein aggregates that are difficult to digest by normal proteasomal pathway. The engagement of TLRs with mycobacterial ligands induces autophagy using both MyD88-dependent and TRIF-dependent pathways [106, 107]. This suggests that autophagy is an effector of innate immune response. The IFN-γ induces IRG proteins that are mainly involved in induction of autophagy and elimination of *M. tuberculosis* bacilli [108] and polymorphism at IRG locus is shown to be associated with resistance to *M. tuberculosis* [109]. Many autophagy-associated proteins are shown to be involved in phagosome-lysosome fusion process [110]. Expression of a number of host genes involved in autophagy is shown to be modulated at the onset of *M. tuberculosis* infection. The pathogen has therefore acquired

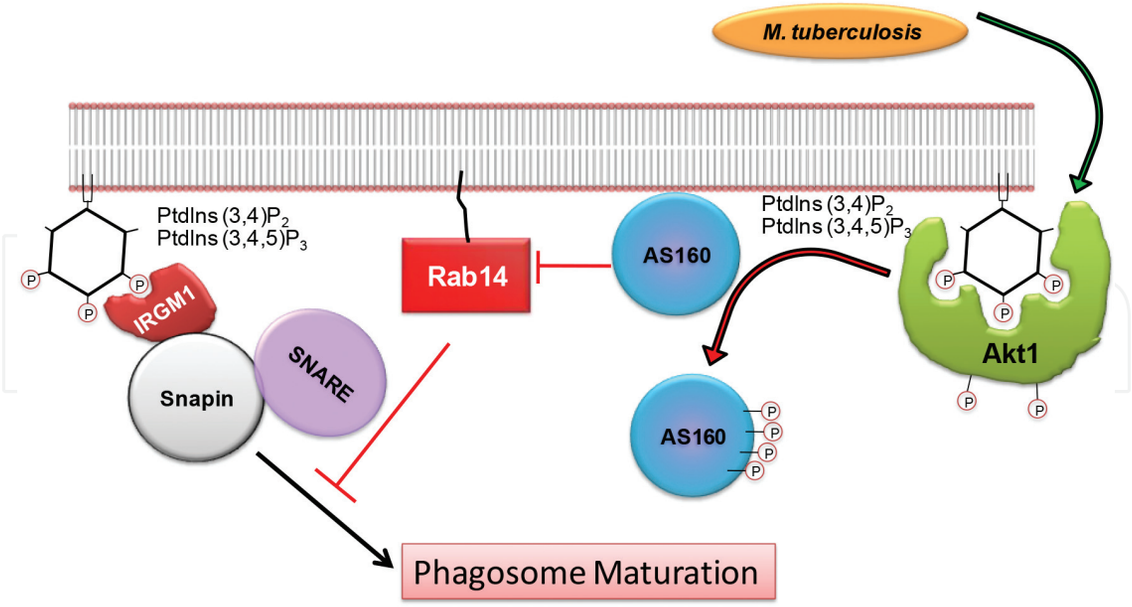

Figure 3. Critical balance between Rab14 and Irgm1 is important to counter *M. tuberculosis* infection. Pathogen results in the production of phosphatidylinositol-3,4-bisphosphate (PtdIns(3,4)P_2) and PtdIns(3,4,5)P_3 which act as a docking site for Akt1. The phosphorylated Akt1 phosphorylates AS160 which is inactive and is unable to deactivate Rab14. The active Rab14 blocks the phagosome maturation. PdlIns also recruit Irgm1 which along with Snapin protein may help in maturation of phagosomes and elimination of intracellular bacteria.

mechanisms to subvert the autophagy to induce a favorable condition for its persistence inside the host [111]. *M. tuberculosis* can also inhibit autophagy by skewing the immune response toward the Th2-type. While proinflammatory cytokines such as TNF-α and IFN-γ promote autophagy, Th2 cytokines such as IL-4 and IL-13 inhibit autophagy in human and murine macrophages, and this is dependent on the Akt-STAT6-signaling pathway [112]. Autophagy is known to help in antigen presentation via both MHC class I and class II pathways and, by inhibiting autophagy, *M. tuberculosis* also achieves the goal of suppression of MHC class I- and class II-mediated antigen presentation [112]. Vaccines that are designed to elicit a strong autophagic response can prove to be effective against latent TB infection and also drugs designed to modulate autophagy can be effective against the drug-resistant strain of *M. tuberculosis* [113].

4.5. Apoptosis

Macrophages use apoptosis as an effector mechanism to eliminate *M. tuberculosis* and to constrain the spreading of infection [114]. The apoptotic vesicles are readily engulfed by the neighboring dendritic cells. The dendritic cells in turn activate $CD8^+$ T cells by cross-presenting the processed antigens in the context of MHC class I. This results in an effective immune response against the bacilli. Necrosis, however, is favorable for the dissemination of bacteria and spread of infection. Hence, *M. tuberculosis* has developed mechanisms to suppress apoptosis and favor necrosis during active infection state [6, 63, 115, 116]. Inhibition of host cell apoptosis by *M. tuberculosis* has been implicated as a potential virulence mechanism. In fact, an inverse correlation between virulence of mycobacterial strains and their capacity to induce apoptosis has been reported [114]. Infection with virulent *M. tuberculosis* strain H37Rv is found to be associated with

reduced expression of several pro-apoptotic genes and increased expression of the anti-apoptotic gene as compared to uninfected macrophages [117]. Studies using H37Rv and H37Ra strains of *M. tuberculosis* to infect alveolar macrophages have indicated that although both could induce apoptosis, the virulent H37Rv induce less apoptosis than the avirulent H37Ra by upregulating expression of the anti-apoptotic gene Bcl-2 in macrophages [118]. TNF-α is shown to play important role in host cell apoptosis infected with avirulent H37Ra strain [114, 119]. Also, the role of Bfl-1/A1 is realized in inhibition of apoptosis by *M. tuberculosis*, as decreased intracellular H37Rv growth was observed in Bfl-1/A1 siRNA-treated macrophages [120]. These cells show enhancement of phagosome-lysosome fusion and Caspase-3 activity indicating that expression of Bfl-1/A1 in H37Rv-infected macrophages provides the bacteria a survival strategy to overcome host defense. Nuclear factor-kappaB (NF-κB) activation is shown to play an essential role in the inhibition of host cell apoptosis by *M. tuberculosis* H37Rv [119]. Infection of macrophages with virulent *M. tuberculosis* confers resistance to apoptotic stimuli like Fas ligand (FasL) or TNF-α by reducing the cell surface expression of Fas receptors or secreting soluble TNF-α-receptor, respectively [121, 122]. Interestingly, Divangahi et al. have shown that virulent *M. tuberculosis* strains inhibit the production of prostaglandin E_2 by interfering with lipoxigenase pathway leading to inhibition of apoptosis and promotion of necrosis [115]. The other mechanism by which *M. tuberculosis* could possibly inhibit apoptotic process is via the *nuoG* gene, which can neutralize the NOX-2-mediated increase in ROS and TNF-α production in phagosomes containing *M. tuberculosis* thereby inhibiting apoptosis [123, 124]. Also, secA2 and pknE play roles in resistance against apoptosis as mutants of these genes induce more apoptosis in macrophages as compared to the wild-type *M. tuberculosis* strains [124–126].

4.6. Reactive oxygen and nitrogen intermediates (ROIs and RNIs)

ROIs and RNIs are produced in cells like macrophages in response to proinflammatory cytokines [127, 128]. ROIs and RNIs being small molecules defuse easily through the membranes and have a detrimental effect on the pathogens been engulfed in phagocytic vacuoles [129]. Studies have indicated that these molecules are important in providing innate host defense against *M. tuberculosis* [4, 129]. ROIs are produced by phagocytes, particularly the polymorphonuclear leukocytes and the activated macrophages, while RNIs are produced mainly by the activated macrophages. ROIs ($O^{2}\bullet^{-}$, H_2O_2, $OH^{\bullet}$,) are generated through the action of phagocyte oxidase (also called NADPH-oxidase). They further react with halides and amines to generate more reactive species (**Figure 4**). The important role of NADPH-oxidase in defense against *M. tuberculosis* and other pathogens is proven by the susceptibility of mice deficient in NADPH oxidase [129–131]. Children carrying mutations in gp91phox subunit of phagocyte oxidase enzyme are found to be more susceptible to TB infection than normal population [132]. In macrophages, IFN-γ in synergy with the TNF-α induces production of nitric oxide (•NO) with the help of inducible nitric oxide synthase (iNOS) (**Figure 4**). Not only does NO exert its effects on its own but also reacts with ROIs and the reaction products like peroxynitrite $^{(OONO-)}$ and other reaction intermediates can be even more toxic [127, 133]. After production, NO is not restricted to the area but readily defuses to other areas by using S-nitrosothiols, S-nitrosylated proteins, and nitrosyl-metal complexes as transport vehicles. NO produces heterogenous and diverse effects owing to its non-specific reactivity with a number of regulatory proteins [129,

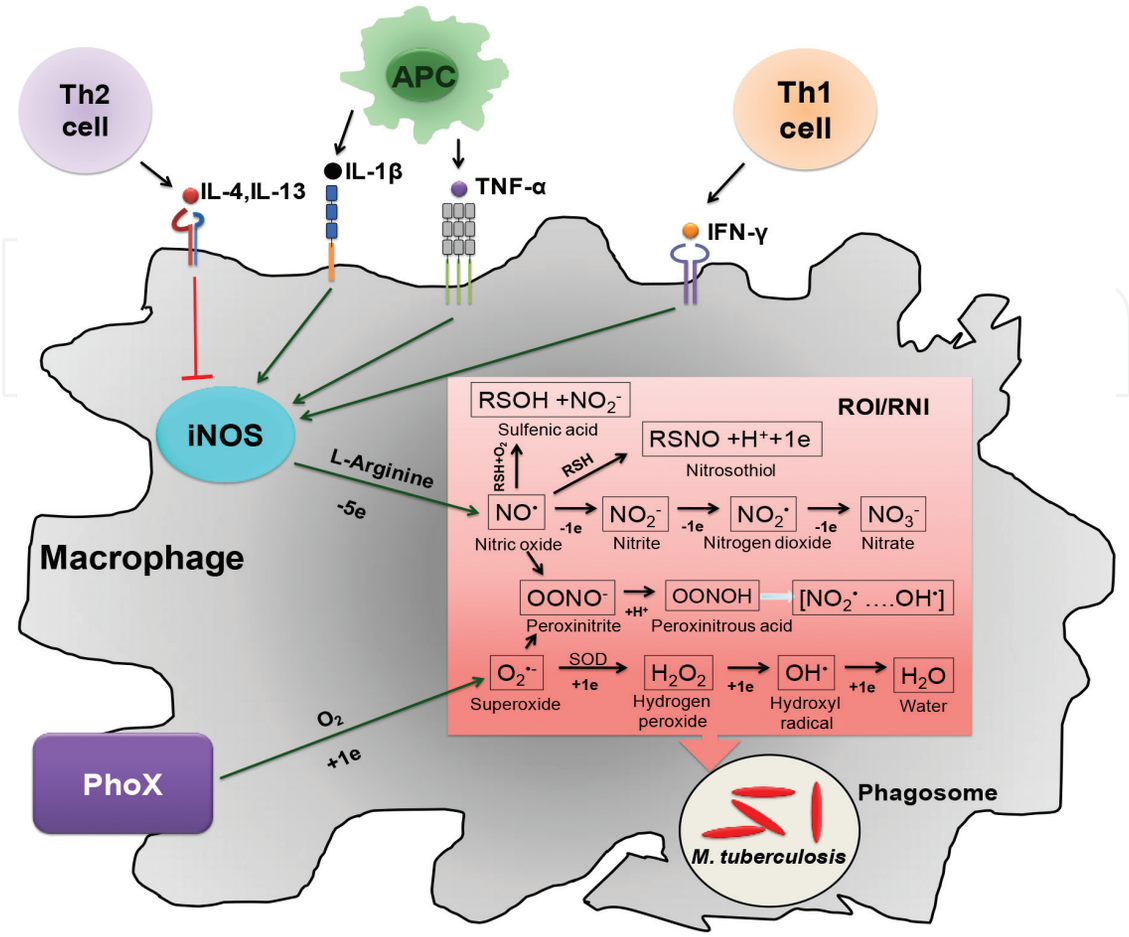

Figure 4. Regulation and function of iNOS during *M. tuberculosis* infection: Th1 cytokines like IFN-γ, TNF-α and IL-1β produced by Th1 T cells or antigen-presenting cells (APC) induce nitric oxide synthase (iNOS) enzyme. IL-4 and IL-13 secreted by Th2 cells negatively regulate iNOS expression. The products of phagocyte oxidase (Phox) and iNOS react and produce even more toxic intermediates like peroxynitrite against *M. tuberculosis*. NO also gives rise to sulfenic acid and nitrosothiols on reaction with sulfhydryl groups.

134]. In mouse macrophages, activation of TLR2 by various bacterial ligands induces iNOS promoter activity, production of NO, and killing of intracellular *M. tuberculosis*. However, protective role of NO in human TB is controversial. An essential role of NO/iNOS in anti-mycobacterial immunity was established by infection studies using iNOS knock-out mice [135, 136]. Mouse homozygous for knock-out allele for iNOS gene $^{(iNOS-/-)}$ when challenged with *M. tuberculosis* showed a very high susceptibility to infection [135]. Although most of the studies were conducted in mouse model, recent studies reveal that NO/iNOS is also important in killing the bacteria in human TB [134, 137, 138]. The iNOS activation through TLR2 pathway was found to enhance the killing of intracellular *M. tuberculosis* [77, 139]. All these studies indicate that probably iNOS also plays crucial role in anti-mycobacterial immunity in human. A comparative study of mice deficient either in phagocyte oxidase or in iNOS showed higher anti-mycobacterial activities by RNIs as compared to ROIs [131]. ROIs and RNIs damage the DNA and react with variety of other chemical moieties such as Fe-S clusters, tyrosyl radicals, hemes, sulfhydryls, thioethers, and alkenes to inactivate important components of the invading pathogens to compromise its survival inside the host.

Like other effector mechanisms, *M. tuberculosis* has evolved effective strategies to counter RNIs- and ROIs-mediated toxicity. The inhibition of the recruitment of iNOS to the infected phagosome would be one of the ideal strategies used by the bacilli [140]. EBP50, a scaffold protein in activated macrophages, targets iNOS to phagosomes [141]. *M. tuberculosis* downregulate EBP50 in the infected macrophages thereby reducing the transport of iNOS to the infected phagosome [141]. The bacteria also induce various genes to protect them from intracellular oxidative stress [109]. For example, mycobacterial catalase peroxidase (*katG*) and alkyl hydroperoxide reductase (*ahpC*) have role in antioxidant defenses, defend bacteria from intracellular oxidative stress [142, 143], and play roles in the virulence of *M. tuberculosis* [144, 145]. It has been shown that virulence of different clinical as well as recombinant strains of *M. tuberculosis* is correlated with varying expression levels of the peroxidase enzymes [143, 144]. NO induces a low oxygen state (hypoxia) in mycobacteria resulting in the overexpression of *katG* and *ahpC* [129, 146]. These genes were also reported to be overexpressed *in vivo* during infection with mycobacteria and studies indicate that these responses are abrogated in the phagosomal environment of $NOS2^{-/-}$ mice [147–149]. Oxidative stress has been established to induce a two-component system called DosR/DosS, consisting of a sensor histidine kinase DosS/DosT and a response regulator DosR [150–152]. The system helps the bacilli to cope oxidative and other stress (S-nitrosoglutathione, ethanol, etc.) conditions by initiating a complex response.

The DosR is characterized as a transcription factor responsible for transcription of the genes in response to oxidative stress. Genes expressed during the stress response, like α-crystallin was shown to carry sequences in the regulatory regions for DosR binding. In response to upstream activation signals, DosR is phosphorylated at Asp54 that results in its binding to DNA via its C-terminal domain and subsequent activation of DosR responsive genes. The sensor kinases, DosS and DosT, respond to redox environment and hypoxia, respectively. Both the proteins contain two GAF domains at N-terminal harboring a heme prosthetic group which interacts with O_2, NO or CO to induce autophosphorylation of the kinases and induce transcription of genes by activating DosR (**Figure 5**). CO is produced by heme oxygenase (HO) enzyme of macrophages. The enzyme is significantly upregulated during *M. tuberculosis* infection, oxidative stress, hypoxia, and stimulation with various cytokines. The enzyme catalyzes degradation of heme to biliverdin, free iron, and produces sufficient physiological amount of CO to induce dormancy program via binding primarily to DosS while DosT plays a little role in CO sensing. Although CO is sufficient to induce dormancy regulon, it was established that iNOS is required for optimal induction of response. Under aerobic condition, both the sensors are blocked and maintain a basal level of DosR responsive genes, while in anaerobic condition, they respond to the ligands and upregulate the DosR responsive genes to help the pathogen to survive in the stress caused by ROIs and RNIs (**Figure 5**) [153–155].

4.7. Vitamin D3

Vitamin D has long been known to be one of the nutritional therapeutic agents with a capacity to modulate the immune system in the case of an *M. tuberculosis* infection [156]. There has been a greater incidence of TB during the spring/summer months in temperate countries like UK [157], which can be correlated to a decrease in Vitamin D production on account of lower sun

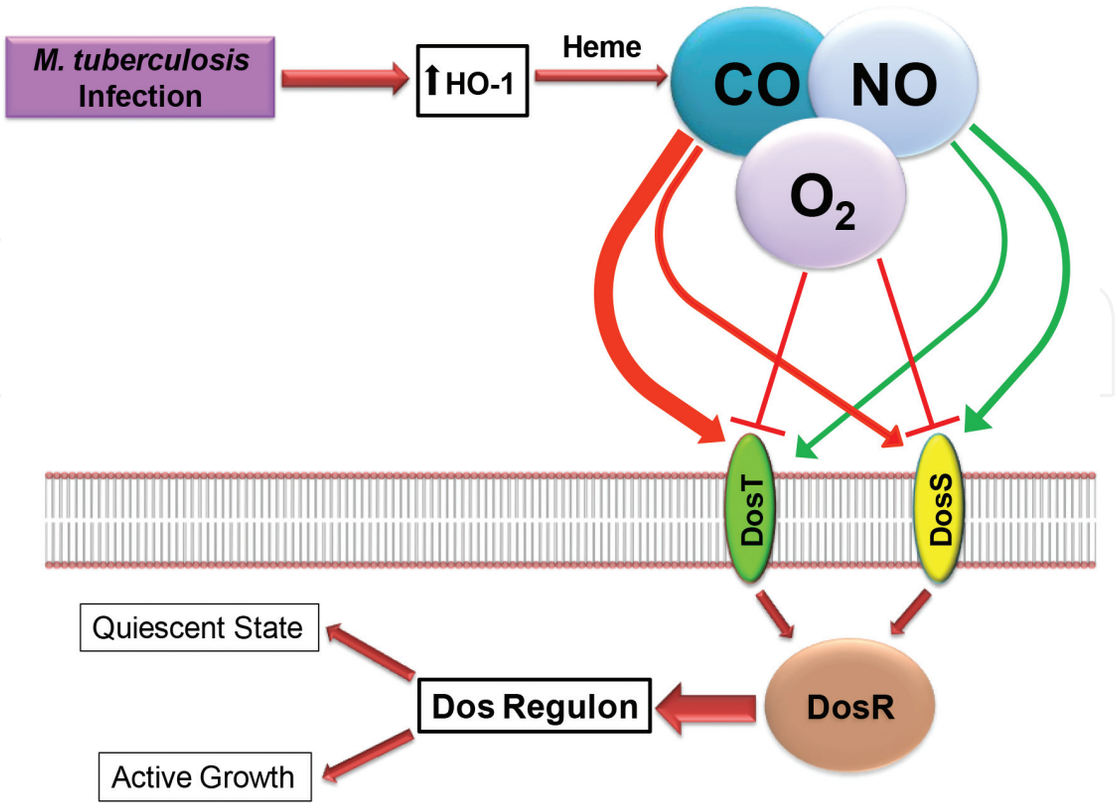

Figure 5. Role of NO, O_2 and CO in regulation of DosR/S/T regulon: NO, O_2, and CO are recognized in conjunction by DosS and DosT in a concentration gradient-dependent manner. CO mostly signals via DosS than DosT, while O_2 inhibits both DosS and DosT. *M. tuberculosis* infection upregulates the production of heme oxygenase-1(HO-1) which metabolizes heme to produce CO.

exposure in the winter months. Dietary composition also seemed to have a bearing with a predisposition toward TB, since a study conducted on an immigrant Gujarati Indian population in UK showed a marked increase in TB cases. It must be mentioned here that this population is a vegetarian one and hence dietary intake of Vitamin D is markedly less [158]. The genetic reason for this preponderance has been worked out to be an association with the VDR polymorphisms associated with the Gujarati Asian population concerned and Vitamin D deficiency [159]. Various studies indicate that Vitamin D can help the body fight against *M. tuberculosis* infections [160]. In a recent study, it has been found that patients administered with Vitamin D in combination with antibiotics recovered from TB more quickly than the patients administered with only antibiotics [161].

The mechanisms for a correlation of Vitamin D and TB are unknown, but it could be the antimicrobial peptides in association of Vitamin D generated by the pattern receptor stimulation in lieu of an infection with *M. tuberculosis* [162]. This stimulation then induces expression of cathelicidin [162] and β-defensin 2 (DEFB4) [163]. Cathelicidin induces phagolysosomal fusion which is necessary for killing of *M. tuberculosis*. In addition, 1,25(OH)2D, which is another downstream biochemical produced in Vitamin D biosynthetic pathway, induces autophagy [160, 164] and downregulates metalloproteinases (MMPs) [165]. All these processes help in the formation of phagolysosomes and the subsequent killing of *M. tuberculosis*. Vitamin D also seems to

affect the adaptive immune system albeit in a regulatory manner. This is ensued as the downstream product 1,25(OH)2D upregulates regulatory responses with a skew toward the Th2-phenotype pattern. This can be ascertained by the anti-proliferative effects of 1,25(OH)2D on $CD4^+$ T cells [166]. It also seems to inhibit Th1 cytokine production [167, 168], while promoting T regulatory function at the same time [169]. It also seemingly upregulates Th2 cytokine production [170]. Studies using 1α,25-dihydroxyvitamin D3 indicate that vitamin D3 increases generation of oxygen intermediates via NADPH-dependent phagocyte oxidase involving the phosphatidylinositol 3-kinase [171]. In addition to this, Vitamin D3 can downregulate transcription of tryptophan-aspartate containing coat protein [172], which is important for the entry and survival of *M. tuberculosis* in human macrophages [173]. Thus, the role of proper nutrition in the control of *M. tuberculosis* is evident. Vitamin D which has otherwise been associated as the "anti-cold" Vitamin seems to have critical roles in the control of *M. tuberculosis* infection at the innate and the adaptive levels of the immune system of the host.

5. Conclusion

TB remains a global pandemic and despite thorough and constructive measures to eradicate TB, it has flourished and continues killing people. It has evolved into various MDR, XDR, and TDR strains, notwithstanding the best healthcare available, which are resistant to the obsolete group of drugs. This necessitates the need to find new drug targets as well as drugs to counter the menace of TB. Therefore, it becomes imperative to understand the biology of *M. tuberculosis* and the host response modulation mechanisms it has evolved. The same can be achieved by dissecting the biochemical processes throughout the life cycle of the pathogen and by understanding the host-pathogen interaction mechanisms in TB, both of which are prerequisites for the development of effective anti-TB vaccines/drugs. More importantly, the processes associated with the so-called "dormant stage" needs to be identified since this stays the biggest challenge in identifying asymptomatic TB patients, and understanding this Trojan can therefore escalate our steps to eradicate this menace by eons. In this chapter, we have attempted to address various host processes that are subverted by *M. tuberculosis* to survive inside its host as well as launch an assault when the host immune defenses are weakened. Right from when *M. tuberculosis* enters inside the body, it counters the host innate defenses by downregulating the oxidative burst inside the macrophages. It also subverts other macrophage effector functions like inhibition of phagolysosomal fusion which is critical for the action of lytic enzymes and therefore forms an important block where it escapes the host defenses. Most importantly, it modulates the host signaling targeting the PAMP receptors, more importantly the TLR2 or to downregulate the proinflammatory signaling cascade known to be detrimental for its intracellular survival. The adaptive responses are similarly affected as one of the major mechanisms, viz. antigen presentation seems to be downregulated. Both MHC class I and class II and even cross presentation are affected. This results in a very less or delayed outcome of protective adaptive immune response which again helps the pathogen to survive very efficiently inside the macrophages. It also affects critical host processes like apoptosis which could clear the pathogen in a controlled manner

without allowing it to spread. Downregulating the proinflammatory signaling cascade also involves skewing the macrophage responses from a protective Th1- to a non-protective Th2-type. This involves most importantly a shift in the cytokine secretion pattern which subverts the host favorable macrophage signaling and effector functions in favor of the host. We have described important pathophysiological events during *M. tuberculosis* infection and the virulence processes by which the bacilli can escape the macrophage surveillance mechanisms, and use it as their safe refuge which may help in designing suitable interventions against *M. tuberculosis* infection.

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References

[1] Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science. 2010;**327**(5966):656-661

[2] Mackaness GB. The immunological basis of acquired cellular resistance. The Journal of Experimental Medicine. 1964;**120**:105-120

[3] Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Frontiers in Bioscience: a Journal and Virtual Library. 2008;**13**:453

[4] Flynn JL, Chan J. Immunology of tuberculosis. Annual Review of Immunology. 2001;**19**: 93-129

[5] Flynn JL, Chan J. Immune evasion by *Mycobacterium tuberculosis*: Living with the enemy. Current Opinion in Immunology. 2003;**15**(4):450-455

[6] Behar SM, Divangahi M, Remold HG. Evasion of innate immunity by *Mycobacterium tuberculosis*: Is death an exit strategy? Nature Reviews Microbiology. 2010;**8**(9):668-674

[7] Jozefowski S, Sobota A, Kwiatkowska K. How *Mycobacterium tuberculosis* subverts host immune responses. BioEssays. 2008;**30**(10):943-954

[8] Tauber AI. Metchnikoff and the phagocytosis theory. Nature Reviews Molecular Cell Biology. 2003;**4**(11):897-901

[9] De Domenico I, McVey Ward D, Kaplan J. Regulation of iron acquisition and storage: Consequences for iron-linked disorders. Nature Reviews Molecular Cell Biology. 2008; **9**(1):72-81

[10] Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature. 2000; **407**(6805):784-788

[11] Ebert R, Florey H. The extravascular development of the monocyte observed in vivo. British Journal of Experimental Pathology. 1939;**20**(4):342

[12] van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. The Journal of Experimental Medicine. 1968;**128**(3):415-435

[13] Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood. 2011;**118**(5):e16-e31

[14] Geissmann F, Gordon S, Hume DA, Mowat AM, Randolph GJ. Unravelling mononuclear phagocyte heterogeneity. Nature Reviews Immunology. 2010;**10**(6):453-460

[15] Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, et al. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. The Journal of Clinical Investigation. 2005; **115**(1):66-75

[16] Adams DO. Molecular interactions in macrophage activation. Immunology Today. 1989; **10**(2):33-35

[17] Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. The Journal of Experimental Medicine. 1983;**158**(3):670-689

[18] O'Shea JJ, Murray PJ. Cytokine signaling modules in inflammatory responses. Immunity. 2008;**28**(4):477-487

[19] Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nature Reviews Immunology. 2008;**8**(12):958-969

[20] Schroder K, Sweet MJ, Hume DA. Signal integration between IFNγ and TLR signalling pathways in macrophages. Immunobiology. 2006;**211**(6–8):511-524

[21] Gordon S, Martinez FO. Alternative activation of macrophages: Mechanism and functions. Immunity. 2010;**32**(5):593-604

[22] Gordon S. Alternative activation of macrophages. Nature Reviews Immunology. 2003; **3**(1):23-35

[23] Goerdt S, Politz O, Schledzewski K, Birk R, Gratchev A, Guillot P, et al. Alternative versus classical activation of macrophages. Pathobiology. 1999;**67**(5–6):222-226

[24] Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends in Immunology. 2002;**23**(11):549-555

[25] Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nature Immunology. 2010;**11**(10):936-944

[26] Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: A marker of alternative immunologic macrophage activation. The Journal of Experimental Medicine. 1992;**176**(1):287-292

[27] Raes G, De Baetselier P, Noel W, Beschin A, Brombacher F, Hassanzadeh Gh G. Differential expression of FIZZ1 and Ym1 in alternatively versus classically activated macrophages. Journal of Leukocyte Biology. 2002;**71**(4):597-602

[28] Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D, et al. Chitin induces accumulation in tissue of innate immune cells associated with allergy. Nature. 2007;**447**(7140):92-96

[29] Raes G, Beschin A, Ghassabeh GH, De Baetselier P. Alternatively activated macrophages in protozoan infections. Current Opinion in Immunology. 2007;**19**(4):454-459

[30] Daley JM, Brancato SK, Thomay AA, Reichner JS, Albina JE. The phenotype of murine wound macrophages. Journal of Leukocyte Biology. 2010;**87**(1):59-67

[31] Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. Journal of Leukocyte Biology. 2006;**80**(6):1298-1307

[32] Dalton DK, Pitts-Meek S, Keshav S, Figari IS, Bradley A, Stewart TA. Multiple defects of immune cell function in mice with disrupted interferon-γ genes. Science. 1993;**259**(5102):1739-1742

[33] Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. Journal of Immunology. 2000;**164**(12):6166-6173

[34] Mosser DM. The many faces of macrophage activation. Journal of Leukocyte Biology. 2003;**73**(2):209-212

[35] Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: Competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. Journal of Immunology. 1998;**160**(11):5347-5354

[36] Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: New molecules and patterns of gene expression. Journal of Immunology. 2006;**177**(10):7303-7311

[37] Hutchinson JA, Riquelme P, Geissler EK, Fandrich F. Human regulatory macrophages. Methods in Molecular Biology. 2011;**677**:181-192

[38] Gerber JS, Mosser DM. Reversing lipopolysaccharide toxicity by ligating the macrophage Fcγ receptors. Journal of Immunology. 2001;**166**(11):6861-6868

[39] Ogmundsdottir HM, Weir DM. Stimulation of phosphatidylinositol turnover in the macrophage plasma membrane: A possible mechanism for signal transmission. Immunology. 1979;**37**(3):689-696

[40] Adams DO, Hamilton TA. The cell biology of macrophage activation. Annual Review of Immunology. 1984;**2**(1):283-318

[41] Russell DG, Vanderven BC, Glennie S, Mwandumba H, Heyderman RS. The macrophage marches on its phagosome: Dynamic assays of phagosome function. Nature Reviews Immunology. 2009;**9**(8):594-600

[42] Huard RC, Fabre M, De Haas P, Lazzarini LCO, van Soolingen D, Cousins D, et al. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. Journal of Bacteriology. 2006;**188**(12):4271-4287

[43] Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proceedings of the National Academy of Sciences. 2002;**99**(6):3684-3689

[44] Alexander KA, Laver PN, Michel AL, Williams M, van Helden PD, Warren RM, et al. Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. Emerging Infectious Diseases. 2010;**16**(8):1296

[45] Murray JF. *Mycobacterium tuberculosis* and the cause of consumption from discovery to fact. American Journal of Respiratory and Critical Care Medicine. 2004;**169**(10):1086-1088

[46] Bifani PJ, Mathema B, Kurepina NE, Kreiswirth BN. Global dissemination of the *Mycobacterium tuberculosis* W-Beijing family strains. Trends in Microbiology. 2002; **10**(1):45-52

[47] Glynn JR, Whiteley J, Bifani PJ, Kremer K, Van Soolingen D. Worldwide occurrence of Beijing/W strains of *Mycobacterium tuberculosis*: A systematic review. Emerging Infectious Diseases. 2002;**8**(8):843

[48] Johnson R, Streicher EM, Louw GE, Warren RM, van Helden PD, Victor TC. Drug resistance in *Mycobacterium tuberculosis*. Current Issues in Molecular Biology. 2006;**8**(2): 97-111

[49] Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. Totally drug-resistant tuberculosis in India. Clinical Infectious Diseases. 2012;**54**(4):579-581

[50] Gillespie SH. Evolution of drug resistance in *Mycobacterium tuberculosis*: Clinical and molecular perspective. Antimicrobial Agents and Chemotherapy. 2002;**46**(2):267-274

[51] Zumla A, Abubakar I, Raviglione M, Hoelscher M, Ditiu L, McHugh TD, et al. Drug-resistant tuberculosis-current dilemmas, unanswered questions, challenges, and priority needs. Journal of Infectious Diseases. 2012;**205**(Suppl 2):S228-S240

[52] Raviglione M. XDR-TB: Entering the post-antibiotic era? The International Journal of Tuberculosis and Lung Disease: the official journal of the International Union against Tuberculosis and Lung Disease. 2006;**10**(11):1185

[53] Sutcliffe IC. Cell envelope composition and organisation in the genus Rhodococcus. Antonie Van Leeuwenhoek. 1998;**74**(1–3):49-58

[54] Minnikin DE, Kremer L, Dover LG, Besra GS. The methyl-branched fortifications of *Mycobacterium tuberculosis*. Chemistry & Biology. 2002;**9**(5):545-553

[55] Smith I. *Mycobacterium tuberculosis* pathogenesis and molecular determinants of virulence. Clinical Microbiology Reviews. 2003;**16**(3):463-496

[56] Rook GA, Dheda K, Zumla A. Immune responses to tuberculosis in developing countries: Implications for new vaccines. Nature Reviews Immunology. 2005;**5**(8):661-667

[57] Bloom BR, editor. Tuberculosis: Pathogenesis, Protection and Control. N. W: American Society for Microbiology; 1994

[58] Tatar D, Senol G, Alptekin S, Karakurum C, Aydin M, Coskunol I. Tuberculosis in diabetics: Features in an endemic area. Japanese Journal of Infectious Diseases. 2009;**62**(6):423-427

[59] Fatkenheuer G, Taelman H, Lepage P, Schwenk A, Wenzel R. The return of tuberculosis. Diagnostic Microbiology and Infectious Disease. 1999;**34**(2):139-146

[60] Lawn SD, Wood R, Wilkinson RJ. Changing concepts of "latent tuberculosis infection" in patients living with HIV infection. Clinical and Developmental Immunology. 2011;**2011**

[61] Narain JP, Raviglione MC, Kochi A. HIV-associated tuberculosis in developing countries: Epidemiology and strategies for prevention. Tubercle and Lung Disease. 1992; **73**(6):311-321

[62] Kaufmann SH. Recent findings in immunology give tuberculosis vaccines a new boost. Trends in Immunology. 2005;**26**(12):660-667

[63] Dannenberg AM. Pathogenesis of Human Pulmonary Tuberculosis: Insights from the Rabbit Model. N. W: American Society for Microbiology; 2006

[64] Nienhaus A, Schablon A, Diel R. Interferon-γ release assay for the diagnosis of latent TB infection–analysis of discordant results, when compared to the tuberculin skin test. PLoS One. 2008;**3**(7):e2665

[65] Miranda C, Tomford JW, Gordon SM. Interferon-γ-release assays: Better than tuberculin skin testing? Cleveland Clinic Journal of Medicine. 2010;**77**(9):606-611

[66] Pai M, Zwerling A, Menzies D. Systematic review: T-cell–based assays for the diagnosis of latent tuberculosis infection: An update. Annals of Internal Medicine. 2008;**149**(3):177

[67] Gannon BW, Hayes CM, Roe JM. Survival rate of airborne *Mycobacterium bovis*. Research in Veterinary Science. 2007;**82**(2):169-172

[68] Hickman C, MacDonald KL, Osterholm MT. Exposure of passengers and flight crew to *Mycobacterium tuberculosis* on commercial aircraft, 1992-1995. Morbidity and Mortality Weekly Report. 1995;**44**(8):137-140

[69] Ernst JD. Macrophage receptors for *Mycobacterium tuberculosis*. Infection and Immunity. 1998;**66**(4):1277-1281

[70] Vergne I, Chua J, Singh SB, Deretic V. Cell biology of *Mycobacterium tuberculosis* phagosome. Annual Review of Cell and Developmental Biology. 2004;**20**:367-394

[71] Pasula R, Downing JF, Wright JR, Kachel DL, Davis Jr TE, Martin W 2nd. Surfactant protein a (SP-A) mediates attachment of *Mycobacterium tuberculosis* to murine alveolar macrophages. American Journal of Respiratory and Critical Care Medicine. 1997;**17**(2):209

[72] Tailleux L, Schwartz O, Herrmann JL, Pivert E, Jackson M, Amara A, et al. DC-SIGN is the major *Mycobacterium tuberculosis* receptor on human dendritic cells. The Journal of Experimental Medicine. 2003;**197**(1):121-127

[73] Schlesinger LS. Macrophage phagocytosis of virulent but not attenuated strains of *Mycobacterium tuberculosis* is mediated by mannose receptors in addition to complement receptors. Journal of Immunology. 1993;**150**(7):2920-2930

[74] Schlesinger LS, Hull SR, Kaufman TM. Binding of the terminal mannosyl units of lipoarabinomannan from a virulent strain of *Mycobacterium tuberculosis* to human macrophages. Journal of Immunology. 1994;**152**(8):4070-4079

[75] Geijtenbeek TBH, Van Vliet SJ, Koppel EA, Sanchez-Hernandez M, Vandenbroucke-Grauls CMJE, Appelmelk B, et al. Mycobacteria target DC-SIGN to suppress dendritic cell function. The Journal of Experimental Medicine. 2003;**197**(1):7-17

[76] Mukhopadhyay S, Nair S, Ghosh S. Pathogenesis in tuberculosis: Transcriptomic approaches to unraveling virulence mechanisms and finding new drug targets. FEMS Microbiology Reviews. 2012;**36**(2):463-485

[77] Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. Science. 2001;**291**(5508):1544-1547

[78] Court N, Vasseur V, Vacher R, Fremond C, Shebzukhov Y, Yeremeev VV, et al. Partial redundancy of the pattern recognition receptors, scavenger receptors, and C-type lectins for the long-term control of *Mycobacterium tuberculosis* infection. Journal of Immunology. 2010;**184**(12):7057-7070

[79] Russell DG. Who puts the tubercle in tuberculosis? Nature Reviews Microbiology. 2007; **5**(1):39-47

[80] Schluger NW, Rom WN. The host immune response to tuberculosis. American Journal of Respiratory and Critical Care Medicine. 1998;**157**(3 Pt 1):679-691

[81] Cooper AM. Cell-mediated immune responses in tuberculosis. Annual Review of Immunology. 2009;**27**:393-422

[82] Mogues T, Goodrich ME, Ryan L, LaCourse R, North RJ. The relative importance of T cell subsets in immunity and immunopathology of airborne *Mycobacterium tuberculosis* infection in mice. The Journal of Experimental Medicine. 2001;**193**(3):271-280

[83] Grotzke JE, Lewinsohn DM. Role of CD8+ T lymphocytes in control of *Mycobacterium tuberculosis* infection. Microbes and Infection. 2005;**7**(4):776-788

[84] Ulrichs T, Kaufmann SH. New insights into the function of granulomas in human tuberculosis. The Journal of Pathology. 2006;**208**(2):261-269

[85] Davis JM, Ramakrishnan L. The role of the granuloma in expansion and dissemination of early tuberculous infection. Cell. 2009;**136**(1):37-49

[86] Capuano SV, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, et al. Experimental *Mycobacterium tuberculosis* infection of cynomolgus macaques closely resembles the various manifestations of human *M. tuberculosis* infection. Infection and Immunity. 2003; **71**(10):5831-5844

[87] Vergne I, Chua J, Lee HH, Lucas M, Belisle J, Deretic V. Mechanism of phagolysosome biogenesis block by viable *Mycobacterium tuberculosis*. Proceedings of the National Academy of Sciences. 2005;**102**(11):4033-4038

[88] Festjens N, Bogaert P, Batni A, Houthuys E, Plets E, Vanderschaeghe D, et al. Disruption of the SapM locus in Mycobacterium bovis BCG improves its protective efficacy as a vaccine against *M. tuberculosis*. EMBO Molecular Medicine. 2011;**3**(4):222-234

[89] Grundner C, Ng HL, Alber T. *Mycobacterium tuberculosis* protein tyrosine phosphatase PtpB structure reveals a diverged fold and a buried active site. Structure. 2005;**13**(11): 1625-1634

[90] Via LE, Deretic D, Ulmer RJ, Hibler NS, Huber LA, Deretic V. Arrest of mycobacterial phagosome maturation is caused by a block in vesicle fusion between stages controlled by rab5 and rab7. Journal of Biological Chemistry. 1997;**272**(20):13326-13331

[91] Deretic V, Singh S, Master S, Harris J, Roberts E, Kyei G, et al. *Mycobacterium tuberculosis* inhibition of phagolysosome biogenesis and autophagy as a host defence mechanism. Cellular Microbiology. 2006;**8**(5):719-727

[92] Pieters J. *Mycobacterium tuberculosis* and the macrophage: Maintaining a balance. Cell Host & Microbe. 2008;**3**(6):399-407

[93] Tan T, Lee WL, Alexander DC, Grinstein S, Liu J. The ESAT-6/CFP-10 secretion system of *Mycobacterium marinum* modulates phagosome maturation. Cellular Microbiology. 2006; **8**(9):1417-1429

[94] Pethe K, Swenson DL, Alonso S, Anderson J, Wang C, Russell DG. Isolation of *Mycobacterium tuberculosis* mutants defective in the arrest of phagosome maturation. Proceedings of the National Academy of Sciences. 2004;**101**(37):13642-13647

[95] Stewart GR, Patel J, Robertson BD, Rae A, Young DB. Mycobacterial mutants with defective control of phagosomal acidification. PLoS Pathogens. 2005;**1**(3):269-278

[96] Ferrari G, Langen H, Naito M, Pieters J. A coat protein on phagosomes involved in the intracellular survival of mycobacteria. Cell. 1999;**97**(4):435-447

[97] Jayachandran R, Sundaramurthy V, Combaluzier B, Mueller P, Korf H, Huygen K, et al. Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin. Cell. 2007;**130**(1):37-50

[98] Blackwell JM, Searle S, Goswami T, Miller EN. Understanding the multiple functions of Nramp1. Microbes and Infection. 2000;**2**(3):317-321

[99] Hackam DJ, Rotstein OD, Zhang W, Gruenheid S, Gros P, Grinstein S. Host resistance to intracellular infection: Mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. The Journal of Experimental Medicine. 1998;**188**(2):351-364

[100] MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-γ-inducible LRG-47. Science. 2003;**302**(5645):654-659

[101] Taylor GA, Feng CG, Sher A. p47 GTPases: Regulators of immunity to intracellular pathogens. Nature Reviews Immunology. 2004;**4**(2):100-109

[102] Taylor GA. IRG proteins: Key mediators of interferon-regulated host resistance to intracellular pathogens. Cellular Microbiology. 2007;**9**(5):1099-1107

[103] Kyei GB, Vergne I, Chua J, Roberts E, Harris J, Junutula JR, et al. Rab14 is critical for maintenance of *Mycobacterium tuberculosis* phagosome maturation arrest. The EMBO Journal. 2006;**25**(22):5250-5259

[104] Tiwari S, Choi HP, Matsuzawa T, Pypaert M, MacMicking JD. Targeting of the GTPase Irgm1 to the phagosomal membrane via PtdIns(3,4)P(2) and PtdIns(3,4,5)P(3) promotes immunity to mycobacteria. Nature Immunology. 2009;**10**(8):907-917

[105] Kuijl C, Neefjes J. New insight into the everlasting host-pathogen arms race. Nature Immunology. 2009;**10**(8):808-809

[106] Deretic V. Autophagy in immunity and cell-autonomous defense against intracellular microbes. Immunological Reviews. 2011;**240**(1):92-104

[107] Harris J, Hope J, Lavelle E. Autophagy and the immune response to TB. Transboundary and Emerging Diseases. 2009;**56**(6–7):248-254

[108] Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science. 2006;**313**(5792):1438-1441

[109] King KY, Lew JD, Ha NP, Lin JS, Ma X, Graviss EA, et al. Polymorphic allele of human IRGM1 is associated with susceptibility to tuberculosis in African Americans. PLoS One. 2011;**6**(1):e16317

[110] Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. Nature. 2011;**469**(7330):323-335

[111] Kumar D, Nath L, Kamal MA, Varshney A, Jain A, Singh S, et al. Genome-wide analysis of the host intracellular network that regulates survival of *Mycobacterium tuberculosis*. Cell. 2010;**140**(5):731-743

[112] Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M, et al. T helper 2 cytokines inhibit autophagic control of intracellular *Mycobacterium tuberculosis*. Immunity. 2007;**27**(3):505-517

[113] Ni Cheallaigh C, Keane J, Lavelle E, Hope J, Harris J. Autophagy in the immune response to tuberculosis: Clinical perspectives. Clinical and Experimental Immunology. 2011;**164**(3):291-300

[114] Keane J, Remold HG, Kornfeld H. Virulent *Mycobacterium tuberculosis* strains evade apoptosis of infected alveolar macrophages. Journal of Immunology. 2000;**164**(4):2016-2020

[115] Divangahi M, Desjardins D, Nunes-Alves C, Remold HG, Behar SM. Eicosanoid pathways regulate adaptive immunity to *Mycobacterium tuberculosis*. Nature Immunology. 2010;**11**(8):751-758

[116] Behar S, Martin C, Booty M, Nishimura T, Zhao X, Gan H, et al. Apoptosis is an innate defense function of macrophages against *Mycobacterium tuberculosis*. Mucosal Immunology. 2011;**4**(3):279-287

[117] Spira A, Carroll JD, Liu G, Aziz Z, Shah V, Kornfeld H, et al. Apoptosis genes in human alveolar macrophages infected with virulent or attenuated *Mycobacterium tuberculosis*: A pivotal role for tumor necrosis factor. American Journal of Respiratory and Critical Care Medicine. 2003;**29**(5):545-551

[118] Sly LM, Hingley-Wilson SM, Reiner NE, McMaster WR. Survival of *Mycobacterium tuberculosis* in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. Journal of Immunology. 2003;**170**(1): 430-437

[119] Dhiman R, Raje M, Majumdar S. Differential expression of NF-κB in mycobacteria infected THP-1 affects apoptosis. Biochimica et Biophysica Acta. 2007;**1770**(4):649-658

[120] Dhiman R, Kathania M, Raje M, Majumdar S. Inhibition of bfl-1/A1 by siRNA inhibits mycobacterial growth in THP-1 cells by enhancing phagosomal acidification. Biochimica et Biophysica Acta. 2008;**1780**(4):733-742

[121] Oddo M, Renno T, Attinger A, Bakker T, MacDonald HR, Meylan PR. Fas ligand-induced apoptosis of infected human macrophages reduces the viability of intracellular *Mycobacterium tuberculosis*. Journal of Immunology. 1998;**160**(11):5448-5454

[122] Balcewicz-Sablinska MK, Keane J, Kornfeld H, Remold HG. Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-α. Journal of Immunology. 1998;**161**(5):2636-2641

[123] Velmurugan K, Chen B, Miller JL, Azogue S, Gurses S, Hsu T, et al. *Mycobacterium tuberculosis* nuoG is a virulence gene that inhibits apoptosis of infected host cells. PLoS Pathogens. 2007;**3**(7):e110

[124] Miller JL, Velmurugan K, Cowan MJ, Briken V. The type I NADH dehydrogenase of *Mycobacterium tuberculosis* counters phagosomal NOX2 activity to inhibit TNF-α-mediated host cell apoptosis. PLoS Pathogens. 2010;**6**(4):e1000864

[125] Jayakumar D, Jacobs WR Jr, Narayanan S. Protein kinase E of *Mycobacterium tuberculosis* has a role in the nitric oxide stress response and apoptosis in a human macrophage model of infection. Cellular Microbiology. 2008;**10**(2):365-374

[126] Hinchey J, Jeon BY, Alley H, Chen B, Goldberg M, Derrick S, et al. Lysine auxotrophy combined with deletion of the SecA2 gene results in a safe and highly immunogenic candidate live attenuated vaccine for tuberculosis. PLoS One. 2011;**6**(1): e15857

[127] Ding AH, Nathan CF, Stuehr DJ. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. Journal of Immunology. 1988; **141**(7):2407-2412

[128] Sato K, Akaki T, Tomioka H. Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α). Clinical and Experimental Immunology. 1998;**112**(1):63-68

[129] Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proceedings of the National Academy of Sciences. 2000;**97**(16):8841-8848

[130] Jackson SH, Gallin JI, Holland SM. The p47phox mouse knock-out model of chronic granulomatous disease. The Journal of Experimental Medicine. 1995;**182**(3):751-758

[131] Adams L, Dinauer M, Morgenstern D, Krahenbuhl J. Comparison of the roles of reactive oxygen and nitrogen intermediates in the host response to *Mycobacterium tuberculosis* using transgenic mice. Tubercle and Lung Disease. 1997;**78**(5):237-246

[132] Lau Y, Chan G, Ha S, Hui Y, Yuen K. The role of phagocytic respiratory burst in host defense. Clinical Infectious Diseases. 1998;**26**(1):226-227

[133] Shiloh MU, Nathan CF. Reactive nitrogen intermediates and the pathogenesis of salmonella and mycobacteria. Current Opinion in Microbiology. 2000;**3**(1):35-42

[134] Chan ED, Chan J, Schluger NW. What is the role of nitric oxide in murine and human host defense against tuberculosis? Current knowledge. American Journal of Respiratory and Critical Care Medicine. 2001;**25**(5):606

[135] MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proceedings of the National Academy of Sciences. 1997;**94**(10):5243-5248

[136] MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. Annual Review of Immunology. 1997;**15**:323-350

[137] Nicholson S, Bonecini-Almeida Mda G, Lapa e Silva JR, Nathan C, Xie QW, Mumford R, et al. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. The Journal of Experimental Medicine. 1996;**183**(5):2293-2302

[138] Wang CH, Lin HC, Liu CY, Huang KH, Huang TT, Yu CT, et al. Upregulation of inducible nitric oxide synthase and cytokine secretion in peripheral blood monocytes from pulmonary tuberculosis patients. The International Journal of Tuberculosis and Lung Disease: the official journal of the International Union against Tuberculosis and Lung Disease. 2001;**5**(3):283-291

[139] 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

[140] Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos M, et al. Mycobacteria inhibit nitric oxide synthase recruitment to phagosomes during macrophage infection. Infection and Immunity. 2004;**72**(5):2872-2878

[141] Davis AS, Vergne I, Master SS, Kyei GB, Chua J, Deretic V. Mechanism of inducible nitric oxide synthase exclusion from mycobacterial phagosomes. PLoS Pathogens. 2007;**3**(12):e186

[142] Master SS, Springer B, Sander P, Boettger EC, Deretic V, Timmins GS. Oxidative stress response genes in *Mycobacterium tuberculosis*: Role of ahpC in resistance to peroxynitrite and stage-specific survival in macrophages. Microbiology. 2002;**148**(Pt 10):3139-3144

[143] Heym B, Zhang Y, Poulet S, Young D, Cole ST. Characterization of the katG gene encoding a catalase-peroxidase required for the isoniazid susceptibility of *Mycobacterium tuberculosis*. Journal of Bacteriology. 1993;**175**(13):4255-4259

[144] Manca C, Paul S, Barry CE 3rd, Freedman VH, Kaplan G. *Mycobacterium tuberculosis* catalase and peroxidase activities and resistance to oxidative killing in human monocytes in vitro. Infection and Immunity. 1999;**67**(1):74-79

[145] Li Z, Kelley C, Collins F, Rouse D, Morris S. Expression of katG in *Mycobacterium tuberculosis* is associated with its growth and persistence in mice and Guinea pigs. Journal of Infectious Diseases. 1998;**177**(4):1030-1035

[146] Hu Y, Butcher PD, Mangan JA, Rajandream MA, Coates AR. Regulation of hmp gene transcription in *Mycobacterium tuberculosis*: Effects of oxygen limitation and nitrosative and oxidative stress. Journal of Bacteriology. 1999;**181**(11):3486-3493

[147] Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, et al. Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: Insights into the Phagosomal environment. The Journal of Experimental Medicine. 2003;**198**(5):693-704

[148] Voskuil MI, Schnappinger D, Visconti KC, Harrell MI, Dolganov GM, Sherman DR, et al. Inhibition of respiration by nitric oxide induces a *Mycobacterium tuberculosis* dormancy program. The Journal of Experimental Medicine. 2003;**198**(5):705-713

[149] Ohno H, Zhu G, Mohan VP, Chu D, Kohno S, Jacobs WR Jr, et al. The effects of reactive nitrogen intermediates on gene expression in *Mycobacterium tuberculosis*. Cellular Microbiology. 2003;**5**(9):637-648

[150] Dasgupta N, Kapur V, Singh KK, Das TK, Sachdeva S, Jyothisri K, et al. Characterization of a two-component system, devR-devS, of *Mycobacterium tuberculosis*. Tubercle and Lung Disease. 2000;**80**(3):141-159

[151] Sherman DR, Voskuil M, Schnappinger D, Liao R, Harrell MI, Schoolnik GK. Regulation of the *Mycobacterium tuberculosis* hypoxic response gene encoding α-crystallin. Proceedings of the National Academy of Sciences. 2001;**98**(13):7534-7539

[152] Kendall SL, Movahedzadeh F, Rison SC, Wernisch L, Parish T, Duncan K, et al. The *Mycobacterium tuberculosis* dosRS two-component system is induced by multiple stresses. Tuberculosis (Edinburgh, Scotland). 2004;**84**(3–4):247-255

[153] Kumar A, Toledo JC, Patel RP, Lancaster JR Jr, Steyn AJ. *Mycobacterium tuberculosis* DosS is a redox sensor and DosT is a hypoxia sensor. Proceedings of the National Academy of Sciences. 2007;**104**(28):11568-11573

[154] Kumar A, Deshane JS, Crossman DK, Bolisetty S, Yan BS, Kramnik I, et al. Heme oxygenase-1-derived carbon monoxide induces the *Mycobacterium tuberculosis* dormancy regulon. Journal of Biological Chemistry. 2008;**283**(26):18032-18039

[155] Shiloh MU, Manzanillo P, Cox JS. *Mycobacterium tuberculosis* senses host-derived carbon monoxide during macrophage infection. Cell Host & Microbe. 2008;**3**(5):323-330

[156] Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: A systematic review and meta-analysis. International Journal of Epidemiology. 2008;**37**(1):113-119

[157] Douglas AS, Strachan DP, Maxwell JD. Seasonality of tuberculosis: The reverse of other respiratory diseases in the UK. Thorax. 1996;**51**(9):944-946

[158] Strachan DP, Powell KJ, Thaker A, Millard FJ, Maxwell JD. Vegetarian diet as a risk factor for tuberculosis in immigrant south London Asians. Thorax. 1995;**50**(2):175-180

[159] Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, et al. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in West London: A case-control study. Lancet. 2000;**355**(9204):618-621

[160] Liu PT, Stenger S, Tang DH, Modlin RL. Cutting edge: Vitamin D-mediated human antimicrobial activity against *Mycobacterium tuberculosis* is dependent on the induction of cathelicidin. Journal of Immunology. 2007;**179**(4):2060-2063

[161] Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, Islam K, et al. Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. Proceedings of the National Academy of Sciences. 2012;**109**(38):15449-15454

[162] 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

[163] Liu PT, Schenk M, Walker VP, Dempsey PW, Kanchanapoomi M, Wheelwright M, et al. Convergence of IL-1β and VDR activation pathways in human TLR2/1-induced antimicrobial responses. PLoS One. 2009;**4**(6):e5810

[164] Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, et al. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host & Microbe. 2009;**6**(3):231-243

[165] Anand SP, Selvaraj P. Effect of 1, 25 dihydroxyvitamin D(3) on matrix metalloproteinases MMP-7, MMP-9 and the inhibitor TIMP-1 in pulmonary tuberculosis. Clinical Immunology. 2009;**133**(1):126-131

[166] Mahon BD, Wittke A, Weaver V, Cantorna MT. The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. Journal of Cellular Biochemistry. 2003;**89**(5):922-932

[167] Vidyarani M, Selvaraj P, Jawahar MS, Narayanan PR. 1, 25 Dihydroxyvitamin D3 modulated cytokine response in pulmonary tuberculosis. Cytokine. 2007;**40**(2):128-134

[168] Imazeki I, Matsuzaki J, Tsuji K, Nishimura T. Immunomodulating effect of vitamin D3 derivatives on type-1 cellular immunity. Biomedical Research. 2006;**27**(1):1-9

[169] Daniel C, Sartory NA, Zahn N, Radeke HH, Stein JM. Immune modulatory treatment of trinitrobenzene sulfonic acid colitis with calcitriol is associated with a change of a T helper (Th) 1/Th17 to a Th2 and regulatory T cell profile. Journal of Pharmacology and Experimental Therapeutics. 2008;**324**(1):23-33

[170] Cantorna MT, Humpal-Winter J, DeLuca HF. In vivo upregulation of interleukin-4 is one mechanism underlying the immunoregulatory effects of 1,25-dihydroxyvitamin D(3). Archives of Biochemistry and Biophysics. 2000;**377**(1):135-138

[171] Sly LM, Lopez M, Nauseef WM, Reiner NE. 1α,25-Dihydroxyvitamin D3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase. Journal of Biological Chemistry. 2001;**276**(38):35482-35493

[172] Anand PK, Kaul D. Vitamin D3-dependent pathway regulates TACO gene transcription. Biochemical and Biophysical Research Communications. 2003;**310**(3):876-877

[173] Anand PK, Kaul D. Downregulation of TACO gene transcription restricts mycobacterial entry/survival within human macrophages. FEMS Microbiology Letters. 2005;**250**(1): 137-144

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