Chapter from the book *Trends in Infectious Diseases*

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1. Introduction

Although the millennium development goal to stop tuberculosis (Tb) epidemic is almost achieved, still in 2012 there were 8.6 million new cases and 1.3 million deaths worldwide [of which 3, 20,000 people were co-infected with HIV too (WHO 2013)]. The rate of new Tb cases has been falling but decline rate at 2 percent per annum is still slow. Progress to handle multidrug resistant tuberculosis (MDR Tb)-defined by resistance to rifampicin and isoniazid (often accompanied by additional resistance), which accounts for 3.6 percent of the new and 20.2 percent of the previously treated Tb cases-is also slow (WHO 2013). Emergence of extremely drug resistant (XDR) strains of Mycobacterium tuberculosis (Mtb) which is about 9.6 percent of the MDR Tb cases (WHO 2013) is a looming threat to the programmes aimed to stop tuberculosis. The XDR strains are resistant to isoniazid and rifampicin (first line drugs); at least to one of the three injectable second line drugs (amikasin, kanamycin or capreomycin) and also to any of the fluoroquinolone drugs to tuberculosis. The MDR and XDR Tb account for much higher death rates among the incident cases.

Induction of autophagy, Vitamin D and arachidonic acid metabolites play a decisive role in determining the susceptibility or resistance to the Mycobacterium tuberculosis infections. Additionally, cytokine responses play a major role. Among the cytokines, important ones are type I/ type II interferons, TNF-α and IL-1β in combination with other cytokines such as IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, IL-22 etc. (Ottenhoff TH, 2012). Mtb has evolved strategies to suppress the immune response mounted against it and even exploits host molecular pathways for its survival benefits. Regulation of apoptosis versus necrosis by Mtb or its host has emerged as a major player in determining the survival or clearance of the pathogen. Many of these pathways have components with opposite roles favouring either host or the pathogen. They...
also are interconnected and the final outcome of the infection depends on the result of their converging effects.

1.1. Tb epidemiology

Tb incidence is generally considered as notifications of the cases with correction for underreporting and non-diagnosis. The total incidence of Tb cases in 2012 was in the range of 8.3-9.0 million globally (WHO 2013). Out of these, children account for 0.5 million cases and 3.1 million cases were among women. According to the WHO report on tuberculosis, most of the cases were from Asia and African regions-India (2.0-2.4 million), China (0.9-1.1 million), South Africa (0.4-0.6 million), Indonesia and Pakistan with 0.45 million and 0.4 million cases respectively, which is dangerously high. The report suggests that it is developing countries and specifically, the poor who are gripped tightly by the disease and who account for the maximum number of new cases and deaths worldwide. Awareness and access to diagnostic labs in developing countries is still low in addition to the lack of availability of affordable treatment. The combination of factors results in the spread of the pathogen to many people before being diagnosed. Diagnostic methods to distinguish latent tuberculosis from active disease and treatment options for latent Tb also are urgently required otherwise tuberculosis cannot be eradicated completely. There is some progress in this area and newer methods to diagnose latent Tb are being developed (Singh SB et al., 2013). In a recent study, Harari A et al. 2011, showed the possibility of discriminating latent Tb from active disease which could not be differentiated by a tuberculin skin test (TST) or interferon gamma release assay (IGRA). They utilized a flow-cytometry based method and analysed the functionality of M tb specific CD4 T cells from cohorts. Their results suggests that CD4 T cells are multifunctional and able to produce IL-2, TNF-α and IFN-γ in latent Tb cases while they dominantly produce TNF-α (single positive) in active disease conditions (Harari A. et al. 2011). Also since BCG is almost 100 years old with varied efficacy, new pre-and post-exposure vaccines are needed to prevent tuberculosis.

1.2. M tb pathogenesis

The genus Mycobacterium originated millions of years ago, but the members of the M tb complex evolved about 15,000-35,000 years back (Gutierrez MC et al., 2005). The noted occurrence of tuberculosis in humans is from their prehistoric remains and from Egyptian mummies dated back to 3000-2400 years (Zink AR et al., 2003). Respiratory tract is the main route of entry of the pathogen as airborne droplets containing the bacterium reaches the lung-a suitable site for this aerobic organism to establish infection. However other tissues and organs viz. lymphatic system, central nervous system, pleura, liver, spleen, bones and joints are also susceptible to infection by M tb and manifestation of the disease (Bloom BR and Small PM, 1998; Golden MP and Vikram HR, 2005). Once in the lung, alveolar macrophages engulf the bacterium, a process facilitated by binding of the lipo-arabinomannan on the bacterial cell wall to the mannose receptors on macrophages. Complement receptors on the macrophage surface also take part in the process of endocytosis of opsonised M tb (Ernst JD, 1998; Kang PB et al., 2005; Kerrigan AM and Brown GD, 2009). These interactions culminate in the release of cytokines which stimulate the adaptive arm of the immune system and eventually leads to
inflammatory response at the site of infection. Other cells of the innate and adaptive immune system migrate to the infection site and try to contain the bacterium by forming a specialized structure called granuloma.

The granuloma plays a major role in preventing the escape of the bacterium to other sites and also creates a localized immune response. If the immune response directed against the pathogen is capable of successfully containing it, granulomas shrink with the formation of the caseous centres, which is the case in immune-competent individuals (Dannenberg AM Jr. and Rook JA, 1994; Doherty TM and Andersen P, 2005). M\(\text{tb}\) is unable to replicate within the caseous centres due to hypoxia, acidic pH and presence of the fatty acids which are toxic to the bacterium but some organisms become dormant and persist for many years (Smith I, 2003). However, if the bacterium is able to survive by utilizing various immune evasion tactics and is able to replicate, necrosis of the infected macrophages takes place. It leads to the degradation of cells composing the granuloma and lipids (especially cholesterol) from the dying cells serve as a rich source of nutrient for the bacterium (Orme IM, 2013). Subsequent destruction of the cells leads to liquefaction within the interior of the granuloma where bacteria lives an apparently extracellular life, probably in the form of a pellicle or biofilms (Ojha AK et al., 2008; Orme IM, 2013) – a part of the bacterial life cycle not well perceived in the scientific arena. At this stage, the pathogen is far from the reach of current drugs available for treating tuberculosis and the components of the immune system modulated by the vaccine to tackle the infection (Orme IM, 2013). The calcification process initiated inside the granuloma after necrosis push the bacterium to the periphery and probable rupture of the membrane leads to dissemination of the bacterium. At this point, M\(\text{tb}\) is capable of dissemination to other organs of the body and is also released to the external environment as aerosol droplets when the host coughs, sneezes, shouts or sings and thus is able to start a new cycle of infection.

Though a high number of people fall prey to the bacterium very often but remain asymptomatic unless there are perturbations in the immune status of the person. The probability of developing active disease after getting infected is high in the initial two years but at least 5-10 percent of the people develop disease during their lifetime (Harada N, 2006; O’Garra A et al., 2013). In this chapter some of the host molecular pathways or their components and the signalling axes which play crucial roles in the inhibition or survival of the M\(\text{tb}\) and some of the recent research in the area has been highlighted.

2. Interferon signalling

Type I interferons e.g. interferon-\(\alpha\) and interferon-\(\beta\) are implicated in progression of the tuberculosis. Mice with impaired type I interferon signalling are better protected from the pathogen (Manca C et al., 2001). In-vitro studies also show that M\(\text{tb}\) infection leads to up regulation of the genes of type I interferon signalling pathways and genes induced by them (Remoli ME et al., 2002). On the other hand interferon-\(\gamma\), a type II interferon is critically important in protection against tuberculosis (Flynn JL et al., 1993; Trinchiari G, 2010). It plays its role by various mechanisms including activation of macrophages, enhances functioning of
CD8 T cells and through reactive nitrogen species (Flynn JL et al., 1993; Green AM et al., 2013). Thus both types of interferons have important and opposite roles in determining the mycobacterial pathogenesis (Teles RM et al., 2013). Berry et al., 2010, found a Tb-specific transcriptional signature in blood which could help in discrimination between latent and active disease and also distinguishes Tb from other infectious and inflammatory diseases. They reported 86 gene signatures which are specific to tuberculosis. These genes are mostly interferon inducible and consists of both type I and type II interferon signalling pathways and could be novel targets for Tb treatment.

3. IL-1β signalling pathway in tuberculosis

IL-1β imparts immunity to tuberculosis and mice lacking in IL-1β or its receptors are susceptible to M tb infection (Mayer-Barber KD, 2010). It is an important factor in host immunity and virulent mycobacteria suppress the IL-1β production which is regulated by type I interferons in macrophages (Novikov A et al., 2011). IL-1β has been shown to possess bactericidal activity in the macrophages derived from murine and humans (Jayaraman P et al., 2013). It upregulates secretion of tumor necrosis factor (TNF) and cell surface expression of TNFR1, thus facilitates TNF signalling which culminates in caspase-3 activation leading to growth inhibition of M tb through apoptosis of the infected macrophage (Jayaraman P et al., 2013). They also showed that this effect of TNF on the M tb infected macrophage is due to autocrine mode of action. In synergy with vitamin D, IL-1β drives transcriptional expression of the antimicrobial peptide genes such as defensin beta 4 (DEFB4), cathepsins, cathelicidins and ubiquitin derived peptides which have M tb killing ability (Alonso S, 2007; Liu PT et al, 2009; Ottenhoff TH, 2012). Results from the work of Liu PT et al. 2009, also suggest that the coherent action of IL-1β and vitamin D is an integral part of the TLR2/1 signalling mediated antimicrobial activity.

IL-1β being a pro-inflammatory cytokine is under tight regulation to prevent the immune-pathology and subsequent tissue damage during chronic infections. Mishra et al., 2013, showed that level of this cytokine is regulated by IFN-γ induced release of nitric oxide (NO) which in turn regulate the inflammasome NLRP3 (nucleotide binding and oligomerization domain-like receptor family pyrin domain containing 3) during M tb infections. This regulation happens at the stage of caspase1 mediated processing of pro-IL-1β to IL-1β and is specifically NLRP3 dependent (Mishra BB. et al., 2013).

4. Inflammasomes in tuberculosis

As briefly discussed above inflammasomes play a regulatory role in tuberculosis and imparts protection if activated. For its survival M tb prevents the activation of inflammasomes, caspase-1 dependent processing of pro-IL-1β and phagosome maturation through its gene zmp1 (Master SS et al., 2008; Lazarevic V and Martinon F, 2008). It has been shown that the production of IL-1β is dependent on the recognition of M tb by pattern recognition receptors
PRR) TLR2/TLR6 and NOD2 (Kleinnijenhuis J et al., 2009). TLR4, the other PRR which is important in Mtb recognition does not play major role in production of IL-1β. The immune adaptor molecule MyD88 has a central role in the transcription of the IL-1β mRNA during Mtb infection (Kleinnijenhuis J et al., 2009).

Absence in melanoma 2 (AIM2) inflammasome is a cytosolic sensor of the DNA and recognises DNA viruses and intracellular bacteria. Co-localisation of Mtb DNA with AIM2 inflammasome has been observed suggesting their direct interaction (Saiga H et al., 2012). AIM2 inflammasomes are involved in activation of macrophages and secretion of IL-1β during infection with pathogenic strain of Mycobacterium bovis suggesting its co-operative role in host immunity (Yang Y, 2013). AIM2 deficient mice are more susceptible to Mtb infection and are defective in production of IL-1β and IL-18 and mount poor Th1 response (Saiga H et al., 2012). These authors also speculated on the role of AIM2 inflammasome in suppressing type I interferons in Mtb infections. NLRP3 inflammasome is implicated in the protective immune response to Mtb infection by facilitating the maturation process of IL-1β (Rathinam VA et al., 2012). However, Mtb suppresses the activation of the NLRP3 inflammasome by inducing IFN-β, while IFN-β induces the AIM2 inflammasome which is detrimental to the pathogen (Fernandes-Alnemri T et al., 2010; Tsuchiya K et al. 2010; Briken V et al., 2013). Thus Mtb balances the level of IFN-β such that NLRP3 inflammasome is kept suppressed and the AIM2 inflammasome is not allowed to be activated. This is done by the ESX-1 secretion system which is dependent on the ESAT6-an RD1 region encoded protein of Mtb (Shah S et al., 2013).

Activating inflammasomes, although critical for protection from Mtb infection and tuberculosis, also need to be regulated to prevent the tissue damage and rampant inflammation. Host regulation of NLRP3 inflammasome is done by nitric oxide which acts as its negative regulator during Mtb infection and consequently controls the level of IL-1β (Mishra BB. et al., 2013).

5. Arachidonic acid metabolites

Mtb on engulfment by macrophages tries to prevent the apoptosis of the harbouring macrophage so that it can establish a niche for itself. It also promotes necrosis of the macrophages in which it resides which help its spread to the neighbouring cells before establishment of the adaptive immune response of the host (Divangahi M et al., 2013). Several lines of research suggest that metabolic products of arachidonic acids such as leukotrienes, lipoxins and eicosanoids play decisive roles by regulating innate and adaptive immunity in the mycobacterial pathogenesis (Divangahi M et al., 2010). The prostaglandins and lipoxins, metabolites of arachidonic acid, have opposite roles. While prostaglandins such as prostaglandin E2 (PGE2) is pro-inflammatory in nature and promotes apoptosis, lipoxins inhibit it and promotes necrosis which results in the spreading of the bacterium (Tobin DM et al., 2010). Lipoxins e.g. Lipoxin A4 and its metabolites are anti-inflammatory in nature, repress TNF-α and stops neutrophil recruitment to the site of infection (Tobin DM et al., 2010). The other metabolite leukotriene B4 (LTB4) enhances level of TNF-α and thus creating a state of hyper-inflammation which is also not a healthy state for the host. Thus, TNF-α is regulated by metabolic products
of arachidonic acid to keep its optimum level so that Mtb infection is controlled while hyper-inflammation is also prevented.

6. Role of vitamin D

Deficiency of vitamin D is associated with higher incidence and manifestation of tuberculosis (Nnoaham KE, 2008; Verway M et al., 2013) and its supplementation helps to overcome this disease. Vitamin D is also able to restore the impaired secretion of TNF-α from macrophages of HIV-positive people (Anandaiah A et al., 2013). It acts as a mediator of innate immune response against Mtb by mediating signals from toll like receptors to the activation of antimicrobial peptides (Liu PT et al., 2006). Liu PT et al. 2006, demonstrated that TLR stimulation by Mtb or lipo-polysaccharide activates vitamin D receptors and subsequent downstream signalling activates transcription and translation of cathelicidin, a peptide with antimicrobial properties and thus creating an antimicrobial state in the human macrophages. Vitamin D has a modulatory role on the levels of cytokines specifically IL-1β and thus aid in immunity to the pathogen (Verway M et al., 2013). It also regulates the role of NLRP3/caspase1 inflammasome leading to regulation of the levels of IL-1β and cross talk between alveolar epithelial cells and macrophages which is required for the synthesis and release of antimicrobial peptides (Verway M et al., 2013).

1, 25-dihydroxyvitamin D3, the active component of vitamin D, plays a major role in induction of autophagy during Mtb infections (Yuk JM et al., 2009). This function of vitamin D3 is performed by activation of transcription of Beclin-1 and Atg5 genes and is mediated by cathelicidins (Yuk JM et al., 2009). Vitamin D3 also helps in the formation of autophagosomes, autophagolysosomes and co-localization of Mtb cells with them, an important step in the killing of the bacterium (Yuk JM et al., 2009).

7. Role of foamy macrophages

After being engulfed by macrophages Mtb dys-regulates its lipid metabolism which leads to lipid accumulation within a subset of these macrophages giving them a characteristic foamy phenotype. The lipid packed foamy macrophages have been associated with several chronic disease conditions such as atherosclerosis and during infections with persistent intracellular pathogens e.g. Mtb, Chlamydia and Toxoplasma (Kalayoglu MV and Byrne GI, 1998; Portugal LR et al., 2008; Galkina E and Ley K, 2009). Triglycerides, phospholipids and cholesterol constitute the low density lipo-proteins (LDL) and in foamy macrophages the influx and efflux of LDLs is dys-regulated (Russell DG, et al., 2009). Cholesterol gets esterified when the macrophage attains foamy phenotype and is retained as lipid droplets (Russell DG et al., 2009). Recently it has been shown that triacylglycerols (TAG) of Mtb are derived from host TAG and are imported by the bacterium for its lipid synthesis (Daniel J et al., 2011). Mtb incorporates host derived lipids directly into its own pool and the accumulation of neutral lipids by the Mtb leads to its lipid fastness (Daniel J et al., 2011).
It is shown that pathogenic mycobacteria synthesize oxygenated mycolic acids which induce foamy cell formation of the macrophages (Peyron P et al., 2008) but this might not hold true under hypoxic conditions (Daniel J et al., 2011). Peyron P et al., 2008 have shown that these lipid droplets serve as nutritional source to the pathogen and help in its non-replicative life cycle and persistence. One recent report also suggested that mycobacterium prevents lipolysis by interfering with the host lipid metabolic pathways, which leads to lipid accumulation inside the macrophage (Singh V et al., 2012). These lipids serve as a source of nutrition and help the pathogen in its dormant lifestyle. The specific presence of foamy macrophages in the necrotic regions has been suggested that they play crucial role in necrosis and hence in spreading of the bacterium (Peyron P et al., 2008). Although macrophages are the frontline innate immune cells, it is clear that their foamy phenotype helps the pathogen in establishing persistent infection and the host innate and adaptive immune response is no more able to eliminate the pathogen once it happens. Thus the ideal way to target the pathogen is before the establishment of the foamy phenotype of the macrophages harbouring the M Tb.

8. Role of autophagy

In addition to the above discussed mechanisms, hosts also try to clear the pathogen by inducing autophagy—an innate defence against M tb (Kumar D et al., 2010; Jo EK, 2013). Nutrient starvation, stress and activation of the specific cytosolic receptors induce autophagy (Ottenhoff TH, 2012). By this process protein aggregates, damaged organelles and cytosolic pathogens are sequestered inside the autophagosomes. The subsequent fusion of the autophagosome with lysosomes leads to degradation of the trapped entities and this process is prominently involved in the clearance of intracellular pathogens including mycobacteria (Gutierrez MG et al., 2004; Alonso S, 2007; Levine B et al. 2011; Cadwell K and Philips JA, 2013). This process is carried out by the product of autophagy related gene (Atg), Beclin 1 in combination with kinase genes PIP3-VPS34 and the GTPase-IGRM (Deretic V, 2010). The role of autophagy is also suggested in inflammation and related phenomenon (Castillo EF, 2012; Deretic V, 2012).

Autophagy regulates innate and adaptive immune pathways viz. antigen presentation to T cells by macrophages and dendritic cells (Jagannath C, 2009; Ottenhoff TH, 2012) and inflammatory responses (Levine B et al. 2011). Thus autophagy plays an effector function during M tb pathogenesis, however this process itself is regulated by vitamin D. Vitamin D up regulates autophagy and plays bridging role between innate and adaptive immune arms (Deretic V, 2005; Yuk JM et al., 2009).

9. Conclusions and future perspectives

M tb, besides evading host immune response against it also delays the onset of the adaptive immunity (Urdahl KB et al., 2011). M tb engulfed by macrophages tries not only to prevent apoptosis of harbouring macrophage but also promotes its necrosis which helps in spread of
the bacterium to the neighbouring cells before establishment of the adaptive immune response (Urdahl KB et al., 2011). Type I interferons regulate the levels of interferon-γ and production of the IL-1β which are critical determinants of immunity to tuberculosis (Novikov A et al., 2011). Eicosanoids play decisive roles in the fate of the infected macrophages (apoptosis versus necrosis) by regulating the level of tumor necrosis factor. Thus, several of the host molecular pathways converge to dictate the delicate balance between host immune response and mycobacterial pathogenesis. We need to further understand their inter-relation and cross regulation in greater details to tackle the mycobacterial infection appropriately. There are intensive research and development activities in progress around the globe to tackle the epidemic of tuberculosis. Bedaquiline—the new drug approved for treatment of the MDR tuberculosis—was released finally in December 2012. According to WHO report on tuberculosis 2013, there are around ten drugs in various phases of clinical trials and many in the preclinical stages (Table 1). Also there are many new TB vaccines in the various phases of clinical development. Whether they are pre-or post-exposure vaccines and other details viz. their immune-therapeutic potential, killed whole cell or extract etc. are nicely elaborated in the WHO report on tuberculosis, 2013.

<table>
<thead>
<tr>
<th>Lead compounds</th>
<th>Cyclopeptides, Diarylquinoline, DprE Inhibitors, InhA inhibitor, LeuRS inhibitor, Macrolides, Mycobacterial Gyrase inhibitors, Pyrazinamide analogs, Riminophenazines, Ruthenium (II) complexes, Spectinamides, Translocase-1 inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclinical development</td>
<td>CPZEN-45, DC-159a, Q203, SQ609, SQ641, TBI-166</td>
</tr>
<tr>
<td>Laboratory toxicity testing</td>
<td>PBTZ-169, TBA-354</td>
</tr>
<tr>
<td>Phase II</td>
<td>AZD5847, Bedaquiline (TMC-207), Linezolid, PA-824, Rifapentine, SQ-109, Sutezolid (PNU-100480)</td>
</tr>
<tr>
<td>Phase III</td>
<td>Delamanid (OPC-67689), Gatifloxacin, Moxifloxacin, Rifapentine</td>
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Note: Four of the drugs in phase II trials (italicized) are novel and part of the combination regimens.

Table 1. Development Pipeline for new TB drugs (WHO, 2013)

The current research is also helping us to better understand the life cycle and survival strategies of the pathogen. Recently, work by Das B et al., 2013, suggested that Mtb persists inside the CD271+/CD45− mesenchymal stem cells in the bone marrow of tuberculosis patients. They showed that the pathogen remains alive even after the full regimen treatment of the patients with anti-TB drugs. The CD271+/CD45− mesenchymal stem cells express drug efflux pumps, produce low levels of reactive oxygen species, are quiescent in nature and have self renewal capability. This makes them an ideal place for Mtb to survive for a long time. The immune-privileged nature of the bone marrow also supports the dormant life of the pathogen and Mtb is able to live a non replicating life inside the bone marrow mesenchymal stem cells (Das B. et al., 2013). The part of the extracellular life of Mtb in the form of pellicle or biofilms inside the liquefied granuloma and alternate hiding places has yet to be clearly elucidated. The
growing knowledge about M tb pathogenesis should help us in targeting tuberculosis more precisely in future.

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