American Journal of Immunology 7 (1): 1-8, 2011
ISSN 1553-619X

Unique Characteristic Features of Mycobacterium Tuberculosis in Relation to Immune System

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Abstract: Problem statement: Tuberculosis is a leading global mortality factor which has not been effectively controlled, with 1.7 million deaths per year and 8.9 million new cases. Aerobic microbe *Mycobacterium Tuberculosis* H37Rv (MTB) is the causative agent of tuberculosis. **Approach:** It is unique among prokaryotes due to its exceptional features contributing to its survival within the hostile environment of macrophages. **Results:** It modifies both its intracellular and local tissue environment and proliferates within macrophages resulting in caseous granulomas, the characteristic lesions of TB. MTB derived cAMP intoxicates host cells and thus enable MTB for long term persistence within macrophages by modifying its intracellular environment. Apart from these, there are several unique structural components of MTB which interfere in the pathways of immune system and thus eluding it from destruction. **Conclusion:** The dormant state of MTB is the major factor which provides this pathogen ability to survive host inflammatory mediators and antibiotic treatment. It is indispensable to delineate the unusual features of MTB that enable its escape from the host immune system, in order to design an efficacious drug against the unpardonable form of tuberculosis.

Key words: Unique characteristic, *mycobacterium tuberculosis*, immune system, survive host, multidrug resistant, virulence factors, resistant tuberculosis, sharply reduced, drug resistant

INTRODUCTION

Tuberculosis is the directing cause of death affecting one third of world's population. It is the most unpardonable infectious disease and the most common one, which easily spreads through the air when the diseased person coughs, sneezes, or spits. Lungs are the most common target, but it may also affect the central nervous system, the gastrourinary system, the lymphatic system, the circulatory system, bones, joints and skin (Kumar and Robbins, 2007) WHO, 2006. A number of treatment and preventive strategies have been employed including BCG (Bacillus Calmette-Guerin) vaccine against TB, but none of them have been proved to be successful so far. Poor administrative control, incomplete and inadequate treatment and spontaneous mutation have led to the emergence of multidrug resistant tuberculosis and later its progression into extensively drug resistant tuberculosis (Jain and Dixit, 2008; Holtz and Cegielski, 2007).

Tuberculosis is caused by *Mycobacterium Tuberculosis* H37Rv (MTB) which is a unique acid fast gram positive bacterium. It is unique because of its high lipid and mycolic acid content of its cell wall. It neither contains phospholipid outer membrane nor retains dye. MTB cell wall bears large amount of glycolipids particularly rich in mycolic acid, peptidoglycan, LAM (lipoarabinomannan), Phosphatidyl Inositol Mannosides (PIM), phthiocerol dimycocerate, cord factor, sulfolipids and wax-D (Alderwick *et al*., 2007; Brennan, 2003; Asano *et al*., 1993; Belisle *et al*., 1997; Fratti *et al*., 2003; Meena and Rajni, 2010; Rajni and Meena, 2010). These unique cell wall components interfere in the host defence pathways and establish its survival within the phagosomes (Alderwick *et al*., 2007; Brennan, 2003; Asano *et al*., 1993; Belisle *et al*., 1997; Fratti *et al*., 2003; Meena and Rajni, 2010; Rajni and Meena, 2010; Kartmann *et al*., 1999). Exported proteins are one of the most important factor responsible for the virulence, in the case of MTB

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Antigen 85 complex is one of those dominant exported proteins which is one of the most powerful and protective antigen of MTB (Belisle *et al*., 1997). MTB is one of the most successful pathogen, which has extraordinary ability to persist within host macrophages through a complex and coordinated process controlled by its special bacterial protein secretion system called as ESX-1 (Raghavan *et al*., 2008; Sorensen *et al*., 1995; Champion and Cox, 2007; Stanley *et al*., 2003; Wel *et al*., 2007; MacGurn *et al*., 2005; Porcelli, 2008).

Tumor Necrosis Factor (TNF) is a cytokine well known for its role in systemic inflammation and the regulation of immnue cells (Flynn *et al*., 1995). It is also an important mediator produced by monocytes against bacterial and parasitic infections. The essentiality of TNF-α during MTB infection has been realized, however its overproduction is associated with deleterious effects (Orme and Collins, 1984; Orme *et al*., 1992; Flory *et al*., 1992 Flynn *et al*., 1992; Rook *et al*., 1986; Mangelsdorf *et al*., 1984; Bevilacqua *et al*., 1986; Nawroth and Stern, 1986; Beutler and Cerami, 1985; 1986; 1989; Dinarello *et al*., 1986; Tracey *et al*., 1988; Cotran *et al*., 1999; Sampaio *et al*., 1991; 1993; Sarno *et al*., 1991).

Granulomas which are known to restrict bacterial spreading actually serve as a nutrient rich reservoir for MTB persistence. Granulomas accumulate at the site of tuberculosis infection and benefits MTB by providing a constant supply of susceptible host cells to infect (Adams, 1976; Egen *et al*., 2008; Peyron *et al*., 2008; Davis and Ramakrishnan, 2009; Bold and Ernst, 2009). This efficient recruitment of uninfected macrophages to the site of infection is controlled by mycobacterial RD1 locus (Davis and Ramakrishnan, 2009). MTB which proliferates within host macrophages modifies its intracellular and local tissue environment causing caseous granulomas with incomplete bacterial sterilization (Baker and Kelly, 2004; Sands and Palmer, 2008; Walburger *et al*., 2004; Axelrod *et al*., 2008; Hunter *et al*., 2007; Agarwal *et al*., 2009; Keane *et al*., 2001; Chakravarty *et al*., 2008). Apart from unique behavior of granulomas there are 17 adenylate cyclase genes present in MTB, among them Rv0386 has been shown to be necessary for virulence. Rv0386 adenylate cyclase also mediates delivery of cAMP derived from bacteria into the macrophage cytoplasm (Agarwal *et al*., 2009).

There are some host factors that initiate and maintain the latent state of TB, Nitric Oxide (NO) is one such host factor which is a product of activated macrophages and exhibits antimycobacterial properties (Cardona and Ruiz-Manzano, 2004). Non-toxic concentration of NO competitively modulates the expression of a 48 gene regulon which expresses to prepare bacilli for survival during long periods of in vitro dormancy. It was suggested that inhibition of respiration by NO production along with oxygen limitation constrains MTB replication rates in persons with latent TB (Cardona and Ruiz-Manzano, 2004; Zhang *et al*., 2001).

***M. tuberculosis* H37rv versus host immune system:**

Cell wall structure: MTB cell wall structure deserves special consideration due to its incomparable characteristics among prokaryotes and it's a major factor for the virulence of the bacterium. The cell wall contains complex lipids apart from peptidoglycan. Mycobacterial cell wall is composed of over 60% of lipids. The lipid fraction of MTB comprises of three major components i.e., mycolic acid, cord factor and wax-D (Alderwick *et al*., 2007; Brennan, 2003). Mycolic acids are unique alpha-branched lipids present in the cell wall of Mycobacterium and Corynebacterium. Mycolic acids are the primary determinant of permeability of mycobacterial cell wall due to its strong hydrophobic nature. Mycolic acid forms a lipid shell around the organism and thus affect permeability properties at the cell surface. Mycolic acids are considered to be an important factor responsible for virulence in MTB, because they defend mycobacteria from the attack of cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They are also known to protect extracellular mycobacteria from complement deposition in serum (Alderwick *et al*., 2007).

Cord factor (trehalose 6, 6'- dimycolate; TDM) is a surface glycolipid, which causes MTB to grow in vitro in serpentine cords. Cord factor is an inhibitor of PMN (Polymorphonuclear neutrophil) migration and is also toxic to mammalian cells. It is most abundantly produced on the surface of virulent strains of MTB while avirulent strains do not have cord factor (Asano *et al*., 1993). The third major lipid 'wax-D' is the major component of freund's complete adjuvant. Apart from wax-D LAM is also an important part of mycobacterial cell wall which has been shown to be involved in phagocytosis of MTB when it is "capped" with short mannose oligosaccharides (ManLAM) (Brennan, 2003; Belisle *et al*., 1997).

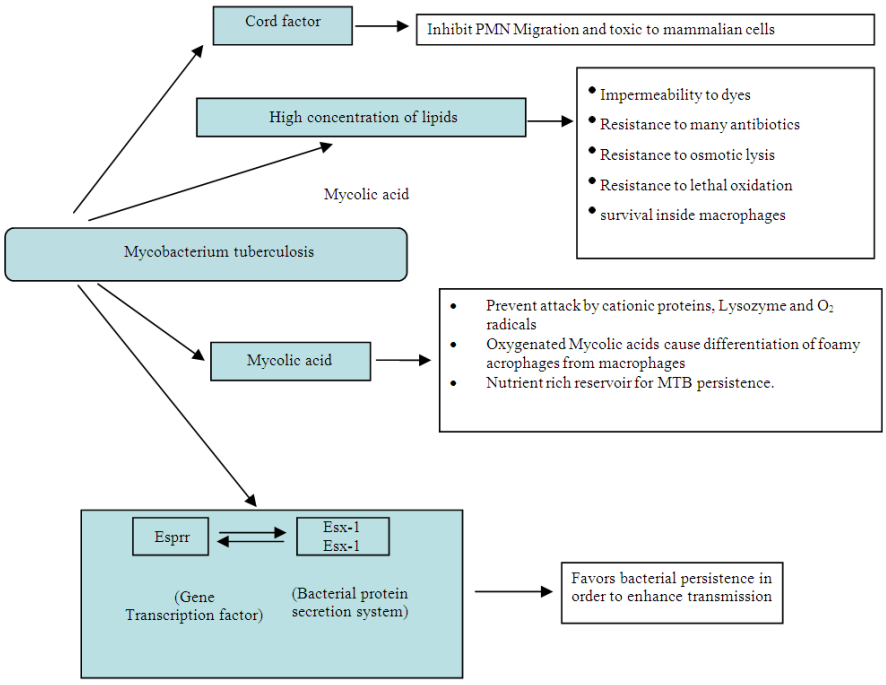

Fig. 1: legend significance of unique characteristic features of *M. tuberculosis* in host immune system

ManLAM is an important regulator of endosomal trafficking since it is known to inhibit phosphatidylinositol 3-kinase and phosphatidlyinositol 3-phosphate binding effectors dependent pathway and thus inhibit phagosome maturation (Fratti *et al*., 2003). Antigen 85 complex is a group of related gene products forming a triad. This complex acts through its fibronectin binding capacities and thus contributes in disease pathogenesis (Meena and Rajni, 2010; Rajni and Meena, 2010). These proteins are also involved in the ultimate stages of mycobacterial cell wall assembly through their mycolyltransferase activity (Belisle *et al*., 1997). Apart from all these unique structural features, porins present in the mycobacterial cell wall also deserves special attention due to its very small pore size compared to other bacteria. These porins act like a diffusion barrier which is 100-1000 times less permeable to hydrophilic molecules and many antibiotics compared to other prokaryotes like *E. coli* (Rajni and Meena, 2010; Kartmann *et al*., 1999). It was suggested that high concentration of lipids in the cell wall of MTB imparts bacterium several properties such as impermeabilty to strains and dyes, resistance to many antibiotics, resistance to killing by acidic and alkaline compounds, resistance to osmotic lysis via complement deposition and resistance to lethal oxidation and survival inside macrophages as shown in Fig. 1 (Alderwick *et al*., 2007; Brennan, 2003).

Modulation of virulence by *M. tuberculosis* h37rv: MTB is one of the most successful pathogens because of its shrewd survival strategy. The extraordinary ability of MTB to persist within host macrophage results from the complex and delicately coordinated process in which it interacts with its host. This process is controlled in part by a specialized bacterial protein-secretion system known as ESX-1. Mycobacteria avoid excessive virulence by maintaining ESX-1 activity, leading to its long term survival (Raghavan *et al*., 2008). Virulence of MTB is mediated by ESX-1, which is also found to occur in other gram positive bacteria. ESAT-6 and CEP-10 are the two proteins secreted by the ESX-1 system. These proteins are the salient targets of the immune system in animals, including humans infected with MTB (Sorensen *et al*., 1995). Studies have shown that during the early phase of infection in mice and in macrophages ESX-1 mutants grow poorly (Patricia *et al*., 2007; Stanley *et al*., 2003). ESX-1 also helps MTB to escape from phagosome vesicles in order to prevent their destruction (Wel *et al*., 2007). It was envisaged that a gene Rv 3849 which is located some distance from the main ESX-1 gene cluster is required for ESX-1 function. EspR is the protein product of Rv3849 which is highly similar to a gene transcription factor of the harmless soil bacterium Bacillus subtilis and is also a DNA-binding transcriptional regulator (MacGurn *et al*., 2005). An important finding has

shown that EspR is itself secreted by ESX-1 (Porcelli, 2008). EspR is a transcription factor that is actively exported from the cell by the same secretion system that it induces. EspR when initially express activate ESX-1 to secrete high levels of virulence promoting proteins, allowing MTB to establish its infection in the host. After infection secretion of EspR reduces its transcriptional activity within the bacterium and diminished ESX-1 activity. This process partially attenuate virulence thus promoting bacterial persistence in order to heightens transmission (Porcelli, 2008).

Role of tumour necrosis factor in the immunopathology of tuberculosis: MTB stimulates variety of immune responses after infection in the host (Flynn *et al*., 1995). CD4 and CD8 T cells have been shown to be responsible for protective immune response to MTB infection (Orme and Collins, 1984; Orme *et al*., 1992; Flory *et al*., 1992; Flynn *et al*., 1992). Different cell types are known to produce TNF-α including macrophages. TNF-α is released upon exposure of macrophages to Lipopolysaccharide (LPS) or other pathogens like mycobacteria (Flynn *et al*., 1995). The release of TNF from macrophages in granulomata occurs via specific sequence of events. Initially IFN-γ is produced by the T-cell mediated response, resulting in the local activation of macrophages. Activated macrophages produces TNF and also gain 1-hydroxylase activity responsible for the conversion of inactive circulating 25-OH vitamin D_3 into the active 1, 25-$(OH)_2$ derivative (Rook *et al*., 1986). This compound fascilitates maturation of macrophages, (Mangelsdorf *et al*., 1984) and heightens the anti- microbial efficacy of human monocytes during MTB infection (Mangelsdorf *et al*., 1984). TNF-α has been associated with potent effects on normal endothelial cells and may also induce intravascular coagulation (Bevilacqua *et al*., 1986; Nawroth and Stern, 1986). Moreover, weight-loss associated with tuberculosis is also due to TNF secretion (Beutler and Cerami, 1986). TNF release is also associated with release of Interleukin-1 (IL-1) which is a pyrogen, (Dinarello *et al*., 1986) and suppresses lipoprotein lipase (Beutler and Cerami, 1986) but induces procoagulant activity in endothelial cells Fig. 2 (Bevilacqua *et al*., 1986). Information obtained from murine models has shown crucial role of TNF in control and survival of MTB infection in mice (Flynn *et al*., 1995).

In view of all these observations it can be envisaged that TNF-α is essential for host immunity. However its overproduction may have severe pathologic consequences, such as fever by direct action of TNF-α on the hypothalamus, (Beutler and Cerami, 1989) marked losses of fat and muscle cells (Tracey *et al*., 1988) weakness, fatigue, nightsweats, lung necrosis and progressive weight loss (Cotran *et al*., 1999). Recently drug thalidomide (alpha-N-phthalimidoglutarimide) has been shown to suppress TNF-α production by human monocytes in vitro, (Sampaio *et al*., 1991; 1993) and it also normalize elevated levels of serum TNF-α in vivo (Sampaio *et al*., 1991; 1993; Sarno *et al*., 1991). Thus there must be a balance in the production of TNF-α to stimulate host immunity.

Granulomas fascilitating spread of *M. tuberculosis* h37rv: Granulomas are the characterisitic lesions of TB, which have long been considered as a host defense mechanism for containing persistent pathogens (Adams, 1976; Egen *et al*., 2008). The first real time images of granulomas was produced in mammals by examining live granulomas in mice infected with Mycobacterium bovis BCG (Egen *et al*., 2008). Their report also identified a role for TNF in maintenance of granulomas. It is also suggested that oxygenated mycolic acids from MTB play an important role in the differentiation of foamy macrophages from macrophages. These foamy macrophages which are granuloma specific cell population are characterized by its high lipid content and could serve as a reservoir for long persistence of tubercle bacillus within its human host (Peyron *et al*., 2008). Recent findings suggest that granulomas commonly known as organized aggregates of immune cells which accumulate at the site of tuberculosis infection are beneficial to the MTB in the early stages of infection. During early stages of granuloma formation macrophages are highly motile and the early granuloma in reality benefits the MTB by recruiting macrophages to the site of infection, thus providing a constant supply of susceptible host cells to infect (Davis and Ramakrishnan, 2009). Uninfected macrophages are actively recruited to the site of infection by the infected macrophages (Bold and Ernst, 2009). Phagocytosis of infected cell remnants and their bacterial contents by uninfected macrophages fascilitates efficient spread and expansion of the bacterial population (Bold and Ernst, 2009). Mycobacterial RD-1 locus has been thought to be responsible for the efficient recruitment of uninfected macrophages to the site of Mycobacterium marium (M. marium) infection (Davis and Ramakrishnan, 2009). It was observed that RD-1 deficient M. marium recruit seven fold less uninfected macrophages to the site of infection than virulent M. marium. MTB is cosidered as one of the most successful bacterium that infects macrophages and dendritic cells and hence is able to counteract and utilize host immune responses (Davis and Ramakrishnan, 2009).

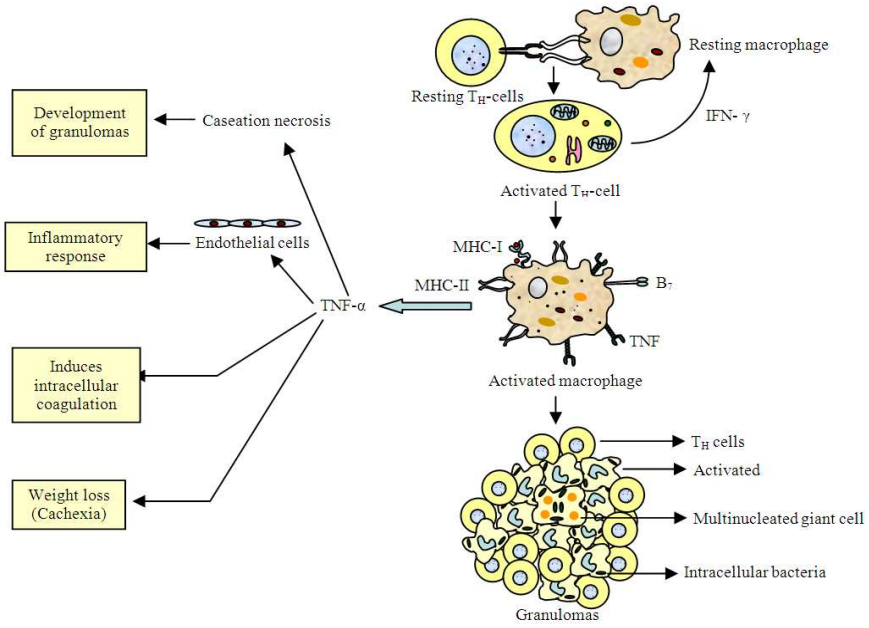

Fig. 2: legend TNF-α in the immunopathology of tuberculosis

Although immune system consisting of adaptive immunity ($CD4^+$ and $CD8^+$ T cell) is able to protect host from foreign particles but its not able to eradicate tuberculosis infection, which results in latent infection (Bold and Ernst, 2009). At the same time MTB also possess mechanisms to confine apoptosis (Davis and Ramakrishnan, 2009). Development of an effective tuberculosis vaccine requires realizing the shortcomings of immune responses to MTB and the virulence mechanisms it employs to circumvent immunity.

***M. tuberculosis* h37rv adenylate cyclase intoxicates macrophages with cyclic AMP:** MTB has evolved highly specialized mechanism to proliferate in the host during infection. Subversion of host cell signal transduction is the common strategy employed by MTB by the introduction of enzymes that modulate the levels of secondary messengers such as cAMP (Baker and Kelly, 2004; Sands and Palmer, 2008). MTB inhibits the host phagosome maturation process to survive intracellularly (Walburger *et al*., 2004; Axelrod *et al*., 2008). It also arouses an immune response giving rise to caseous necrosis and the formation of solid granulomas (Hunter *et al*., 2007). It was suggested that these lesions may fascilitate chronic contained infection (Baker and Kelly, 2004; Sands and Palmer, 2008; Walburger *et al*., 2004). It was observed that infection with the MTB mutant lacking the adenylate cyclase gene Rv0386 resulted in cAMP levels that were fivefold lower than those observed with wild type MTB (Agarwal *et al*., 2009). Data has shown that Rv0386 is required for full virulence in the mouse model and loss of this adenylate cyclase gene results in a macrophage infection that is devoid of a cAMP burst. Rv0386 mutant elicited sharply reduced CREB phosphorylation signals after macrophage infection, which is equivalent to cAMP levels in uninfected macrophages. It also resulted in reduced TNF-α secretion, which is 40-50% below wild type levels. Low levels of TNF-α are associated with immune containment of latent MTB, (Keane *et al*., 2001; Chakravarty *et al*., 2008) while in active disease high levels of TNF-α cause caseation necrosis leading to the development of granulomas.

Dormancy in *M. tuberculosis* h37rv: MTB latent bacilli are microorganisms that are able to survive within the hostile environment of macrophages and adapt themselves to the stressful conditions generated by host defense system. By slowing metabolism and becoming dormant MTB counterbalances these conditions and appear as silent to the immune system (Cardona and Ruiz-Manzano, 2004). The dormant form of tubercle bacilli is the major block for tuberculosis treatment. Evidence from murine models of tuberculosis have shown progressive infection in animals unable to produce the inducible isoform of NO synthase and in animals treated with a NO synthase inhibitor. It was concluded that O_2 and low, nontoxic concentration of NO competitively modulate the expression of a 48- gene regulon, which expresses in

vivo and prepares bacilli for survival during long periods of in vitro dormancy. According to data obtained from experiments it is postulated that inhibition of respiration by NO production and O_2 limitation within granulomas restricts MTB replication rates in individuals with latent tuberculosis (Zhang *et al*., 2001). Gradual reduction in O_2 concentration leads to a non replicating persistent state characterized by bacteriostasis and metabolic, chromosomal and structural changes in the dormant bacteria. It was observed that even after long periods of hypoxia induced bacteriostasis, provision of O_2 results in resuscitation of the dormant bacteria (Zhang *et al*., 2001).

CONCLUSION

It is quite striking how MTB has developed so much variation in its cell wall from other gram positive bacteria. These unique cell wall components have different functions after recruitment into host cell. The high lipid content of cell wall imparts impermeability to various compounds including resistance to numerous drugs. Moreover, MTB bacilli also release mycobacterial products which directly or indirectly can affect local immune responses. Apart from these, dormant state of MTB is one of the most remarkable feature of this pathogen which is closely associated with the ability of the pathogen to survive host inflammatory responses and antibiotics. Establishment of this dormant state requires unique regulatory mechanisms and signals to enter and exit dormancy. The more we will understand the processes involved in cell wall metabolism, cell division and growth signaling, the more we will be able to design a novel drug effective against the dormant MTB.

ACKNOWLEDGEMENT

We thank Dr. Rajesh S. Gokhale for making this work possible. The authors acknowledge financial support from GAP0050 of the DST (Department of Science and Technology) and CSIR (Council of Scientific and Industrial Research).

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