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Miscellaneous Paths induced by *Mycobacterium Tuberculosis* for its Reactivation and Dissemination from the Freckled Site –Granuloma

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***Abstract*— In present times, emergence of Extra pulmonary tuberculosis (EPTB) are serious hurdle in diagnosis and control of human tuberculosis (hTB) cases. Regulation of disease especially in cases of EPTB (disseminated/ localized), requires the understanding of potent mechanisms induced by *Mycobacterium tuberculosis* (*M.tb)* and therapeutic strategies targeting dissemination mechanisms induced by *M.tb*. Pathogenesis of tuberculosis is characterized by formation of granuloma, site from where *M.tb* either get regulated or reactivated. Though cell- mediated immunity (CMI) of the host is enough to control progression of the disease, however, it fails to exert 'sterile eradication'. *M.tb* resides inside the macrophages and is relatively resistant to microbicidal mechanisms that efficiently eliminate other phagocytosed bacteria. TB bacilli escape macrophage attack and migrate to extracellular regions. Present paper summarizes various mechanisms and genes involved in the dissemination of *M.tb* to other organs and reviews strategies for the regulation of dissemination of *M.tb* infection in humans.**

***Keywords*— Extra pulmonary tuberculosis; granuloma; Immune system; Chemokines; Tumor necrosis factor-alpha**

INTRODUCTION

So far researchers have been focusing on the morphology and hypoxic condition of granuloma, which influences the physiological mechanisms of the bacilli. Initiation of granuloma formation has been relatively well-described in the literature but very little attention has been paid on the functional aspects of granuloma. Ineffective granuloma formation with the development of necrosis, liquefaction of necrotic areas and subsequent merging of mycobacterial rich granulomatous contents in the airways leads to dissemination of disease. But still there are many unanswered questions related to the adaptation mechanisms of *M.tb* that assist in the dissemination and reactivation. Though bacillus is an obligate aerobe how it survives in the anaerobic conditions and is able to differentiate is a big question. After granuloma formation and going in to the latent phase, how bacillus reactivates during un-favourable conditions?

In the anaerobic condition bacilli can survive in the latent condition but not able to replicate, if the number of bacilli gets increased at the site of infection, since supply of oxygen is essential for the survival[1]. Reduced bacterial growth due to decreased oxygen availability may contribute to the overall reduction in the burden of bacilli[2]. It is believed that *M.tb* adapts itself or induce the mechanisms in such a way that it gets oxygen supply and becomes capable to differentiate as well as escapes from the granuloma. *M.tb* adapts so many strategies to stay alive, differentiate and disseminate.

IMMUNE RESPONSE AGAINST *MYCOBACTERIUM TUBERCULOSIS*

It is clear that the immune system reacts efficiently in cases of the vast majority of infections. This is particularly evident in the case of TB, where most people infected by the tubercle bacillus (90%) do not develop the disease throughout their lifetimes. Nevertheless, the risk of developing the disease increases considerably when TB infection co-exists with an alteration in the immune system, such as co-infection and late response following the dissemination of infection to other organs from lung. Even though macrophages are the main targets of infection by *M.tb* bacilli, it has been proposed that other cell populations

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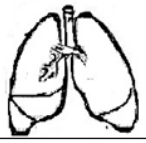
can also be infected by mycobacteria and therefore may be important in the development of the disease. Neutrophils have well-characterized microbicidal mechanisms such as those dependent on oxygen and the formation of neutrophil extracellular traps[3]. Therefore, these cells are thought to contribute to the control of infection through production of chemokines, induction of granuloma formation[4] and transference of their own microbicidal molecules to infected macropages[5]. Regardless of bacterial virulence factors involved, two specific mechanisms are known to increase the likelihood of reactivation, first quantitative and qualitative depletion of CD4+ T cells, other is represented by impairment of TNF-α signaling.

HOST FACTORS RESPONSIBLE FOR DISSEMINATION

Nine to eleven days after aerosol inoculation in mice, *M.tb.*, bacilli disseminate to the pulmonary lymph nodes (LN), where *M.tb.*, specific T cells are detected within 2 to 3 days. This indicates that initial spread of bacteria occurs via lymphatic drainage and acquired T-cell immune response is generated in the draining LN[6]. Dissemination to peripheral sites, such as spleen and liver takes place from 11 to 14 days post infection and is followed by the appearance of *M.tb* specific T cells in lungs and spleen[7].Mature dendritic cells at chronic hypoxia (4 days, 1% O2) that promotes the onset of highly proinflammatory gene expression[8] which have an increased ability to induce neo-vascularization and inflammation compared to their normoxic part[9]. The activity of TNF-α induces a cytokine storm, which supports the release of chemokines that recruit blood-borne monocytes, T-cells, B-cells, fibroblasts and other cells[10]. Anti-TNF-α agent, adalimumab causes disseminated TB in non-human primates. The alteration of macrophage function and reduction of CD8+CCR7+CD45RA+ effector memory T-cells are likely mechanisms involved in the promotion of TB disease during TNF neutralization treatment[11] also leads to the dissemination. In human, cavitation (extensive necrosis) occurs in lung apices where there is high chance of dissemination of bacilli from lung[12].Wall of the cavity consists of an external zone of collagen, the cavity's capsule, and an internal zone of softening caseum where, because of the direct connection with the airways, the high oxygen content favors the intense multiplication of tubercle bacilli. *M.tb*., residing in the cavity walls, where their active replication is promoted by increased access to oxygen, can not only be disseminated outside the host with sputum coughed out but also reach the lung parts distant from cavities and, probably, other organs, migrating with alveolar macrophages[13]. At one extreme bacilli discharged from the cavity are ingested by non-activated macrophages, in which they temporarily grow until tissue-damaging hypersensitivity kills the bacillus-containing macrophages and destroys nearby tissues[14]. However, in a host with fine cell mediated immunity, immunologically specific T cells and their lymphokines activate macrophages, which are then able to kill intracellular bacilli without excessive tissue damage. Inflammatory dendritic cells involves in the dissemination of granulomatous inflammation[15]. Lower IFN-γ levels, in particular, critically impacts on macrophage responses by suppressing phagosome maturation and promoting mycobacterial survival[16]. It is generally thought that lungs and their draining lymph nodes are the source of extra-pulmonary dissemination. However abdominal lymph nodes might also play a role in the dissemination of bacilli[17].

Table-1

M.tb through droplets from infected one enters into the lung



Lungs contain about 40 types of cells. Only macrophages and dendritic cells are activated against *M.tb* primarily

Dendritic and macrophages cells carry mycobacteria to lymphnodes via lymphatic vessels.

Naïve T cells are activated to mycobacterial antigens and immune cells migrate to the infection site to form granuloma

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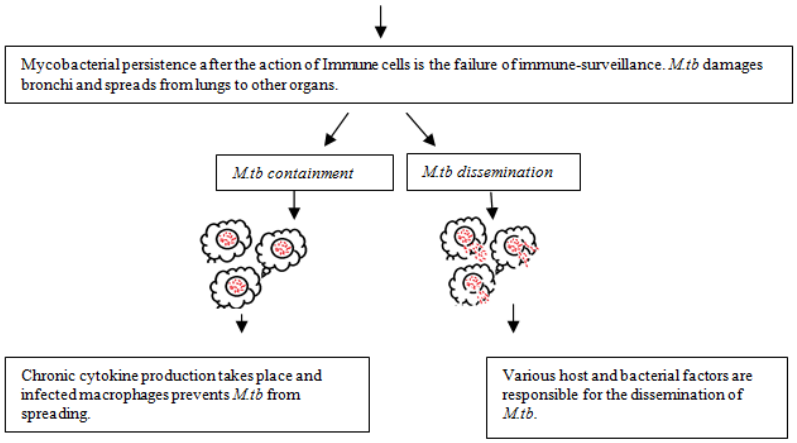

S.No	Host factors involved in dissemination of TB.	mechanism	References
1	*MBP, (IL)-1p/IL-1R, TIRAP* genotype C558T, *P2X7, IL-10, TLR2 genotype T597C, NRAMP1, STAT-1, IFN-γR1,*	Due to genetic polymorphism and mutations	52,55,56,57
2	CD8+CCR7+CD45RA+ effector memory T-cells	By neutralizing TNF-α	11
3	*VEGF*	Drives angiogenesis	23
4	ELR+ chemokines	Drives angiogenesis	37
S.No	**Bacterial factors involved in dissemination of TB.**	**mechanism**	
1	mce 2 and mce 3	Unknown mechanism	62
2	hbhA	Responsible for reducing the bacterial adherence to cells.	58
3	Rv0311,Rv0805,Rv0931c, Rv0986, Rv0805 and Rv0986	Unknown mechanism but dissemination is seen in these mutant strains infected individuals.	60
4	WhiB4	Sense oxidative stress response, modulate survival in macrophages and helps in dissemination	59
5	*Mel2*	Increases susceptibility to ROS	60
6	*PKS derived PGL*	Unknown mechanism	61
7	*Igr* region	Deletion of this region induces dissemination. This region containing six genes are shows defect in bacterial intracellular growth.	62
8	*TDM*	Cyclopropane modification, limits phagolysosome fusion drives angiogenesis.	28
9	AM-Ag85b	Inhibits antigen presentation	29

VASCULARIZATION AS ONE OF THE DISSEMINATION FACTOR

Tuberculous granulomas are extensively vascularized[18]. Angiogenesis triggered by mycobacterial granulomas might have possibly important consequences for the pathology of infection and progression. Angiogenesis and lymphangiogenesis are classical features of granuloma formation in pulmonary tuberculosis[19]. Pathogenic angiogenesis possesses a complex relationship with TNF. Short TNF pulses early in inflammation can prime endothelial cells for sprouting and proliferation while simultaneously blocking *VEGFR* signaling, delaying but ultimately promoting angiogenesis[21]. Metabolic demands are thought to regulate vascularizaton 2of tissues and tumours (which are thought to be similar to granulomas). Hypoxia upregulates *vegf* owing both to increased transcription mediated by hypoxia inducible factor-1 and an increase in VEGF mRNA stability dependent on 3' region

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of mRNA[22]. VEGF is responsible for angiogenesis in granulomatous inflammation and is produced by macrophages to induce an immune recruitment response when mycobacteria enter the airway can be used as a biomarker[23]. As granulomas mature, they induce angiogenesis and vascular permeability that aids mycobacterial growth[24]. Anti-VEGF antibody for cancer and eye diseases is able to create more structurally and functionally normal granuloma vasculature and improve the delivery of a low-molecular-weight tracer[25]. By administering the VEGFR antagonist pazopanib established infections could be treated therapeutically, with reductions in vascularization and bacterial burdens[26] and limited granuloma vascularization diminished bacterial dissemination from established infections. First, it appears that a VEGF-mediated, granuloma-induced angiogenic program is ultimately beneficial to mycobacteria. Just as bacterial induction of granulomas may provide a new niche for cell-to-cell spread, so does the induction of angiogenesis favor bacterial replication by providing additional oxygen source. Necrotic granulomas, typically associated with worse disease outcome, had decreased burden in animals treated with anti-angiogenic agents.

MECHANISMS INDUCED BY PATHOGEN *(M.TB)* FACTORS

Mycobacterialy induced angiogenesis at the granuloma promotes bacterial growth *in-vivo*, nascent blood vessels associated with early granulomas may provide oxygen and nutrients and can create a permissive replication niche for the bacteria. By counteracting the *M.tb*-induced pro-inflammatory leukocyte response, platelets may protect against excessive tissue damage, but may also compromise the production of protective cytokines, such as IFN-γ and TNF-α. TDM in *M.tb* is an adjuvant and is associated with mycobacterial disease pathogenesis, including phagolysosome fusion and activation of matrix metalloproteases[27]. TDM can induce angiogenesis in rat corneal mode. Role of TDM in angiogenesis driven mycobacterial expansion via *Vegf* induction and identifying the proximal cis-cyclopropyl modification of mycolic acid tails is as crucial as TDM-mediated angiogenesis. It is becoming increasingly evident that a multi-faceted mechanism is involved in blood vessel development[28]. Specific antibodies to AM and Ag85b contribute to control bacillary dissemination[29]. Microbial products stimulate mast cells via two members of toll-like receptor family, TLR-2 and TLR-4. It was demonstrated that there is an interaction between mast cells and *M.tb.*, through CD48 molecule. This interaction triggers release of preformed mediators which are involved respectively in the activation of neutrophils and maintenance of integrity of granuloma. In contrast to the normal phagocytosis, during which phagosomal content is degraded upon fusion with lysosomes, mycobacteria block this process[30].

BACTERIAL FACTORS RESPONSIBLE FOR DISSEMINATION

M. tb may have virulence factors that promote CNS dissemination, distinct from those required for pulmonary TB. Two products of *M. tb.*, RD1 gene locus, early secretory antigenic target 6 kDa (ESAT-6) and culture filtrate protein 10 kDa (CFP-10), have been linked to cell lysis and may enable bacilli to invade and spread within alveolar epithelium[31]. Recent studies in embryonic zebrafish indicate ESAT-6 may also stimulate trafficking of infected macrophages within granulomas, thereby promoting early dissemination of bacilli. 28-kDa heparin-binding haemagglutinin adhesin (HBHA), required for extrapulmonary dissemination of the bacilli, *M. tuberculosis* HBHA induces receptor-mediated endocytosis and epithelial transcytosis, which may represent a macrophage-independent extra-pulmonary dissemination mechanism leading to systemic infection by *M.tb*. *M. tb.*, mel2 locus plays a role in persistence within lungs and dissemination or survival within extrapulmonary tissues. *mel2* mutant does not differ from the wild type in survival/ growth in lungs during the first 4 weeks post infection but does display a defect in persistence. Interestingly, bacillary numbers in spleen at 4 weeks were less for the *mel2* mutant than the wild type, suggesting that *mel2* plays a role in the dissemination and/or susceptibility to extrapulmonary immune components[32]. WhiB4 has been postulated to act as a sensor of oxidative stress. *M. tb* ΔwhiB4 showed a defect in dissemination to guinea-pig spleen, suggesting that whiB4 is essential for successful dissemination. It is likely WhiB4 regulates oxidative stress response to modulate survival in macrophages, and thus helps bacterial dissemination (Chawla et al., 2012).

TNF- α ROLE IN REACTIVATION AND DISSEMINATION OF *M.TB*

Failure to sustain focused bactericidal activity within granulomas is accompanied by reduction in overall cytokine profile that include IFN-γ, IL-12p70 and IL-10. Clinically, similar findings were noted in reactivating tuberculosis patients on anti-TNF therapy, who presented with a decreased T cell activation profile and reductionin IFN-γ and IL-10 synthesis[33]. Macrophages are key in establishing latency via TNF production, while T cell–derived TNF is essential, but not sufficient, for protection against *M.tb.*, infection[34].There is limited data indicating fraction of TNF

that is cleaved into sTNF in vivo. Considering relative transient expression of tmTNF in vitro, it was assumed that cleaved TNF is approximately 95% (i.e., only 5% TNF is transmembrane) so that the majority of tmTNF is cleaved and released in its bioactive soluble form[35]. Different percentages of sTNF effect on bacteria load, System gradually shifts to higher bacterial loads with decreasing amounts of sTNF. This transition arises through oscillations that push the system to active TB when sTNF is almost completely deleted (where sTNF is,3% of total TNF). System reactivates when almost no sTNF is released. This suggests sTNF is necessary to maintain latency, likely because of its crucial role in lymphocyte and macrophage recruitment, and that tmTNF is not sufficient to maintain latency in humans, as seen in mice[36]. sTNF is necessary to maintain latency. Intracellular bacilli released after macrophages death induced by antibody binding to tmTNF (whether dependent on tmTNF reverse signaling or complement cascade) can only facilitate bacterial clearance by the host and does not enhance dissemination. But till now it is not clear that how the solTNF functions during the active tuberculosis.

ANGIOGENIC ELR+ CHEMOKINES

ELR+ motif containing CXC chemokines are best known for its chemotactic activity towards neutrophils and monocytes/macrophages is also a potent angiogenic factor[37]. Macrophages are known to form columns and tubes *in-vitro* and *in-vivo* frequently preceding neo-vessel formation. NF-kappaB is activated in endothelial cells exposed to VEGF, and if the activity of NF-kappaB is blocked, the expression of CXC chemokines such as CXCL-8 and CXCL-1 is down regu. It regulates the expression of CXC chemokines that have critical roles in the regulation of angiogenesis during many pathological processes. NF-kappaB decoy ODN(drug) treatment down-regulated CXCL-1, CXCR2, VEGFR2 mRNA in endothelial cells and decreased VEGF protein expression in lesions[39]. In cattle infected intranasally with *Mycobacterium bovis*[40], lesions were not observed in the draining lymph nodes until 14 days post infection. It may be concluded that lymph nodes become involved early in the infection, even at a low dose (7 cfu), highlighting the importance of early dissemination of tuberculous bacilli via lymphatic system to the hilar lymph node[41]. One mechanism of disease progression in extra pulmonary-TB may be a less effective balance of IL-10 in the presence of elevated proinflammatory chemokines CCL2 and CXCL9[42]. Cxcr3.2 deficiency limited the macrophage-mediated dissemination of mycobacteria. Furthermore, the loss of Cxcr3.2 function attenuated the formation of granulomatous lesions and led to a reduction in the total bacterial burden. Prevention of mycobacterial dissemination by targeting the CXCR3 pathway might represent a host-directed therapeutic strategy for treatment of tuberculosis. The delay of adapted immune response permits bacterial growth and dissemination in the late stages of disease[43]. Direct target or complete inhibition of such host factors might show the adverse effect in spite of decreasing the disease progression. Strong suppression of Reg3g and the inflammatory chemokines Ccl2 and Cxcl5 and activation of classical complement pathway factors C1r, C1s, C2, and C3 occur with dexamethasone treatment, effects absent with anti-Vegf treatment[44]. CXCR4/ CXCL12 signaling axis can induce angiogenesis and progression of tumors by increasing expression of VEGF through the activation of PI3K/Akt pathway[45]. CXCR4 antagonist TN14003 blocked the CXCL12 induced phosphorylation of Akt in a dose-dependent manner.

DISSEMINATION THROUGH SPECIFIC STRAIN

There is evidence that bacteria from the Beijing lineage, many of which do not produce PGL, are more capable of causing severe, disseminated tuberculosis in humans. C allele of TLR-2 T597C allele were more likely to have tuberculosis caused by the East-Aian/Beijing genotype than other individuals[46]. It is evident that *M.tb.,* genotype influences clinical disease phenotype and demonstrates, for the first time, significant interaction between host and bacterial genotypes and development of tuberculosis. Some strains of *M. tb.*, commonly found in Europe and America are less likely to cause tuberculous meningitis in Vietnamese adults than strains predominantly found in Asia. We then looked at the interaction between *M. tb.*, strains and mutations in human immune genes and show that particular mutation, TLR2 T597C, is more commonly found in patients infected with the East-Asian/Beijing strains of *M. tb*[47]. CDC1551 disseminate to the CNS more than the H37Rv strains[48]. Extra-pulmonary disease is prominent feature of the guinea pig model and dissemination to organs not normally assayed such as heart and adrenal glands should be taken into account in the assessment of disease process. While lungs are the first organ to be affected by *M. tb.*, infection spreads rapidly to extra-pulmonary sites by lymphatic and hematogenous dissemination 10.55AWBeijing strain, HN878, responsible for an outbreak of tuberculosis in Texas, USA, was found to be hypervirulent in mice and induced low concentrations of TNF-α from macrophages[49]. High amount of both G-CSF and GM-CSF by the F15/LAM4/KZN and F28 strains may be responsible for extensive lung damage, leading to increased infectivity and dissemination of pathogen in the lungs, high concentrations of IL-6, IFN-γ, TNF-α and G-CSF at 48 h, and IL-8, IFN-γ, TNF-α, G-CSF and GM-CSF

at 72 h, were induced by F28 and F15/LAM4/KZN strains, respectively. PTGS2 induction in response to virulent *H37Rv* compared with avirulent *H37Ra*, which, combined with our observations, may implicate reduced efficacy of the downstream sequelae of glycolytic reprogramming in increased susceptibility to the virulent strain. *Lta4h* knockdown increased burden and limited angiogenesis along decreased dissemination, suggesting a role for angiogenesis in dissemination independent of burden[51].

DISCUSSION

Various immune cells surround the infected alveolar macrophages to form a mycobacterial granuloma, which acts as a "physical barrier" limiting bacillary dissemination. In spite of this host and the bacterial factors are responsible for the escape of *M.tb* from the granuloma of lung to other body organs. Targeted therapy of these host and bacterial factors along with vascular normalization in combination with anti-TB drugs has the potential to enhance treatment in patients with extrapulmonary TB. This will be helpful in decreasing bacterial load, decreasing extra pulmonary TB cases and maintaining constructive granuloma structure.

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