Impact of diabetes on mechanisms of immunity against Mycobacterium tuberculosis

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Abstract
Tuberculosis (TB), caused by infection with mycobacterium tuberculosis, is a major source of morbidity and mortality in Pakistan. Diabetes caused by imbalance in glycaemic control is also highly prevalent in the country. The co-incidence of both diseases results in worsening outcomes of TB, making treatment and management more difficult. Both innate and adaptive arms of the host immune response are required for protection against M. tuberculosis infection. Host immunity is modified in diabetes mellitus type 2 where key pathways such as, the T cell driven interferon-gamma responses to M. tuberculosis antigens and other T cell and macrophage activating cytokines are suppressed. This makes diabetes with TB a more severe disease and results in worse treatment outcomes. Effective coordination between T cells and host macrophages is required for control of TB infection. Therefore, early identification of diabetes and management of hyperglycaemia during TB treatment is essential for favourable outcomes.

Keywords: Host immunity, Tuberculosis, Diabetes, Latent TB.

Introduction
Tuberculosis (TB) remains an important cause of morbidity and mortality worldwide, with 10 million new cases having been reported in 2016 and a mortality rate of 17/100,000 population globally.1 Morbidity due to TB is increased in the presence of diabetes. Combatting this double burden seems daunting as mechanisms underlying this increased susceptibility of bacterial infection to diabetics remain elusive.2 Clinical evidence suggests that patients with TB and diabetes (DM) co-morbidity are likely to have cavitary lung lesions and four-fold increased risk of relapse when compared to patients without risk factors.3 The increased risk of developing TB is attributed to multiple mechanisms that occur in diabetic patients. This review focuses on the modulation of host protective responses that occurs due to diabetes.

Host Immunity against Mycobacterium tuberculosis
Upon infection of the respiratory tract by mycobacterium (M) tuberculosis, the host mounts an inflammatory response, causing the recruitment of neutrophils, macrophages, monocytes, natural killer (NK) cells and dendritic cells in order to contain the bacteria.4 Alveolar macrophages in the lower respiratory tract phagocytose the bacteria by engulfing it in a membrane-bound vacuole. This phagocytosis and subsequent antigen presentation then allows for the development of the adaptive immune response, mediated through T cells; cluster of differentiation (CD)8+ cytotoxic and CD4+ type 1 T helper (Th1) cells. All these factors undergo complex interactions in order to form granulomas surrounding the mycobacterium.5

Each component of the early immune response acts to protect the host from M. tuberculosis (Mtb) infection. Activated neutrophils use the rapid release of reactive oxygen species (ROS) and proteolytic granules in order to both directly attack invading pathogens and indirectly active neutrophil extracellular traps to kill the bacteria.6 They also regulate surface receptor expression and cytokine and chemokine secretion, thereby affecting the downstream host immune response. However, during chronic Mtb infection, neutrophils may delay the clearance of bacteria.7

In response to infection, circulating monocytes are recruited to the affected site through the action of neutrophil-induced cytokines and chemokines where they differentiate into macrophages.8 Macrophages use phagocytic and anti-microbial mechanisms to defend the host, as well as specific cytokine profiles which induce different cell-mediated immune responses. M1 macrophages induce up-regulation of pro-inflammatory factors such as inducible nitric oxide synthase (iNOS) and inflammatory cytokines.9 These cytokines, particularly interleukin (IL)-12 and IL-8 subsequently elicit an interferon gamma (IFN-γ) response from NK and T cells, thereby establishing Th1 cell-mediated immunity. Further macrophages are activated and along with iNOS and nicotinamide adenine dinucleotide phosphate (NADPH)
oxidase, act to kill the invading bacteria. NK cells act at the site of infection to increase IFN-γ production and lysis of Mtb infected cells. They modulate T cells in favour of the Th1 response, thereby enabling the host to fight off the invading infection through CD8+ T cell-derived IFN-γ production and cytolytic activity.

Dendritic cells act at the site of infection in order to uptake the bacteria and process, and upon maturation, present the antigens. They produce immune-regulatory cytokines such as IL-12 and IL-18, thereby aiding the Th1 response. Mtb can evade the immune response by suppressing the traffic of dendritic cells to the lymph nodes, thereby preventing their maturation.

An early Th1 response offers significant protection against Mtb infection. CD4+ T cells regulate the immune response and contribute to inflammation in order to protect the host. Th1 and Th17 cells are the main effector CD4+ cells in the response to infection by Mtb. Th1 cells activate the anti-mycobacterial properties of macrophages and secrete IFN-γ whilst Th17 cells activate neutrophilic inflammation and act to mediate tissue damage.

**Diabetes**

Diabetes Mellitus (DM) is a multifactorial metabolic disease primarily characterised by insulin resistance, glucose intolerance and hyperglycaemia. The aetiology involves a complex repertory of several genetic and environmental factors that predispose to higher blood glucose levels and free fatty acids. Advances in understanding the aetiology of type-2 DM (T2DM) have established the involvement of immune system and have questioned the classification of T2DM as a purely metabolic disease.

Studies have shown that several alterations occur in glucose and lipid metabolism leading to shift from an anti-inflammatory to pro-inflammatory milieu which makes the diabetic host more susceptible to bacterial infections.

Glucose and lipid metabolism is altered in adipocytes and hepatocytes, leading to a pro-inflammatory state. Hyperglycaemia accelerates formation of advanced glycation end-products (AGE) leading to an increase in the production of ROS. The increased levels of ROS are regulated poorly in T2DM individuals due to a decrease in reduced glutathione (GSH), which is exacerbated by the decreased ability to regenerate GSH caused by increased consumption of NADPH, a co-factor essential for this regeneration. This chronic low grade inflammatory state is worsened by increased levels of pro-inflammatory cytokines which are released by stressed adipocytes and macrophages present in visceral adipose tissue as well as by neutrophils and monocytes. These cytokines include tumour necrosis factor-alpha (TNF-γ), C-reactive protein (CRP), IL-1β, IL-6, IL-8 and IL-12. These heightened levels, along with the increased IFN-γ expression found in adipose tissue, lead towards the development of insulin resistance, which contributes to the shift of the anti-inflammatory M2 macrophages towards the M1 pro-inflammatory subset.

These changes mainly include adipocyte hypertrophy which eventually reduces the production of anti-inflammatory adiponectin, increased levels of pro-inflammatory cytokines (IL-6, TNF-γ and IL-1β) and increase infiltration of M1 macrophages, CD8+, CD4+ T-cells in the adipose tissue. This pro-inflammatory cytokine spillover eventually leads to immuno-compromise and has a significant role in the development of insulin resistance. These mechanisms are related to hyperglycaemia and insulinopenia, thereby indirectly causing immune dysfunction by indirectly affecting lymphocyte and macrophage dysfunction.

The altered immune response in individuals with DM is associated with an increased susceptibility to TB, leading to an increasing double burden of these diseases, particularly in endemic countries. Aside from affecting both the innate and adaptive immune responses DM leads to a failure to mount a robust response to invading bacterial infections. These factors may alter the predisposition of DM individuals towards active disease.

**Immune Dysfunction in Diabetic Patients**

Diabetes causes a dysfunction in the early immune response to infection. The neutrophil response is predominantly suppressed, and the impaired activity of glutathione (GSH) reductase leads to a decrease in the efficiency of neutrophil-based ROS production. Monocytes and macrophages in DM patients have poorer phagocytic and antimicrobial functions against Mtb and produce less cytokines in response to infection. However, inflammatory cytokine production in unstimulated macrophages is increased, thereby adding to the pro-inflammatory state. These changes may cause poor containment of the bacteria in initial infection stages as well as alterations in T cell immunity. Poor glycaemic control leading to an increase in high glucose levels inhibits lectin binding, thus causing poorer pathogen recognition and phagocytosis in DM patients.

The pathogenesis and complications of DM are closely associated with defective glucose metabolism, obesity, cardiovascular disease and inability to launch an effective immune response.
immune response. Toll like receptors (TLRs) and inflammatory mediators such as IL-6, TNF-\(\gamma\) and IL-1\(\beta\) dysfunction have been associated with T2DM. It has been shown that where there is good glycaemic control, cytokine and TLR gene expression are improved compared to patients who do not have good glycaemic control.\(^{31}\)

**Impact of diabetes on immunity against M. tuberculosis**

The primary effector cells required to limit Mtb are phagocytes and lymphocytes. Diabetes is known to affect the chemotaxis, phagocytosis, activation and antigen presentation by phagocytes when exposed to Mtb. Studies have shown that patients with poor glycaemic control (glycosylated haemoglobin [HbA1c]>7%) had an increased risk of TB activation compared to patients with normal glycaemic level.\(^{24}\)

In DM hosts, there is a delay in activation of Th1 cell-mediated immunity, leading to dysfunction in the adaptive immune response.\(^{28}\) In co-morbid TB-DM patients, increased levels of Th1 and Th17 cytokines are found.\(^{32}\) The efficacy of the Th1 response is reduced by impaired cellular interactions and the inability to mount a response during infections in DM hosts. The unregulated increase in Th17 response cytokines leads to an increase in the dissemination of the bacteria through IL-17 secretion and neutrophil recruitment.\(^{33}\) Elevated levels of regulatory T cells (Treg) and Th2 type cytokines in TB-DM patients lead to a shift towards the Th2 responses which contributes to the susceptibility of the DM host to infection by reducing the helpful Th1 responses and allowing for increased bacterial persistence.\(^{34}\)

Patients with DM are at increased risk of developing TB.\(^{35}\) It has been hypothesised that susceptibility to mycobacterial infection is due to defective Th-1 cytokine response.\(^{25}\) Ex-vivo whole blood assays have shown that patients with active TB have lower IFN-\(\gamma\) levels and higher production of other pro-inflammatory cytokines and IL-4. However, diabetic patients without TB showed reduced non-specific IFN-\(\gamma\) production, which is essential for inhibition of initial growth of Mtb. Hence, it is thought that defective non-specific immune response in DM may contribute to increased susceptibility to develop TB.\(^{35}\)

Furthermore, in order to assess the molecular dynamics between the Mtb and the innate immune system, in-vitro studies have compared the interaction of Mtb with human blood monocytes in patients with and without DM. These studies were conducted in the presence of autologous serum containing the Mtb, which can enter monocytes via both serum-dependant and independent pathways. The findings in the study indicated reduced binding and phagocytosis of Mtb by monocytes from DM cases relative to non-DM controls. This difference was higher in samples with a higher concentration of autologous serum, suggesting that DM monocytes are defective in restricting Mtb entry. It is thought that these defects lie in the serum complement factors leading to component 3B (C3B) / inactive component 3B (iC3b) opsonisation or its receptor.\(^{2}\)

Moreover comparison of monocyte surface marker expression between TB-DM and TB-no DM reveals a higher expression of chemokine (C-C motif) receptor 2 (CCR2) in TB-DM patients which is in concert with the up-regulation of its ligand chemokine (C-C motif) Ligand 2 (CCL2).\(^{36}\) Despite this correlation, further research is required to determine if higher expression of CCR2 has a mechanistic role in restraining the diabetic monocytes to the site of Mtb infection in the lung.

Vitamin D is important for appropriate immune function and, hence, plays a role in immunity against infections.\(^{37}\) Low serum D levels in patients with TB have been associated with lower C-X-C motif chemokine 10 (CXCL10) responsiveness to Mtb infection.\(^{38}\) Recent reports have associated low vitamin D levels in T2DM patients with a reduced immune response against Mtb.\(^{39}\)

Anti-microbial peptides (AMPs; cathelicidin LL37, human neutrophil peptide-1 and human beta defensins) are a key component of innate immunity against pathogens and play a role in the destruction of pathogens engulfed by phagocytic cells.\(^{40}\) AMPs are activated by Mtb infection.\(^{41}\) Studies have revealed reduced expression of AMPs in DM patients with latent and active TB when compared with non-DM patients.\(^{42}\) These indicate an association between down-regulation of AMPs and their impact on DM and TB susceptibility.

**Pre-Diabetes and TB**

Cytokine interactions that characterise pulmonary tuberculosis (PTB) coincident with pre-diabetes mellitus (PDM) are still not known. There are a limited number of studies which have focussed on coincident cytokine levels in individuals with PDM who have PTB.\(^{43}\) Circulating levels of cytokines in plasma of TB patients with and without PDM have shown elevated circulating levels of type 1, type 17 and other pro-inflammatory cytokines in TB-PDM. Also TB-PDM is characterised by high systemic levels of type 2 (IL-5) and regulatory (IL-10 and Transforming growth factor beta [TGF-\(\beta\)]) cytokines. Overall, it shows that PDM is characterised by heightened cytokine responses.\(^{44}\)
Diabetes and Latent TB

One-third of the world population is estimated to be latently infected with Mtb and is at high risk of developing active disease. Latent tuberculosis (LTB) is assessed through a Mantoux test reaction which investigates hypersensitivity to Mtb antigens in individuals. Studies focussing on CD8+ and CD4+ T cell makes those with DM more prone to developing active the issue to host protection against Mtb infection and who do not have PDM.43

However, due to cross-reactivity with bacillus calmette-guérin (BCG), a more accurate assessment of Mtb infection can be made based on T cell-based IFN-γ release assays (IGRAs) such as the QuantiFERON-Gold test (QFT) (Cellestis, Germany) or the T-SPOT TB (Oxford Immunotech, UK).20,46,47 Studies on LTB have shown that individuals have a strong protective IFN-γ response to Mtb antigens.48 However, it has been shown that in DM there is down-modulation of Th1, Th2 and Th17 responses in cases with LTB infection.49 This compounds the issue to host protection against Mtb infection and makes those with DM more prone to developing active TB.34 Studies focussing on CD8+ and CD4+ T cell cytokines in LTB with coincident PDM have shown that LTB-PDM is characterised by diminished levels of mono and dual functional CD4+ Th1 and Th17 and mono-functional Th2 cells in comparison to individuals with LTB who do not have PDM.43

Conclusions

The mechanisms that result in exacerbation of TB in the presence of diabetes are not completely clear. However, it is evident that multiple immune parameters are modified in the Mtb infected host due to DM. Both innate and adaptive immunity is affected. These alterations affect Mtb antigen presentation, secretion of anti-microbial peptides and peripheral blood monocyte trafficking downstream; thereby increasing Mtb susceptibility in DM patients. Overall, an increased awareness of DM and early diagnosis of TB is essential for better treatment and prevention of both diseases.

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References