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Influence of diabetes mellitus on immunity to human tuberculosis

Pavan Kumar Nathella[1,2] and Subash Babu[1,3]

[1]*National Institutes of Health—International Centre for Excellence in Research, Chennai,* [2]*National Institute for Research in Tuberculosis, Chennai, India and* [3]*Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA*

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Correspondence: Dr Subash Babu, NIH-ICER, National Institute for Research in Tuberculosis, Chennai 600031, India.
Email: sbabu@mail.nih.gov
Senior author: Dr Subash Babu

Summary

Type 2 diabetes mellitus(DM) is a major risk factor for the development of active pulmonary tuberculosis (TB), with development of DM pandemic in countries where TB is also endemic. Understanding the impact of DM on TB and the determinants of co-morbidity is essential in responding to this growing public health problem with improved therapeutic approaches. Despite the clinical and public health significance posed by the dual burden of TB and DM, little is known about the immunological and biochemical mechanisms of susceptibility. One possible mechanism is that an impaired immune response in patients with DM facilitates either primary infection with *Mycobacterium tuberculosis* or reactivation of latent TB. Diabetes is associated with immune dysfunction and alterations in the components of the immune system, including altered levels of specific cytokines and chemokines. Some effects of DM on adaptive immunity that are potentially relevant to TB defence have been identified in humans. In this review, we summarize current findings regarding the alterations in the innate and adaptive immune responses and immunological mechanisms of susceptibility of patients with DM to *M. tuberculosis* infection and disease.

Keywords: B cells; diabetes mellitus; latent tuberculosis; monocytes and dendritic cells; T cells; tuberculosis.

Introduction

The relationship between type 2 diabetes mellitus (DM) and tuberculosis (TB) and their combined role in causing human disease has been accepted for a long time but has only lately become a priority issue of clinical and fundamental research.[1] Type 2 DM and pulmonary TB (PTB) are two of the most common co-morbid conditions in many parts of the world, and the union of these diseases appears to pose a serious threat to health care worldwide. A variety of clinical and epidemiological studies have identified DM as a risk factor for the development of active TB.[2] Besides, DM also appears to be associated with a greater severity of TB disease among the infected population and to have a harmful effect on both disease presentation and response to treatment.[1,3] A meta-analysis of 13 observational studies on the risk for TB disease in patients with DM determined that diabetic patients were 3·1 times more likely to have TB than non-diabetic individuals.[2] Subsequently, over 40 different studies, including four prospective studies, 16 retrospective studies and 17 case–control studies, have confirmed the increased susceptibility of individuals with DM to TB disease.

In six high TB-burden countries, India, Indonesia, China, Nigeria, Pakistan and South Africa, the burdens of DM are 18%, 14%, 22%, 5%, 15% and 15%, respectively. The spectrum of the TB and DM problem in India, the country with one of the highest burdens of both diseases, was the focus of a study in 2011 by Viswanathan *et al.*[4] The authors of that study performed oral glucose-tolerance tests on 827 patients newly diagnosed with TB in Chennai. In that cohort, 25·3% of patients with TB were confirmed to have DM and 24·5% had pre-diabetes.[4] Another study conducted in Kerala reported that nearly half of patients with TB in Kerala had DM, and approximately half of these patients were newly-diagnosed during the study.[5]

The immunological source of susceptibility to TB among those with DM is not well understood. Enhanced susceptibility to TB in patients with DM has been attributed to several factors, including direct effects related to hyperglycaemia and insulin resistance and indirect effects related to macrophage and lymphocyte function.[1,6,7] The

impaired immune response in patients with DM, which facilitates either primary infection with TB or reactivation of latent TB, may be the possible reason for these defective immune responses.[8] Studies probing the innate and adaptive immune response to microbial antigens in patients with DM suggest that these responses are compromised, particularly in patients with chronic hyperglycaemia.[9–11] Whether this applies to TB infection remains unclear.

Innate immunity to TB in patients with DM

Blood monocytes also play a key role in TB and undergo prompt migration to the lung upon initial *Mycobacterium tuberculosis* infection, where they differentiate into macrophages and dendritic cells for antigen presentation and secretion of cytokines. Furthermore, *M. tuberculosis* can enter and replicate (or be contained) within monocytes.[12] The role of monocytes in TB-naive individuals was assessed by Gomez *et al.*[13] The association of *M. tuberculosis* with monocytes was significantly lower in patients with DM than in those without. Multivariate analysis controlling for host socio-demographics, DM characteristics and serum lipids indicated that male gender and poorly controlled DM were significantly associated with the lower interaction of *M. tuberculosis* with monocytes. Serum heat-inactivation reduced the association of *M. tuberculosis* to similar levels in both study groups, suggesting alterations in the complement pathway of patients with DM.[13] In addition, Kumar *et al.*[14] reported that coincident DM resulted in significantly lower frequencies of classical and intermediate monocytes in individuals with PTB. However, after completion of anti-TB treatment, examination of the phenotype of monocyte subsets revealed significantly increased frequencies of classical and intermediate monocytes, indicating that alterations in frequencies of monocytes in TB-DM co-morbidity is reversible following anti-TB therapy.[15] TB-DM was also associated with increased CCR2 expression, which may restrain monocyte traffic to the lung, indicating that DM might also influence the migratory capacity of monocytes in patients with DM.[16]

The examination of alveolar macrophages in TB-DM patients has revealed the presence of hypodense alveolar macrophages, which are less activated and are correlated with the severity of disease, implying that they might contribute to the increased susceptibility to *M tuberculosis* infection.[17] In addition, Arce-Mendoza *et al.*[18] reported that DM also affects the expression of receptors like CD64, CD206 and RAGE in monocytes. Upon *M. tuberculosis* antigen stimulation, all three receptors were significantly diminished in TB-DM compared with TB alone, whereas RAGE expression was increased in patients with TB as well as in those with DM compared with controls. *In vitro* studies, using human macrophages from healthy donors and stimulated with *M. tuberculosis* under hyperglycaemic conditions, have revealed increased production of cytokines, including tumour necrosis factor (TNF-α), interleukin-1β (IL-1β), IL-6 and IL-10.[19] However, examination of soluble markers of monocyte/macrophage activation, including sCD163 and sCD14, has not revealed any significant differences in the plasma levels between TB-DM and TB.[20] Hence, the role of monocyte/macrophages in TB-DM co-morbidity needs further exploration.

Dendritic cells (DC) are one of the crucial players in linking innate and adaptive immune responses through their role in capturing, processing and presenting antigens. Studies have reported that migration of DC to the draining lymph node is essential for the activation of naive T cells in TB infection[21] and that at the initiation of the infection, DC are highly represented at sites of *M. tuberculosis* infection.[22,23] To elucidate the influence of DM on DC, Kumar *et al.*[14] examined the *ex vivo* phenotypic profile of the DC subset in patients with TB with or without DM. TB-DM individuals exhibited significantly lower frequencies of both myeloid DC and plasmacytoid DC compared with individuals with TB. However, their contribution in the pathogenesis of this co-morbidity and the effect of anti-TB treatment on the *ex vivo* phenotype of DC subsets are poorly understood. To address this, Kumar *et al.*[24] examined the frequency of DC subsets in individuals with TB with DM or without coincident DM (NDM) at baseline and at 2 months and 6 months of anti-TB treatment and found that individuals with TB-DM were characterized by diminished frequencies of myeloid DC and plasmacytoid DC at baseline and after 2 months of treatment but not following 6 months of treatment in comparison with those with TB-NDM. Therefore, the changes in the percentages of DC subsets in TB-DM individuals are reversed by anti-TB treatment.[24] Hence, hyperglycaemia and its related factors potentially function as the primary influence driving alterations in the frequency of DC subsets in TB.

Neutrophils are the other innate cell type that play an important role in pathogenesis or protection against TB infection and disease. Neutrophils are a critical component of the innate immune response to TB and are believed to contribute to disease protection through oxidative killing of mycobacteria.[25] These cells can also promote pathology in conditions of high bacterial load, which favours neutrophil accumulation,[26,27] and a neutrophil-dominant interferon signature in whole blood has been shown to correlate with active TB disease.[28] TB-DM co-morbidity is characterized by heightened levels of absolute neutrophil counts.[20] However, recent studies have reported an impairment in the function of neutrophils in these individuals, with a decreased capacity to phagocytose *M. tuberculosis* or other *M. tuberculosis*-related molecules.[29,30] Finally, neutrophil counts have

been identified at the nexus between DM and TB using Bayesian Network modelling of transcriptional analysis of TB-DM and TB patients. These data indicate that neutrophilic inflammation is a central feature of TB-DM and that incident TB disease elevates levels of biomarkers associated with macrovascular complications above the levels found in DM without co-morbid TB (unpublished data).

Natural killer (NK) cells are also effector cells of innate immunity. The effector functions of NK cells are regulated by a sequence of inhibitory or activating receptors.[31] During early infection, NK cells are capable of activating phagocytic cells at the site of infection. NK cells, which are large granular circulating lymphocytes, are recruited to the sites of bacterial infections, where they specialize in recognizing and destroying infected host cells.[32] NK cell production of interferon-γ (IFN-γ), IL-17 and IL-22 is thought to play an important role in host defence against mycobacterial infection.[33] A recent study found that TB-DM is characterized by expanded frequencies of TB antigen-stimulated NK cells expressing type 1 (TNF-α) and type 17 (IL-17A and IL-17F) cytokines. In contrast, NK cells were associated with significantly decreased antigen-specific expression of CD107a in TB-DM patients.[34] In addition, Zhang *et al.*[35] reported that NKT cells from peripheral blood mononuclear cells in TB patients with or without DM were significantly increased compared with levels in non-TB diabetic patients and healthy controls.

Antimicrobial peptides (AMPs) are a key component of the innate immunity to pathogens and are present mainly in phagocytic cells of the immune system, where they act by killing engulfed pathogens.[36] Studies have shown that AMPs have high anti-mycobacterial activity but low immunogenicity and are therefore promising therapeutic agents.[37,38] Among the AMP family, four of the most prominent are cathelicidin (LL37), human β-defensin 2 (HBD2), human neutrophil peptide 1–3 (HNP1–3) and granulysin.[39] Our unpublished data report that heightened levels of cathelicidin, HBD2 and HNP1–3 and diminished levels of granulysin are present in individuals with TB-DM and TB compared with individuals with latent TB (LTB) or non-TB-infected individuals (NTB). In addition, there was an association of cathelicidin and HBD2 with the extent and severity of lung disease and with bacterial burdens, as well as correlations of AMPs with glycaemic parameters. Finally, our unpublished data revealed that there was a major reversal in the systemic concentrations of these AMPs after anti-TB treatment in both TB-DM and TB individuals. In addition, Gonzalez-Curiel *et al.*[40] reported that AMP gene expression is increased during active TB compared with LTB but in contrast to our unpublished data they reported lower expression of AMPs in DM, in both the LTB and the TB groups.[18] In summary, patients with DM appear to have alterations in innate immunity, with decreased *M. tuberculosis* phagocytosis and decreased expression of genes that contribute to *M. tuberculosis* containment.

Adaptive immunity to TB in DM patients

Immunity to *M. tuberculosis* requires T-helper type 1 (Th1) responses and (to a lesser extent) Th17 responses.[41,42] Interleukin-2, IFN-γ and TNF-α as well as IL-17 and IL-23, all play important roles in the induction and maintenance of protective immune responses against TB.[43–48] Cytokines of the innate and adaptive immune systems orchestrate the immune response to TB infection, with type 1, type 17 and the IL-1 family of cytokines having been implicated in protection against TB disease.[49] An increasing number of immunological studies in patients with DM who have developed TB indicate a paradoxical hyper-inflammatory response. Restrepo *et al.*[50] demonstrated that purified protein derivative stimulation of whole blood in TB-DM patients resulted in higher production of IFN-γ, IL-2, TNF-α and granulocyte–macrophage colony-stimulating factor compared with non-diabetic patients with TB. This finding was confirmed and extended by Kumar *et al.*[51] To study the influence of DM on $CD4^+$ T-cell responses in active PTB, these authors examined baseline, *M. tuberculosis* antigen-specific (CFP-10 and ESAT-6), and polyclonal induction of single, double and triple-cytokine-producing cells of the Th1 and Th17 subsets in individuals with active TB and coincident DM and compared them with individuals with active TB without DM. The patients with TB-DM exhibited elevated frequencies of single- and double-cytokine-producing $CD4^+$ Th1 cells, as well as increased frequencies of Th17 subsets following *M. tuberculosis* antigen stimulation, but lower frequencies of regulatory T (Treg) cells, in comparison with individuals with TB and without DM.[51]

A subsequent study of plasma cytokine levels in patients with DM by Kumar *et al.*[52] confirmed this finding by studying the influence of type 2 DM on active PTB. The authors examined levels of a large panel of type 1, type 2, type 17, regulatory, and other pro-inflammatory cytokines and chemokines in individuals with active TB and coincident DM and compared them with those in individuals with active TB but without DM. They also examined *M. tuberculosis* antigen-stimulated levels of certain cytokines in the whole blood of individuals with TB and DM and in individuals with TB but without DM. TB-DM is characterized by elevated circulating levels of type 1 (IFN-γ, TNFα and IL-2), type 2 (IL-5) and type 17 (IL-17A) cytokines but decreased circulating levels of IL-22. This was also associated with increased systemic levels of other pro-inflammatory cytokines (IL-1β, IL-6 and IL-18) and an anti-inflammatory cytokine (IL-10) but not type 1 IFNs. Moreover, *M. tuberculosis* antigen-stimulated

whole blood also showed increased levels of pro-inflammatory cytokines. The type 1 and type 17 cytokines in plasma exhibit a significant positive correlation with haemoglobin A1C levels, indicating that impaired control of DM is associated with this pro-inflammatory milieu. Multivariate analysis revealed that the association of pro-inflammatory cytokines with DM was not influenced by age, sex or other metabolic parameters.[52] Other studies also have compared the IFN-γ cytokine responses of TB patients with or without DM following *in vitro* stimulation of purified mycobacterial antigens. Walsh *et al.*[53] reported that IFN-γ secretion was significantly higher in TB-DM when compared with patients with TB but no DM. However, not all studies have uniformly reported an increase in the type 1 or other pro-inflammatory cytokines in TB-DM. Gan *et al.*[54] reported no significant differences in the levels of TB-antigen-specific IFN-γ between diabetic and non-diabetic patients with culture confirmed TB. Stalenhoef *et al.*[55] in measuring the production of IFN-γ and other pro-inflammatory cytokines from whole blood of Indonesian TB patients with or without DM stimulated with *M. tuberculosis* sonicate, or *Escherichia coli* lipopolysaccharide, or phytohaemagglutinin, found no difference in the expression of pro-inflammatory cytokines, including IFN-γ. The only significant difference found in this study was diminished IL-10 production in the diabetic group. Similarly, Faurholt-Jepsen *et al.*[56] also reported that DM is associated with reduced mycobacterial antigen-specific IFN-γ production in TB patients. These results contrast with data from Restrepo *et al.*[50] and Kumar *et al.*,[51,52] probably reflecting differences in the populations studied or in methods such as the source of *M. tuberculosis* antigen. Individuals with DM have been shown to exhibit a characteristic deficiency in intracellular glutathione (GSH) levels, resulting in impaired production of IL-12p70 and IFN-γ in *M. tuberculosis*-infected peripheral blood mononuclear cells.[35] This impairment is reversible upon addition of GSH to the cell cultures. However, this study failed to examine the role of GSH in TB-DM directly.

Both $CD4^+$ and $CD8^+$ T cells and their memory subsets like central memory and effector memory T cells have been shown to play important roles in protective immune responses in animal models of vaccination.[57] To characterize the influence of DM on memory T-cell subsets, Kumar *et al.*[14] examined the *ex vivo* phenotypic profile of $CD4^+$ and $CD8^+$ memory T-cell subsets in TB patients with or without DM. TB-DM individuals exhibited significantly lower frequencies of naive but not central or effector memory in $CD4^+$ memory T cells and lower frequency of naive and effector memory but significantly higher frequency of central memory in $CD8^+$ memory T cells compared with PTB individuals.[14] However, their involvement in the pathogenesis of this co-morbidity and the effect of anti-TB treatment on the phenotype of the T-cell subsets is poorly understood. To address this, Kumar *et al.*[24] examined the frequency of different T-cell subsets in individuals with TB-DM or without coincident DM (NDM) at baseline and at 2 months and 6 months of anti-TB treatment and found that TB-DM is characterized by enhanced frequencies of central memory $CD4^+$ and $CD8^+$ T cells and diminished frequencies of naive, effector memory and/or effector $CD4^+$ and $CD8^+$ T cells at baseline and after 2 months of treatment but not following 6 months of treatment, in comparison with TB-NDM. Central memory $CD4^+$ and $CD8^+$ T-cell frequencies displayed a positive correlation with fasting blood glucose and HbA1c levels. Hence, DM appears to exert a profound effect on the frequencies of central memory, effector memory and naive T cells, which normalizes following anti-TB therapy.[24]

$CD8^+$ T cells are also important producers of pro-inflammatory cytokines, including type 1 and type 17 cytokines in TB,[58] but their contribution to the cytokine environment in TB-DM co-morbidity is not known. Similarly, expression of cytotoxic granule release mediators – Perforin, Granzyme B and CD107a – is an important component of the cytotoxic function of these cells.[59] $CD8^+$ T cells are known to play a protective role in the immune response to murine TB and *M. tuberculosis*-specific $CD8^+$ T cells have also been found in humans.[60] These cells have the capacity to activate macrophage defence mechanisms by secreting IFN-γ and TNF-α and also help in eliminating the bacteria by the granule exocytosis pathway.[41] A recent study to determine the role of these lymphocytes in TB-DM found elevated frequencies of mycobacterial antigen-stimulated $CD8^+$ T cells expressing type 1 (IFN-γ and IL-2) and type 17 (IL-17F) cytokines in the TB-DM group.[34] In contrast, the authors also reported that $CD8^+$ T cells were associated with significantly diminished expression of the cytotoxic markers Perforin, Granzyme B and CD107a, both at baseline and following antigen or anti-CD3 stimulation. The data, therefore, reveal an important association of altered cytotoxic T-cell potential with the pathogenesis of TB-DM co-morbidity.[34]

One of the major contributing factors for the exaggerated $CD4^+$ and $CD8^+$ T-cell response in TB-DM could potentially be due to decreased frequency and function of natural Treg cells. Indeed, Kumar *et al.*[61] have reported a decrease in the frequency of Treg cells in TB-DM patients compared with those with PTB alone. However, this study failed to address the function of the Treg cells in the periphery. In contrast, Treg cells were found to be increased in frequencies at the site of infection in TB-DM individuals with enhanced IL-10 and diminished IFN-γ production.[62] Hence, compartmentalization of Treg cells could potentially be an important driver of the enhanced T-cell responses in TB-DM. The overall adaptive immune

response evidence suggests that the hyper-reactive antigen-specific T-cell response in TB-DM is significantly greater than the response of TB-NDM patients and that this expanded population could possibly contribute to lung pathology in diabetic individuals. This difference from TB-NDM patients provides indirect support for dysfunctional immunity in DM patients, which leads to TB susceptibility. The effect of DM on $CD4^+$ and $CD8^+$ T cells in PTB is summarized in Fig. 1.

Latent tuberculosis in DM patients

Type 1 and type 17 cytokines and the IL-1 family of cytokines are known to influence susceptibility to TB in both humans and animal models.[41,58] Kumar *et al.*[63] postulated that DM could alter the normal homeostatic levels of these cytokines in latent infection. To this end, the authors compared levels of a panel of type 1, type 17, IL-1 family and other relevant pro-inflammatory cytokines

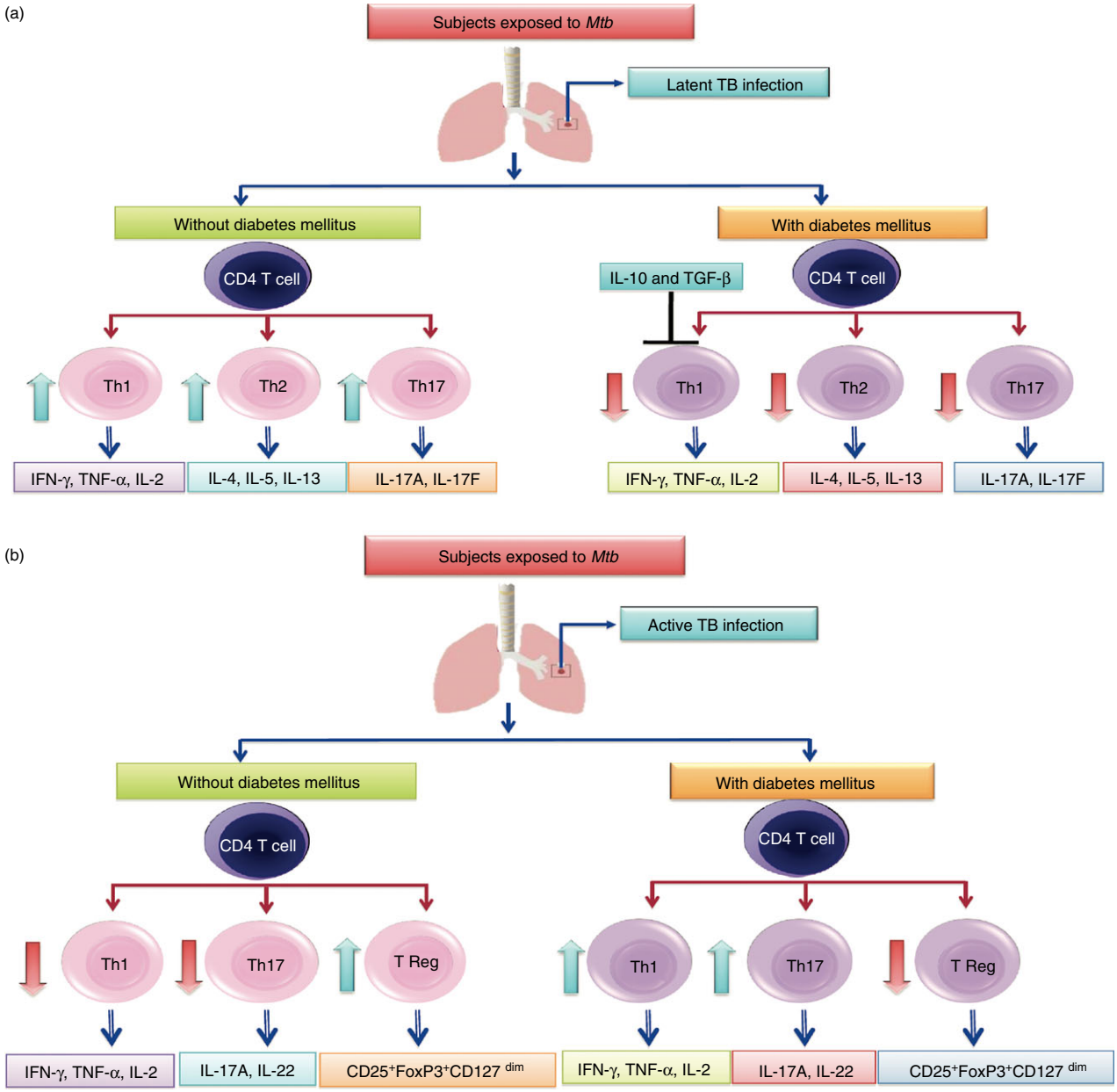

Figure 1. Influence of diabetes mellitus (DM) on latent and active tuberculosis (TB). The $CD4^+$ T-cell responses to mycobacterial antigens in individuals with latent TB with or without DM is summarized in terms of T helper type 1 (Th1), Th2 and Th17 profiles (a). The $CD4^+$ T-cell responses to mycobacterial antigens in individuals with active TB with or without DM is summarized in terms of Th1, Th17 and regulatory T (Treg) cell profiles (b).

in individuals with latent infection and coincident DM levels in individuals with latent infection but without DM. They show that LTB-DM individuals exhibit diminished circulating levels of type 1 (IFN-γ, IL-2 and TNF-α) and type 17 (IL-17F) cytokines. This was associated with decreased systemic levels of other pro-inflammatory cytokines (IL-1β and IL-18) and the anti-inflammatory cytokine IL-10 but not with decreased systemic levels of type 2 cytokines. Moreover, latently infected individuals with DM had diminished levels of spontaneous and *M. tuberculosis* antigen-specific levels of type 1 and type 17 cytokines when antigen-stimulated whole blood was examined.[63] Hence, these data on the diminution of pro-inflammatory cytokine production both at steady state and following antigen stimulation in the context of latent infection potentially suggest a profound impact of DM on immune responses in LTB. This study also examines the important modulatory role played by poor glucose control in latent infection and offers important insights into the potential mechanism by which DM could influence the progression from latent infection to active TB.

A subsequent study of $CD4^+$ cell frequencies in LTB-DM patients by Kumar *et al.*[64] examined mycobacteria-induced immune responses in the whole blood of individuals with LTB-DM and compared them with responses of individuals without DM (LTB-NDM). Authors of the study show that baseline and antigen-specific $CD4^+$ T-cell response from LTB-DM are characterized by diminished frequencies of mono- and dual-functional $CD4^+$ Th1, Th2 and Th17 cell compared with LTB-NDM. This alteration was at least partially related to IL-10 and transforming growth factor-β, as neutralization of either cytokine resulted in significantly augmented frequencies of Th1 and Th2 cells but not Th17 cells in LTB-DM individuals. Although $CD8^+$ T cells were originally considered to be less important in the immune response to *M. tuberculosis* infection, it is now clear that $CD8^+$ T cells play a fundamental role in this immune response.[65] Like $CD4^+$ T cells, $CD8^+$ T cells are able to produce IFN-γ, TNF-α and IL-2, which are known to have critical functions during *M. tuberculosis* infection.[65,66] To explore the influence of DM on $CD8^+$ T-cell responses during LTB infection, Kumar *et al.*[67] estimated the cytokine and cytotoxic marker expression pattern on $CD8^+$ T cells in individuals with LTB-DM and compared them with responses of individuals without DM (LTB-NDM). Individuals with LTB-DM had diminished frequencies of $CD8^+$ Tc1, Tc2 and Tc17 cells following *M. tuberculosis* antigen stimulation. In contrast, enhanced frequencies of $CD8^+$ T cells expressing cytotoxic markers were present in LTB-DM compared with those without DM. In summary, DM alters the immune response in latent TB leading to a suboptimal induction of protective $CD4^+$ and $CD8^+$ T-cell responses, thereby providing a possible mechanism for increased susceptibility to active disease.

To characterize the phenotypic profile of leucocyte subsets at homeostasis, Kumar *et al.*[14] examined the *ex vivo* phenotypic profile of T-cell, B-cell, DC and monocyte subsets in individuals with LTB and non-TB-infected individuals with or without DM (Fig. 2). LTB-DM individuals exhibited significantly lower frequencies of effector memory $CD4^+$ T cells compared with LTB individuals, whereas NTB-DM individuals exhibited significantly lower frequencies of naive and central memory $CD4^+$ T cells compared with NTB individuals. In addition, LTB-DM individuals exhibited significantly higher frequencies of activated memory and atypical B cells but significantly lower frequencies of naive B cells compared with LTB individuals. On the other hand, NTB-DM individuals exhibited significantly lower frequencies of naive and immature B cells compared with NTB individuals. LTB-DM and NTB-DM individuals exhibited significantly lower frequencies of both myeloid DC and plasmacytoid DC compared with LTB and NTB individuals. Finally, both LTB-DM and NTB-DM exhibited significantly lower frequencies of classical and intermediate monocytes and significantly higher frequencies of non-classical monocytes in comparison to LTB and NTB individuals. This suggests that although the effect of coincident DM on phenotypic profile of T-cell and B-cell subsets is only moderate, it profoundly alters the frequencies of monocyte and DC subsets in LTB.[14]

In diabetic people, progression of freshly acquired infection to TB disease could result from impaired sentinel function of alveolar macrophages regardless of previous exposure. Although this mechanism has not yet been studied directly, evidence that 10–20% of patients with LTB have a peripheral blood transcriptional signature of active TB disease[68] supports the idea that a subgroup of people with LTB may have clinically imperceptible but biologically active foci of infection at high risk for progression to clinically evident TB. It will be interesting to see if DM increases the occurrence of active TB peripheral blood transcriptional signatures in LTB.

Effect of pre-diabetes in TB patients

Pre-diabetes (PDM) or intermediate hyperglycaemia is a high risk state for DM that is defined by glucose values that are higher than normal, but lower than diabetic thresholds.[69] The prevalence of PDM is increasing worldwide, and it is estimated that over 470 million people will have PDM by 2030.[70] PDM is linked with the coexisting insulin resistance and pancreatic β-cell dysfunction – with both abnormalities apparent before changes in glycaemic control are detectable.[69,71] An association of PDM with TB risk is not understood but would not be surprising in the light of clinical evidence linking susceptibility to poor glycaemic control.[72] TB disease might promote insulin resistance and induce PDM as a

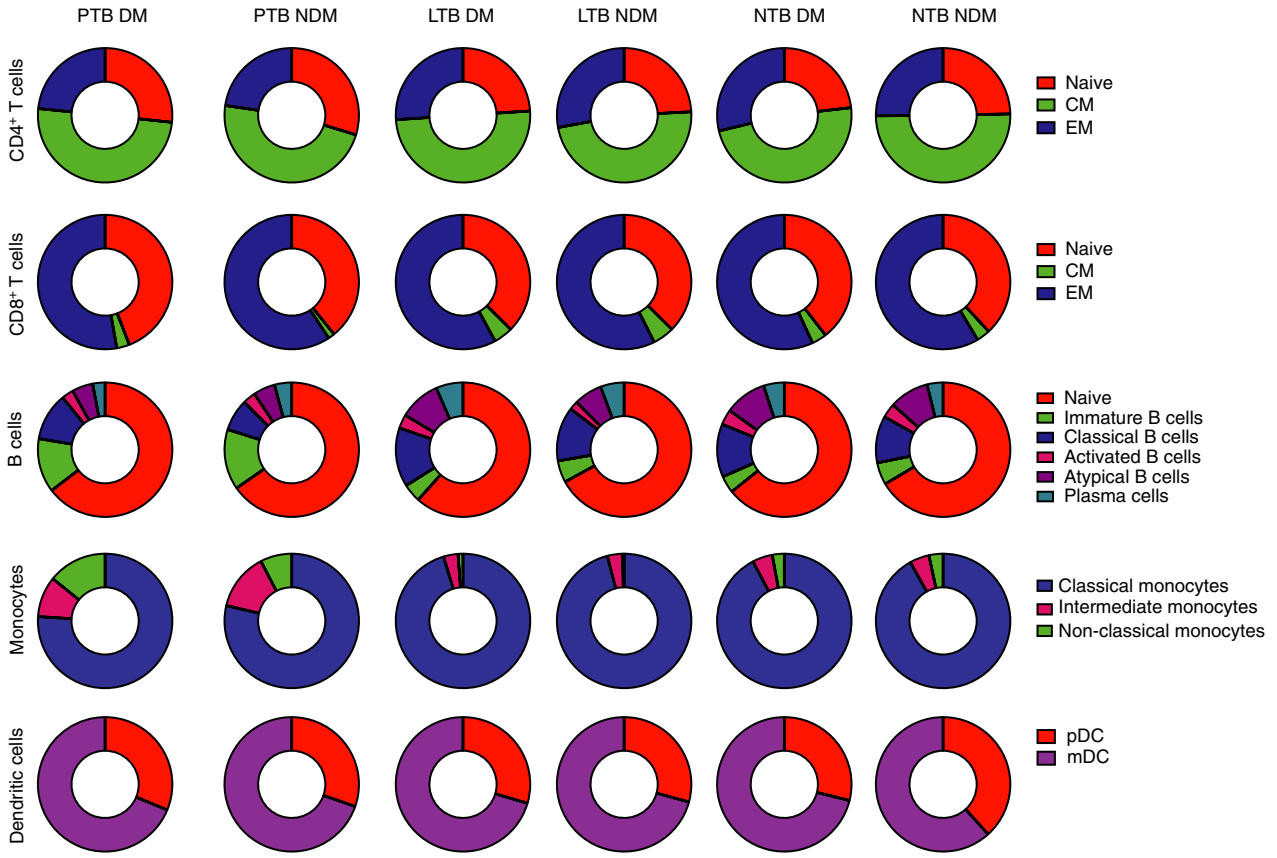

Figure 2. Alterations in the frequencies of $CD4^+$ T-cell, $CD8^+$ T-cell, B-cell, monocyte and dendritic cell subsets in tuberculosis-diabetes (TB-DM) co-morbidity. Frequency distribution of $CD4^+$ naive, central memory (CM) or effector memory (EM) subsets in active pulmonary TB with DM (PTB-DM) or without DM (PTB-NDM); latent TB with DM (LTB-DM) or without DM (LTB-NDM) and no TB with DM (NTB-DM) or without DM (NTB-NDM) is depicted as pie-charts. Similarly, frequency distributions of $CD8^+$ T-cell subsets, B-cell subsets, monocyte subsets and dendritic cell subsets are also depicted.

consequence of inflammatory stress. In that scenario, TB would represent a significant factor contributing to disordered glucose metabolism with implications for bi-directional screening.[73,74] Pre-diabetes could develop as an effect rather than a cause of TB, or TB risk might be increased in individuals with PDM by a factor other than hyperglycaemia, such as dyslipidaemia. Pre-DM is also associated with the presence of insulin resistance and B-cell dysfunction – abnormalities that start before changes in blood glucose levels become detectable.[75] To understand the impact of PDM in active PTB, Kumar *et al.*[76] examined levels of a large panel of type 1, type 2, type 17, regulatory and other pro-inflammatory cytokines in individuals with active TB and coincident PDM, and compared them with those in individuals with active TB but without DM. The authors found that TB with PDM is characterized by elevated circulating levels of type 1 and type 17 and other pro-inflammatory and anti-inflammatory cytokines, indicating that a balanced pro- and anti-inflammatory cytokine milieu is a feature of PDM-TB co-morbidity.[76]

To further characterize the role of $CD4^+$ and $CD8^+$ T-cell cytokines in LTB with coincident PDM, Kumar *et al.*[77] studied the baseline, mycobacterial antigen and mitogen-stimulated T-cell cytokine responses in LTB individuals with (LTB-PDM) or without (LTB-NDM) concomitant PDM. The authors found that LTB-PDM is characterized by diminished frequencies of mono- and dual functional $CD4^+$ Th1, Th2 and Th17 cells at baseline and following mycobacterial antigen stimulation in comparison with LTB-NDM. LTB-PDM is also characterized by diminished frequencies of single producing $CD8^+$ Tc1, Tc2 and Tc17 cells at baseline and following *M. tuberculosis* antigen stimulation in comparison with LTB-NDM. LTB-PDM is therefore characterized by diminished frequencies of Th1/Tc1, Th2/Tc2 and Th17/Tc17 cells, indicating that PDM is associated with alterations of the immune response in latent TB leading to compromised $CD4^+$ and $CD8^+$ T-cell function. Our data also suggest that this interaction might also have an effect on TB disease, potentially shifting the balance of metabolic regulation from PDM to overt DM.

Biomarkers associated with TB-DM co-morbidity

To understand the pathogenesis in TB with DM, Andrade *et al.*,[78] performed an exploratory study assessing a series of biological parameters like haem oxygenase-1 (HO-1), acute phase proteins, tissue metalloproteinases and tissue inhibitors of metalloproteinase (TIMPs) as well as cytokines and chemokines. Cross-sectional analyses were performed in plasma samples from individuals with active PTB or with coincident TB and DM from South India. Compared with patients with TB without DM, those with coincident DM exhibited increased *M. tuberculosis* bacillary loads in sputum. Plasma levels of HO-1 but not of other acute phase proteins were higher in patients with TB and DM than in patients without DM, independent of bacillary sputum loads. HO-1 concentrations also positively correlated with random plasma glucose, circulating glycosylated haemoglobin and low-density lipoprotein levels. Moreover, patients with coincident TB and DM exhibited increased plasma levels of tissue inhibitor of metalloproteinase (TIMP-4) and elevated peripheral blood neutrophil counts, which, when considered together with HO-1, resulted in increased power to discriminate individuals with active TB with and without DM. The authors conclude that elevated plasma levels of HO-1 and TIMP-4, in addition to increased absolute neutrophil counts in the blood, are potential markers of pathogenesis in TB with DM.[78]

TB-DM co-morbidity is characterized by increased inflammation with elevated circulating levels of inflammatory cytokines and other angiogenic factors are also intricately involved in the angiogenesis-inflammation nexus. To study the association of angiogenic factors with TB-DM, Kumar *et al.*[79] examined the systemic levels of angiogenic factors like vascular endothelial growth factor A (VEGF-A), VEGF-C, VEGF-D, VEGF-R1, VEGF-R2 and VEGF-R3 and angiopoietins like angiopoietin 1, angiopoietin 2 and Tie 2 receptor (unpublished data) in individuals with either TB-DM or TB alone. All the angiogenic factors were significantly higher in TB-DM compared with TB individuals, whereas in contrast angiopoietins like angiopoietin 1 and angiopoietin 2 were significantly diminished in TB-DM compared with TB individuals (unpublished data). Moreover, the levels of VEGF-A, -C, -R2 and/or -R3 were significantly enhanced in TB-DM with bilateral or cavitary disease or with haemoptysis, suggesting an involvement with both disease severity and adverse clinical presentation. The levels of these angiogenic factors also revealed a significant positive relationship with bacterial burdens and HbA1c levels. Finally, the circulating levels of angiogenic factors were significantly diminished and in contrast angiopoietins were increased (unpublished data) following successful anti-TB treatment at 6 months. The authors suggest that the factors mentioned above could serve as accurate biomarkers for monitoring therapeutic responses in TB-DM co-morbidity.[79]

The term adipocytokine is used to describe cytokines that are mostly produced by adipose tissue. The adipocytokines, adiponectin and leptin have been defined as the most essential adipocyte products, thereby redefining adipose tissue as a key element not only of the endocrine system, but also of the immune system.[80] Adipose tissue is a central inflammatory source in obesity and type 2 DM, not only because of cytokines produced from the adipocyte itself, but also because of infiltration by pro-inflammatory macrophages.[80,81] To study the influence of DM on active PTB and LTB, Kumar *et al.*[82] examined circulating levels of adipocytokines in the plasma of individuals with PTB-DM or LTB-DM and compared them with those without DM (PTB or LTB). PTB-DM or LTB-DM is characterized by diminished circulating levels of adiponectin and adipsin and/or heightened circulating levels of leptin, visfatin and PAI-1. In addition, adiponectin and adipsin display a significant negative correlation, whereas leptin, visfatin and plasminogen activator inhibitor-1 (PAI-1) show a significant positive correlation with HbA1C levels and random blood glucose levels. The authors suggest that alterations in the systemic levels of adipocytokines indicate that altered adipose tissue inflammation underlying type 2 DM potentially contributes to the pathogenesis of TB disease.[82]

The IL-20 subfamily of cytokines consists of five members: IL-19, IL-20, IL-22, IL-24 and IL-26 and they play an important role in both host defence mechanisms and glucose metabolism. The IL-20 subfamily of cytokines exerts profound effects on host innate immune responses, including promoting the production of antimicrobial peptides, strengthening barrier function at epithelial and mucosal surfaces and facilitating recruitment of leucocytes and their activation at the site of inflammation.[83–85] Since the interface between TB and DM involves both of the above processes, Kumar *et al.*[86] examined the relationship of the IL-20 subfamily of cytokines in TB-DM co-morbidity. They examined circulating plasma cytokine levels in individuals with PTB-DM or LTB-DM and compared them with those without DM (PTB or LTB). PTB-DM is characterized by diminished circulating levels of IL-19, IL-20, IL-22 and IL-24 but increased levels of IL-10. Similarly, LTB-DM was also characterized by diminished circulating levels of IL-10, IL-19, IL-20 and IL-24 but increased levels of IL-22. Moreover, there was a significant negative correlation of IL-10, IL-19, IL-20, IL-22 and IL-24 levels with haemoglobin A1C (HbA1c) levels in both PTB and/or LTB individuals. Coincident DM in either PTB or LTB was characterized by diminished production of the IL-20 subfamily of cytokines, which suggests that the IL-20 subfamily of cytokines is associated with the regulation of both host immunity and metabolic processes in the context of co-morbidity.[86]

The blood transcriptome offers a glimpse of immunological events in the lung and a consensus gene expression signature of active TB is evolving from observational studies in non-diabetic TB patients in Africa, China, Europe and Indonesia.[87] To gain insights into mechanisms of TB susceptibility in human DM and to assess the impact of TB disease on diabetic complications, we leveraged data and blood samples from TB-DM and TB-NDM patients. The authors performed an integrative analysis of whole blood gene expression and plasma analytes, comparing South Indian TB patients with and without DM to diabetic and non-diabetic individuals without TB. Integrative analysis showed a high degree of comparability in the blood transcriptional response to TB between diabetic and non-diabetic participants but a distinct signature of plasma cytokine and growth factor levels and an association of TB-DM with neutrophilic inflammation.[88] The data further show that co-morbid TB activates a range of pathways associated with diabetic complications, above the levels observed with DM alone. Hence, neutrophilic inflammation and diabetic complication pathways may be useful targets for host-directed therapies to improve TB treatment and outcomes in this growing patient population.[88]

Immunological mechanisms of susceptibility in animal models

Several studies have shown increased susceptibility to TB in animal models of TB-DM co-morbidity. Saiki *et al.*[89] used streptozotocin to exhaust insulin-producing cells and cause hyperglycaemia. High doses of *M. tuberculosis* Schacht were administered intravenously to hyperglycaemic mice and untreated controls and after 3 months post infection, > 90% of hyperglycaemic mice had died versus < 10% of the euglycaemic controls.[89] To examine the TB susceptibility, Martens *et al.*[90] used streptozotocin-treated C57BL/6 mice that were hyperglycaemic for < 4 weeks (acute) or > 12 weeks (chronic) before low-dose aerosol challenge with *M. tuberculosis* Erdman. Chronic diabetic mice displayed >1 log higher bacterial burden and more inflammation in the lung compared with euglycaemic mice. The adaptive immunity was delayed in mice with chronic DM and showed a reduced expression of IFN-γ in the lung and fewer *M. tuberculosis* antigen-specific (ESAT-6) T-cell responses compared with euglycaemic mice during the first month of infection. However, after 2 months of TB disease, pro-inflammatory cytokine levels were significantly enhanced in chronic diabetic mice compared with euglycaemic mice. Also, hyperglycaemic mice displayed enhanced absolute numbers of $CD4^+$ and $CD8^+$ T cells, macrophages and neutrophils in the lung during 16 weeks post infection compared with euglycaemic controls.[90] Vallerskog *et al.*[91] tested the hypothesis that DM leads to delayed priming of adaptive immunity in the lung-draining lymph nodes following low-dose aerosol challenge with virulent *M. tuberculosis*. The authors demonstrated that *M. tuberculosis* antigen-specific IFN-γ production by T cells takes place at a later stage with a delay in recruitment of these cells to the lung in the lymph nodes of diabetic mice compared with control mice. Dissemination of *M. tuberculosis* from lung to lymph nodes was also delayed in diabetic mice, although they showed no defect in DC trafficking from lung to lymph nodes after lipopolysaccharide

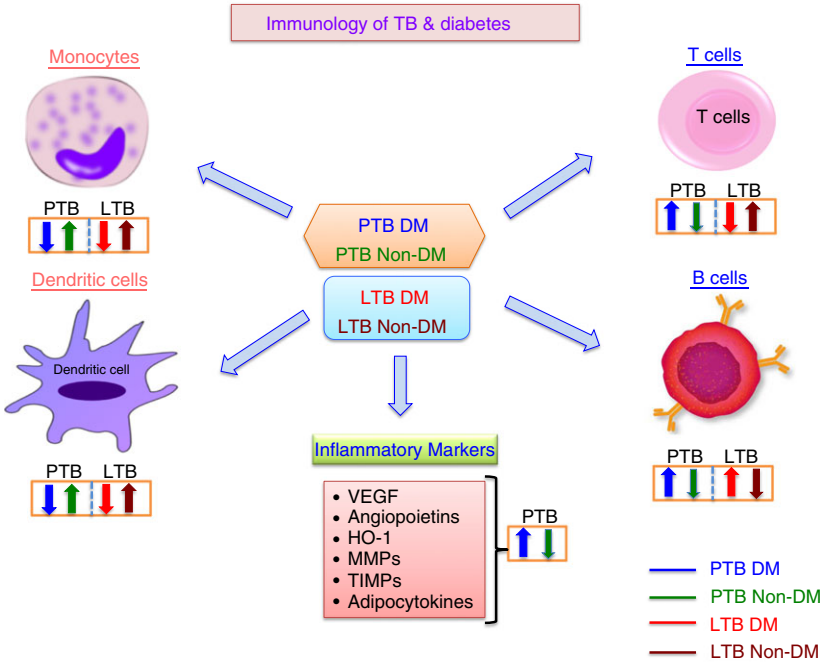

Figure 3. Summary of the immunological interactions between tuberculosis (TB) and diabetes (DM). The effects of DM on the different arms of the innate and adaptive immune systems is represented by arrows indicating increased or decreased function compared with healthy control individuals.

stimulation. Hence, authors concluded that late delivery of antigen-bearing antigen-presenting cells to the lung-draining lymph nodes and delayed priming of the adaptive immune response occur in the presence of DM and this results in delayed initiation of the immune responses necessary to confine *M. tuberculosis* replication.[91]

As DM is associated with increased inflammation and susceptibility to TB and overproduction of several T-cell-associated cytokines has been reported both in animal and human experiments, Martinez *et al.*[92] investigated the effects of hyperglycaemia on T-cell responses upon T-cell receptor stimulation in the absence of infection. The authors of the study report that T cells from hyperglycaemia have heightened proliferation and cytokine production in response to anti-CD3e monoclonal antibody or antigen stimulation and they also report that naive T cells from mice with chronic hyperglycaemia have a significantly increased frequency of decondensed nuclei, after primary activation on initial encounter with antigen. They therefore, propose that chronic hyperglycaemia causes epigenetic modification of naive T cells by a p38 mitogen-activated protein kinase-dependent chromatin decondensation and that this mechanism may contribute to pathological inflammation associated with DM.[92] Subsequently, Martinez *et al.*[93] also tested whether alveolar macrophages from diabetic mice would phagocytose *M. tuberculosis ex vivo*. Alveolar macrophages from diabetic mice had diminished expression of CD14 and also displayed reduced phagocytosis of *M. tuberculosis*.[93] Similarly, in a diet-induced model of type 2 DM, the authors observed that macrophages from diabetic mice exhibited reduced phagocytosis of mycolic-acid-coated beads, reduced internalization and killing of mycobacteria and altered cytokine responses.[94]

Cheekatla *et al.*[95] developed a mouse model of DM using streptozotocin and nicotinamide factors, which increase the susceptibility of diabetic mice during *M. tuberculosis* challenge. Using this model, the authors report that NK and $CD11c^+$ cell interactions in *M. tuberculosis*-infected diabetic mice displayed augmented IL-6 production and reduced survival of *M. tuberculosis* -infected diabetic mice.[95] In another study, Podell *et al.*[96] developed a guinea pig model of type 2 DM-TB co-morbidity to study TB-DM interactions because unlike murine models, lipid metabolism in the guinea pig more closely resembles that of humans, making it ideal for studying *M. tuberculosis* infection. The authors report that *M. tuberculosis* infection of diabetic guinea pigs resulted in severe TB with reduced survival and a higher bacterial burden compared with non-diabetic controls and also displayed an exacerbated pro-inflammatory cytokine and chemokine response in the lung and spleen. These results suggest that a guinea pig model of type 2 DM-TB co-morbidity will be helpful in understanding the complex pathogenesis of TB in patients with DM.[96]

Conclusions

The human data from TB-DM studies suggest an impaired innate immune response to *M. tuberculosis*, followed by hyper-reactive adaptive immune responses. Our review has summarized the immunological alterations in PTB-DM and LTB-DM (Fig. 3). The severity of TB disease increasing the risk of DM creates a significant negative impact on public health, especially in the countries where both diseases are highly endemic. Very few human studies have been performed in this area and the few that have been done provide important insights into the influence of poorly controlled type 2 DM on the pathogenesis of TB, supporting the effects of an excessive but otherwise intact adaptive immune response to *M. tuberculosis* during DM. These studies provide a rational basis for testing combined antimicrobial and anti-inflammatory therapies in diabetic patients with TB. A better understanding of the immunological basis of TB susceptibility in DM will help in the rational development of therapeutic strategies to alleviate the dual burden of these diseases.

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