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In the present study we aimed to understand the T cell responses in pulmonary and extra-pulmonary tuberculosis with or without type 2 diabetes mellitus. T cell mediated immune responses play a major role in protective immune responses to tuberculosis. The ability of CD4⁺ T cells to produce Type 1 cytokines (especially INFγ), that can activate phagocytes to contain/constrain the intracellular mycobacterial pathogen, is crucial in host protection other CD4⁺ T cell subsets other than the Th1-type may also play a role in protection from tuberculous disease most notably the IL-17 producing CD4⁺ T cells (Th17 cells) that have been shown to mediate the recruitment of protective Th1 cells in lungs.

To study roles of T cell cytokines and potential regulatory factors, we examined Mtb antigen-specific induction of Type 1, 2, and 17 responses as well as production of IL-10 and TGFβ in pulmonary TB (PTB), tuberculous lymphadenitis (TBL) and latent TB (LTB) individuals in an area highly endemic for tuberculosis. We observed that active pulmonary TB was characterized by a diminution of spontaneous and antigen-specific production of Type 1, 2 and 17 cytokines. TBL individuals, in contrast to those with PTB, exhibited a reduction only in the production of Type 1 (but not Type-2 or -17) cytokines. The suppression of cytokine responses in PTB was primarily mediated by IL-10.

TB lymphadenitis (TBL) is the commonest form of extra-pulmonary TB and, in the face of HIV/AIDS, is becoming a major health problem worldwide. In addition, TBL adds a layer of complexity in the field of TB due to the difficulty in diagnosis and treatment. To study the role of Th1, Th17, and Th22 cell in the pathogenesis of TBL, we examined baseline, antigen-specific, and polyclonal induction of Th1, Th17 and Th22 cells in TBL and compared them to those in
PTB individuals. We show that TBL individuals have elevated frequencies of single, double, and triple cytokine-producing CD4+ Th1 and Th17 cells, both at baseline and following mycobacterial antigen stimulation, in comparison with PTB patients. We also show that frequencies of natural Tregs (nTregs) in individuals with TB disease were inversely related to frequencies of mono- and multifunctional Th1 but not Th17 cells.

While the roles of T and NK cells are well understood in the context of active pulmonary TB, the role of these cells in extra-pulmonary TB is less well understood. Since TBL is felt to reflect a hematogenous disseminated form of TB, we postulated that T and NK cells might play a different role in TBL compared to PTB. To this end, we examined the frequencies of CD4+ and CD8+ T and NK cells expressing Type 1 and Type 17 cytokines in TBL, both at baseline and following mycobacterial antigen stimulation and have demonstrated that in TBL there is an expansion of these cell types that is mediated in part by IL-1 and IL-6.

Tuberculosis (TB) is associated with oxidative stress and the induction of host antioxidants to counteract this response. Heme oxygenase-1 (HO-1) is a critical promoter of cytoprotection in diverse disease models including mycobacterial infection. Nevertheless, the pattern of expression of HO-1 in human tuberculosis has not been studied. Here, we examine expression of HO-1 in M. tuberculosis-exposed and -infected individuals and test its ability to distinguish active from latent and successfully treated TB cases. Systemic levels of HO-1 were dramatically increased in individuals with active pulmonary and extra-pulmonary tuberculosis and particularly those with bilateral lung lesions and elevated bacillary loads in sputum. HO-1 levels effectively discriminated active from latent tuberculosis with higher predictive values than either C-reactive protein or serum amyloid protein. Moreover, there was a marked reduction in
HO-1 levels in active TB cases following anti-tuberculous therapy but not in those who failed treatment. These findings establish HO-1 levels as a potentially useful parameter for distinguishing active from latent or treated pulmonary tuberculosis.

Type 2 diabetes mellitus is a major risk factor for the development of active tuberculosis, although the biological basis underlying this susceptibility remains poorly characterized. To identify the influence of coincident diabetes mellitus on cytokine levels in pulmonary tuberculosis, we examined circulating levels of a panel of cytokines and chemokines in the plasma of individuals with tuberculosis with diabetes and compared them with those of individuals without diabetes. Tuberculosis with diabetes is characterized by elevated circulating levels of type 1, type 2, and type 17. This was associated with increased systemic levels of other proinflammatory cytokines and an antiinflammatory cytokine but not type 1 IFNs. Moreover, tuberculosis antigen–stimulated whole blood also showed increased levels of proinflammatory cytokines. Finally, type 1 and type 17 cytokines in plasma exhibit a significant positive correlation with hemoglobin A1C levels, indicating that impaired control of diabetes is associated with this proinflammatory milieu. Multivariate analysis revealed that the association of proinflammatory cytokines with diabetes mellitus was not influenced by age, sex, or other metabolic parameters.

To characterize the role of Th1 and Th17 cells in tuberculosis with coincident diabetes (DM), we examined mycobacteria-specific immune responses in the whole blood of individuals who had tuberculosis with DM and compared them to those in individuals who had tuberculosis without DM. Tuberculosis coincident with DM is characterized by elevated frequencies of monofunctional and dualfunctional CD4+ Th1 cells following Mycobacterium tuberculosis antigen stimulation and elevated frequencies of Th17 subsets at both baseline and following
antigen stimulation. This was associated with increased systemic (plasma) levels of both Th1 and Th17 cytokines and decreased baseline frequencies of natural regulatory T cells but not interleukin 10 or transforming growth factor β.

To identify the role of CD8⁺ T and NK cells in pulmonary TB with diabetes, we examined mycobacteria–specific immune responses in the whole blood of individuals with TB-DM and compared them with those without DM (TB-NDM). TB-DM is characterized by elevated frequencies of mycobacterial - antigen stimulated CD8⁺ T cells expressing Type 1 (IFNγ and IL-2) and Type 17 (IL-17F) cytokines. TB-DM is also characterized by expanded frequencies of TB - antigen stimulated NK cells expressing Type 1 (TNF-α) and Type 17 (IL-17A and IL-17F) cytokines. This was not associated with alterations in CD8⁺ T cell or NK cell numbers or subset distribution.

Understanding the impact of type 2 diabetes mellitus (T2DM) on TB and the determinants of comorbidity is critical in responding to this growing public health problem. Here, we have tested a series of candidate biomarkers that could be utilized for tracking susceptibility to TB-T2DM. Cross-sectional analysis of levels of heme oxygenase-1 (HO-1), acute phase proteins, tissue metalloproteinases (MMPs) and their inhibitors (TIMPs) as well as cytokines and chemokines were performed in plasma samples from individuals with active pulmonary TB and with coincident TB-T2DM. Plasma levels of HO-1 were higher in TB-T2DM patients than in non-diabetics, independent of bacillary sputum loads. HO-1 concentrations positively correlated with markers associated with poor glucose control, such as random plasma glucose levels, percent of circulating glycosylated hemoglobin and levels of low-density lipoprotein cholesterol. Moreover, circulating levels of HO-1, but not of other acute phase proteins, could distinguish diabetic from non-diabetic individuals with active TB. Patients with
coincident TBT2DM also exhibited increased plasma levels of TIMP-4 and elevated peripheral blood neutrophil counts; when these markers were considered together with HO-1 there was an increased ability to discriminate between the diabetic and non-diabetic individuals with active TB. Principal component analysis confirmed, within a large panel of biomarkers of inflammation and tissue damage/remodeling, that simultaneous measurement of HO-1, TIMP-4 and neutrophils can distinguish diabetic from non-diabetic patients with active TB. Intriguingly, there were no significant associations between either HO-1 or TIMP-4 and a variety of different cytokines, chemokines and other immune markers based on correlation analyses.

To identify the influence of coincident DM on cytokine levels in pulmonary TB, we examined circulating levels of adipocytokines cytokines, pancreactic hormones and gut hormones in the plasma of individuals with TB-DM and compared them with those without DM (TB-NDM), our data reveal that TB-DM is characterized by diminished adipocytokines and highted responsiveness, indicating that chronic inflammation underlying Type 2 diabetes potentially contributes to increased immune pathology and poor control in TB infection.