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BRIEF REPORT

Impaired Cytokine but Enhanced Cytotoxic Marker Expression in *Mycobacterium tuberculosis*–Induced $CD8^+$ T Cells in Individuals With Type 2 Diabetes and Latent *Mycobacterium tuberculosis* Infection

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Type 2 diabetes mellitus (DM) is a risk factor for tuberculosis among individuals with latent *Mycobacterium tuberculosis* infection. To explore the influence of DM on $CD8^+$ T-cell responses during latent *M. tuberculosis* infection, we estimated the cytokine and cytotoxic marker expression pattern in individuals with latent *M. tuberculosis* infection with DM and those with latent *M. tuberculosis* infection without DM. Among individuals with latent *M. tuberculosis* infection, those with DM had diminished frequencies of $CD8^+$ T-helper type 1 (Th1), Th2, and Th17 cells following stimulation by *M. tuberculosis* antigen and enhanced frequencies of $CD8^+$ T cells expressing cytotoxic markers, compared with those without DM. Thus, our results suggest that coincident DM modulates $CD8^+$ T-cell function during latent *M. tuberculosis* infection.

Keywords. latent *M. tuberculosis* infection; diabetes mellitus; $CD8^+$ T cells; cytokines.

Diabetes mellitus (DM) is a major risk factor for the development of tuberculosis, conferring a >3-fold increase in the risk of this disease [1]. DM is also a major contributor to the severity of lung disease, poor outcomes of tuberculosis treatment, and drug resistance in diseased individuals [2]. It is also now estimated that 15% of the tuberculosis burden worldwide is attributable to DM [3]. The biological basis for the increased susceptibility to tuberculosis among individuals with DM is still largely unknown, although there is recent evidence that innate and adaptive immune responses to *M. tuberculosis* antigens are affected in individuals with DM [3]. Thus, exaggerated production of proinflammatory cytokines, systemic immune activation markers, and markers of inflammation, as well as heightened activation of $CD4^+$ T cells, $CD8^+$ T cells, and natural killer cell responses are highly characteristic of tuberculosis and DM comorbidity [4–7]. However, very few data are available on the innate and adaptive immune interaction between DM and latent *M. tuberculosis* infection.

Although $CD8^+$ T cells were initially considered to be less important in the immune response to *M. tuberculosis* infection, it is now clear that $CD8^+$ T cells play a vital role in this immune response [8]. Like $CD4^+$ T cells, $CD8^+$ T cells are able to produce interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and interleukin 2 (IL-2), which are known to have critical functions during *M. tuberculosis* infection [8, 9]. $CD8^+$ T-cell–expressing T-helper type 1 (Th1) cytokines can be monofunctional (defined as expression of 1 cytokine) or multifunctional (defined as expression of >1 cytokine). In addition, $CD8^+$ T cells can also produce other cytokines, such as interleukin 17A (IL-17A), IL-17F, interleukin 4 (IL-4), interleukin 5 (IL-5), and interleukin 13 (IL-13), which can also have a protective or pathogenic effect on *M. tuberculosis* infection [8, 9]. Finally, $CD8^+$ T cells have cytolytic functions to kill *M. tuberculosis*–infected cells via processes mediated by granules (ie, perforin, granzyme, and granulysin) [8, 9]. Expression of cytotoxic molecules, including CD107a, perforin, and granzyme B, is an indirect indicator of the ability of $CD8^+$ T cells to kill target cells.

To study the influence of DM on $CD8^+$ T-cell responses in latent *M. tuberculosis* infection, we compared baseline, antigen-stimulated, and polyclonal induction of $CD8^+$ T-cell–expressed cytokines and cytotoxic markers in individuals with latent *M. tuberculosis* infection and coincident DM to findings in those without diabetes. We show that individuals with latent *M. tuberculosis* infection and DM have diminished frequencies of cytokine-expressing $CD8^+$ T cells and elevated frequencies of cytotoxic marker–expressing $CD8^+$ T cells. Thus, our data demonstrate that DM profoundly alters the $CD8^+$ T-cell response to *M. tuberculosis* antigens and possibly contributes to increased susceptibility to active disease.

METHODS

Study Population

We studied 44 individuals with latent *M. tuberculosis* infection, of whom 22 had and 22 did not have concomitant DM. Latent *M. tuberculosis* infection was diagnosed on the basis of positive results of both the tuberculin skin test (induration diameter, >12 mm) and the Quantiferon TB Gold In-Tube assay (Qiagen), the absence of pulmonary symptoms, and the presence of normal findings of chest radiography (Supplementary

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Table 1). DM was diagnosed on the basis of an elevated glucose level (ie, >200 mg/dL) in a random blood sample and an elevated glycated hemoglobin level (HbA1c; ie, >6.5%). All individuals with DM had recently received their DM diagnosis and were not receiving antidiabetic medication. All individuals were negative for human immunodeficiency virus (HIV). Compared with subjects with latent *M. tuberculosis* infection and without diabetes, those with diabetes and latent *M. tuberculosis* infection had higher fasting blood glucose, HbA1c, alanine aminotransferase, serum cholesterol, low-density lipoprotein cholesterol, and triglyceride levels but lower high-density lipoprotein cholesterol levels (Supplementary Table 1). The groups did not differ significantly in age, sex, or body mass index (Supplementary Table 1). All individuals were examined as part of a clinical protocol approved by the Institutional Review Board of the National Institute of Research in Tuberculosis (clinical trials registration NCT00375583), and informed written consent was obtained from all participants.

Antigens

M. tuberculosis antigens used were purified protein derivative (PPD; Statens Serum Institute), ESAT-6 peptide pools, and CFP-10 peptide pools (both from BEI resources, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH]), HIV Gag peptide pools (AIDS Reagent Program, Division of AIDS, NIAID, NIH) was used as a control antigen. Final concentrations were 10 μg/mL for PPD, ESAT-6, CFP-10 and HIV Gag peptide pools. Phorbol myristoyl acetate and ionomycin (P/I) were used at concentrations of 12.5 ng/mL and 125 ng/mL, respectively.

In Vitro Culture

Whole blood specimens were diluted 1:1 with Roswell Park Memorial Institute 1640 medium supplemented with penicillin/streptomycin (100 U/100 mg/mL), L-glutamine (2 mM), and HEPES (10 mM; all from Invitrogen) and distributed in 12-well tissue culture plates (Costar). The cultures were then stimulated with PPD, ESAT-6, CFP-10, P/I, or medium alone in the presence of the costimulatory molecules CD49d/CD28 at 37°C for 6 hours. Brefeldin A (10 μg/mL) was added after 2 hours. After 6 hours, centrifugation, washing, and red blood cell lysis was performed. The cells were fixed using cytofix/cytoperm buffer (BD Biosciences) and cryopreserved at −80°C.

Intracellular Cytokine and Cytotoxic Marker Staining

The cells were thawed, washed, and then stained with surface antibodies for 30–60 minutes. Surface antibodies used were CD3 and CD8. The cells were washed and permeabilized with BD Perm/Wash buffer (BD Biosciences) and stained with intracellular cytokines for an additional 30 minutes before washing and acquisition. Cytokine antibodies used were IFN-γ, TNF-α, IL-2, IL-17F, IL-17A, IL-4, IL-5, and IL-13. Cytotoxic marker antibodies used were CD107a, perforin, and granzyme B. Eight-color flow cytometry was performed on a FACSCanto II flow cytometer with FACSDiva software, version 6 (Becton Dickinson). The lymphocyte gating was set by forward and side scatter, and 100 000 lymphocytes events were acquired. Data were collected and analyzed using FlowJo software (TreeStar). All data are depicted as frequencies of $CD8^+$ T cells expressing cytokine(s) or cytotoxic markers. Baseline values following stimulation by medium are depicted as the baseline frequency, while frequencies following stimulation with antigens are depicted as net frequencies (with baseline values subtracted).

Statistical Analysis

Data analyses were performed using GraphPad PRISM (GraphPad Software). Geometric means were used for measurements of central tendency. Statistically significant differences between 2 groups were analyzed using the nonparametric Mann–Whitney *U* test.

RESULTS

Latent *M. tuberculosis* Infection With DM Is Associated With Decreased Frequencies of $CD8^+$ T Cells Expressing Cytokines After Antigen Stimulation

To determine the influence of DM on $CD8^+$ Th1, Th2, and Th17 cells in latent *M. tuberculosis* infection, we measured the frequencies $CD8^+$ T cells expressing IFN-γ, IL-2, TNF-α, IL-4, IL-5, IL-13, IL-17A, and IL-17F at baseline and following stimulation with either mycobacterial antigens or P/I. A representative contour plot showing the baseline, ESAT-6–stimulated, and P/I-stimulated frequencies of $CD8^+$ Th1, Th2, and Th17 cells is shown in Supplementary Figure 1. As shown in Figure 1*A*, individuals with latent *M. tuberculosis* infection and DM exhibited significantly reduced frequencies of $CD8^+$ Th1 (IFN-γ) cells at baseline. Similarly, in response to ESAT-6 (Figure 1*B*) or CFP-10 (Figure 1*C*) or PPD (Supplementary Figure 3*A*), individuals with latent *M. tuberculosis* infection and DM exhibited significantly decreased frequencies of $CD8^+$ Th1, Th2, and Th17 cells, as well. In contrast, these individuals did not exhibit any significant difference in the frequencies of $CD8^+$ Th1, Th2, or Th17 cells in response to control antigen (HIV Gag; Supplementary Figure 3*B*) or P/I (Figure 1*D*).

Latent *M. tuberculosis* Infection With DM Is Associated With Increased Frequencies of $CD8^+$ T Cells Expressing Cytotoxic Markers After Antigen Stimulation

To determine the influence of DM on $CD8^+$ T cells expressing cytotoxic markers during latent *M. tuberculosis* infection, we measured the frequencies $CD8^+$ T cells expressing CD107a, perforin, and granzyme B at baseline and following stimulation with either mycobacterial antigens or P/I. A representative contour plot showing the baseline, ESAT-6–stimulated, and P/I-stimulated cytotoxic marker expression on $CD8^+$ T cells is shown in Supplementary Figure 2. As shown in Figure 2*A*, individuals with latent *M. tuberculosis* infection and DM exhibited significantly increased frequencies of CD107a-expressing $CD8^+$ T cells at baseline. However, in response to ESAT-6

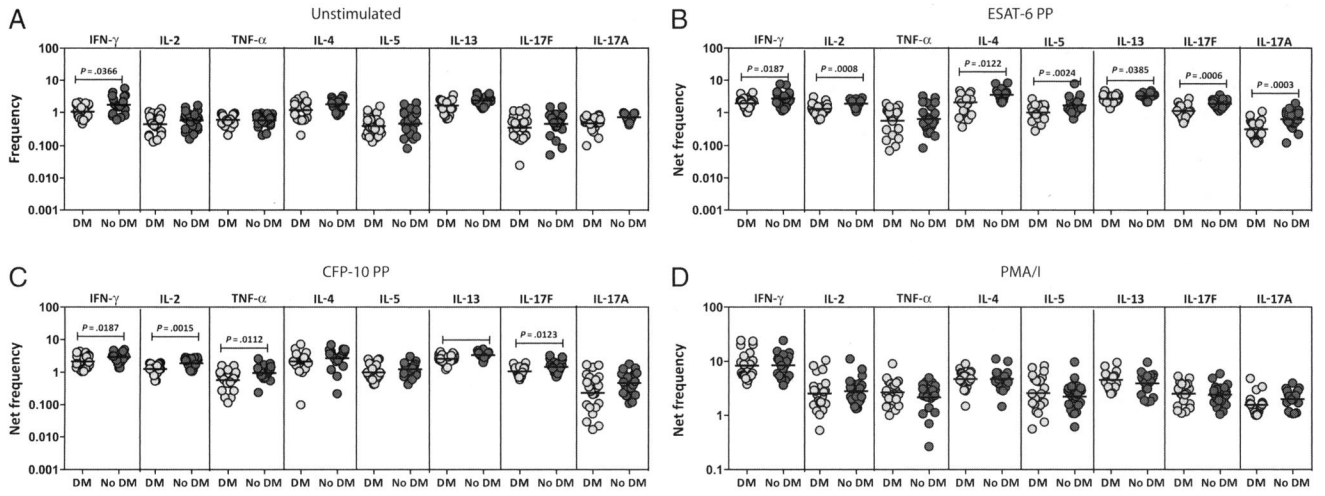

Figure 1. Latent *Mycobacterium tuberculosis* infection with type 2 diabetes mellitus (DM) is associated with decreased antigen-induced frequency of $CD8^+$ T-helper type 1 (Th1), Th2, and Th17 cells. Whole-blood specimens were cultured with medium alone or mycobacterial antigens or phorbol myristoyl acetate and ionomycin (P/I) for 6 h, and the baseline and antigen-stimulated frequencies of $CD8^+$ Th1, Th2, and Th17 cells were determined. The baseline (*A*) as well as ESAT-6 peptide pool–stimulated (*B*), CFP-10 peptide pool–stimulated (*C*), and P/I-stimulated (*D*) frequencies of $CD8^+$ Th1, Th2, and Th17 cells in 22 individuals with latent *M. tuberculosis* infection and DM and 22 with latent *M. tuberculosis* infection without DM are shown. Each circle represents a single individual, and the bars represent the geometric mean values. Net frequencies were calculated by subtracting baseline frequencies from the antigen-induced frequencies for each individual. *P* values were calculated using the Mann–Whitney *U* test. Abbreviations: IFN-γ, interferon γ; IL-2, interleukin 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-13, interleukin 13; IL-17, interleukin 17; TNF-α, tumor necrosis factor α.

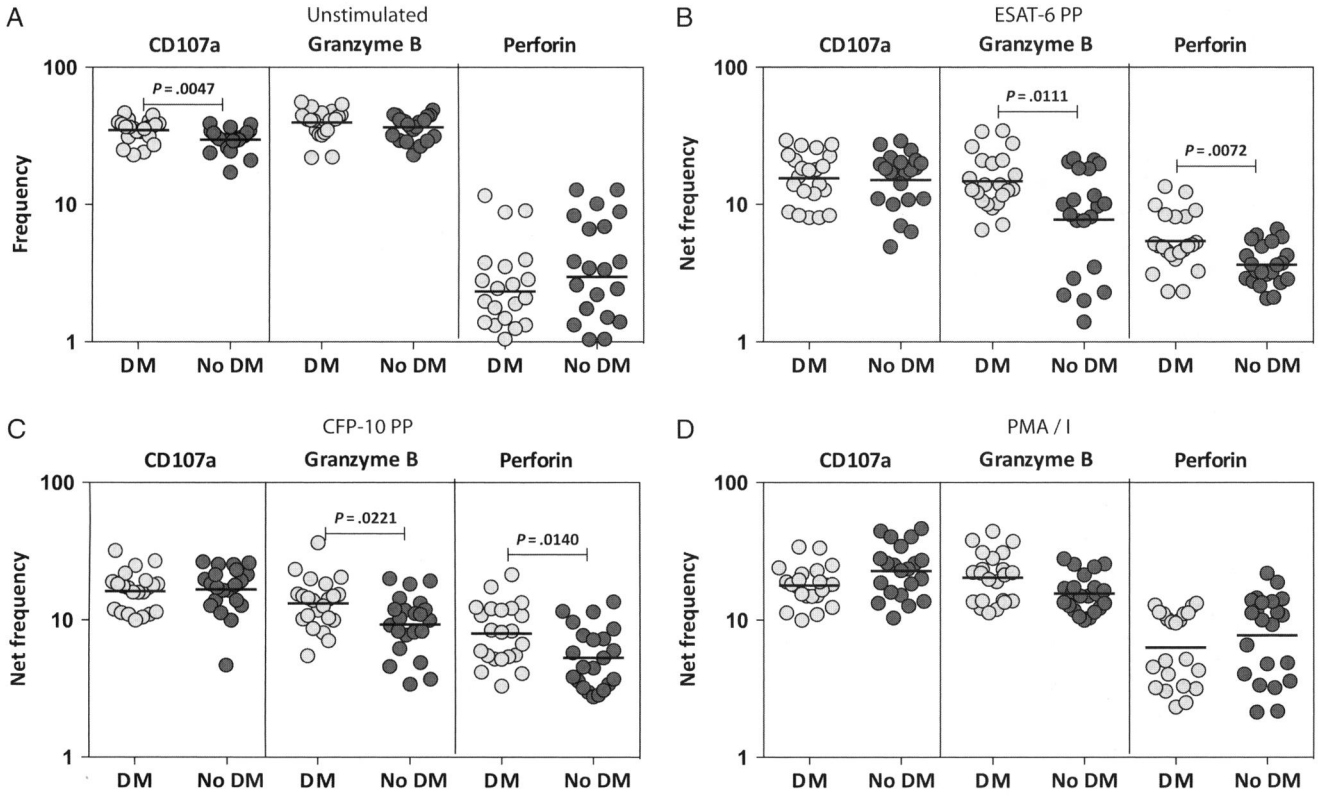

Figure 2. Latent *Mycobacterium tuberculosis* infection with type 2 diabetes mellitus (DM) is associated with increased antigen-induced frequency of perforin- and granzyme B–expressing $CD8^+$ T cells. Whole blood specimens were cultured with medium alone, mycobacterial antigens, or phorbol myristoyl acetate and ionomycin (P/I) for 6 hours, and the baseline and antigen-stimulated frequencies of $CD8^+$ T cells expressing CD107a, perforin, and granzyme B were determined. The baseline (*A*), ESAT-6 peptide pool–stimulated (*B*), CFP-10 peptide pool–stimulated (*C*), and P/I-stimulated (*D*) frequencies of $CD8^+$ T cells expressing the above markers in 22 individuals with latent *M. tuberculosis* infection and DM and 22 individuals with latent *M. tuberculosis* infection without DM are shown. Each circle represents a single individual, and the bars represent the geometric mean values. Net frequencies were calculated by subtracting baseline frequencies from the antigen-induced frequencies for each individual. *P* values were calculated using the Mann–Whitney *U* test.

(Figure 2*B*), CFP-10 (Figure 2*C*), or PPD (Supplementary Figure 4*A*), individuals with latent *M. tuberculosis* infection and DM exhibited significantly increased frequencies of $CD8^+$ T cells expressing perforin and granzyme B. In contrast, these individuals did not exhibit any significant difference in the frequencies of $CD8^+$ T cells expressing any cytotoxic markers in response to control antigen (HIV Gag; Supplementary Figure 4*B*) or P/I (Figure 2*D*).

DISCUSSION

Tuberculosis comorbidity with noncommunicable diseases such as DM is highly prevalent in tuberculosis-endemic areas, and estimates of population-attributable tuberculosis risk due to DM are very high [2]. Although several studies have investigated adaptive immune responses in tuberculosis-DM comorbidity, very few studies have studied the adaptive immune responses in cases of latent *M. tuberculosis* infection and DM [3]. We have previously shown that individuals with latent *M. tuberculosis* infection and DM are characterized by diminished systemic and antigen-specific Th1 and Th17 cytokine responses [10]. In addition, we have also previously shown that latent *M. tuberculosis* infection with DM is not associated with any significant alterations in the proportion of naive, effector, central memory, and effector memory $CD8^+$ T cells but is associated with significantly lower proportions of myeloid and plasmacytoid dendritic cells, as well as classical and intermediate monocytes, compared with latent *M. tuberculosis* infection alone [11]. The alterations in the frequency of major antigen-presenting cells in cases of latent *M. tuberculosis* infection with DM led us to hypothesize that it would reflect in the activation of $CD8^+$ T cells.

Our findings reveal a dichotomy in the expression and function of $CD8^+$ T cells during latent *M. tuberculosis* infection with DM. While cytokine expression on antigen-stimulated $CD8^+$ T cells is clearly diminished, the expression of cytotoxic markers reflecting the capacity for degranulation and killing activity are clearly enhanced in these individuals. This is in marked contrast to the expression profile of $CD8^+$ T cells in individuals with tuberculosis and concomitant DM, in whom cytokine expression was enhanced and cytotoxic marker expression was diminished, as reported previously [6]. This is also in contrast to the data showing impaired expression of CD107a in $CD8^+$ T cells in HIV-infected individuals with latent *M. tuberculosis* infection [12]. It is therefore likely that DM induces a phenotypic change that is different during latent *M. tuberculosis* infection, compared with that during tuberculosis, and is also different from that during HIV coinfection. Thus, DM appears to play a significant regulatory role in the expression and function of $CD8^+$ T cells, depending on the status of *M. tuberculosis* infection or disease. Interestingly, the impairment of cytokine function and enhancement of cytotoxic marker expression is augmented upon stimulation with *M. tuberculosis* antigens, PPD, ESAT-6, and CFP-10 peptides and is also pathogen induced, since the differences between the 2 groups of latent *M. tuberculosis* infection are almost completely abolished upon stimulation with a control antigen or polyclonal stimulus.

Although less well studied, modulation of $CD8^+$ T-cell function is a major characteristic of human tuberculosis [13]. Diminished Th1 cytokine production by antigen-stimulated $CD8^+$ T cells appears to be typical of individuals with pulmonary tuberculosis, compared with those with latent *M. tuberculosis* infection [14]. Similarly, both peripheral and local $CD8^+$ T cells have been reported to exhibit reduced cytotoxic activity in tuberculosis, compared with latent *M. tuberculosis* infection [15]. Our data indicate that individuals with latent *M. tuberculosis* infection and DM appear to resemble the phenotypic and functional profile associated with tuberculosis, rather than latent infection. Thus, DM is associated with altered $CD8^+$ T-cell expression and function in the setting of latent infection. Our study also is the first study to examine the expression profile of Th2 and Th2 cytokines in $CD8^+$ T cells. While Th17 responses are important in memory responses and immunity to *M. tuberculosis* infection, Th2 responses might actively promote the acquisition of overt disease. Our data on the association of $CD8^+$ T cells expressing these cytokines appears to suggest that DM is associated with a global diminution in the antigen-stimulated frequencies of most $CD8^+$ T-cell subsets, irrespective of their cytokine status. Our study thus provides important insights into the influence of DM on the pathogenesis of *M. tuberculosis* infection and disease, by influencing the role of antigen-stimulated $CD8^+$ T cells in maintaining latency and causing perturbations in this functional arm of the immune system. Our data support evidence that $CD8^+$ T-cell responses are associated with protective immunity to tuberculosis and that deficiency in this arm of the immune system in the presence of DM could predispose individuals to develop tuberculosis. Our study did not obtain information regarding smoking history, alcohol use, socioeconomic status, or other potential confounding variables that could affect $CD8^+$ T-cell responses. Our study also suffers from being an associative study but nevertheless provides impetus for future studies examining the role of $CD8^+$ T cells as correlates of protective immunity in latent infection and its alteration in the presence of metabolic abnormalities.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med **2008**; 5:e152.
2. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. Lancet Infect Dis **2009**; 9:737–46.
3. Ronacher K, Joosten SA, van Crevel R, Dockrell HM, Walzl G, Ottenhoff TH. Acquired immunodeficiencies and tuberculosis: focus on HIV/AIDS and diabetes mellitus. Immunol Rev **2015**; 264:121–37.
4. Kumar NP, Sridhar R, Banurekha VV, et al. Type 2 diabetes mellitus coincident with pulmonary tuberculosis is associated with heightened systemic type 1, type 17, and other proinflammatory cytokines. Ann Am Thorac Soc **2013**; 10:441–9.
5. Kumar NP, Sridhar R, Banurekha VV, Jawahar MS, Nutman TB, Babu S. Expansion of pathogen-specific T-helper 1 and T-helper 17 cells in pulmonary tuberculosis with coincident type 2 diabetes mellitus. J Infect Dis **2013**; 208:739–48.
6. Kumar NP, Sridhar R, Nair D, Banurekha VV, Nutman TB, Babu S. Type 2 diabetes mellitus is associated with altered CD8($^{+}$) T and natural killer cell function in pulmonary tuberculosis. Immunology **2015**; 144:677–86.
7. Restrepo BI, Fisher-Hoch SP, Pino PA, et al. Tuberculosis in poorly controlled type 2 diabetes: altered cytokine expression in peripheral white blood cells. Clin Infect Dis **2008**; 47:634–41.
8. Lin PL, Flynn JL. CD8 T cells and *Mycobacterium tuberculosis* infection. Semin Immunopathol **2015**; 37:239–49.
9. Grotzke JE, Lewinsohn DM. Role of CD8^{+} T lymphocytes in control of *Mycobacterium tuberculosis* infection. Microbes Infect **2005**; 7:776–88.
10. Kumar NP, George PJ, Kumaran P, Dolla CK, Nutman TB, Babu S. Diminished systemic and antigen-specific type 1, type 17, and other proinflammatory cytokines in diabetic and prediabetic individuals with latent *Mycobacterium tuberculosis* infection. J Infect Dis **2014**; 210:1670–8.
11. Kumar NP, Moideen K, Dhakshinraj SD, et al. Profiling leucocyte subsets in tuberculosis - diabetes co-morbidity. Immunology **2015**; 146:243–50.
12. Kalokhe AS, Adekambi T, Ibegbu CC, Ray SM, Day CL, Rengarajan J. Impaired degranulation and proliferative capacity of *Mycobacterium tuberculosis*-specific CD8^{+} T cells in HIV-infected individuals with latent tuberculosis. J Infect Dis **2015**; 211:635–40.
13. Jasenosky LD, Scriba TJ, Hanekom WA, Goldfeld AE. T cells and adaptive immunity to *Mycobacterium tuberculosis* in humans. Immunol Rev **2015**; 264: 74–87.
14. Day CL, Abrahams DA, Lerumo L, et al. Functional capacity of *Mycobacterium tuberculosis*-specific T cell responses in humans is associated with mycobacterial load. J Immunol **2011**; 187:2222–32.
15. Andersson J, Samarina A, Fink J, Rahman S, Grundstrom S. Impaired expression of perforin and granulysin in CD8^{+} T cells at the site of infection in human chronic pulmonary tuberculosis. Infect Immun **2007**; 75:5210–22.