

Type 2 Diabetes Mellitus Coincident with Pulmonary Tuberculosis Is Associated with Heightened Systemic Type 1, Type 17, and Other Proinflammatory Cytokines

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Abstract

Rationale: 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.

Objectives and Methods: 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.

Measurements and Main Results: Tuberculosis with diabetes is characterized by elevated circulating levels of type 1 (IFN- γ , tumor necrosis factor- α , and IL-2), type 2 (IL-5), and type 17 (IL-17A) cytokines but decreased circulating levels of IL-22. This was associated with increased systemic levels of other proinflammatory cytokines (IL-1 β , IL-6, and IL-18) and an antiinflammatory cytokine

(IL-10) 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.

Conclusions: Our data reveal that tuberculosis with diabetes is characterized by heightened cytokine responsiveness, indicating that chronic inflammation underlying type 2 diabetes potentially contributes to increased immune pathology and poor control in tuberculosis infection.

Keywords: bacterial; cytokines; chemokines; tuberculosis; diabetes mellitus

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Among the many risk factors for the development of tuberculosis, type 2 diabetes mellitus is one that has long been recognized through clinical and epidemiological studies (1). There is growing evidence that type 2 diabetes mellitus is an important risk factor for developing active pulmonary tuberculosis (2). In fact, a meta-analysis of

13 observational studies on the risk for tuberculosis disease in diabetics determined that diabetic patients were 3.1 times more likely to have tuberculosis than nondiabetic individuals (2). In addition to increasing the risk for reactivating latent tuberculosis infection, there is also evidence that type 2 diabetes mellitus is also associated with

greater severity of tuberculosis disease affecting both disease presentation and response to treatment. Despite the clinical and public health significance posed by the dual burden of tuberculosis and diabetes mellitus, little is known about the immunological and biochemical mechanisms of susceptibility.

Enhanced susceptibility to tuberculosis in patients with diabetes mellitus has been ascribed to several factors including those related directly to hyperglycemia and insulin resistance as well as to indirect effects on macrophage and lymphocyte function (1). Several early studies reported reduced proinflammatory cytokines in patients with diabetes after infection with *Mycobacterium tuberculosis* (3, 4). Two studies, however, reported the elevation of helper T-cell type 1 (Th1) cytokines in tuberculosis-infected diabetic hosts; one was conducted in streptozocin-treated mice (5) and another in diabetic and nondiabetic patients with tuberculosis (6). In addition, we have previously shown that type 2 diabetes mellitus in tuberculosis disease is associated with elevated frequencies of Th1 and Th17 cells and cytokines (7). Cytokines of the innate and adaptive immune systems orchestrate the immune response to tuberculosis infection, with type 1, type 17, and the IL-1 family of cytokines having been implicated in protection against tuberculosis disease (8) in murine systems whereas type 2 and antiinflammatory cytokines along with type 1 IFNs have been associated with either increased susceptibility to disease and/or enhanced pathology (8).

To study the influence of type 2 diabetes mellitus on active pulmonary tuberculosis, we examined levels of a large panel of type 1, type 2, type 17, regulatory, and other proinflammatory cytokines and chemokines in individuals with active tuberculosis and coincident diabetes and compared them with those in individuals

with active tuberculosis but without diabetes. We also examined tuberculosis antigen-stimulated levels of certain cytokines in the whole blood of individuals with tuberculosis and diabetes and in individuals with tuberculosis without diabetes. We show that those with tuberculosis and diabetes have increased systemic levels of most of the proinflammatory cytokines as well as IL-5 and IL-10 but have decreased levels of IL-22 and type 1 IFN. In addition, tuberculosis antigen-stimulated levels of most of these cytokines were also increased in individuals with tuberculosis and diabetes. Thus, our data suggest that active tuberculosis with coincident diabetes mellitus is associated with heightened cytokine levels, levels possibly contributing to immune-mediated pathology in tuberculosis.

Methods

Study Population

We studied a group of 88 individuals with active pulmonary tuberculosis attending the Tuberculosis Clinic at Stanley Medical Hospital in Chennai, India—the first consecutive 44 with diabetes and the first 44 without. Active pulmonary tuberculosis was diagnosed on the basis of sputum smear and culture positivity. Type 2 diabetes mellitus was diagnosed on the basis of glycated hemoglobin (HbA1c) levels and random blood glucose, according to the American Diabetes Association criteria (all type 2 diabetes individuals had HbA1c

levels > 6.5% and random blood glucose > 200 mg/dl). All the individuals were HIV negative. The two groups did not differ significantly in terms of radiological extent of disease or the site of disease as assessed by chest X-ray readings from three independent experts. The two groups did differ significantly in smear grades, with the diabetes group having worse smear grades as assessed by the Mann-Whitney test, corrected for ties ($P = 0.005$), indicating higher bacillary burdens. All individuals were anti-tuberculous treatment naive. Anthropometric measurements, including height, weight, and waist circumference, and biochemical parameters, including plasma glucose, lipid profile, and HbA1c, were obtained by standardized techniques as detailed elsewhere (9). Hematology was performed on all individuals, using the Ac·T 5diff hematology analyzer (Beckman Coulter, Brea, CA). This study comprised a separate set of individuals compared with our previous study on tuberculosis and diabetes (7). All individuals were examined as part of a clinical protocol approved by the Institutional Review Board of the National Institute of Research in Tuberculosis (NCT01154959), and informed written consent was obtained from all participants.

ELISA

Plasma cytokines and chemokines were measured with a Bio-Plex multiplex cytokine assay system (Bio-Rad, Hercules, CA). The parameters analyzed were IFN- γ , tumor necrosis factor (TNF)- α , IL-2,

Table 1. Demographics of individuals with tuberculosis and diabetes and of individuals with tuberculosis without diabetes

	TB-DM (n = 44)	TB-NDM (n = 44)	P Value
Age, yr	45 (33–70)	43.5 (20–70)	0.0016
Sex, M/F	31/13	38/6	NS
BMI, kg/m ²	23.90 (19.56–33.38)	22.16 (14.01–31.22)	NS
Duration of diabetes, yr	4.5 (1–28)	—	
Systolic blood pressure, mm Hg	115 (82–152)	117 (90–169)	NS
Diastolic blood pressure, mm Hg	70 (59–90)	79.5 (40–112)	NS
Random glucose, mg/dl	287 (200–653)	101.5 (76–177)	<0.0001
Glycated hemoglobin, %	11.3 (8.01–16.16)	5.6 (4.46–6.30)	<0.0001
Total cholesterol, mg/dl	215 (124–259)	182.5 (134–296)	0.0079
Serum triglycerides, mg/dl	182 (57–679)	84.5 (57–433)	<0.0001
High-density lipoprotein cholesterol, mg/dl	38 (22–82)	47 (34–86)	0.0002
Low-density lipoprotein cholesterol, mg/dl	124 (47–185)	112 (65–205)	0.1196

Definition of abbreviations: BMI = body mass index; F = female; M = male; NS = not significant; TB-DM = tuberculosis with diabetes mellitus; TB-NDM = tuberculosis without diabetes mellitus.

Values represent geometric means and range (except for age, for which median and range are shown). *P* values were calculated by Mann-Whitney test (except for sex, which was tested by Fisher exact test).

IL-17A, IL-4, IL-5, IL-13, IL-10, IL-6, IL-12, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8, chemokine ligand-11 (CCL11), granulocyte colony-stimulating factor (G-CSF), chemokine ligand-2 and -4 (CCL2 and CCL4, respectively), and platelet-derived growth factor (PDGF). Plasma levels of transforming growth factor (TGF)- β , IL-1 α , IL-1 β , IL-18, IFN- γ -inducible protein-10 (IP-10) (all R&D Systems); IL-17F (BioLegend, San Diego, CA); IL-22 (eBioscience, San Diego, CA); type 1 IFNs—IFN- α (multiple subtypes) and IFN- β (PBL Interferon Source, Piscataway, NJ)—were measured by ELISA.

QuantiFERON Supernatant ELISA

Whole blood from a subset of individuals with tuberculosis and diabetes and individuals with tuberculosis without diabetes ($n = 22$ each) was incubated with either no antigen or tuberculosis antigen (ESAT-6, CFP-10, TB 7.7) or mitogen (phorbol ester/ionomycin) according to the manufacturer's instructions, using a QuantiFERON In-Tube Gold kit (Qiagen, Valencia, CA). Unstimulated or tuberculosis antigen-stimulated whole blood supernatants were then used to analyze levels of IFN- γ , TNF- α , IL-17, IL-10, IL-1 β , and IL-18, using DuoSet ELISA kits from R&D Systems (Minneapolis, MN).

Statistical Analysis

Geometric means were used for measurements of central tendency. Statistically significant differences between two groups were analyzed by nonparametric Mann-Whitney U test. Correlations were calculated by Spearman rank correlation. For discriminating diabetes from nondiabetes, using cytokines, we used a Holm's adjusted stepwise logistic modeling procedure with 24 of the cytokines and chemokines. Briefly, the Holm's adjusted stepwise logistic modeling procedure is a forward stepwise procedure in the spirit of Holm's multiple comparison correction: for $k = 24$ variables, include the first variable if its $P < 0.05/k$, include second if its $P < 0.05/(k - 1)$, and so on, stopping when no new variable may be included. This procedure ensures that the overall probability of selecting any significant variable by chance is bounded at the 0.05 level. Because highly correlated variables with selected variables may not be selected

by the procedure, we also used logistic regression for each variable alone. We estimated the percentage of correct prediction by leave-one-out cross validation, and give the Holm's adjusted P values, which correct for the multiple comparisons. Analyses were performed with GraphPad PRISM version 5.01 (GraphPad Software, San Diego, CA)

(Mann-Whitney and correlations) or R version 2.15.2.

Results

Study Population Characteristics

The baseline characteristics including demographics and clinical and biochemical

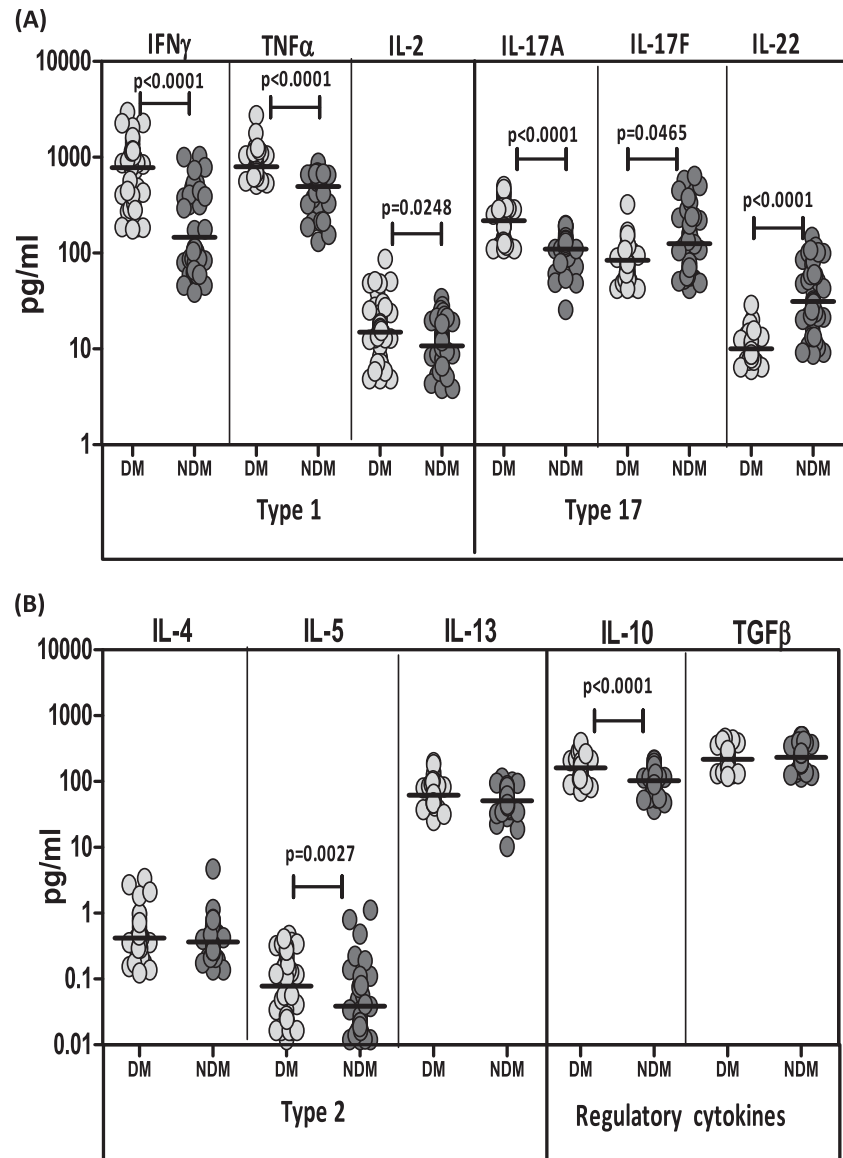


Figure 1. Elevated systemic levels of type 1 and type 17 cytokines as well as IL-5 and IL-10 in tuberculosis with diabetes. Plasma levels of (A) type 1 (IFN- γ , tumor necrosis factor [TNF]- α , IL-2) and type 17 (IL-17A, IL-17F, IL-22) cytokines and (B) type 2 (IL-4, IL-5, IL-13) and regulatory (IL-10, transforming growth factor [TGF]- β) cytokines were measured by ELISA in individuals with tuberculosis and diabetes ($n = 44$) and in individuals with tuberculosis with no diabetes ($n = 44$). Data are represented as scatter plots, with each circle representing a single individual (light gray, diabetes mellitus [DM]; dark gray, non-diabetes mellitus [NDM]). P values were calculated by Mann-Whitney test.

features of the study population are shown in Table 1. As can be seen, compared with subjects without diabetes, those with diabetes and tuberculosis had higher fasting blood glucose, glycated hemoglobin, serum cholesterol, low-density lipoprotein and triglyceride levels but lower high-density lipoprotein cholesterol levels. The groups did not significantly differ in their baseline hematological parameters (data not shown).

Tuberculosis with Diabetes Is Associated with Increased Circulating Levels of Type 1 and Type 17 Cytokines

To determine the influence of diabetes mellitus on type 1 and type 17 cytokines in active tuberculosis, we measured the circulating levels of IFN- γ , TNF- α , and IL-2 as well as IL-17A, IL-17F, and IL-22 in individuals with tuberculosis with or without diabetes (Figure 1). As shown in Figure 1A, the systemic levels of all three type 1 cytokines—IFN- γ (geometric mean of 780.5 pg/ml in tuberculosis with diabetes vs. 146.3 pg/ml in tuberculosis with no diabetes), TNF- α (geometric mean of 796.7 vs. 496.3 pg/ml), and IL-2 (geometric mean of 15.02 vs. 10.73 pg/ml)—were significantly higher in diabetic compared with nondiabetic individuals. Similarly, the systemic level of the prototypical type 17 cytokine IL-17A was also significantly higher in diabetic compared with nondiabetic individuals with tuberculosis (geometric mean of 218.3 vs. 110.2 pg/ml). In contrast, two other cytokines associated with the Th17 response—IL-17F (geometric mean of 84.4 pg/ml vs. 125.1 pg/ml) and IL-22 (geometric mean of 10.1 pg/ml vs. 31.3 pg/ml)—were found to be present at significantly lower levels in diabetic compared with nondiabetic individuals with tuberculosis. Thus, tuberculosis with diabetes is associated with heightened levels of type 1 cytokines and IL-17A at the time of presentation of active pulmonary tuberculosis.

Tuberculosis with Diabetes Is Associated with Increased Circulating Levels of IL-5 and IL-10

To determine the influence of diabetes on type 2 and regulatory cytokines in active tuberculosis, we measured the circulating levels of IL-4, IL-5, and IL-13 as well as IL-10 and TGF- β in diabetic and nondiabetic individuals with tuberculosis

(Figure 1). As shown in Figure 1B, the systemic levels of IL-5 (geometric mean of 78 vs. 38 pg/ml) but not IL-4 and IL-13 were significantly higher in diabetic compared with nondiabetic individuals. Similarly, the systemic levels of the regulatory cytokine IL-10 (geometric mean of 161.8 vs. 102.8 pg/ml) were also significantly higher in diabetic compared with nondiabetic individuals. In contrast, the circulating levels of the other potent regulatory cytokine, TGF- β , was not significantly different between the two groups. Thus, tuberculosis with diabetes is not associated with a concomitant decrease in type 2 or regulatory cytokine levels at the time of presentation with active pulmonary tuberculosis.

Tuberculosis with Diabetes Is Associated with Increased Circulating Levels of IL-1 Family and Other Proinflammatory Cytokines

To determine the influence of diabetes on the IL-1 family and other proinflammatory cytokines as well as type 1 IFNs in active tuberculosis, we measured circulating levels of these in diabetic and nondiabetic individuals with tuberculosis (Figure 2). As shown in Figure 2, systemic levels of the IL-1 family cytokines IL-1 β (geometric

mean of 125.2 vs. 101.7 pg/ml) and IL-18 (geometric mean of 23.3 vs. 10.2 pg/ml), but not IL-1 α , were significantly higher in diabetic compared with nondiabetic individuals. Similarly, the systemic levels of IL-6 (geometric mean of 731.8 vs. 565.4 pg/ml) but not IL-12 or GM-CSF were also significantly higher in diabetic compared with nondiabetic individuals. In contrast, the levels of IFN- β (geometric mean of 6.1 vs. 10.8 pg/ml) but not IFN- α were found to be present at significantly lower levels in diabetic compared with nondiabetic individuals. Thus, tuberculosis with diabetes is associated with heightened levels of proinflammatory cytokines but not type 1 IFNs at the time of presentation.

Tuberculosis with Diabetes Is Associated with Increased Tuberculosis Antigen–Stimulated Levels of Type 1 and Other Proinflammatory Cytokines

To determine the influence of diabetes on tuberculosis antigen–stimulated levels of type 1 and other proinflammatory cytokines in active tuberculosis, we measured circulating levels of these cytokines after stimulation with no antigen or a cocktail of tuberculosis antigens

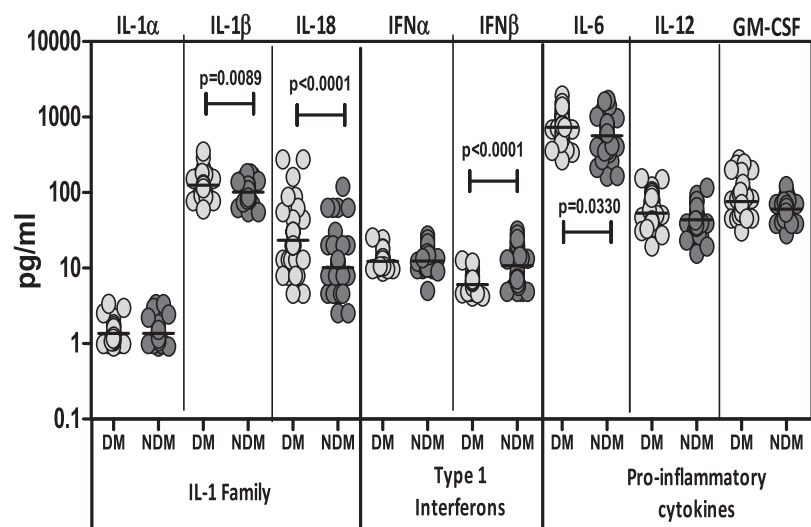


Figure 2. Elevated systemic levels of IL-1 family and other proinflammatory cytokines in tuberculosis with diabetes. Plasma levels of IL-1 family members (IL-1 α , IL-1 β , IL-18), type 1 IFNs (IFN- α and IFN- β), and other proinflammatory (IL-6, IL-12, granulocyte-macrophage colony-stimulating factor [GM-CSF]) cytokines were measured by ELISA in individuals with tuberculosis and diabetes ($n = 44$) and in individuals with tuberculosis with no diabetes ($n = 44$). Data are represented as scatter plots, with each circle representing a single individual (light gray, diabetes mellitus [DM]; dark gray, non-diabetes mellitus [NDM]). P values were calculated by Mann-Whitney test.

(ESAT-6, CFP-10, TB 7.7) in a subset (n = 22 each) of diabetic and nondiabetic individuals with tuberculosis (Figure 3). As shown in Figure 3A, the spontaneously produced levels of IFN- γ (geometric mean of 93.7 vs. 46.7 pg/ml), TNF- α (geometric mean of 30.7 vs. 20.1 pg/ml), IL-17A (geometric mean of 8.8 vs. 6.2 pg/ml), and IL-1 β (geometric mean of 743.9 vs. 401.3 pg/ml) were significantly higher in diabetic compared with nondiabetic individuals. Similarly, as shown in Figure 3B, the tuberculosis antigen-stimulated (net cytokine) levels of IFN- γ (geometric mean of 948 vs. 389.3 pg/ml), TNF- α (geometric mean of 36.9 vs. 23.4 pg/ml), IL-17A (geometric mean of 15.3 vs. 6.9 pg/ml), and IL-10 (geometric mean of 84.7 vs. 61.6 pg/ml) were also significantly higher in diabetic compared with nondiabetic individuals. Mitogen stimulation showed elevated levels of all cytokines, but these were not significantly different between the two groups (data not shown). Thus, tuberculosis with diabetes is associated with heightened levels of tuberculosis antigen-stimulated proinflammatory cytokines at the time of presentation.

Tuberculosis with Diabetes Is Not Associated with Altered Circulating Levels of Chemokines

To determine the influence of diabetes on chemokines in active tuberculosis, we measured the circulating levels of IL-8, G-CSF, CCL2, CCL4, CCL11, PDGF, and CXCL-10 in diabetic and nondiabetic individuals with tuberculosis. As shown in Figure 4, the systemic levels of all the chemokines examined were not significantly different between the two groups. Thus, tuberculosis with diabetes is not associated with altered levels of circulating chemokines.

Relationship between Systemic Cytokines and HbA1c Levels

HbA1c is an accurate indicator of the level of diabetic control and increased values reflect poor control. Thus, to examine the relationship between the systemic levels of type 1, type 17, and regulatory cytokines with the degree of diabetic control, we assessed the association of IFN- γ , TNF- α , IL-17A, and IL-10 with HbA1c levels (in %) in all the individuals in the study. As shown in Figure 5, the systemic levels of IFN- γ , TNF- α , IL-17A, and IL-10 each exhibited

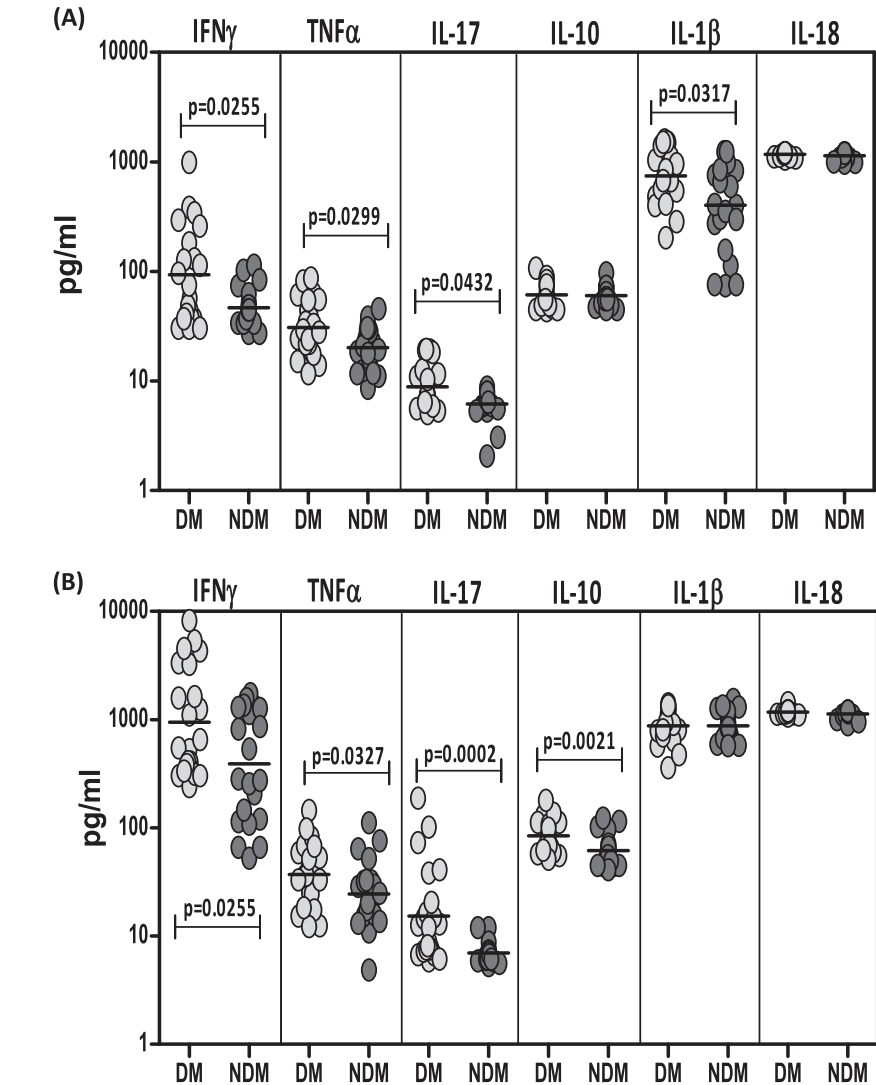


Figure 3. Elevated tuberculosis (TB) antigen-stimulated and unstimulated levels of type 1, type 17, and other proinflammatory cytokines in tuberculosis with diabetes. (A) Unstimulated or (B) TB antigen-stimulated levels of type 1, type 17, and other proinflammatory cytokines were measured by ELISA in whole blood of a subset of individuals with tuberculosis and diabetes (n = 22) and in individuals with tuberculosis with no diabetes (n = 22). TB antigen-stimulated cytokines are shown as net cytokines with the baseline subtracted out. Data are represented as scatter plots, with each circle representing a single individual (light gray, diabetes mellitus [DM]; dark gray, non-diabetes mellitus [NDM]). P values were calculated by Mann-Whitney test.

a significant positive association with the HbA1c levels in the tuberculosis-infected individuals.

Discriminating Diabetes from Nondiabetes in Active Pulmonary Tuberculosis

Because the systemic levels of a variety of pro- and antiinflammatory cytokines were significantly altered in tuberculosis with diabetes, we wanted to estimate the contribution of any of these cytokines

toward our ability to discriminate diabetes from nondiabetes in individuals with tuberculosis. Using a Holm's adjusted forward stepwise modeling procedure and a P value less than 0.05, we concluded that IFN- γ and IL-22 significantly discriminate diabetes from nondiabetes. This is plotted in Figure 6A, with the lines (contours from the model) representing the probability that an individual is a diabetic. Interestingly, even after correcting for age, sex, and smear grade,

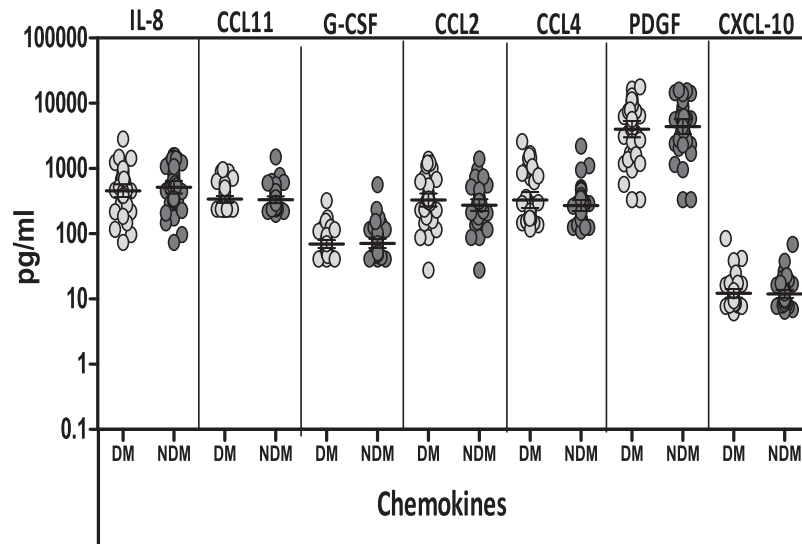


Figure 4. No alterations in systemic levels of common chemokines in tuberculosis with diabetes. Plasma levels of some of the common chemokines—IL-8, chemokine ligand-11 (CCL11), granulocyte colony-stimulating factor (G-CSF), chemokine ligand-2 and -4 (CCL2 and CCL4, respectively), platelet-derived growth factor (PDGF), and chemokine (C-X-C motif) ligand-10 (CXCL-10)—were measured by ELISA in individuals with tuberculosis and diabetes ($n = 44$) and in individuals with tuberculosis with no diabetes ($n = 44$). Data are represented as scatter plots, with each circle representing a single individual (light gray, diabetes mellitus [DM]; dark gray, non-diabetes mellitus [NDM]). P values were calculated by Mann-Whitney test.

IFN- γ levels continue to be a significant predictor in this model. To demonstrate the value of the other parameters, we represent the discriminatory power from each of the logistic regressions for each cytokine alone, using a heat map for the cytokines whose adjusted P value is less than 0.05 (Figure 6B). Thus, our data suggest that multiple cytokines can be useful discriminators of diabetes in tuberculosis-infected individuals, including IL-17A (adjusted $P = 0.0001478$); IFN- β (adjusted $P = 0.0005502$); TNF- α (adjusted $P = 0.0099083$); IL-10 (adjusted $P = 0.0014021$), and IL-18 (adjusted $P = 0.0073989$) with IFN- γ (adjusted $P = 0.0000821$) and IL-22 (adjusted $P = 0.0000078$) being the most powerful and therefore potentially associated diabetes-mediated effects in the pathogenesis of active pulmonary tuberculosis.

Discussion

Components of the immune system are altered in type 2 diabetes mellitus, including altered levels of specific cytokines and chemokines, changes in the number and activation state of various immune cell

subsets, and increased apoptosis and tissue fibrosis (10). Together, these changes suggest that inflammation participates in the pathogenesis of type 2 diabetes, but how these changes affect the immune response to bystander antigens or newly acquired infections remains unclear. The immunological basis for susceptibility to tuberculosis among those with diabetes mellitus is not well understood. One possible mechanism is that an impaired immune response in diabetic patients facilitates either primary infection with tuberculosis or reactivation of latent tuberculosis (11). Studies examining the innate and adaptive immune response to microbial antigens in diabetic patients suggest that these responses are compromised, particularly in patients with chronic hyperglycemia (12–14). Whether this applies to tuberculosis infection remains unclear.

Cytokines are known to play a major role in determining the outcome of infection in host defense against mycobacterial infections (8). Of major importance are IFN- γ and TNF- α , the functions of which have been well documented in both mouse models and human infections (15–17). The prototypical type 17 cytokine, IL-17A,

has been shown to play a central role in mediating immunity to both extra- and intracellular bacteria, including *Mycobacterium tuberculosis* (18, 19). However, the role of IL-17F, another member of the type 17 cytokine family, has not been examined. On the other hand, IL-22 has been shown not to play an important role in resistance to mycobacterial infection (20). Our data on individuals with tuberculosis and concomitant diabetes reveal an interesting pattern of cytokine expression at baseline. First, the cytokines known to be involved in protection against infection and/or disease, that is, IFN- γ , TNF- α , and IL-17A, are all present at much higher levels in diabetic individuals. Second, cytokines not known to play a role in resistance, that is, IL-17F and IL-22, are both present at much lower levels. Whereas IL-17A is the prototypical cytokine marking CD4⁺ T cells as Th17 cells, IL-22 is the main cytokine produced by Th22 cells. The regulation of these subsets as well as CD4⁺ T cells producing IL-17F is not well understood (21). Our data would therefore suggest that in tuberculosis with diabetes, IL-17A is differentially regulated in comparison with IL-17F and IL-22. T cell-mediated immune responses are known to be altered in individuals with insulin resistance, which could indicate that T-cell factors are linked to disturbed insulin sensitivity. Indeed, it has been previously demonstrated that CD4⁺ T cells producing IL-17A and IFN- γ are increased in frequency and magnitude and promote inflammation and that several cytokines produced by Th1 or Th17 cells have been linked to insulin resistance (22–24). In addition, patients with type 2 diabetes also have been shown to exhibit decreased frequencies of natural regulatory T cells, indicating the presence of an unbalanced proinflammatory milieu in type 2 diabetes (7, 24). Thus, underlying poorly controlled diabetes by itself could contribute to the increase in type 1 and 17 cytokines observed in individuals with tuberculosis and diabetes.

Interestingly, the enhanced production of type 1 and 17 cytokines is associated with significantly higher systemic levels of IL-1 family and other proinflammatory cytokines. Cytokines of the IL-1 family, especially IL-1 α and IL-1 β , are important for resistance to infection, with mice deficient in IL-1 α , IL-1 β , and IL-1 receptor

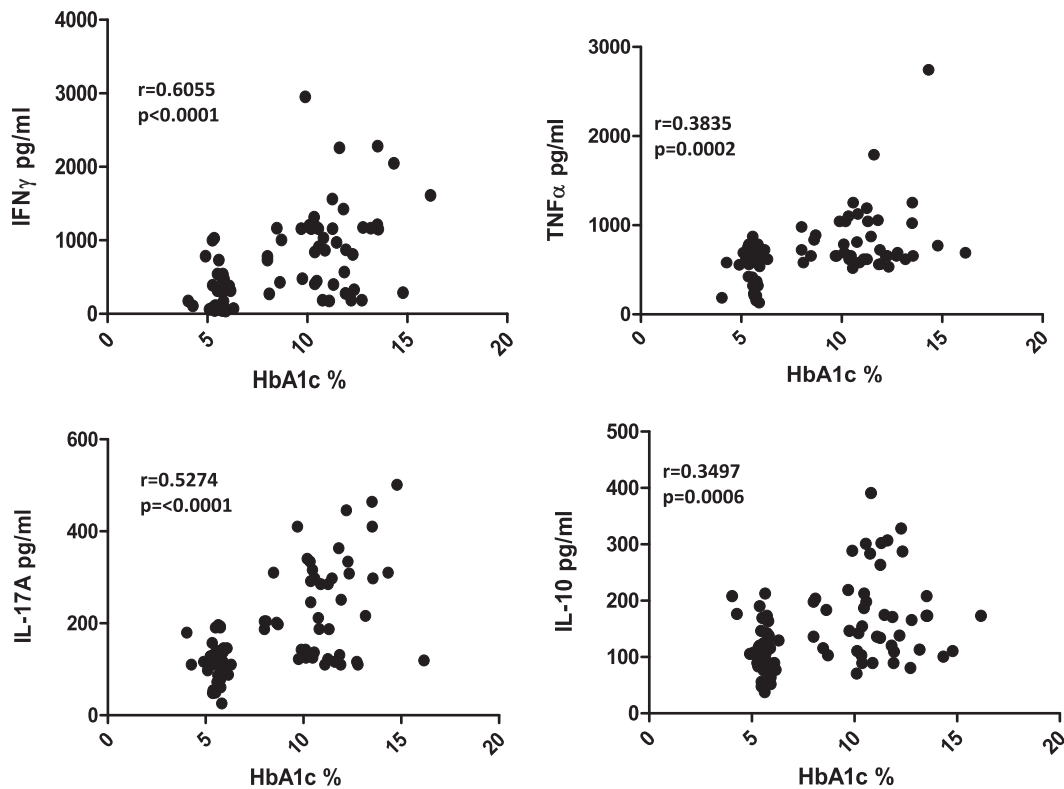


Figure 5. Positive relationship between systemic levels of cytokines and glycated hemoglobin (HbA1c) levels in tuberculosis-infected individuals. The relationship between the plasma levels of IFN- γ , tumor necrosis factor (TNF)- α , IL-17A, and IL-10 and HbA1c levels was examined in tuberculosis-infected ($n = 88$) individuals. Data are represented as scatter plots, with each circle representing a single individual. P values were calculated by Spearman rank correlation.

all showing greater susceptibility to tuberculosis (25, 26). In addition, IL-18 has also been shown to play a role in protective immunity to tuberculosis (27). Similarly, IL-12 is also known to be crucial in immunity to tuberculosis both in mouse models and human infections (28, 29). More recently, IL-6 has been shown to mediate inhibition of disease progression (30). Therefore, we examined a whole panel of proinflammatory cytokines expecting to observe impaired induction of one or more of these host protective cytokines. However, our data suggest that contrary to our initial hypothesis, host-protective cytokines do not exhibit impaired production at homeostasis but in fact are present at significantly heightened levels in individuals with tuberculosis and diabetes. Therefore, tuberculosis with diabetes is not associated with a down-modulation of host-protective proinflammatory cytokines. Whether this is the cause of increased susceptibility to tuberculosis or the result of increased bacterial burden or severity of disease in diabetic individuals remains to be

ascertained. Our data did reveal minor differences in smear grades of tuberculous disease between the two groups (data not shown); however, more detailed analysis of the bacterial burden or severity of disease was not performed in this study. Finally, chemokines including CCL2, CCL4, IL-8, and CXCL-10 are all known to play a role in recruitment of immune cells to the mycobacteria-infected lung (31). In addition, CCL2 is also known to play a role in resistance to infection (32). However, our data reveal that alteration in the systemic levels of these chemokines is not a major feature of tuberculosis with diabetes.

One potential mechanism for the increase in baseline levels of proinflammatory cytokines in individuals with tuberculosis and diabetes could be a concomitant decrease in the levels of systemic type 2 or regulatory cytokines. However, as revealed by the increased systemic levels of IL-5 and IL-10 and unaltered levels of IL-4, IL-13, and TGF- β , it is not only cytokines with inflammatory potential that are elevated in diabetic

individuals but also cytokines with immune-regulatory potential. Moreover, both type 2 cytokines and regulatory cytokines are known to play a role in enhancing susceptibility to infection (33). For example, IL-10 is elevated in samples obtained from patients with tuberculosis (34), and an increased ability to innately produce IL-10 is associated with increased incidence of active tuberculosis (35). Our data demonstrating increases in both pro- and antiinflammatory cytokines suggest that the alteration of the balance between these groups of cytokines is not clearly associated with diabetes in tuberculous disease. Other cytokines known to enhance susceptibility to infection and/or disease are the type 1 IFNs. Indeed, type 1 IFN receptor-deficient mice show lower bacterial burdens compared with wild-type controls (36), whereas mycobacteria-infected mice treated with substances known to induce type 1 IFN exhibit exacerbated lung pathology and increased bacterial burden (37). Therefore, we examined the baseline expression of IFN- α and IFN- β in tuberculosis with

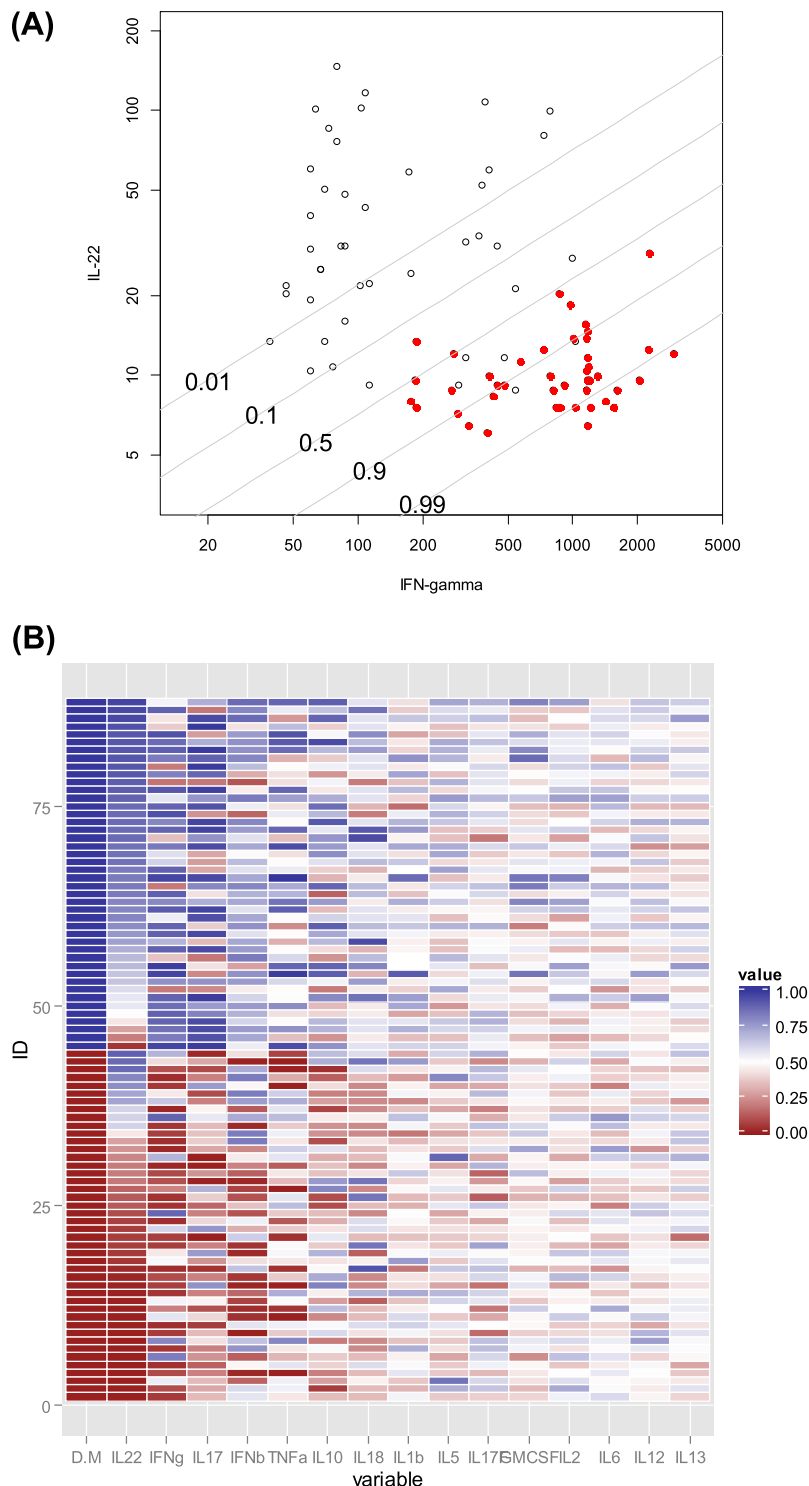


Figure 6. Discriminatory value of cytokines in tuberculosis-infected individuals. (A) Contribution of IFN- γ and IL-22 to the discrimination of diabetes from no diabetes in tuberculosis-infected individuals ($n = 88$) at a P value less than 0.05 is plotted, using a logistic model. The red dots are those with diabetes and active pulmonary tuberculosis. The lines are contours from the model, representing the probability that an individual is diabetic. (B) Heat map of predicted values from logistic regressions with a single cytokine predicting diabetes. The first column is the diabetes mellitus (DM) status (dark blue, DM; white, no DM). The rows are subjects sorted first by diabetes status, and then by predicted diabetes status by IL-22, the best predictor. We have shown only cytokines with unadjusted P values less than 0.05. GM-CSF = granulocyte-macrophage colony-stimulating factor; TNF- α = tumor necrosis factor- α .

diabetes and showed that type 1 IFNs are not elevated in these individuals, indicating a minimal (if any) role for type 1 IFN in diabetes-mediated immune pathology in active tuberculosis.

The major limitation in analyzing the immune parameters in circulation is the nonspecific nature of the analysis. We have therefore sought to corroborate the plasma cytokine results by measuring the levels of a subset of pro- and antiinflammatory cytokines in tuberculosis antigen-stimulated whole blood supernatants. Our data on both pro- and antiinflammatory cytokine responses after tuberculosis antigen stimulation clearly show that diabetes has a profound effect on altering antigen-specific innate and adaptive immune responses in tuberculosis. Thus, elevation in circulating levels of type 1 and type 17 cytokines as well as IL-10 is reflected by similar elevations of *in vitro*-measured cytokines after stimulation with mycobacterial antigens in tuberculosis with diabetes.

Our study provides important insights into the influence of poorly controlled type 2 diabetes mellitus on the pathogenesis of pulmonary tuberculosis. Our data supporting the effects of an excessive inflammatory response in diabetes provide a rational basis for testing combined antimicrobial and antiinflammatory therapies in patients with diabetes and tuberculosis. Our study also provides an impetus to perform longitudinal studies examining the role of immunological biomarkers in the development of tuberculosis in diabetic patients, especially the roles of IFN- γ and IL-22 as predictors of diabetes influence on active tuberculosis. Finally, by dissecting the innate and adaptive cytokine response at homeostasis in diabetic individuals with tuberculosis, our study also suggests that immunomodulatory strategies already in clinical trials for the treatment of type 2 diabetes mellitus, such as IL-1 antagonists (10), could potentially aid in combating the increased susceptibility to exogenous infections and their subsequent pathology. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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