M. tuberculosis infection in diabetics is associated with increased inflammatory cytokine but decreased Suppressor of cytokine signaling (SOCS)-3 responses

Kiran Iqbal[1], Muhammad Irfan[1], Qamar Masood[1], Maliha Yameen[1], Nanik Ram[1], Bushra Jamil[1], Shoaib Rao[1], Martin Rottenberg[2], and Zahra Hasan[3]

[1]Aga Khan University
[2]Karolinska Institutet
[3]The Aga Khan University

April 28, 2020

Abstract

Introduction Tuberculosis (TB) infections and latent Mycobacterium tuberculosis (MTB) infection (LTBi) remain prevalent globally. Type 2 diabetes mellitus (DM) worsens TB outcomes but the immune mechanisms that cause this are not yet clear. We investigated a role of suppressor of cytokine signaling molecules (SOCS1 and SOC3) in regulating host cytokine responses in the diabetic host infected with MTB. Materials and Methods We studied peripheral blood cells from health endemic controls (EC), LTBi cases, diabetics with and without LTBi and TB patients. Mycobacterial antigen-stimulated cytokine secretion was determined using the Th1/Th2 11 plex cytokine assay. Antigen-induced gene expression of IFNγ, TNFα, IL6 and SOCS3 was determined by reverse-transcription PCR. Results Purified protein derivative (PPD) antigen stimulation induced higher levels of, IL-6, IL-2, TNFα and GM-CSF levels in DM-LTB as compared with EC and LTB cases. IL-13 levels were raised in DM-LTB cases as compared with DM cases. PPD-induced IFNγ and IL-6 transcripts were raised in DM-LTBi as compared with EC. TNFα mRNA levels were raised in DM-LTBi as compared with LTBi. SOCS3 mRNA levels were reduced in DM-LTBi as compared with LTBi. SOCS3 transcripts were higher in LTBi as compared with EC and TB groups. Discussion We found co-occurrence of LTBi with DM to be associated with an increased release of proinflammatory IL-6, IL-2 and TNF-α but reduced SOCS3 mRNA levels. SOCS3 protects against MTB infection therefore, reduced levels in DM-LTB may be contribute to progression from LTBi to active TB in individuals infected with MTB.

M. tuberculosis infection in diabetics is associated with increased inflammatory cytokine and decreased Suppressor of cytokine signaling (SOCS)-3 responses

Kiran Iqbal Masood[1], Muhammad Irfan[2], Qamar Masood[2], Maliha Yameen[1], Bushra Jamil[2], Nanik Ram[2], Shoaib Rao[1], Martin Rottenberg[3], Zahra Hasan[1]*

[1]Department of Pathology and Laboratory Medicine, The Aga Khan University Karachi, Pakistan; [2]Department of Medicine, The Aga Khan University Karachi, Pakistan;[3]Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Sweden.

Running Title: Increased inflammation in diabetics with latent TB is associated with reduced SOCS3 gene expression

Scientific heading: Clinical and Human Immunology

*Corresponding Author:

Zahra Hasan, PhD, FRCPath

Professor

Department of Pathology and Laboratory Medicine

The Aga Khan University

Stadium Road, P.O.Box 3500

Karachi 74800, Pakistan

Email: zahra.hasan@aku.edu

Contact: +92 21 3486 4527

Word count: 5079

Summary

Introduction

Tuberculosis (TB) infections and latent *Mycobacterium tuberculosis* (MTB) infection (LTBi) remain prevalent globally. Type 2 diabetes mellitus (DM) worsens TB outcomes but the immune mechanisms that causes this are not yet clear. We investigated a role of suppressor of cytokine signaling molecules (SOCS1 and SOC3) in regulating host cytokine responses in the diabetic host infected with MTB.

Materials and Methods

We studied peripheral blood cells from health endemic controls (EC), LTBi cases, diabetics with and without LTBi and TB patients. Mycobacterial antigen-stimulated cytokine secretion was determined using the Th1/Th2 11 plex cytokine assay. Antigen-induced gene expression of IFNγ, TNFα, IL6 and SOCS3 was determined by reverse-transcription PCR.

Results

Purified protein derivative (PPD) antigen stimulation induced higher levels of, IL-6, IL-2, TNFα and GM-CSF levels in DM-LTB as compared with EC and LTB cases. IL-13 levels were raised in DM-LTB cases as compared with DM cases. PPD-induced IFNγ and IL-6 transcripts were raised in DM-LTBi as compared with EC. TNFα mRNA levels were raised in DM-LTBi as compared with LTBi. SOCS3 mRNA levels were reduced in DM-LTBi as compared with LTBi. SOCS3 transcripts were higher in LTBi as compared with EC and TB groups.

Discussion

We found co-occurrence of LTBi with DM to be associated with an increased release of proinflammatory IL-6, IL-2 and TNF-α but reduced SOCS3 mRNA levels. SOCS3 protects against MTB infection therefore, reduced levels in DM-LTB may be contribute to progression from LTBi to active TB in individuals infected with MTB.

Key words: MTB, latent TB, diabetes, SOCS3

Introduction

One third of the people suffering with type 2 diabetes mellitus (DM) reside in developing countries many of which are endemic for Tuberculosis (TB). In Pakistan, the prevalence of diabetes is reported to be 9.8% and 3% of all national deaths are thought to be associated with DM and DM-related conditions (1). As Pakistan also ranks 5th amongst high TB burden countries, it is an additional challenge to manage patients with TB and DM comorbidity (2). Different risk factors may account for an important proportion of TB cases in the population. Diabetes (DM) occurs as the individual becomes resistant to insulin, resulting in poor metabolism of glucose. Individuals with DM have a threefold increased risk of developing TB (3,

4). DM has also a negative impact on treatment outcomes in patients with tuberculosis (TB) (5). Latent, asymptomatic infection or immune reactivity with *M. tuberculosis* (LTBi) occurs in around one fourth of the world population (6), and the association between LTBi and DM-2 has been suggested (7).

Studies on host immunity of individuals with LTBi indicate that CD4 T cell responses specific to MTB antigens are raised in this group (8). Which is the premise of the MTB-specific T cell -derived IFNγ assay testing for LTBi diagnosis (9). Additional cytokines and chemokines such as IL-10, TNFα, CXCL10, CXCL9 are also found to be raised in LTBi as compared with healthy endemic controls (EC) (10). Gene expression studies have identified particular biomarker expression patterns within LTBi individuals that discriminate them from those who are healthy and those who have active TB (11) (12). Heterogeneity of LTBI was further recognised by epidemiological differences in the risk of TB between those with recent and remote infection (13). It is well understood that immune regulatory factors such as, IL-12, IFN-γ and TNF-α confer protective immunity against MTB infection, the understanding of factors which lead to disease progression from LTBi to TB is very limited (14).

DM is associated with altered cellular and humoral immune responses with modulation of macrophage and lymphocyte function (15). Delayed management of DM can lead to diverse complications including susceptibility to infections (16). Inflammatory mediators including, proinflammatory cytokines such as IFNγ, TNFα, IL-6, and downregulatory cytokines IL-10, TGFβ together with adipokines, which affect leptin regulation and impact metabolism in glycogen stores, are all raised in DM cases (17). Individuals with LTBi and DM have been shown to sub-optimal CD4 T cell responses to MTB-specific antigens and increased production of the Th 2 cytokines IL-10 and TGF-beta (18).

Host innate and adaptive responses are both crucial in protection against TB. Suppressor of cytokines signaling (SOCS) molecules regulate key cytokine drivers of activation of both innate and adaptive immunity. SOCS has eight member molecules including cytokine-inducible regulator of signaling (CIS), SOCS1-7 (19). SOCS1 and 3 are found to play a role in the pathogenesis of TB and diabetes (20). SOCS1 suppresses STAT1, the transcription factor involved in IFN-γ and IFN-α/β signaling, while SOSC3 has different targets, including STAT3 activation via certain receptors as the IL6-receptor family (21). SOCS1 and 3 molecules are modulated by MTB for its own survival, play a pivotal role in controlling infection outcomes (19, 22, 23).

Separately, SOCS1 and SOCS3 inhibit the insulin receptor substrates 1 and -2 (IRS-1 and IRS-2) protein, hindering insulin responses and insulin growth factor (IGF)-1 signaling, thereby affecting glucose metabolism (24). Here we investigated the impact of diabetes on progression of MTB infection to active disease through the study of mycobacterial antigen-induced cytokine activation and their association of regulatory SOCS1 and SOCS3.

Materials and Methods

Ethical approval

This study was approved by the Ethical Review Committee of The Aga Khan University Karachi, Pakistan. Written informed consent was taken from all study participants.

Subject selection

Study subjects were recruited from Aga Khan University and Hospital (AKUH), Karachi, Pakistan using a cross-sectional study design with a consecutive convenience sampling method. Study subjects were recruited within a given study period based on the given inclusion criteria.

All study subjects were aged 18 – 65 years. Thirty-nine asymptomatic healthy volunteers at AKUH were recruited for the study. We recruited health care workers who have contact with TB patients as possible LTB cases in addition to household contacts of TB patients. Thirty-three patients with DM were recruited from Department of Medicine clinics. Inclusion criteria for DM cases were: patients with previously confirmed Type 2 DM. Exclusion criteria were pregnancy, comorbid conditions such as HIV infection, active TB, chronic

renal failure, chronic liver disease or corticosteroid therapy. For potential DM-LTBi cases we recruited household contacts of TB patients who had DM.

In total, ten ml of whole blood sample was drawn from all study subjects. Random blood glucose (RBS) and glycosylated hemoglobin (HbA1c) levels were tested. DM was defined by HbA1c [?] 6.4 %.

A positive reaction in a Quantiferon TB Gold in Tube assay (QFT-GIT) test was performed to classify individuals which are otherwise are healthy and asymptomatic as LTBi. As per manufacturer's instructions, a threshold of [?]0.35 IU/mL was considered for LTBi. Hence after QFT-GIT testing, the healthy control group was categorized into endemic controls (EC n=24) or latent TB cases (LTBi, n=15). Further, subjects recruited in the DM group were further categorized into QFT-GIT negative (DM, n=16) and positive (DM-LTBi, n=17).

Fifteen patients with pulmonary TB (PTB) were recruited. Patients with confirmed diagnosis of TB who had not received anti-tuberculous therapy (ATT); male or female; between 18–65 years of age were included in the study. Exclusion criteria were pregnancy, comorbid conditions (such as HIV infection, DM, chronic renal failure, chronic liver disease or corticosteroid therapy) and patients with relapsed TB. All PTB patients were diagnosed by clinical examination, chest X-ray and had a positive sputum acid-fast bacillus (AFB) microscopy and/ or AFB culture. They were classified as PTB as per WHO guidelines (25).

Stimulation of PBMCs with mycobacterial antigens

Five ml of blood was used for isolation of peripheral blood mononuclear cells (PBMCs). Briefly, PBMCs were separated using Ficoll-Histopaque as described (26) and plated at 10^6 cells/ well in a 24 well plate. Cells were incubated with purified protein derivative antigen (PPD) at 10 µg/ml (BeiResources, Colorado State University, USA) or left unstimulated for 18h at 37°C. Cellular supernatants were collected after centrifugation. Cells were washed and the pellets were snap-frozen at -80°C until tested. Cell monolayers were lysed and harvested using TriZol reagent (Invitrogen, USA) and stored at -70°C until processed further.

Measurement of cytokines by Th1/Th2 Cytokine 11-Plex Human ProcartaPlex assay

Cytokines were measured in serum collected from whole blood. Also, in cellular supernatants of PBMC monolayers after 18 h of incubation with and without PPD antigen stimulation. Concentrations of IFN-γ, IL-12, IL-2, TNF-α, GM-CSF, IL-1, IL-13, IL-4, IL-5, IL-6 and IL-18 were measured in cell supernatants with a Th1/Th2 Cytokine 11-Plex Human ProcartaPlex assay from ThermoFischer Scientific, USA. Readings were taken on a Luminex 100 instrument (Luminex technologies, USA).

Real Time PCR

Total RNA was isolated from PBMCs using Trizol reagent. RNA (1 µg) was reverse transcribed using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) as described by the manufacturer. Real time PCR was performed in duplicate 20 µl reactions containing Platinum SYBR Green qPCR Supermix-UDG (Thermofisher scientific, USA), 150 nM forward and reverse primers, and 2 µl of cDNA. HuPO (human acidic ribosomal protein) primer sequences were obtained from published reports as described previously (27). IFN-γ, TNF-α, IL-6, SOCS1 and SOCS3 sequence specific primers were used. Two-fold dilutions of cDNA samples were amplified to control amplification efficiency and to determine the optimal concentration required for each primer pair. HuPO was used as a control gene to calculate the ΔCt values for individual samples. The relative amount of cytokine/HuPO transcripts was calculated using the $2^{-[?][?]cT}$ method as described (28). These values were then used to calculate the relative expression of cytokine mRNA in each of the samples tested.

Statistical analysis

Data is depicted as median values for each group with the IQR (inter quartile range 25th to 75th percentile) indicated in each case. Comparison of non-parametric data between the groups was performed using the Mann–Whitney U test. Analysis was performed and data plotted using GraphPad PRISM Version 5 (GraphPad Software, San Diego, CA, USA).

Results

Characteristics of study subjects

The age (mean ± SD; EC, 41.3± 11.1 y; DM, 50.4 ± 7.77 y) and gender distribution (Male/Female; EC, 18/21; DM, 20/13) of EC and DM study subjects was comparable (Table 1).

Patients with TB had a mean age of 40 y and comprised 11: 4 Male/Female cases. We used the QuantiFERON-gold In-Tube assay (QFT-GIT) to determine the latent TB status of each case and found that 15 of 39 healthy controls and 17 of 33 DM cases had LTBi.

Mycobacterial antigen-induced pro-inflammatory cytokines upregulated in DM-LTB cases

To investigate the effect of latent MTB infection on host immunity, we compared the levels of proinflammatory cytokines in the supernatants from purified protein derivative (PPD)- stimulated healthy and diabetic individuals with and without LTBi. Specifically, IFNγ, IL-6, IL-2, IL-12, TNF-α, GM-CSF, IL-1β and IL-18 levels were measured in supernatants of PPD-stimulated PBMCs in EC, LTBi, DM and DM-LTB groups.

PPD-stimulated IL-6 (p=0.011), IL-2 (p=0.0131), TNF-α (p=0.027) and GM-CSF (p=0.0075) levels were found to be significantly different between the study groups, Table 2. These cytokines were found to be raised in DM-LTB study subjects as compared with EC and/or LTBi and DM groups. IL-6 levels were significantly raised in DM-LTBi as compared with EC (p=0.0015) and LTB (p=0.0142). DM-LTBi cases had raised IL-2 levels as compared with EC (p.0.0015), LTBi (p=0.0187) and DM (p=0.0388) cases. TNF-α levels were raised in DM-LTBi as compared with EC (p=0.0098), LTBi (p=0.0141) and DM (p=0.0118) cases. In DM-LTBi cases, GM-CSF levels were significantly raised as compared with EC (p=0.0014), LTBi (p=0.0394). GM-CSF levels were also raised in TB as compared with EC (p=0.0075) and LTBi (p=0.038). IL-1β levels were significantly raised in DM-LTB as compared with EC (p=0.0020). IL-18 levels were increased in DM-LTB as compared with EC (p=0.0090).

We observed that levels of IFN-γ showed an increased trend in DM-LTB as compared with EC, DM and LTB groups however, these were not significantly different.

Mycobacterial antigen-induced IL-13 and IL-5 levels are raised in DM-LTB cases

To investigate the effect of mycobacterial antigens on type 2 cytokines we measured PPD-induced secretion of IL-13, IL-5 and IL-4 in DM and EC cases with and without LTBi. PPD- stimulated IL-5 (p=0.025) levels were found to be differ between study groups (Table 3). PPD-stimulated IL-5 levels were also raised in DM-LTBi as compared with EC (p=0.0288). IL-13 levels were significantly increased in DM-LTB as compared with DM (p=0.0093). IL-4 secretion was not detected in the supernatants studied.

Διφφερεντιαλ ΙΦΝ-γ, ΤΝΦ-α, ΙΛ-6 ανδ ΣΟΣ3 μΡΝΑ εξπρεσσιον λεελς ιν ηεαλτηψ ςοντρολς ανδ διαβετιςς ωιτη ανδ ωιτηουτ λατεντ ΤΒ

To understand cytokine activation in our study subjects in the context of immune-regulatory molecules, we compared gene transcription levels and the key regulatory molecules SOCS1 and SOCS3 in healthy controls and diabetics to investigate any effect of latent TB infection.

We measured determined PPD- stimulated mRNA levels in peripheral blood cells from EC, LTBi, DM, DM-LTBi and TB groups. We observed increased IFN-γ mRNA titers in DM-LTBi (p=0.033) and TB (p=0.046) as compared with EC (Fig 1A). PPD-stimulated TNFα mRNA expression was increased in DM-LTB as compared with EC (p=0.035, Fig. 1B). PPD-stimulated IL-6 (p=0.009, Fig 1C) mRNA expression was increased in DM-LTBi as compared with EC.

SOCS3 mRNA expression was found to be significantly reduced in PPD-stimulated cells of DM-LTBi (p=0.0422), TB (p=0.019) and EC (p=0.025) as compared with LTBi cases (Fig 1D). SOCS1 mRNA expression level was comparable in all the study groups (data not shown).

Basal levels of mRNA transcripts for IFN-γ, TNF-α, IL-6, SOCS1 and SOCS3 mRNA were determined in all groups were similar.

Discussion

The association between LTBi and DM-2 has implications on global TB control: a mathematical model predicts that elimination of TB by the year 2035 might be hampered if the incidence of DM-2 continues to increase worldwide (29). An elevated glucose or HbA1c levels has been shown to be related with an increased risk of developing LTBi (30).

The aim of our study is to contribute to understanding the impact of diabetes on host immunity during mycobacterial infection and disease. Diabetes has been shown to be associated with altered cellular and humoral immune responses. It has been suggested that when DM co-occurs with TB, host protective immune function is affected (31). This leads to more un-favourable outcomes of both TB and DM (31), with a slower recovery and increased chance of TB relapse (32). Less is known about the alterations of host immune responses in DM in LTBi individuals. Limited reports indicate that there is an IL-10 and TGF-beta driven decrease in host protective CD4 T cell responses (10).

We studied PPD-triggered cytokine responses in healthy subjects with and without LTBi, diabetics with and without LTBi and also patients with TB. We observed increased PPD-induced IL-2, TNF-α and IL-6 protein levels in DM-LTBi as compared with EC, LTBi and DM. PPD- stimulated GM-CSF levels were also raised in DM-LTBi as compared with EC and LTBi groups.

IL-5 protein titers were higher in DM-LTBi compared with EC cases and IL-13 levels were raised in DM-LTBi as compared with DM as well, suggesting heightened levels of some Th2 cytokines in diabetics with latent TB. As the latter are markers of alternate macrophage activation (11), this suggests a possible shift to macrophage dysfunction in diabetics who are latently infected with MTB.

The increased susceptibility to *M. tuberculosis* infection is regulated by alterations in of IFN-γ, TNF-α, IL1-β, IL-6 and IL-10 levels (33, 34). Studies in diabetic mice have shown reduced levels of IFN-γ in lungs after infection with *M. tuberculosis* and increased bacterial load as compared with the non-diabetic ones (35).

An impaired trafficking of immune cells, resulting in delayed T cell responses was observed to the site of infection in diabetic mouse model resulting in increased susceptibility to infection with *M. tuberculosis* (36, 37). TNF-α is a major regulator of granuloma formation and hence containment of *M. tuberculosis* infection. However, dysregulation in TNF-α levels also leads to the rupture of granulomas resulting in dissemination of granulomas (38).

We observed increased TNF-α gene expression at the protein and mRNA levels in DM-LTB as compared with EC and LTBi. Elevated levels of TNF-α have been reported in patients with chronic diabetes (39). Raised TNFα levels have also observed in patients with raised HbA1c levels (39). IL-2 and GM-CSF were shown to be raised in DM-LTB as compared with EC. These findings are in line with previous studies (40, 41). Patients with diabetes are known to exhibit a persistent pro-inflammatory state linked with the development of insulin resistance and pathology linked with diabetes (42).

We found IL-6 protein levels to be significantly raised in DM-LTB cases as compared with both EC and LTBi subjects. Similarly, we found PPD-induced IL-6 mRNA expression to be raised in DM-LTB subjects as compared with EC. Raised levels of IL-6 in plasma has been shown to be associated with metabolic disorders (43). Raised IL-6 mRNA levels have been reported in insulin resistant humans (43). In another study, a positive association has been reported between elevated levels of IL-6 and poorly controlled diabetes (39). In TB, IL-6 has been shown to play a vital role in pro-inflammatory responses including promoting T cell and B cell responses (44). IL-6 deficient mice have been shown to be susceptible to TB infection (45).

We also observed IL-5 levels to be raised in DM-LTB as compared with healthy controls. Previously, TB with diabetes has been associated with an increase in circulating IL-5 levels(46) . Further, PPD-induced IL-13 secretion was raised in DM-LTB cases as compared with those with DM only. IL-13 has previously been

shown to be raised in TB patients with pre-DM (47). IL-13 regulation is required for effective glucogenesis (48).

Our observation that individuals with PPD-induced SOCS1 was upregulated the most in TB patients is concordant with previous reports (49). Previously, it has been shown that *M. bovis* BCG (50) and *M. tuberculosis* infection upregulates SOCS1 expression (51). We also observed an increase in SOCS1 upregulation in diabetics. Previous reports showed that SOCS1 inhibit insulin receptor substrate (IRS) proteins thereby causing insulin resistance (52).

After PPD-stimulation of PBMCs, SOCS3 expression was found to be the highest in LTBi cases and reduced in EC, DM, DM-LTBi and TB cases. Upregulation of SOCS3 in response to *M. tuberculosis* infection has been shown previously (23). SOCS3 is required for protection against MTB infection and mice deficient in SOCS3 are susceptible to mycobacterial infections (53). Upregulation of SOCS3 in macrophages has been shown to provide protection against infection by intracellular pathogens (23). We have previously found reduced SOCS3 gene expression in patients with far advanced PTB (54). In this case, the association of downregulated SOCS3 together with raised inflammatory cytokines in DM-LTBi may be suggestive of less protective immune state in the host, making it more prone to disease progression.

This work further points to the importance of early identification of diabetes and of latent TB both as important independent predisposing factors for TB progression. It emphasizes the need to identify health interventions and treatments which may aid management of the individual prior to progression towards active TB disease.

Author Contributions

Conception and design: ZH and MR; Analysis and interpretation: ZH, MR, KI, SR, MY; Drafting the manuscript for important intellectual content: ZH, MR, KI: Patient selection: MI, QM, BJ, NR

Acknowledgments

This work received financial support from a VR grant, Swedish Research Council, Sweden and the Higher Education Commission Pakistan, National Program for Research Universities project 5218. We thank Maliha Yameen and Amina Ajmal for assistance with patient recruitment and technical assistance. Thanks to clinical colleagues involved in patient recruitment

Conflict of Interest Statement

None declared

References

1. World Health Organization. Global Report on Diabetes. 2016.

2. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: a systematic review of 13 observational studies. PLoS Med. 2008;5(7):e152.

3. World Health Organisation. Global Tuberculosis Report. 2019.

4. Restrepo BI. Diabetes and Tuberculosis. Microbiol Spectr. 2016;4(6).

5. Dooley KE, Tang T, Golub JE, Dorman SE, Cronin W. Impact of diabetes mellitus on treatment outcomes of patients with active tuberculosis. Am J Trop Med Hyg. 2009;80(4):634-9.

6. Cohen A, Mathiasen VD, Schon T, Wejse C. The global prevalence of latent tuberculosis: a systematic review and meta-analysis. Eur Respir J. 2019;54(3).

7. Lee MR, Huang YP, Kuo YT, Luo CH, Shih YJ, Shu CC, et al. Diabetes Mellitus and Latent Tuberculosis Infection: A Systematic Review and Metaanalysis. Clin Infect Dis. 2017;64(6):719-27.

8. Tully G, Kortsik C, Hohn H, Zehbe I, Hitzler WE, Neukirch C, et al. Highly focused T cell responses in latent human pulmonary Mycobacterium tuberculosis infection. J Immunol. 2005;174(4):2174-84.

9. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, et al. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. Clin Microbiol Rev. 2014;27(1):3-20.

10. Jeong YH, Hur YG, Lee H, Kim S, Cho JE, Chang J, et al. Discrimination between active and latent tuberculosis based on ratio of antigen-specific to mitogen-induced IP-10 production. J Clin Microbiol. 2015;53(2):504-10.

11. Sampath P, Moideen K, Ranganathan UD, Bethunaickan R. Monocyte Subsets: Phenotypes and Function in Tuberculosis Infection. Front Immunol. 2018;9:1726.

12. Jacobsen M, Repsilber D, Gutschmidt A, Neher A, Feldmann K, Mollenkopf HJ, et al. Candidate biomarkers for discrimination between infection and disease caused by Mycobacterium tuberculosis. J Mol Med (Berl). 2007;85(6):613-21.

13. Barry CE, 3rd, Boshoff HI, Dartois V, Dick T, Ehrt S, Flynn J, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol. 2009;7(12):845-55.

14. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. Annu Rev Immunol. 2013;31:475-527.

15. Hodgson K, Morris J, Bridson T, Govan B, Rush C, Ketheesan N. Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. Immunology. 2015;144(2):171-85.

16. Casqueiro J, Casqueiro J, Alves C. Infections in patients with diabetes mellitus: A review of pathogenesis. Indian J Endocrinol Metab. 2012;16 Suppl 1:S27-36.

17. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. Front Endocrinol (Lausanne). 2013;4:71.

18. Kumar NP, Moideen K, George PJ, Dolla C, Kumaran P, Babu S. Coincident diabetes mellitus modulates Th1-, Th2-, and Th17-cell responses in latent tuberculosis in an IL-10- and TGF-beta-dependent manner. Eur J Immunol. 2016;46(2):390-9.

19. Carow B, Rottenberg ME. SOCS3, a Major Regulator of Infection and Inflammation. Front Immunol. 2014;5:58.

20. Suchy D, Labuzek K, Machnik G, Kozlowski M, Okopien B. SOCS and diabetes–ups and downs of a turbulent relationship. Cell Biochem Funct. 2013;31(3):181-95.

21. Ronn SG, Billestrup N, Mandrup-Poulsen T. Diabetes and suppressors of cytokine signaling proteins. Diabetes. 2007;56(2):541-8.

22. Masood KI, Pervez S, Rottenberg ME, Umar B, Hasan Z. Suppressor of cytokine signaling-1 and chemokine (C-X-C Motif) receptor 3 expressions are associated with caseous necrosis in granulomas from patients with tuberculous lymphadenitis. J Microbiol Immunol Infect. 2016;49(6):984-7.

23. Rottenberg ME, Carow B. SOCS3 and STAT3, major controllers of the outcome of infection with Mycobacterium tuberculosis. Semin Immunol. 2014;26(6):518-32.

24. Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. J Biol Chem. 2002;277(44):42394-8.

25. World Health Organization. Guidelines for treatment of tuberculosis. 2010. WHO/HTM/TB/2009.420.

26. Masood KI, Rottenberg ME, Carow B, Rao N, Ashraf M, Hussain R, et al. SOCS1 Gene Expression is Increased in Severe Pulmonary Tuberculosis. Scand J Immunol. 2012;76(4):398-404.

27. Dheda K, Huggett JF, Bustin SA, Johnson MA, Rook G, Zumla A. Validation of housekeeping genes for normalizing RNA expression in real-time PCR. Biotechniques. 2004;37(1):112-4, 6, 8-9.

28. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8.

29. Pan SC, Ku CC, Kao D, Ezzati M, Fang CT, Lin HH. Effect of diabetes on tuberculosis control in 13 countries with high tuberculosis: a modelling study. Lancet Diabetes Endocrinol. 2015;3(5):323-30.

30. Martinez L, Zhu L, Castellanos ME, Liu Q, Chen C, Hallowell BD, et al. Glycemic Control and the Prevalence of Tuberculosis Infection: A Population-based Observational Study. Clin Infect Dis. 2017;65(12):2060-8.

31. Nathella PK, Babu S. Influence of diabetes mellitus on immunity to human tuberculosis. Immunology. 2017;152(1):13-24.

32. Lee PH, Lin HC, Huang AS, Wei SH, Lai MS, Lin HH. Diabetes and risk of tuberculosis relapse: nationwide nested case-control study. PLoS One. 2014;9(3):e92623.

33. Cooper AM. Cell-mediated immune responses in tuberculosis. Annu Rev Immunol. 2009;27:393-422.

34. O'Garra A. Systems approach to understand the immune response in tuberculosis: an iterative process between mouse models and human disease. Cold Spring Harb Symp Quant Biol. 2013;78:173-7.

35. Martens GW, Arikan MC, Lee J, Ren F, Greiner D, Kornfeld H. Tuberculosis susceptibility of diabetic mice. Am J Respir Cell Mol Biol. 2007;37(5):518-24.

36. Vallerskog T, Martens GW, Kornfeld H. Diabetic mice display a delayed adaptive immune response to Mycobacterium tuberculosis. J Immunol. 2010;184(11):6275-82.

37. Shaler CR, Horvath C, Lai R, Xing Z. Understanding delayed T-cell priming, lung recruitment, and airway luminal T-cell responses in host defense against pulmonary tuberculosis. Clin Dev Immunol. 2012;2012:628293.

38. Filley EA, Bull HA, Dowd PM, Rook GA. The effect of Mycobacterium tuberculosis on the susceptibility of human cells to the stimulatory and toxic effects of tumour necrosis factor. Immunology. 1992;77(4):505-9.

39. Mirza S, Hossain M, Mathews C, Martinez P, Pino P, Gay JL, et al. Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. Cytokine. 2012;57(1):136-42.

40. 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(5):739-48.

41. Surendar J, Mohan V, Pavankumar N, Babu S, Aravindhan V. Increased levels of serum granulocyte-macrophage colony-stimulating factor is associated with activated peripheral dendritic cells in type 2 diabetes subjects (CURES-99). Diabetes Technol Ther. 2012;14(4):344-9.

42. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. Nat Rev Endocrinol. 2012;8(12):709-16.

43. Qu D, Liu J, Lau CW, Huang Y. IL-6 in diabetes and cardiovascular complications. Br J Pharmacol. 2014;171(15):3595-603.

44. Van Snick J. Interleukin-6: an overview. Annu Rev Immunol. 1990;8:253-78.

45. Ladel CH, Blum C, Dreher A, Reifenberg K, Kopf M, Kaufmann SH. Lethal tuberculosis in interleukin-6-deficient mutant mice. Infect Immun. 1997;65(11):4843-9.

46. Kumar NP, Sridhar R, Banurekha VV, Jawahar MS, Fay MP, Nutman TB, 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(5):441-9.

47. Hasan Z, Irfan M, Masood Q, Ahmed O, Moosajee US, Rao S, et al. Raised levels of IFN-gamma and IL-13 are associated with pre-diabetes amongst newly diagnosed patients with Tuberculosis. J Pak Med Assoc. 2019;69(4):468-73.

48. Stanya KJ, Jacobi D, Liu S, Bhargava P, Dai L, Gangl MR, et al. Direct control of hepatic glucose production by interleukin-13 in mice. J Clin Invest. 2013;123(1):261-71.

49. Masood KI, Rottenberg ME, Carow B, Rao N, Ashraf M, Hussain R, et al. SOCS1 gene expression is increased in severe pulmonary tuberculosis. Scand J Immunol. 2012.

50. Mizuno S, Soma S, Inada H, Kanuma T, Matsuo K, Yasutomi Y. SOCS1 Antagonist-Expressing Recombinant Bacillus Calmette-Guerin Enhances Antituberculosis Protection in a Mouse Model. J Immunol. 2019;203(1):188-97.

51. Masood KI, Rottenberg ME, Carow B, Rao N, Ashraf M, Hussain R, et al. SOCS1 gene expression is increased in severe pulmonary tuberculosis. Scand J Immunol. 2012;76(4):398-404.

52. Dumpati R, Ramatenki V, Vadija R, Vellanki S, Vuruputuri U. Structural insights into suppressor of cytokine signaling 1 protein- identification of new leads for type 2 diabetes mellitus. J Mol Recognit. 2018;31(7).

53. Schmok E, Abad Dar M, Behrends J, Erdmann H, Ruckerl D, Endermann T, et al. Suppressor of Cytokine Signaling 3 in Macrophages Prevents Exacerbated Interleukin-6-Dependent Arginase-1 Activity and Early Permissiveness to Experimental Tuberculosis. Front Immunol. 2017;8:1537.

54. Masood KI, Rottenberg ME, Salahuddin N, Irfan M, Rao N, Carow B, et al. Expression of M. tuberculosis-induced suppressor of cytokine signaling (SOCS) 1, SOCS3, FoxP3 and secretion of IL-6 associates with differing clinical severity of tuberculosis. BMC Infect Dis. 2013;13:13.

Table Legends

Table 1. Characteristics of study subjects. EC, Endemic control; LTBi, latent TB; DM, diabetes mellitus; DM-LTBi, latently infected Diabetics; TB, tuberculosis; RBS, random blood sugar.

Table 2. Differential pro-inflammatory cytokine secretion between healthy controls, diabetics and LTBi cases. EC, Endemic control; LTBi, latent TB; DM, diabetes mellitus; DM-LTBi, latently infected Diabetics; TB, tuberculosis. Supernatants were collected and tested for IFN-γ, IL-12p70, IL-2, TNF-α, GM-CSF, and IL-1β by Th1 Th2 11 plex procarta plex assay (Thermofischer, USA). Data is depicted as median with IQR. Kruskal Wallis test was run between the groups to see the difference in secretion levels and P values are included in the table. Individual groups were also compared using Mann–Whitney U-test. *Denotes a significant difference ([?]0.05) as compared with DM-LTBi; # as compared with EC; NS, not significantly different, p>0.05.

Table 3. Differential type 2 cytokine secretion between healthy controls, diabetics and LTBi cases. EC, Endemic control; LTBi, latent TB; DM, diabetes mellitus; DM-LTBi, latently infected diabetics; TB, tuberculosis. Supernatants were collected and tested for IL-13, IL-4, IL-5, IL-6 and IL-18 by Th1 Th2 11 plex procarta plex assay (Thermofischer, USA). Data is depicted as median with IQR. Kruskal Wallis test was run between the groups to see the difference in secretion levels and P values are included in the table. Individual groups were also compared using Mann–Whitney U-test. *Denotes a significant difference ([?]0.05) as compared with DM-LTBi; NS, not significantly different, p>0.05.

Figure Legend

Fig 1. Raised proinflammatory responses in diabetics with latent TB. EC, Endemic control; LTBi, latent TB; DM, diabetes mellitus; DM-LTBi, latently infected diabetics; TB, tuberculosis. Total cellular RNA was extracted from PBMCs subjected to RT-PCR using sequence-specific primers to IFN-γ, TNF-α, IL-6, and SOCS-3. All mRNA expression levels were normalized to the housekeeping gene HuPO, and data calculated as median Target gene / HuPO by the relative quantification 2ΔΔC t method. Data is depicted as bar graphs for each group with median values indicated by a horizontal line. *Denotes a significant difference ([?]0.05) between the groups using Mann–Whitney U-test. Figure shows A. IFN-γ, B. TNF-α, C. IL-6 and D. SOCS-3.

Hosted file

`Tables and figures_TB-DM.docx` available at https://authorea.com/users/309125/articles/440108-m-tuberculosis-infection-in-diabetics-is-associated-with-increased-inflammatory-cytokine-but-decreased-suppressor-of-cytokine-signaling-socs-3-responses