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IMMUNOLOGICAL ASPECTS

# High body mass index is associated with heightened systemic and mycobacterial antigen – Specific pro-inflammatory cytokines in latent tuberculosis☆

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SUMMARY

High body mass index (HBMI) has been shown to be protective against active tuberculosis (TB), although the biological mechanism underlying this protection is poorly understood. The immunological association between HBMI and latent TB has never been examined. In order to study the association of HBMI with latent TB, we examined the circulating and TB- antigen or mitogen stimulated levels of a large panel of cytokines in individuals with latent TB (LTB) and high or normal body mass index (HBMI or NBMI). HBMI is characterized by heightened circulating levels of pro-inflammatory (IFNγ, TNFα, IL-22, IL-1α, IL-12 and GM-CSF) cytokines but decreased circulating levels of anti-inflammatory cytokines (IL-4, IL-5 and TGFβ). This systemic cytokine profile is associated with elevated TB-antigen and mitogen stimulated levels of IFNγ, TNFα, IL-2 and IL-1α and diminished levels of IL-10 and TGFβ. In addition, we also observed a positive correlation between the circulating levels of IFNγ, TNFα, IL-22, IL-1α with BMI and a negative correlation between the circulating levels of IL-10, TGFβ and BMI. Our data, therefore, suggest the modulation of protective and regulatory cytokines might underlie the protective effect of HBMI against the development of active TB.

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*Mycobacterium tuberculosis* (Mtb) infection usually results in a latent, asymptomatic state (LTB) and only about 5–10% of these infected individuals actually progress to active TB during their lifetime [1,2]. It is well known that cytokines of the innate and adaptive immune systems orchestrate the immune response to Mtb, with pro-inflammatory cytokines such as IFNγ, TNFα, IL-1α, IL-β and GM-CSF having been implicated in protection against TB disease in murine models [3,4], whereas anti-inflammatory cytokines have been shown to be common risk factors driving increased susceptibility to disease [5,6]. Mounting evidence suggests that being overweight or obese might decrease the risk of TB. A meta-analysis of different studies examining the relationship between nutritional status and TB suggested that there was log-linear inverse relationship between TB incidence and BMI, within the BMI range of 18.5–30 kg/m$^2$ [7]. Consequently, people who are overweight have a lower risk of developing active TB than people with normal weight for height. Subsequently, other studies from both high and low TB endemic settings have confirmed this association [8,9]. Data from animal studies clearly reveal an important role for protective cytokines, especially IFNγ and TNFα in protection against TB; and the regulatory cytokines, IL-10 and TGFβ in increased susceptibility to TB in nutritionally manipulated animal models [10]. We have also shown that latent TB individuals with low BMI exhibit perturbations in their systemic and TB-antigen specific cytokine responses [11].

We therefore hypothesized that high BMI would be predominantly associated with heightened protective cytokine responses and thereby potentially at decreased risk of developing active TB in latent TB individuals (LTB). To study the influence of high BMI on LTB, we examined the plasma levels of a large panel of pro- and anti-inflammatory cytokines in individuals with LTB and coincident

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high BMI (HBMI) and compared them to those with LTB but with normal BMI (NBMI). We show that those with HBMI have increased systemic levels of most of the pro-inflammatory cytokines but decreased levels of anti-inflammatory cytokines, a pattern that is reflected upon TB antigen and mitogen stimulation. Thus, our data suggest that high BMI is associated with heightened protective cytokine levels and diminished regulatory cytokine levels in LTB, possibly contributing to a plausible biological mechanism for increased protection against active TB.

## 1. Materials and methods

### 1.1. Study population

We studied a group of 60 individuals with latent TB infection − 30 with high BMI and 30 with normal BMI (Table 1). LTB was diagnosed on the basis of being Tuberculin skin test (TST) positive and Quantiferon TB Gold (QFT) positive with no symptoms or signs of active TB, no history of previous TB and normal chest radiographs. Anthropometric measurements, including height, weight and biochemical parameters, including plasma glucose, plasma lipids and HbA1c were obtained using standardized techniques. Low and normal BMI were defined on the basis of the 2013 American Heart Association/American College of Cardiology guidelines (HBMI between 25 and 29.9 and NBMI between 18.5 and 24.9 kg/m$^2$). All the individuals were non-diabetic and HIV negative. They were also not hypertensive, dyslipidemic or obese. The age, sex or socio-economic status of the two groups were not significantly different (Table 1). We also studied a control group of LTBI negative individuals (n = 30). All individuals were examined as part of a natural history protocol approved by the Institutional Review Board of the National Institute of Research in Tuberculosis (NCT00375583), and informed written consent was obtained from all individuals.

### 1.2. ELISA

Plasma cytokines were measured using a Bioplex multiplex cytokine assay system (Bio-Rad). The parameters analyzed were IFNγ, TNFα, IL-2, IL-17A, IL-4, IL-5, IL-13, IL-10, IL-6, IL-12 and GM-CSF. Plasma levels of TGFβ, IL-1α, IL-1β, IL-18, IL-22 (all R& D Systems) and IL-17F (Biolegend) were measured by ELISA.

### 1.3. QFT ELISA

Whole blood from HBMI or NBMI individuals was incubated with either no antigen or with a cocktail of TB antigens (ESAT-6, CFP-10, TB 7.7) or mitogen in vitro, according to the manufacturers instructions using the QFT kit (Qiagen). The unstimulated or TB antigen or mitogen stimulated whole blood supernatants were then used to analyze the levels of IFNγ, TNFα, IL-2, IL-1α, IL-10 and TGFβ using the Duo-set ELISA kits from R& D systems.

**Table 1**
Demographic profile of the study population.

| Parameter | HBMI n = 30 | NBMI n = 30 |
|---|---|---|
| No. of males/no. of females | 14/16 | 18/12 |
| Age (Yr) | 28 (19–62) | 29 (19–61) |
| Body mass index | 33 (25.9–38.7) | 22 (18.90–24.97) |
| Albumin (g/dl) | 4.6 (3.6–4.9) | 4.2 (3.7–4.8) |
| Random blood glucose (mg/dl) | 98.8 (60–258) | 92.3 (60.129) |
| HbA1c (%) | 5.7 (4.7–10) | 5.7 (4.9–6.1) |
| Urea (mg/dl) | 23 (13–36) | 21.9 (11–42) |
| Creatinine (mg/dl) | 0.75 (0.4–1.1) | 0.80 (0.4–1.3) |
| ALT (U/liter) | 35.5 (10–126) | 22.4 (7–92) |
| AST (U/liter) | 29.9 (14–79) | 24.7 (11–68) |

The values represent geometric means and ranges (except for age, for which medians and ranges are shown, and number of males and females).

### 1.4. Statistical analysis

Geometric means (GM) were used for measurements of central tendency. Statistically significant differences between two groups were analyzed using the nonparametric Mann–Whitney *U* test with Holm's correction for multiple comparisons. Correlations were calculated using Spearman rank correlation. Analyses were performed using GraphPad PRISM Version 5.01.

## 2. Results

### 2.1. HBMI is associated with increased circulating levels of Type 1 and other pro-inflammatory cytokines but decreased anti-inflammatory cytokines

To determine the influence of BMI on Type 1, Type 2, Type 17, other pro- and anti-inflammatory cytokines in LTB, we measured the circulating levels of these cytokines in HBMI and NBMI individuals with concomitant LTB (Figure 1). As shown in Figure 1A and C, the systemic levels of Type 1 (IFNγ and TNFα), Type 17 (IL-22) and other pro-inflammatory (IL-1α, IL-12 and GM-CSF) cytokines were significantly increased in HBMI compared to LBMI individuals. In contrast, the systemic levels of Type 2 (IL-4 and IL-5) and other anti-inflammatory (TGFβ) cytokines were significantly decreased. Thus, HBMI is associated with altered levels of circulating cytokines at homeostasis in LTB individuals.

### 2.2. HBMI is associated with increased TB antigen stimulated levels of pro-inflammatory cytokines and decreased levels of anti-inflammatory cytokines

To determine the influence of BMI on TB antigen stimulated cytokine production in LTB, we measured circulating levels of these cytokines following stimulation of whole blood with no antigen or a cocktail of TB antigens (ESAT-6, CFP-10, TB 7.7) or mitogen in HBMI and NBMI individuals with concomitant LTB (Figure 2). As shown in Figure 2A, the spontaneously produced levels of IFNγ, TNFα, IL-2 and IL-1α were significantly higher and levels of IL-10 and TGFβ significantly lower in HBMI compared to NBMI individuals. Similarly, as shown in Figure 2B and C, the TB antigen and mitogen stimulated levels of IFNγ, TNFα, IL-2 and IL-1α were significantly higher and levels of IL-10 and TGFβ significantly lower in HBMI compared to NBMI individuals. Thus, HBMI is associated with enhanced levels of TB antigen stimulated pro-inflammatory cytokines and diminished levels of anti-inflammatory cytokines.

### 2.3. LTB individuals exhibit significantly higher levels of both pro- and anti-inflammatory cytokines compared to LTB negative controls

To examine the role of cytokines in latent TB infection, we measured the systemic levels of Type 1, Type 2, Type 17, other pro- and anti-inflammatory cytokines in LTB$^+$ NBMI individuals and compared them to LTB negative controls. As shown in Table 2, the systemic levels of Type 1 (IFNγ, IL-2 and TNFα), Type 17 (IL-22) and other pro-inflammatory (IL-18, IL-12 and GM-CSF) cytokines were significantly increased in LTB$^+$ compared to LTB negative individuals. Similarly, the systemic levels of regulatory (IL-10 and TGFβ) cytokines were also significantly increased in LTB$^+$ compared to LTB negative individuals. Thus, latent TB infection per se is associated with increased levels of circulating cytokines.