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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 (IFN γ , TNF α , IL-22, IL-1 α , IL-12 and GM-CSF) cytokines but decreased circulating levels of anti-inflammatory cytokines (IL-4, IL-5 and TGF β). This systemic cytokine profile is associated with elevated TB-antigen and mitogen stimulated levels of IFN γ , TNF α , IL-2 and IL-1 α and diminished levels of IL-10 and TGF β . In addition, we also observed a positive correlation between the circulating levels of IFN γ , TNF α , IL-22, IL-1 α with BMI and a negative correlation between the circulating levels of IL-10, TGF β 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.

Keywords

Tuberculosis; BMI; Overweight; Obesity; Cytokines; Immune response

Mycobacterium tuberculosis (Mtb) infection usually results in a latent, asymptomatic state (LTB) and only about 5e10% 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 IFN γ , TNF α , IL-1 α , IL- β and GM-CSF having been implicated in protection against TB

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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 metaanalysis 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/m² [7]. Consequently, people who are over weight 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 IFN γ and TNF α in protection against TB; and the regulatory cytokines, IL-10 and TGF β 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 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 e 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/m²). 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 IFN γ , TNF α , IL-2, IL-17A, IL-4, IL-5, IL-13, IL-10, IL-6, IL-12 and GM-CSF. Plasma levels of TGF β , IL-1 α , IL-1 β , 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 IFN γ , TNF α , IL-2, IL-1 α , IL-10 and TGF β using the Duo-set ELISA kits from R&D systems.

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 (IFN γ and TNF α), Type 17 (IL-22) and other pro-inflammatory (IL-1 α , IL-12 and GM-CSF) cytokines were significantly increased in HBMI compared to NBMI individuals. In contrast, the systemic levels of Type 2 (IL-4 and IL-5) and other anti-inflammatory (TGF β) 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 IFN γ , TNF α , IL-2 and IL-1 α were significantly higher and levels of IL-10 and TGF β significantly lower in HBMI compared to NBMI individuals. Similarly, as shown in Figure 2B and C, the TB antigen and mitogen stimulated levels of IFN γ , TNF α , IL-2 and IL-1 α were significantly higher and levels of IL-10 and TGF β 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 (IFN γ , IL-2 and TNF α), 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 TGF β) 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.

2.4. Relationship between systemic cytokines and BMI

To examine the relationship between the systemic levels of pro-and anti-inflammatory cytokines with BMI, we assessed the association of IFN γ , TNF α , IL-2, IL-1 α , IL-10 and TGF β with BMI (in kg/m²) in all the individuals in the study. As shown in Table 3, the systemic levels of IFN γ , TNF α , IL-2 each exhibited a significant positive association with BMI, while the systemic levels of TGF β exhibited a significant negative association in LTB individuals. No significant relationship between the systemic levels of other cytokines was observed with BMI in LTB individuals.

3. Discussion

Previous studies have demonstrated a strong inverse relationship between BMI and TB risk. These include studies conducted in both high endemic—(China [12], India [13] and South Africa [9]) and low endemic—(Norway [14] and USA [8,15]) countries. While nutritional differences between HBMI and NBMI individuals is obviously a major biological driver for the protective effect against active disease, the mechanism by which this is manifest immunologically is not known. Cytokines are known to play a major role in determining the outcome of infection in host defense against mycobacterial infections [3,4]. Typically, Type 1 and Type 17 cytokines are associated with protection against infection, while other pro-inflammatory cytokines, especially IL-1 α , IL-1 β , IL-6, IL-12, IL-18 and GM-CSF are also considered important for resistance to infection in animal models [3,5]. Our examination of the above mentioned cytokines clearly reveals an exaggerated production of most of the above resulting in significantly increased circulating levels. In addition to the heightened systemic cytokine levels, LTB individuals with high BMI also appear to mount enhanced protective cytokine responses to TB antigens. Therefore, the ability to produce increased levels of IFN γ , TNF α , IL-2 and IL-1 α both at baseline and following TB-antigen or mitogen stimulation, suggests that LBMI individuals are potentially capable of mounting an adequate protective immune response against Mtb.

In contrast, both Type 2 cytokines and other anti-inflammatory cytokines are known to play a role in enhancing susceptibility to infection [16,17]. Our examination clearly reveals a

diminution in the production levels of some of these cytokines systemically and in response to TB antigens or mitogens. Therefore, HBMI also appears to be associated with a lack of increased induction of the regulatory network of cytokines that typically promote susceptibility to disease. While in the setting of active TB disease, it is generally considered that both pro-and anti-inflammatory cytokines promote TB establishment and progression, it is now thought that these cytokines play a different role in latent infection [5,6]. Thus, alterations in the balance between pro-and anti-inflammatory cytokines has been shown to promote the transition from latent to active infection in non-human primate models of TB infection. Also, perturbations of cytokine responses appears to represent a pivotal checkpoint of the response against Mtb since most clinical studies show that the risk of developing TB is highest during the first 6 months of treatment involving biologicals targeting the TNF α /IL-12/IL-23 axis [18,19]. Similarly, deleterious mutations in genes encoding the IL-12/IFN γ signaling pathway are also known to enhance susceptibility to severe infection and disease [20].

Our data would therefore indicate that alteration of the balance between pro-and anti-inflammatory cytokines plays a pivotal role in the establishment of cytokine responses in LTB. Finally, our data also offers direct evidence on the association of BMI with the perturbations in plasma cytokine levels in LTB individuals with a positive relationship between BMI and the Type 1 cytokines (IFN γ , TNF α and IL-2) and a negative relationship with TGF β . In addition, we had adjusted for most of the known confounders including age, sex, socioeconomic status, obesity, diabetes, HIV and previous treatment. This suggests that BMI per se independent of other co-variables in the system regulates systemic cytokine levels in TB infection. While our study certainly has limitations (cross-sectional, small sample size, relying on systemic cytokine measurement), it nevertheless highlights an important biological mechanism that could potentially underlie the increased protection shown by elevated BMI against TB disease.

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Figure 1.

Altered systemic levels of Type 1, Type 2, Type 17, other pro-and anti-inflammatory cytokines in HBMI individuals. (A) The plasma levels of Type 1 (IFN γ , TNF α , IL-2) and Type 17 (IL-17A, IL-17F and IL-22) cytokines were measured by ELISA in HBMI (n = 30) and NBMI (n = 30) individuals with LTb. (B) The plasma levels of Type 2 (IL-4, IL-5, IL-13) and other anti-inflammatory (IL-10 and TGF β) cytokines were measured by ELISA in HBMI (n = 30) and NBMI (n = 30) individuals with LTb. (C) The plasma levels of IL-1 family (IL-1 α , IL-1 β , IL-18) and other pro-inflammatory (IL-6, IL-12 and GM-CSF)

cytokines were measured by ELISA in HBMI (n = 30) and NBMI (n = 30) individuals with LTB. The data are represented as scatter plots with each circle representing a single individual (light grey – HBMI and dark grey – NBMI). P values were calculated using the Manne–Whitney test.

Figure 2.

Heightened unstimulated, TB antigen and mitogen stimulated and levels of pro-inflammatory cytokines and diminished levels of anti-inflammatory cytokines in HBMI individuals. The unstimulated (A) or TB antigen stimulated (B) or mitogen stimulated (C) levels of IFN γ , TNF α , IL-2, IL-10, TGF β and IL-1 α were measured by ELISA in whole blood of a HBMI (n = 30) and NBMI (n = 30) individuals with LTb. The data are represented as scatter plots with each circle representing a single individual (light grey – HBMI and dark grey – NBMI). P values were calculated using the Manne–Whitney test.

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).

Table 2

Comparison of systemic levels between LTBI positive and LTBI negative group.

Cytokines	Value (GM [range])		P value*
	LTBI positive (n = 30)	LTBI negative (n = 30)	
Type 1 cytokines			
IFN γ	2083 (948.47–8209.04)	1528 (280.72–6372.44)	0.0231
TNF α	2255 (777.46–6620.98)	311.3 (136.74–524.17)	<0.0001
IL-2	304.7 (123.28–894.05)	227.4 (124.15–621.55)	0.0214
Type 17 cytokines			
IL-17A	280.7 (19.47–704.93)	225.1 (35.31–624.99)	0.1149
IL-17F	1.497 (0.463–3.296)	1.334 (0.31–2.82)	0.4876
IL-22	129.2 (33.77–358.14)	68.35 (23.17–141.46)	0.0146
Type 2 cytokines			
IL-4	81.56 (24.01–148.84)	92.28 (32.05–144.95)	0.2062
IL-5	167.7 (104.27–300.22)	164.9 (119.29–349.42)	0.6305
IL-13	182 (107.91–625.64)	198.3 (120.29–621.33)	0.2342
Regulatory cytokines			
IL-10	214.8 (132.06–328.29)	149.9 (102.12–269.45)	<0.0001
TGF β	490.1 (230.89–968.1)	376.4 (172.27–709.73)	0.0205
Pro-inflammatory cytokines			
IL-1 α	1510 (168–6451)	1433 (169.57–6189.16)	0.838
IL-1 β	374.4 (123.99–1035.54)	328.1 (74.74–1082.78)	0.5205
IL-18	6520 (3985–8254)	6095 (3239.93–8592.44)	0.0015
IL-6	1429 (367.69–5747.34)	1412 (333.74–5754.92)	0.9093
IL-12	312.4 (151.58–494.94)	276.5 (191.91–432.63)	0.0173
GM-CSF	200.2 (119.15–307.06)	170.9 (71.54–979.48)	0.0304

Significant p values are highlighted in bold.

* P value calculated using Mann–Whitney analysis.

Table 3

Correlation of BMI and different cytokines.

Cytokines	P value *	r value
Type 1 cytokines		
IFN γ	0.0003	0.4499
TNF α	<0.0001	0.7556
IL-2	0.045	0.2642
Type 17 cytokines		
IL-17A	0.8472	-0.2541
IL-17F	0.966	-0.0056
IL-22	0.0006	0.4578
Type 2 cytokines		
IL-4	0.0006	-0.4304
IL-5	0.0064	-0.3481
IL-13	0.5584	0.077
Regulatory cytokines		
IL-10	0.0848	0.2244
TGF β	<0.0001	-0.6146
Pro-inflammatory cytokines		
IL-1 α	0.4231	-0.1072
IL-1 β	0.8693	-0.0217
IL-18	0.0417	0.2638
IL-6	0.6962	0.5146
IL-12	0.0198	0.3001
GM-CSF	0.1267	-0.1994

Significant p values are highlighted in bold.

* P value and r-value calculated using spearman rank correlation.