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MECHANISMS OF PATHOGENESIS

Modulation of pro- and anti-inflammatory cytokines in active and latent tuberculosis by coexistent *Strongyloides stercoralis* infection

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SUMMARY

Helminth infections are known to induce modulation of both innate and adaptive immune responses in active and latent tuberculosis (TB). However, the role of helminth infections in modulating systemic cytokine responses in active and latent tuberculosis (LTB) is not known. To define the systemic cytokine levels in helminth-TB coinfection, we measured the circulating plasma levels of Type 1, Type 2, Type 17, other pro-inflammatory and regulatory cytokines in individuals with active TB (ATB) with or without coexistent *Strongyloides stercoralis* (Ss) infection by multiplex ELISA. Similarly, we also measured the same cytokine levels in individuals with LTB with or without concomitant Ss infection in a cross-sectional study. Our data reveal that individuals with ATB or LTB and coexistent Ss infection have significantly lower levels of Type 1 (IFNγ, TNFα and IL-2) and Type 17 (IL-17A and IL-17F) cytokines compared to those without Ss infection. In contrast, those with ATB and LTB with Ss infection have significantly higher levels of the regulatory cytokines (IL-10 and TGFβ), and those with LTB and Ss infection also have significantly higher levels of Type 2 cytokines (IL-4, IL-5 and IL-13) as well. Finally, those with LTB (but not ATB) exhibit significantly lower levels of other pro-inflammatory cytokines (IFNα, IFNβ, IL-6, IL-12 and GM-CSF). Our data therefore reveal a profound effect of Ss infection on the systemic cytokine responses in ATB and LTB and indicate that coincident helminth infections might influence pathogenesis of TB infection and disease.

1. Introduction

Helminth infections and tuberculosis are both major public health problems worldwide with tuberculosis (TB) afflicting nearly 10 million new cases annually [1] and helminths infecting over 2 billion people [2]. In addition, TB and helminth infections share considerable geographical overlap with both infections affecting mostly lower and some middle-income countries worldwide [3]. Furthermore, the larvae of many intestinal helminths migrate through the lungs, thereby providing a biological pathway for these helminths to influence the host immune response to TB [3]. Typically, TB manifests itself as a clinical spectrum ranging from asymptomatic, latent infection to clinically active pulmonary or extra-pulmonary disease. After initial infection, most individuals control bacterial replication and enter a period of infectious latency known as latent tuberculosis (LTB). Approximately, 5–10% of those with LTB progress to active tuberculosis (ATB) in their lifetime, a progression reflecting the failure of host immune responses in containing bacterial replication [1]. While the adaptive immune system is pivotal in the pathogenesis of TB disease, it is also abundantly clear that systemic inflammatory and cytokine responses also significantly influence disease activity and severity [4,5].

Helminth parasites are commonly characterized by their ability to establish chronic infections in humans, sometimes lasting decades. Although helminth infections are rarely lethal, they can

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contribute to morbidity in adults and impair physical and cognitive development in children [6]. Among the helminth parasites, *Strongyloides stercoralis* (Ss) the causative agent of strongyloidiasis, infects ~50–100 million people worldwide [7,8]. Ss infection is often clinically asymptomatic and longstanding due, in large part, to the parasites' autoinfective lifecycle and their ability to modulate or evade the host immune system [2,9]. Recent, epidemiological and experimental evidence provide evidence that helminths (both systemic and intestinal) have a negative regulatory role in the immune response to TB infection and disease, although no evidence exists for an impact on clinical outcomes [3,10,11].

Helminth infections are known to induce modulatation of T cell mediated immune responses to TB antigens in both LTB and ATB [12]. However, their role in modulating systemic cytokine responses in coincident LTB and ATB has not been fully explored. We therefore hypothesized that the regulatory networks established during chronic Ss infection could potentially modulate the cytokine response to TB infection and disease. Thus, we examined the systemic levels of a variety of systemic cytokines, shown to influence either susceptibility or resistance to active disease in LTB or systemic or local pathology in ATB. We find that coexistent Ss infection has a major impact on the innate and adaptive cytokine responses in both LTB and ATB, with a much more pronounced effect on LTB.

2. Materials and methods

2.1. Ethics statement

All individuals were examined as part of a natural history study protocol approved by Institutional Review Boards of the National Institute of Allergy and Infectious Diseases (USA) and the National Institute for Research in Tuberculosis (India), and informed written consent was obtained from all participants.

2.2. Study population

We studied a group of 88 individuals with active pulmonary TB, 42 of whom were infected with *S. stercoralis* (hereafter ATB + Ss) infection and 46 of whom had active TB alone (ATB) (Table 1). We also studied another group of 88 individuals with latent TB, 44 of whom were infected with *S. stercoralis* (hereafter LTB + Ss) infection and 44 of whom had active TB alone (LTB). All the study individuals were recruited from patients and their relatives attending the outpatient clinic at the Stanley Medical Hospital, Chennai. This was a cross-sectional study and a convenient sampling methodology was used. Samples were collected at the time of diagnosis of ATB and LTB. ATB was diagnosed microbiologically on the basis of being culture positive for Mtb by solid cultures in LJ medium and all were sputum smear positive. LTB individuals were asymptomatic with positive Quantiferon Gold-in-tube tests and normal chest radiographs. Ss infection was diagnosed by the presence of IgG antibodies to the 31-kDa recombinant NIE antigen by the Luciferase Immunoprecipitation System Assay (LIPS), as described previously [13]. This test has been reported previously to be the most accurate serologic test for diagnosis of Ss infection [14]. We used a cutoff of 10,000 light units as determined by positive and negative controls previously, which had used stool microscopy as the reference standard. All individuals were also negative for filarial infection by filarial antigen tests (ICT card test and TropBio ELISA) but stool microscopy for intestinal helminths was not done. All individuals were HIV negative (determined by rapid card test), non-diabetic (determined by fasting blood glucose) and anti-tuberculous and antihelmintic (self-reported) treatment naive. The two groups of active TB individuals did not differ significantly in bacillary burden (as estimated by smear grades at the time of diagnosis following Ziehl-Nielsen staining). Moreover, the individuals in this study were different from the group of individuals described in our previous studies [15,16].

2.3. ELISA

Plasma cytokines were measured using a Bioplex multiplex cytokine assay system (Bio-Rad, Hercules, CA). The parameters analyzed were IFNγ, TNFα, IL-2, IL-17A, IL-4, IL-5, IL-10, IL-6, IL-12p70 and GM-CSF. Plasma levels of TGFβ, IL-1α, IL-1β (all R& D Systems); IL-17F (Biolegend); IL-22 (eBioscience); Type 1 interferons (IFNs) – IFNα (multiple subtypes) and IFNβ (PBL Interferon Source) were measured by ELISA. All samples were run in duplicates.

2.4. Statistical analysis

Data analyses were performed using GraphPad PRISM (GraphPad Software, Inc., San Diego, CA, USA). Geometric means (GM) were used for measurements of central tendency. Comparisons were made using either by Mann–Whitney U test for comparison between 2 groups or by Kruskal–Wallis test with Dunn's multiple comparisons for multiple groups or by Student's t-test and adjusted by Benjamin-Hochberg Procedure for the heatmaps. R software package was used to plot the heat map for log2 transformed values of plasma levels.

3. Results

3.1. Study population characteristics

The baseline characteristics including demographic and hematological features of the study population are shown in Table 1. As

Table 1
Demographics and hematological parameters of the study population.

	LTB	LTB + Ss	p Value	ATB	ATB + Ss	p Value
	n = 44	n = 44		n = 46	n = 42	
Age	35 (23–60)	48 (28–64)	NS	36 (18–65)	42 (18–65)	NS
M/F	21/23	20/24	NS	35/11	33/09	NS
Smear grade: 1+/2+/3+	nil	nil	NA	25/10/11	20/18/4	NS
NIE LIPS	Neg	Pos	NA	Neg	Pos	NA
WBC 10^3/uL	7.56 (4–15.1)	7.96 (5.6–17.7)	NS	9.95 (4.3–20.3)	9.78 (4.1–15)	NS
Hb g/dL	13.1 (6–17.7)	13.42 (9.1–17.6)	NS	11.9 (7.4–19.6)	11.5 (3.7–20.2)	NS
Neutrophil 10^3/uL	3.87 (2.26–8.26)	4.02 (2.31–7.56)	NS	6.9 (3.65–17.74)	6.31 (2.14–12.58)	NS
Lymphocytes 10^3/uL	2.34 (1.11–4.61)	2.54 (1.56–4.61)	NS	1.7 (0.64–3.92)	1.87 (1.03–3.81)	NS
Monocytes 10^3/uL	0.51 (0.23–1.32)	0.52 (0.27–1.06)	NS	0.7 (0.18–1.33)	0.74 (0.25–1.81)	NS
Eosinophils 10^3/uL	0.48 (0.13–2.27)	0.44 (0.16–1.95)	NS	0.23 (0.06–1.06)	0.29 (0.04–4.34)	NS

Values represent the geometric mean or median (and range) and the p values were calculated using the Mann–Whitney U test.

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