The Journal of Immunology

Coexistent Helminth Infection–Mediated Modulation of Chemokine Responses in Latent Tuberculosis

Anuradha Rajamanickam, Saravanan Munisankar, Yukthi Bhootra, Chandra Kumar Dolla, Thomas B. Nutman and Subash Babu

J Immunol 2019; 202:1494-1500; Prepublished online 16 January 2019;
doi: 10.4049/jimmunol.1801190
http://www.jimmunol.org/content/202/5/1494

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Coexistent Helminth Infection–Mediated Modulation of Chemokine Responses in Latent Tuberculosis

Anuradha Rajamanickam,* Saravanan Munisankar,* Yukthi Bhootra,* Chandra Kumar Dolla,† Thomas B. Nutman,‡ and Subash Babu*,‡

Coexistent helminth infections are known to modulate T cell and cytokine responses in latent infection with *Mycobacterium tuberculosis*. However, their role in modulating chemokine responses in latent tuberculosis (LTB) has not been explored. Because chemokines play a vital role in the protective immune responses in LTB, we postulated that coexistent helminth infection could modulate chemokine production in helminth-LTB coinfection. To test this, we measured the levels of a panel of CC and CXC chemokines at baseline and following mycobacterial Ag or mitogen stimulation in individuals with LTB with (*Strongyloides stercoralis*$^+$LTB$^+$) or without *S. stercoralis* (*S. stercoralis*$^-$LTB$^+$) infection and in individuals without both infections, healthy controls (HC). At baseline (in the absence of a stimulus), *S. stercoralis*$^+$LTB$^+$ individuals exhibited significantly diminished production of CCL1, CCL2, CCL4, CCL11, CXCL9, CXCL10, and CXCL11 in comparison with *S. stercoralis*$^-$LTB$^+$ and/or HC individuals. Upon mycobacterial Ag stimulation, *S. stercoralis*$^+$LTB$^+$ individuals exhibited significantly diminished production of CCL1, CCL2, CCL4, CCL11, CXCL2, CXCL9, and CXCL10 in comparison with *S. stercoralis*$^-$LTB$^+$ and/or HC individuals. No differences were observed upon mitogen stimulation. Finally, after anthelmintic treatment, the baseline levels of CCL1, CCL2, CCL4, CCL11, and CXCL11 and mycobacterial Ag–stimulated levels of CCL1, CCL2, CCL11, CXCL2, and CXCL10 were significantly increased in *S. stercoralis*$^+$LTB$^+$ individuals. Thus, our data demonstrate that *S. stercoralis*$^+$LTB$^+$ individuals are associated with a compromised ability to express both CC and CXC chemokines and that this defect is at least partially reversible upon treatment. Hence, coexistent helminth infection induces downmodulation of chemokine responses in LTB individuals with likely potential effects on tuberculosis pathogenesis. ***The Journal of Immunology*****, 2019, 202: 1494–1500.**

Helminth infections are major modulators of immune responses with the ability to regulate both innate and parasite-specific adaptive immune responses and, perhaps as a consequence, to establish long-standing infection. With chronic helminth infections, it has been demonstrated that the regulatory environment driven by parasite Ags drives modulation of the immune response to bystander Ags. For example, helminth infections are known to modulate *Mycobacterium tuberculosis*–specific T cell and cytokine responses in both latent and active tuberculosis (TB). Moreover, this bystander modulation of *M. tuberculosis*–specific responses can be reversed, to some degree, with anthelmintic treatment.

Chemokines are felt to play a major role in latent TB (LTB) infection as they appear to be critical in the formation and maintenance of quiescent granulomas (1) and in the recruitment of cells from the periphery for positioning within the granuloma (2). Establishment of the TB granuloma is controlled by the synchronized expression of various chemokines. This synchronization has recently been shown to be critical in control of TB disease along with CD4$^+$ T cells recruitment into the lung parenchyma (3–5). CCL1, CCL2, CCL3, and CCL4 have been shown to be increased in *M. tuberculosis* infection and these chemokines also mediate dendritic cell trafficking to the lymph nodes (6, 7). CXCL9, 10, and 11 play a potential role in granuloma formation and maintenance as well (8, 9). Human data support a protective role for CXCR3-binding chemokines as CXCL9 levels correlate with disease severity (10, 11). Moreover, chemokine levels in the circulation are known to influence the ability of the host immune response to maintain protective immunity in LTB (12). Finally, defects in the production of certain chemokines have been associated with increased susceptibility to *M. tuberculosis* in animal models (13).

We hypothesized that helminth infection would modulate the levels of protective chemokine responses in LTB and thereby predispose to an increased risk of developing active TB. We thus examined the levels of the CC and CXC family of chemokines in LTB with or without *Strongyloides stercoralis* infection. Our data clearly demonstrate the modulation by *S. stercoralis* of *M. tuberculosis*–specific chemokine responses and its reversal following anthelmintic therapy.

Materials and Methods

Study population

We studied a group of 40 individuals with LTB infection and *S. stercoralis* coinfection (hereafter, *S. stercoralis*$^+$LTB$^+$), 40 with LTB infection without *S. stercoralis* infection (hereafter, *S. stercoralis*$^-$LTB$^+$) and 30 healthy controls (HC). This study was conducted in South India, and the individuals were screened as part of a protocol in a rural village in the suburbs of Chennai. All enrolled individuals were between 18 and 65 y old. The recruited individuals did not have diabetes, HIV, or other parasitic

*National Institutes of Health—National Institute for Research in Tuberculosis—International Center for Excellence in Research, Chennai, India 600031; †National Institute for Research in Tuberculosis, Chennai, India 600031; and ‡Laboratory of Parasitic Diseases, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

ORCIDs: 0000-0002-8143-5502 (A.R.); 0000-0001-6887-4941 (T.B.N.); 0000-0001-9783-8042 (S.B.).

Received for publication August 27, 2018. Accepted for publication December 14, 2018.

This work was supported by the Division of Intramural Research, National Institute for Allergy and Infectious Diseases, National Institutes of Health.

Address correspondence and reprint requests to Dr. Subash Babu, National Institutes of Health—National Institute for Research in Tuberculosis—International Center for Excellence in Research, National Institute for Research in Tuberculosis, No. 1 Sathiyamurthy Road, Chetpet, Chennai 600031, India. E-mail address: sbabu@mail.nih.gov

The online version of this article contains supplemental material.

Abbreviations used in this article: HC, healthy control; LTB, latent TB; post-T, posttreatment; pre-T, pretreatment; QFT, QuantiFERON; TB, tuberculosis.

infections. LTB was diagnosed based on positive results of a tuberculin skin test and QuantiFERON (QFT) TB Gold in tube, with no symptoms or signs of active TB, no history of previous TB, and normal chest radiographs. Tuberculin skin test was performed using two tuberculin units of Tuberculin Purified Protein Derivative RT 23 Serum Statens Institute. A positive skin test was defined as an induration of at least 12-mm diameter based on the previously determined cutoff norms for South India (14). QFT was performed according to the manufacturer's instructions (Qiagen). Hematology was performed on all individuals using the Act5 Diff Hematology Analyzer (Beckman Coulter). *S. stercoralis* infection was diagnosed by the presence of IgG Abs to the recombinant NIE Ag as described previously (15, 16, 20). This was further confirmed by stool microscopy. A single stool sample was obtained and examined for intestinal helminth infection by Kato–Katz technique. Stool samples found to be negative for other intestinal helminths by stool microscopy and positive for *S. stercoralis* infection by serology were then subjected to specialized stool examination with nutrient agar plate cultures. Only the individuals who were positive for *S. stercoralis* infection by both serology and nutrient agar culture plate technique were selected for this study. Also, filarial infections were excluded by TropBio ELISA. All individuals were from the same socioeconomic status. All *S. stercoralis*–infected individuals were treated with a single dose of ivermectin (12 mg) and albendazole (400 mg), and follow-up blood draws were obtained 6 mo later. Following anthelmintic treatment, parasitological examinations were repeated after 6 mo to confirm successful chemotherapy. All individuals were examined as part of a clinical protocol approved by the Institutional Review Board of the National Institute for Allergy and Infectious Diseases and the National Institute of Research in Tuberculosis (NCT00375583 and NCT00001230), and informed written consent was obtained from all individuals.

QFT ELISA

Whole blood obtained from *S. stercoralis*$^+$LTB$^+$, *S. stercoralis*$^-$LTB$^+$, and HC individuals was incubated in vitro with media alone (unstimulated) or a mixture of TB Ags (ESAT-6, CFP-10, and TB 7.7 [TB Ag]) or mitogen (PHA) for 18 h, according to the manufacturer's instructions using the QFT kit (Qiagen). The baseline (unstimulated) or TB Ag or mitogen-stimulated whole blood supernatants were then used to analyze the levels of chemokine panel using a Human Magnetic Luminex Assay Kit from R&D Systems. The parameters analyzed were CCL1, CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11. The net chemokine levels were calculated by TB Ag stimulation or mitogen minus unstimulated levels.

Statistical analysis

Geometric means and/or medians were used for measurements of central tendency.

Statistically significant differences between the three groups were analyzed using the Kruskal–Wallis test with Dunn post hoc for multiple comparisons. Statistical differences before and after treatment were analyzed using the Wilcoxon signed rank test, followed by Holm correction for multiple comparisons.

Results

Study population characteristics

The baseline demographics of the study population and hematological parameters are shown in Table I. There were no differences in age or sex or body mass index between the groups or in their hematological parameters, with the exception of the absolute eosinophil counts in which the *S. stercoralis*$^+$LTB$^+$ had higher eosinophil counts when compared with *S. stercoralis*$^-$LTB$^+$ and HC ($p = 0.0354$). In contrast, the *S. stercoralis*$^+$LTB$^+$ individuals had a significantly smaller purified protein derivative skin test induration in comparison with *S. stercoralis*$^-$LTB$^+$ individuals (Table I).

*LTB/*S. stercoralis *coinfection is associated with diminished baseline (unstimulated) levels of CC and CXC chemokines*

To determine the influence of *S. stercoralis* infection at baseline levels of CC and CXC chemokines in LTB individuals, we measured the baseline (unstimulated) levels of CCL1, CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11 in *S. stercoralis*$^+$LTB$^+$, *S. stercoralis*$^-$LTB$^+$ and HC individuals. As shown in Fig. 1, the *S. stercoralis*$^+$LTB$^+$ group had significantly lower spontaneous production of CCL1 (Fold change of 0.63), CCL2 (0.11), CCL4 (0.63), CCL11 (0.15), CXCL9 (0.32), CXCL10 (0.69) and CXCL11 (0.38) when compared with the *S. stercoralis*$^-$LTB$^+$ group. The baseline levels of CCL3, CXCL1, and CXCL2 did not show any significant difference between the *S. stercoralis*$^+$LTB$^+$ and *S. stercoralis*$^-$LTB$^+$ individuals. Thus, LTB/*S. stercoralis* coinfection is associated with diminished levels of certain CC and CXC chemokines.

S. stercoralis *coinfection alters CC and CXC chemokine production in response to TB Ag stimulation*

To determine the influence of *S. stercoralis* infection on Ag-stimulated levels of CC and CXC chemokines in LTB individuals, we measured the TB Ag–stimulated levels of CCL1, CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11 in *S. stercoralis*$^+$LTB$^+$, *S. stercoralis*$^-$LTB$^+$ and HC individuals (Fig. 2). The net chemokine levels were calculated by TB Ag stimulation minus unstimulated levels. As shown in Fig. 2, *S. stercoralis* infection was associated with significantly lower levels of CCL2 (fold change of 0.41), CCL4 (0.59), CCL11 (0.24), CXCL2 (0.24), CXCL9 (0.32), and CXCL10 (0.56) in the TB Ag–stimulated cultures in comparison with those from the *S. stercoralis*$^-$LTB$^+$ group. The TB antigenic stimulated levels of CCL1, CCL3, CXCL1, and CXCL11 were not significantly different between *S. stercoralis*$^+$LTB$^+$ and *S. stercoralis*$^-$LTB$^+$ individuals. We also assessed the changes in chemokine levels following TB Ag stimulation in comparison with unstimulated levels at the individual level in *S. stercoralis*$^+$LTB$^+$ individuals. As shown in Supplemental Fig. 1A, the levels of all the CC and CXC chemokines were significantly increased upon TB Ag stimulation in *S. stercoralis*$^+$LTB$^+$ individuals. Thus, LTB/*S. stercoralis* coinfection is associated with altered levels of CC and CXC chemokines.

Chemokine response to mitogens is similar across all groups

To determine the influence of *S. stercoralis* infection on mitogen-stimulated levels of CC and CXC chemokines in LTB individuals,

Table I. Baseline demographics of study population

Study Groups	*S. stercoralis*$^+$LTB$^+$	*S. stercoralis*$^-$LTB$^+$	HC	*p* Value
Number	40	40	30	
Gender (male/female)	20/20	20/20	15/15	
Median age (range)	38 (20–60)	36 (20–60)	35 (30–60)	
BMI	19 (18.6–21.5)	21 (18.8–23.4)	23 (19.0–24.5)	
Tuberculin skin test (mm)	14 (12–18)	19 (14–24)	<5 (1–4)	0.0264
QFT ELISA	Positive (≥0.35 IU/ml)	Positive (≥0.35 IU/ml)	Negative (≤0.35 IU/ml)	
WBC 10^3/ml GM (range)	12.14 (7.1–21.2)	11.52 (7.2–19.1)	10.37 (6.9–15.4)	NS
Hb gm/dl GM (range)	11.9 (8.4–13.3)	14.28 (10–21.8)	12.11 (9.1–14.6)	NS
Eosinophil count, cells/ml GM (range)	550 (80–3880)	380 (75–3185)	280 (70–2710)	0.0354

The values shown represent the geometric mean and range, except for age (median and range).
BMI, body mass index; GM, geometric mean; gm, gram; Hb, hemoglobin.

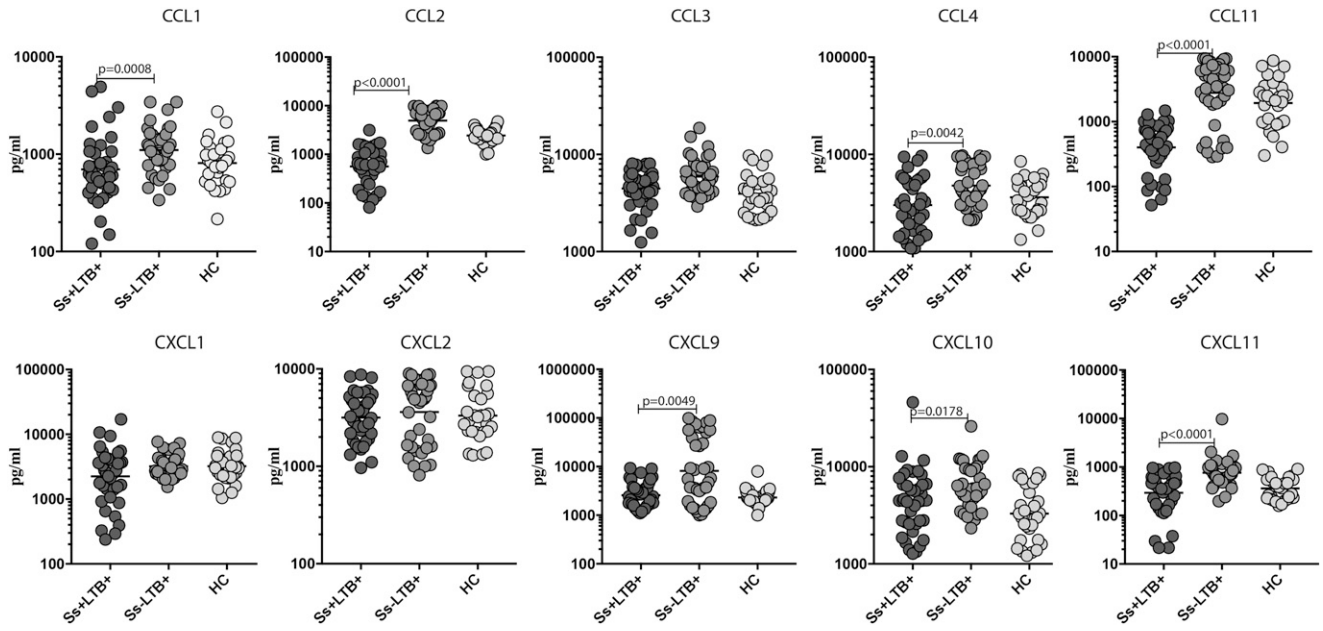


FIGURE 1. LTB/*S. stercoralis* coinfection is associated with diminished baseline (unstimulated) levels of CC and CXC chemokines. The baseline (unstimulated) levels of CC (CCL1, CCL2, CCL3, CCL4, and CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11) chemokines were measured by multiplex ELISA in *S. stercoralis*$^{+}$LTB^{+} (n = 40), *S. stercoralis*$^{-}$LTB^{+} (n = 40), and HC (n = 30) individuals. The data are represented as scatter plots, with each circle representing a single individual (dark gray, *S. stercoralis*$^{+}$LTB^{+}; light gray, *S. stercoralis*$^{-}$LTB^{+}; pale gray, HC). All p values were calculated using the Kruskal–Wallis test.

we measured the PHA-stimulated levels of CCL1, CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11 in *S. stercoralis*$^{+}$LTB^{+}, *S. stercoralis*$^{-}$LTB^{+}, and HC individuals. The net chemokine levels were calculated by mitogen stimulation minus unstimulated levels. As shown in Fig. 3, the PHA-stimulated levels of the CCL1, CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11 chemokines were not significantly different among the two groups. We also assessed the changes in chemokine levels following mitogen stimulation in comparison with unstimulated levels at the individual level in *S. stercoralis*$^{+}$LTB^{+} individuals. As shown in Supplemental Fig. 1B, the levels of all the CC and CXC chemokines were significantly increased upon mitogen stimulation in *S. stercoralis*$^{+}$LTB^{+} individuals.

Anthelmintic treatment significantly increases the spontaneously produced CC and CXC chemokine levels in S. stercoralis^{+}*LTB*$^{+}$ *individuals*

To assess whether the diminished chemokine levels associated with *S. stercoralis* infection in LTB could be reversed following anthelmintic therapy, we measured the CC and CXC chemokines in

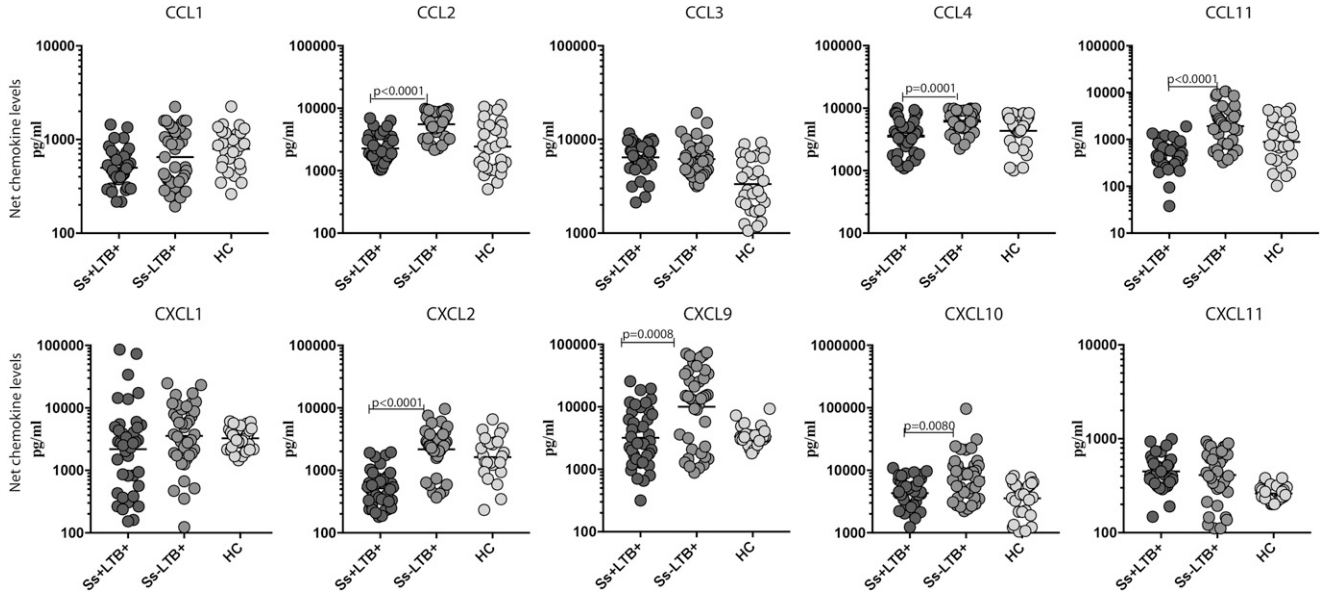


FIGURE 2. *S. stercoralis* coinfection alters CC and CXC chemokine production in response to TB Ag stimulation. The TB Ag–stimulated levels of CC (CCL1, CCL2, CCL3, CCL4, and CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11) chemokines were measured by multiplex ELISA in *S. stercoralis*$^{+}$LTB^{+} (n = 40), *S. stercoralis*$^{-}$LTB^{+} (n = 40), and HC (n = 30) individuals. The data are represented as scatter plots, with each circle representing a single individual (dark gray, *S. stercoralis*$^{+}$LTB^{+}; light gray, *S. stercoralis*$^{-}$LTB^{+}; pale gray, HC). All p values were calculated using the Kruskal–Wallis test. The net chemokine levels were calculated by TB Ag stimulation minus unstimulated levels.

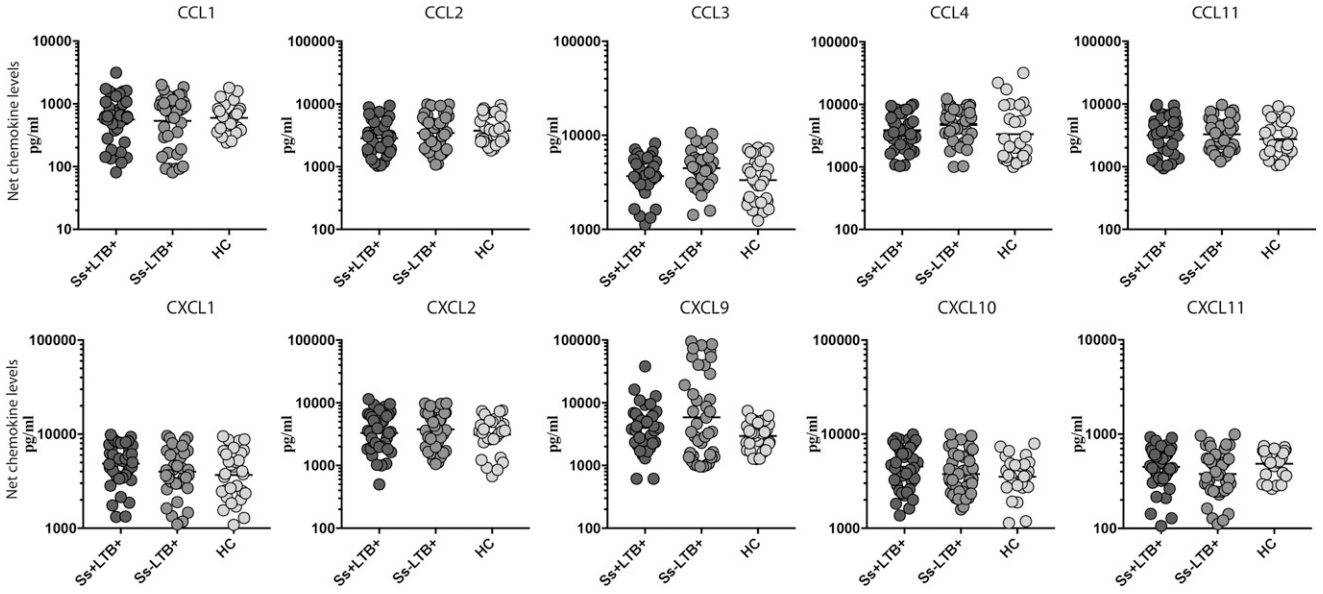


FIGURE 3. Chemokine response to mitogens is similar across all groups. The mitogen-stimulated levels of CC (CCL1, CCL2, CCL3, CCL4, and CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11) chemokines were measured by multiplex ELISA in *S. stercoralis*$^{+}$LTB^{+} (n = 40), *S. stercoralis*$^{-}$LTB^{+} (n = 40), and HC (n = 30) individuals. The data are represented as scatter plots, with each circle representing a single individual (dark gray, *S. stercoralis*$^{+}$LTB^{+}; light gray, *S. stercoralis*$^{-}$LTB^{+}; pale gray, HC). All p values were calculated using the Kruskal–Wallis test. The net chemokine levels were calculated by mitogen stimulation minus unstimulated levels.

S. stercoralis^{+}LTB^{+} before (pretreatment [pre-T]) and 6 mo after anthelmintic therapy (posttreatment [post-T]). As shown in Fig. 4, CCL1 (p = 0.0166), CCL2 (p = 0.0427), CCL4 (p = 0.0030), CCL11 (p = 0.0003), and CXCL11 (p = 0.0003) levels are significantly increased at post-T compared with pre-T levels. The levels of CCL3, CXCL1, CXCL2, CXCL9, and CXCL10 were not significantly different between pre-T and post-T. Thus, anthelmintic therapy of *S. stercoralis* infection

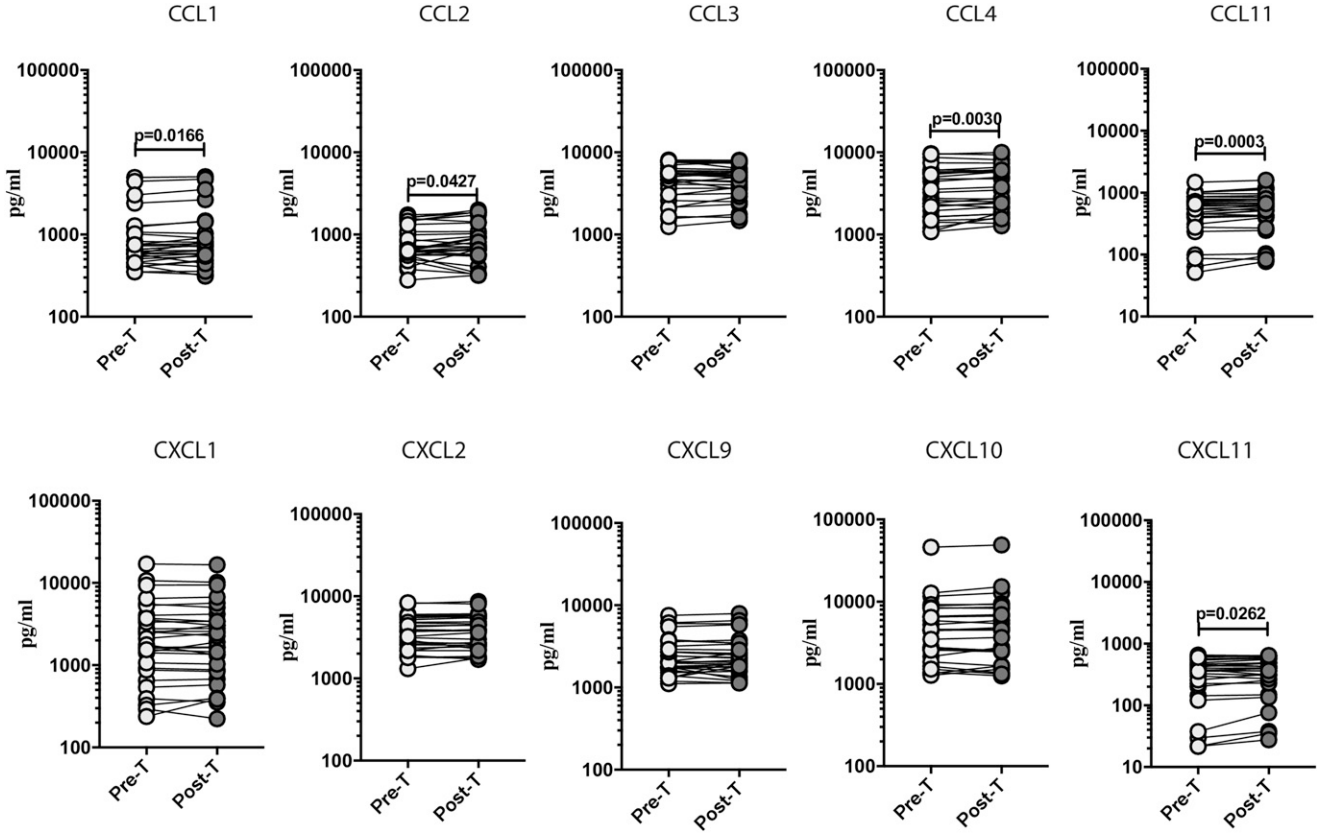


FIGURE 4. Anthelmintic treatment significantly increases the spontaneously produced CC and CXC chemokine levels in *S. stercoralis*$^{+}$LTB^{+} individuals. The baseline (unstimulated) levels of CC (CCL1, CCL2, CCL3, CCL4, and CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11) chemokines were measured by multiplex ELISA in *S. stercoralis*$^{+}$LTB^{+} individuals at pre-T (n = 40) and 6 mo post-T (n = 40) time points. The data are represented as line graphs, with each line representing a single individual. All p values were calculated using the Wilcoxon signed rank test.

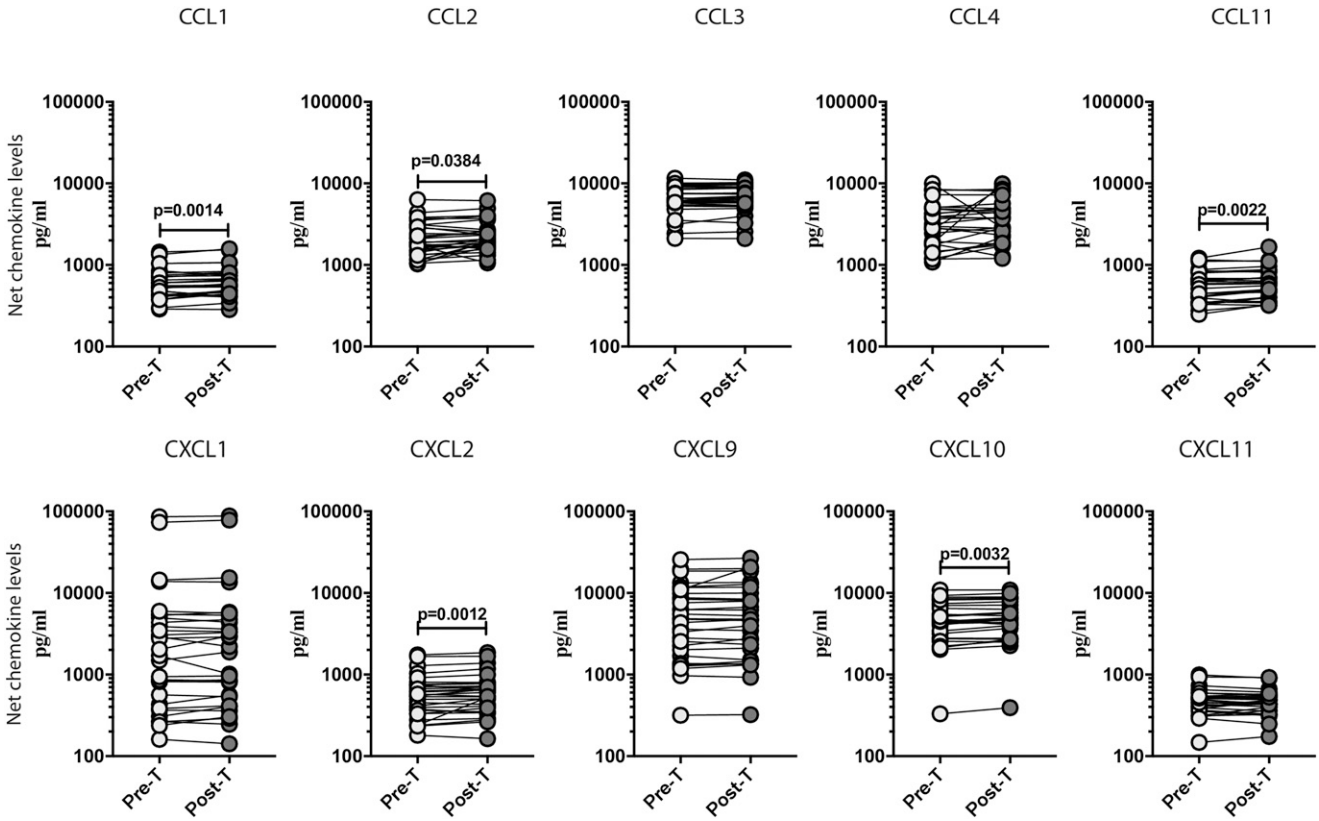


FIGURE 5. Anthelmintic treatment significantly increases the CC and CXC chemokine levels upon TB Ag stimulation in *S. stercoralis*$^+$LTB$^+$ individuals. The TB Ag–stimulated levels of CC (CCL1, CCL2, CCL3, CCL4, and CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11) chemokines were measured by multiplex ELISA in *S. stercoralis*$^+$LTB$^+$ individuals at pre-T (n = 40) and 6 mo post-T (n = 40) time points. The data are represented as line graphs, with each line representing a single individual. All p values were calculated using the Wilcoxon signed rank test. The net chemokine levels were calculated by TB Ag stimulation minus unstimulated levels.

results in increased levels of spontaneously expressed CC and CXC chemokines in *S. stercoralis*$^+$LTB$^+$ individuals following treatment.

Anthelmintic treatment significantly increases the CC and CXC chemokine levels upon TB Ag stimulation in S. stercoralis$^+$*LTB*$^+$ *individuals*

To examine the effect of treatment on chemokines in *S. stercoralis*$^+$LTB$^+$ individuals upon *M. tuberculosis* Ag stimulation following anthelmintic therapy, we examined the TB Ag–stimulated levels of CC and CXC chemokines in *S. stercoralis*$^+$LTB$^+$ at baseline and 6 mo after anthelmintic therapy. The net chemokine levels were calculated by TB Ag stimulation minus unstimulated levels. As shown in Fig. 5, *S. stercoralis*$^+$LTB$^+$ individuals had significantly increased levels of CCL2 (p = 0.0384), CCL11 (p = 0.0022), CXCL2 (p = 0.0037), and CXCL10 (p = 0.0032) at post-T compared with pre-T levels. There was no significant differences between the levels of CCL3, CCL4, CXCL1, and CXCL9 between pre-T and post-T. Thus, anthelmintic therapy of *S. stercoralis* infection results in increased levels of TB Ag–stimulated CC and CXC chemokines in *S. stercoralis*$^+$LTB$^+$ individuals following treatment.

No significant alterations following anthelmintic treatment upon mitogen stimulation in S. stercoralis$^+$*LTB*$^+$ *individuals*

To examine the effect of treatment on chemokines in *S. stercoralis*$^+$LTB$^+$ individuals following mitogenic stimulation following anthelmintic therapy, we examined the mitogen-stimulated levels of CC and CXC chemokines in *S. stercoralis*$^+$LTB$^+$ at baseline and 6 mo after anthelmintic therapy. The net chemokine levels were calculated by mitogen stimulation minus unstimulated levels. As shown in Fig. 6, the levels of CC and CXC chemokines were not significantly different between pre-T and post-T individuals.

Discussion

Helminth infections are potent modulators of the immune response, and various studies have been performed to determine the potential effect of helminth infection on the antimycobacterial protective immune response (17–19). Geographically, *S. stercoralis* infection commonly overlaps with *M. tuberculosis* (20), and mice infected with *S. stercoralis* have been shown to exhibit impaired immune responses to TB infection (21). The TB granuloma is the prototypical characteristic of tuberculous disease and generates an immune micro environment, which contains a number of cell types including macrophages, neutrophils, lymphocytes, and B cells, among others (22). Development of the TB granuloma is controlled by the harmonized expression of the chemotactic cytokines denoted as chemokines. Chemokine expression creates a chemical gradient that allows for mobilization and recruitment of cells from peripheral organs to the site of infection and within the granuloma (3). Chemokines, categorized within four families (C, CC, CXC, and CX3C), are essential in regulating inflammation, leukocyte recruitment, and antimicrobial immunity (23, 24).

M. tuberculosis infection of macrophages results in the production of CC chemokines, and these are essential for inhibition of bacterial growth (6). Previous studies have shown that CCL2 and CCL11 levels were lower in TB patients when compared with LTB individuals, a diminution that can stabilize following completion of antituberculous treatment (25). The levels of CCL2

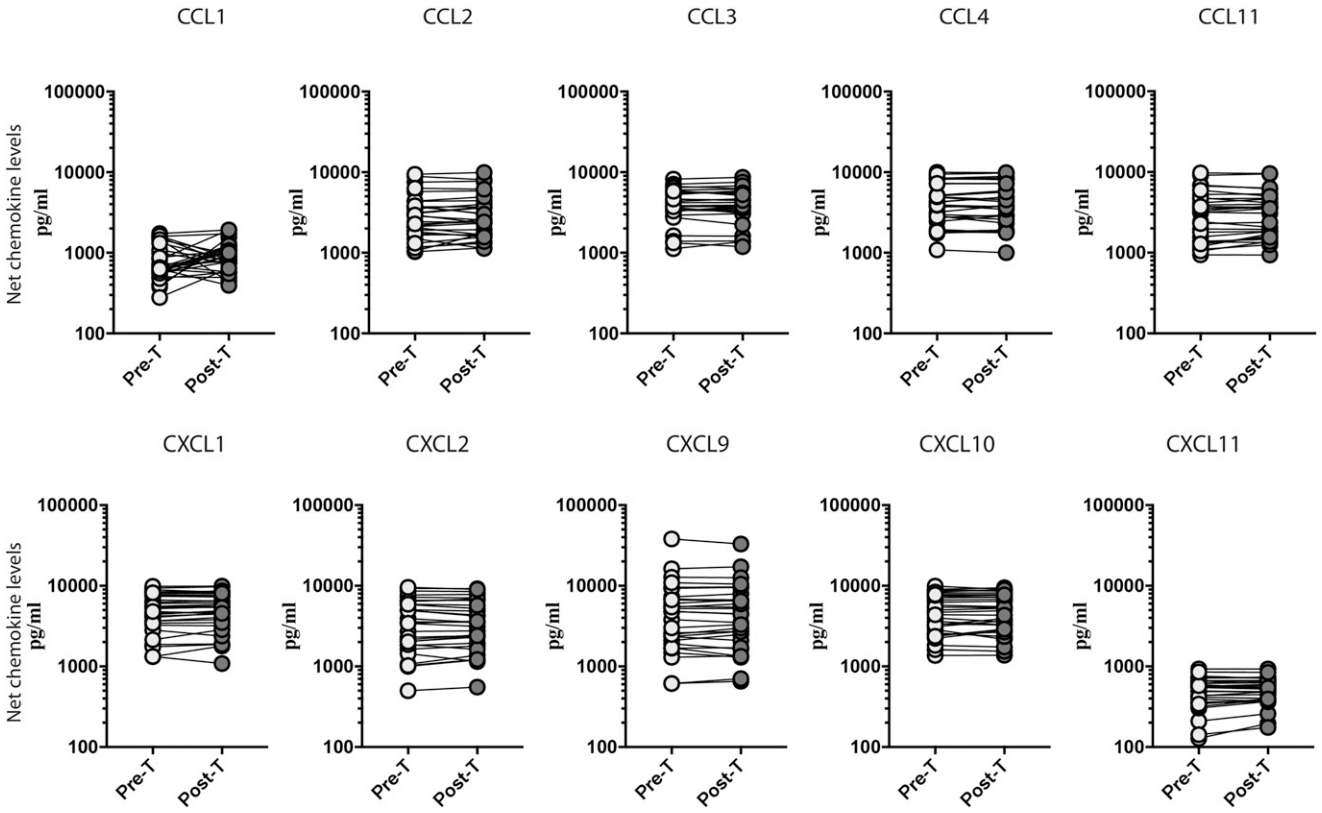


FIGURE 6. No significant alterations of chemokines following anthelmintic treatment upon mitogen stimulation in *S. stercoralis*$^+$LTB$^+$ individuals. The mitogen-stimulated levels of CC (CCL1, CCL2, CCL3, CCL4, and CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, and CXCL11) chemokines were measured by multiplex ELISA in *S. stercoralis*$^+$LTB$^+$ individuals at pre-T ($n = 40$) and 6 mo post-T ($n = 40$) time points. The data are represented as line graphs, with each line representing a single individual. All p values were calculated using the Wilcoxon signed rank test. The net chemokine levels were calculated by mitogen stimulation minus unstimulated levels.

were diminished in coinfected individuals, and CCL2 appears to play a vital role in retaining the architecture of granuloma in latent infection (26) and plays a crucial role in protective immunity against *M. tuberculosis* (27–29). Thus, depressed levels of CCL2 might impair immunity in *S. stercoralis*$^+$LTB$^+$ individuals. CXC chemokines have been shown to be involved in the accumulation of activated memory T cells, aid in promoting Th1-associated immune responses (30), and are involved in granuloma formation (31, 32). CXCL2 levels were diminished in *S. stercoralis*$^+$LTB$^+$ individuals, which is known to recruit neutrophils to the site of infection (33, 34) and is also one of the important regulators of host innate immune response during mycobacterial infection (35). Thus, diminished levels of CXCL2 might lead to impairment in the immunity to latent infection. In our study, the levels of CC chemokine (such as CCL1, CCL3, CCL4, and CCL11) and CXC chemokine (such as CXCL2, CXCL9, CXCL10, and CXCL11) levels were significantly diminished in *S. stercoralis*$^+$LTB$^+$ individuals. The CC chemokines (CCL3, CCL4, CCL5, and CCL8) as well as the CXC chemokines (CXCL9, CXCL10, and CXCL11) are upregulated in *M. tuberculosis*–infected mice (12, 36). These chemokines play an important role in the recruitment of T cells and other cells to the lung during early infection. These data suggested that stimulation of chemokines in response to inflammatory signals produced by *M. tuberculosis* infection could facilitate the control of *M. tuberculosis* infection (36). In contrast, in our study, the levels of CCL3, CCL4, CXCL9, CXCL10, and CXCL11 levels were significantly diminished in coinfected individuals, which might affect the recruitment of T cells and other cells to the lung and thereby facilitate susceptibility to TB infection.

In our study, the levels of CC chemokines and CXC chemokines were significantly diminished in *S. stercoralis*$^+$LTB$^+$ individuals when compared with *S. stercoralis*$^-$LTB$^+$. This suggests that *S. stercoralis* infection could cause perturbations in the recruitment of immune cells to the site of infection, which, in turn, could lead to compromised immune responses. Interestingly, the post-T data also confirm a direct association of helminth infections on the modulation in the levels of most of the above-mentioned chemokines in that they increased following successful anthelmintic treatment. Our data also indicate that altered levels of these CC chemokines could potentially modify the recruitment of monocytes and T cells and the diminished levels of the CXC chemokines might be due to compromised ability to drive T cell recruitment to the lungs in *S. stercoralis*$^+$LTB$^+$ individuals. *S. stercoralis* infection might induce perturbation in neutrophil recruitment and have the ability to modulate granuloma maintenance and integrity by altering the levels of CXCR3 ligands, which reflect as diminished levels of CXC chemokines. In addition, our data suggest that *S. stercoralis* infection–induced alteration in chemokines levels in response to TB Ags could have an indirect effect on the maintenance and stability of granulomas in helminth-TB coinfections. This is directly validated by data from mouse models of TB infection in which alterations in chemokine levels underlie disruption of protective immunity to TB (37). Finally, our data on mitogen-stimulated chemokines provides evidence that LTB individuals with or without *S. stercoralis* coinfection do not differ in the ability to produce chemokines per se, but, rather, express certain chemokines at differential levels upon latent infection or in vitro restimulation.

Because distinct chemokines govern the specific recruitment of diverse immune cells to the lung, our study showing alterations

of multiple chemokines (more specifically diminution) leads us to speculate that anti-TB immune responses engendered in LTB individuals might be compromised as a result of helminth coinfection. This speculation is reinforced by our data on the reversibility (at least partially) of the levels of different chemokines following anthelmintic therapy. In summary, our study clearly demonstrates that *S. stercoralis* infection is associated with altered levels of chemokines at baseline and up on TB antigenic stimulation. Although our study does not prove a causal relationship, it does propose evidence of a significant effect of *S. stercoralis* infection on the modulation of chemokines expression and function. Our data add to the mounting list of immunological mechanisms by which coexistent helminth infections can modulate responses in LTB. Our data also suggest that treatment of helminth infection would be advantageous in the conduct of TB vaccine trials in TB and helminth coendemic countries.

Acknowledgments

We thank Dr. M. Satiswaran and Prabbu Balakrishnan for valuable assistance in collecting the clinical data for this study. We thank the staff of the Department of Epidemiology, National Institute for Research in Tuberculosis, for valuable assistance in recruiting the patients for this study.

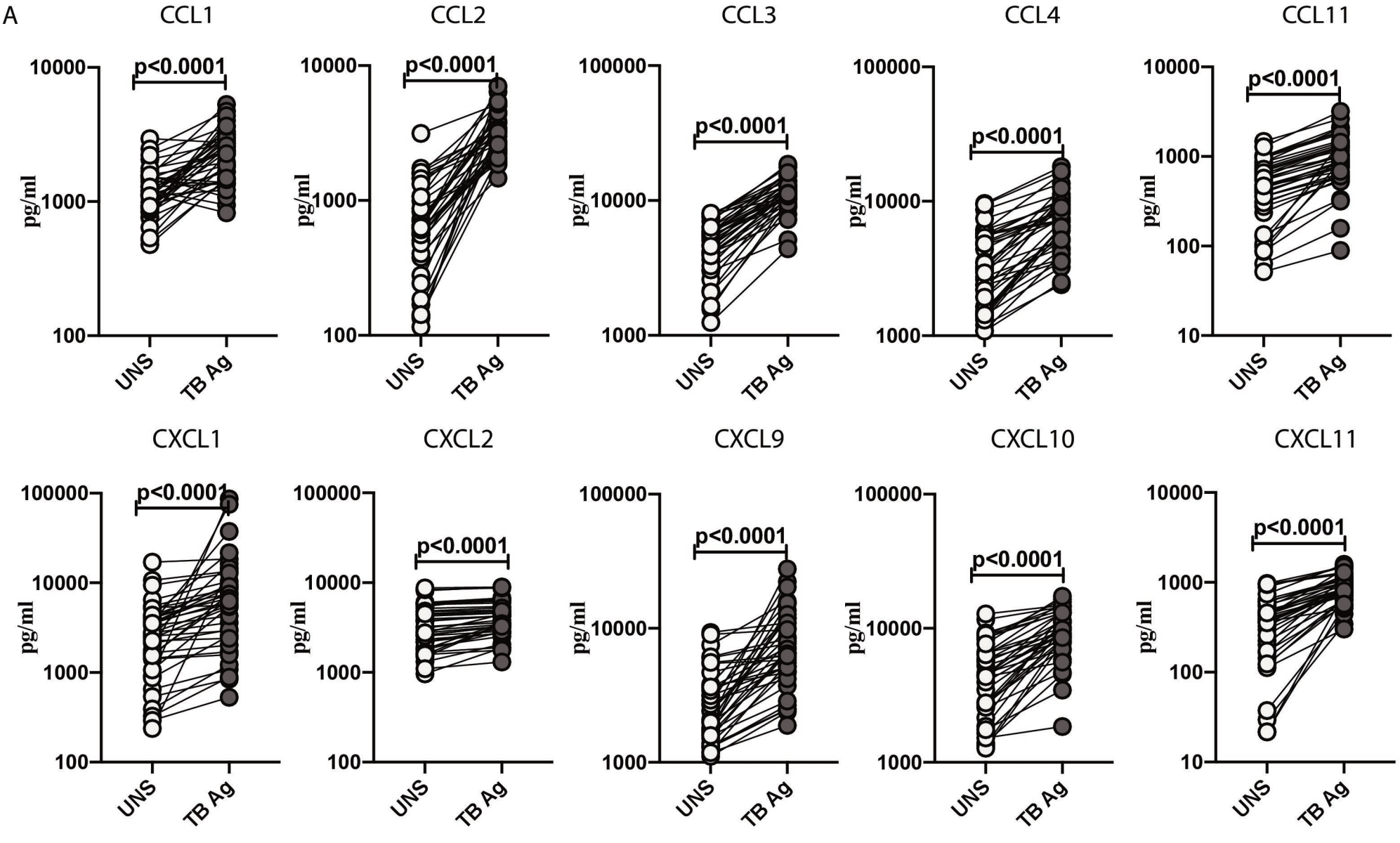
Disclosures

The authors have no financial conflicts of interest.

References

1. Saunders, B. M., and A. M. Cooper. 2000. Restraining mycobacteria: role of granulomas in mycobacterial infections. *Immunol. Cell Biol.* 78: 334–341.
2. Khader, S. A., G. K. Bell, J. E. Pearl, J. J. Fountain, J. Rangel-Moreno, G. E. Cilley, F. Shen, S. M. Eaton, S. L. Gaffen, S. L. Swain, et al. 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8: 369–377.
3. Sakai, S., K. D. Kauffman, J. M. Schenkel, C. C. McBerry, K. D. Mayer-Barber, D. Masopust, and D. L. Barber. 2014. Cutting edge: control of *Mycobacterium tuberculosis* infection by a subset of lung parenchyma-homing CD4 T cells. *J. Immunol.* 192: 2965–2969.
4. Moguche, A. O., S. Shafiani, C. Clemons, R. P. Larson, C. Dinh, L. E. Higdon, C. J. Cambier, J. R. Sissons, A. M. Gallegos, P. J. Fink, and K. B. Urdahl. 2015. ICOS and Bcl6-dependent pathways maintain a CD4 T cell population with memory-like properties during tuberculosis. *J. Exp. Med.* 212: 715–728.
5. Torrado, E., J. J. Fountain, M. Liao, M. Tighe, W. W. Reiley, R. P. Lai, G. Meintjes, J. E. Pearl, X. Chen, D. E. Zak, et al. 2015. Interleukin 27R regulates CD4+ T cell phenotype and impacts protective immunity during *Mycobacterium tuberculosis* infection. *J. Exp. Med.* 212: 1449–1463.
6. Saukkonen, J. J., B. Bazydlo, M. Thomas, R. M. Strieter, J. Keane, and H. Kornfeld. 2002. Beta-chemokines are induced by *Mycobacterium tuberculosis* and inhibit its growth. *Infect. Immun.* 70: 1684–1693.
7. Algood, H. M., and J. L. Flynn. 2004. CCR5-deficient mice control *Mycobacterium tuberculosis* infection despite increased pulmonary lymphocytic infiltration. *J. Immunol.* 173: 3287–3296.
8. Seiler, P., P. Aichele, S. Bandermann, A. E. Hauser, B. Lu, N. P. Gerard, C. Gerard, S. Ehlers, H. J. Mollenkopf, and S. H. Kaufmann. 2003. Early granuloma formation after aerosol *Mycobacterium tuberculosis* infection is regulated by neutrophils via CXCR3-signaling chemokines. *Eur. J. Immunol.* 33: 2676–2686.
9. Chakravarty, S. D., J. Xu, B. Lu, C. Gerard, J. Flynn, and J. Chan. 2007. The chemokine receptor CXCR3 attenuates the control of chronic *Mycobacterium tuberculosis* infection in BALB/c mice. *J. Immunol.* 178: 1723–1735.
10. Hasan, Z., B. Jamil, J. Khan, R. Ali, M. A. Khan, N. Nasir, M. S. Yusuf, S. Jamil, M. Irfan, and R. Hussain. 2009. Relationship between circulating levels of IFN-gamma, IL-10, CXCL9 and CCL2 in pulmonary and extrapulmonary tuberculosis is dependent on disease severity. *Scand. J. Immunol.* 69: 259–267.
11. Hasan, Z., B. Jamil, M. Ashraf, M. Islam, M. Dojki, M. Irfan, and R. Hussain. 2009. Differential live *Mycobacterium tuberculosis*-, M. bovis BCG-, recombinant ESAT6-, and culture filtrate protein 10-induced immunity in tuberculosis. *Clin. Vaccine Immunol.* 16: 991–998.
12. Monin, L., and S. A. Khader. 2014. Chemokines in tuberculosis: the good, the bad and the ugly. *Semin. Immunol.* 26: 552–558.
13. Slight, S. R., and S. A. Khader. 2013. Chemokines shape the immune responses to tuberculosis. *Cytokine Growth Factor Rev.* 24: 105–113.
14. Radhakrishna, S., T. R. Frieden, and R. Subramani, Tuberculosis Research Centre (ICMR). 2003. Association of initial tuberculin sensitivity, age and sex with the incidence of tuberculosis in south India: a 15-year follow-up. *Int. J. Tuberc. Lung Dis.* 7: 1083–1091.
15. Bisoffi, Z., D. Buonfrate, M. Sequi, R. Mejia, R. O. Cimino, A. J. Krolewiecki, M. Albonico, M. Gobbo, S. Bonafini, A. Angheben, et al. 2014. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. *PLoS Negl. Trop. Dis.* 8: e2640.
16. Buonfrate, D., M. Sequi, R. Mejia, R. O. Cimino, A. J. Krolewiecki, M. Albonico, M. Degani, S. Tais, A. Angheben, A. Requena-Mendez, et al. 2015. Accuracy of five serologic tests for the follow up of Strongyloides stercoralis infection. *PLoS Negl. Trop. Dis.* 9: e0003491.
17. Metenou, S., S. Babu, and T. B. Nutman. 2012. Impact of filarial infections on coincident intracellular pathogens: *Mycobacterium tuberculosis* and *Plasmodium falciparum*. *Curr. Opin. HIV AIDS* 7: 231–238.
18. Rafi, W., R. Ribeiro-Rodrigues, J. J. Ellner, and P. Salgame. 2012. 'Coinfection-helminthes and tuberculosis'. *Curr. Opin. HIV AIDS* 7: 239–244.
19. Babu, S., and T. B. Nutman. 2016. Helminth-tuberculosis co-infection: an immunologic perspective. *Trends Immunol.* 37: 597–607.
20. Lipner, E. M., P. G. Gopi, R. Subramani, C. Kolappan, K. Sadacharam, P. Kumaran, D. R. Prevots, P. R. Narayanan, T. B. Nutman, and V. Kumaraswami. 2006. Coincident filarial, intestinal helminth, and mycobacterial infection: helminths fail to influence tuberculin reactivity, but BCG influences hookworm prevalence. *Am. J. Trop. Med. Hyg.* 74: 841–847.
21. Salgame, P., G. S. Yap, and W. C. Gause. 2013. Effect of helminth-induced immunity on infections with microbial pathogens. *Nat. Immunol.* 14: 1118–1126.
22. Dorhoi, A., and S. H. Kaufmann. 2016. Pathology and immune reactivity: understanding multidimensionality in pulmonary tuberculosis. *Semin. Immunopathol.* 38: 153–166.
23. Murphy, P. M., M. Baggiolini, I. F. Charo, C. A. Hébert, R. Horuk, K. Matsushima, L. H. Miller, J. J. Oppenheim, and C. A. Power. 2000. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol. Rev.* 52: 145–176.
24. Murphy, P. M. 2002. International union of pharmacology. XXX. Update on chemokine receptor nomenclature. *Pharmacol. Rev.* 54: 227–229.
25. Yang, Q., Y. Cai, W. Zhao, F. Wu, M. Zhang, K. Luo, Y. Zhang, H. Liu, B. Zhou, H. Kornfeld, and X. Chen. 2014. IP-10 and MIG are compartmentalized at the site of disease during pleural and meningeal tuberculosis and are decreased after antituberculosis treatment. *Clin. Vaccine Immunol.* 21: 1635–1644.
26. Hussain, R., A. Ansari, N. Talat, Z. Hasan, and G. Dawood. 2011. CCL2/MCP-I genotype-phenotype relationship in latent tuberculosis infection. *PLoS One* 6: e25803.
27. Peters, W., H. M. Scott, H. F. Chambers, J. L. Flynn, I. F. Charo, and J. D. Ernst. 2001. Chemokine receptor 2 serves an early and essential role in resistance to *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. USA* 98: 7958–7963.
28. Scott, H. M., and J. L. Flynn. 2002. *Mycobacterium tuberculosis* in chemokine receptor 2-deficient mice: influence of dose on disease progression. *Infect. Immun.* 70: 5946–5954.
29. Hasan, Z., J. M. Cliff, H. M. Dockrell, B. Jamil, M. Irfan, M. Ashraf, and R. Hussain. 2009. CCL2 responses to *Mycobacterium tuberculosis* are associated with disease severity in tuberculosis. *PLoS One* 4: e8459.
30. Pokkali, S., S. D. Das, and L. R. 2008. Expression of CXC and CC type of chemokines and its receptors in tuberculous and non-tuberculous effusions. *Cytokine* 41: 307–314.
31. Chiu, B. C., C. M. Freeman, V. R. Stolberg, E. Komuniecki, P. M. Lincoln, S. L. Kunkel, and S. W. Chensue. 2003. Cytokine-chemokine networks in experimental mycobacterial and schistosomal pulmonary granuloma formation. *Am. J. Respir. Cell Mol. Biol.* 29: 106–116.
32. Lande, R., E. Giacomini, T. Grassi, M. E. Remoli, E. Iona, M. Miettinen, I. Julkunen, and E. M. Coccia. 2003. IFN-alpha beta released by *Mycobacterium tuberculosis*-infected human dendritic cells induces the expression of CXCL10: selective recruitment of NK and activated T cells. *J. Immunol.* 170: 1174–1182.
33. Rittner, H. L., S. A. Mousa, D. Labuz, K. Beschmann, M. Schäfer, C. Stein, and A. Brack. 2006. Selective local PMN recruitment by CXCL1 or CXCL2/3 injection does not cause inflammatory pain. *J. Leukoc. Biol.* 79: 1022–1032.
34. De Filippo, K., A. Dudeck, M. Hasenberg, E. Nye, N. van Rooijen, K. Hartmann, M. Gunzer, A. Roers, and N. Hogg. 2013. Mast cell and macrophage chemokines CXCL1/CXCL2 control the early stage of neutrophil recruitment during tissue inflammation. *Blood* 121: 4930–4937.
35. Boro, M., V. Singh, and K. N. Balaji. 2016. *Mycobacterium tuberculosis*-triggered Hippo pathway orchestrates CXCL1/2 expression to modulate host immune responses. *Sci. Rep.* 6: 37695.
36. Kang, D. D., Y. Lin, J. R. Moreno, T. D. Randall, and S. A. Khader. 2011. Profiling early lung immune responses in the mouse model of tuberculosis. *PLoS One* 6: e16161.
37. O'Garra, A., P. S. Redford, F. W. McNab, C. I. Bloom, R. J. Wilkinson, and M. P. Berry. 2013. The immune response in tuberculosis. *Annu. Rev. Immunol.* 31: 475–527.

Supplementary Figure.1



Supplementary Figure 1. LTBI/Ss co-infection is associated with increased levels of CC and CXC chemokines after antigenic stimulation.
(A) The unstimulated and TB-antigen stimulated levels of CC (CCL1, CCL2, CCL3, CCL4, CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, CXCL11) chemokines were measured by in Ss+LTB+ (n=40) individuals and the levels are shown for each individual. (B) The mitogen stimulated levels of CC (CCL1, CCL2, CCL3, CCL4, CCL11) and CXC (CXCL1, CXCL2, CXCL9, CXCL10, CXCL11) chemokines were measured by in Ss+LTB+ (n=40) individuals and the levels are shown for each individual. The data are represented as line graphs with each line representing a single individual.
(light grey – Ss +LTB+ UNS (Unstimulated) and dark grey – Ss +LTB+ TB-antigen stimulation. P values were calculated using the Wilcoxon signed rank test.