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Helminth Coinfection Alters Monocyte Activation, Polarization, and Function in Latent *Mycobacterium tuberculosis* Infection

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Helminth infections are known to influence T and B cell responses in latent tuberculosis infection (LTBI). Whether helminth infections also modulate monocyte responses in helminth–LTBI coinfection has not been fully explored. To this end, we examined the activation, polarization, and function of human monocytes isolated from individuals with LTBI with (n = 25) or without (n = 25) coincident *Strongyloides stercoralis* infection (*S. stercoralis*–positive and *S. stercoralis*–negative respectively). Our data reveal that the presence of *S. stercoralis* infection is associated with lower frequencies of monocytes expressing CD54, CD80, CD86 at baseline (absence of stimulation) and in response to mycobacterial-Ag stimulation than monocytes from *S. stercoralis*–negative individuals. In contrast, *S. stercoralis* infection was associated with higher frequencies of M2-like monocytes, as determined by expression of CD206 and CD163. Monocytes from *S. stercoralis*–positive individuals had a reduced capacity to phagocytose or exhibit respiratory burst activity following mycobacterial-Ag or LPS stimulation and were less capable of expression of IL-1β, TNF-α, IL-6, and IL-12 at baseline and/or following Ag stimulation compared with those without *S. stercoralis* infection. In addition, definitive treatment of *S. stercoralis* infection resulted in a significant reversal of the altered monocyte function 6 mo after anthelmintic therapy. Finally, T cells from *S. stercoralis*–positive individuals exhibited significantly lower activation at baseline or following mycobacterial-Ag stimulation. Therefore, our data highlight the induction of dampened monocyte activation, enhanced M2 polarization, and impaired monocyte function in helminth–LTBI coinfection. Our data also reveal a different mechanism by which helminth infection modulates immune function in LTBI. ***The Journal of Immunology*****, 2020, 204: 1274–1286.**

Monocytes are critical players in the immunity to *Mycobacterium tuberculosis* infection and disease. Monocytes and macrophages are the primary target of *M. tuberculosis* and their innate capacity to control *M. tuberculosis* defines the early progression of infection (1). Although *M. tuberculosis* has traditionally been thought to survive and replicate in macrophages, recent work has revealed that *M. tuberculosis* infects multiple subsets of mononuclear phagocytes, including recruited monocytes in vivo and in vitro (2). Monocytes are also involved in transporting bacteria from the lungs to the local lymph nodes and presenting Ags for activation of $CD4^+$ T cells (3–5). Finally, blood monocytes are the main sources of recruited monocytes to the lung during *M. tuberculosis* infection (5). Monocytes are typically elevated in active tuberculosis (TB) and are polarized to the M1 phenotype (upregulating inducible NO synthase [iNOS] to produce NO) (6, 7).

Helminths are considered to be a risk factor for the development of active TB in individuals with latent TB infection (LTBI) (8–10). This is due to the ability of helminth infection to modulate both innate and adaptive immune responses in LTBI, with major effects on T and B cells. With regards to helminth–TB coinfection, very few studies have examined the role of helminth infections in modulating monocyte function in the context of *M. tuberculosis* coinfection (11, 12). Helminth infections are known to induce arginase-1 in macrophages, which promotes lung inflammation and disease severity in TB (13), and to dampen phagolysosomal maturation in macrophages (14). Helminth infections can modulate TLR expression and function on monocytes (15) and induce alternative activation of macrophages in the setting of *M. tuberculosis* coinfection (16). However, very little is known is about the effect of helminth infections and subsequent anthelmintic treatment on monocyte function, in particular activation (as determined by expression of CD154, CD80 and CD86), M2 polarization (as determined by CD206 and CD163 expression), and function (as determined by phagocytosis, respiratory burst response, and cytokine response) in LTBI.

Therefore, we sought to examine the activation, polarization, and function of monocytes in a helminth–LTBI coinfection.

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Abbreviations used in this article: GM, geometric mean; iNOS, inducible NO synthase; LTBI, latent TB infection; P/I, PMA/ionomycin; Post-T, 6 mo following anthelmintic treatment; PPD, purified protein derivative; Pre-T, pretreatment frequency; qPCR, quantitative PCR; TB, tuberculosis; WCL, whole cell lysate.

To this end, we studied the monocyte populations in LTBI individuals with or without *Strongyloides stercoralis*, a common helminth infection, affecting ~30–100 million people worldwide. Our data show that *S. stercoralis* infection is associated with dampened monocyte activation, enhanced M2 polarization, impaired monocyte function, and finally decreased T cell activation in LTBI individuals and that this altered function can be at least partially reversed by anthelmintic chemotherapy.

Materials and Methods

Ethics statement

All participants were examined as part of a natural history study protocol (12-I-073) approved by institutional review boards of the National Institute of Allergy and Infectious Diseases and the National Institute for Research in Tuberculosis (Chennai, India). Informed written consent was obtained from all participants.

Study population

We recruited a group of 50 individuals with LTBI, 25 with *S. stercoralis* infection (hereafter *S. stercoralis*–positive), and 25 without *S. stercoralis* infection (hereafter *S. stercoralis*–negative). Fifteen individuals in each group were used for the in vitro stimulation of monocytes for activation marker and cytokine expression and T cell activation. Ten individuals in each group were used for the functional assay experiments, and 12 individuals were used for quantitative PCR (qPCR) experiments. This study was conducted in a rural village in the suburbs of Chennai. All enrolled individuals were between 18 and 65 y old. None had previous anthelmintic treatment, a known history of helminth infections, or HIV. LTBI was diagnosed based on positive results of 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 PPD RT 23 SSI (Serum Statens Institut). A positive skin test was defined as an induration of at least 12 mm in diameter, based on the previously determined cut off norms for South India (17). Quantiferon was performed according to the manufacturer's instructions (Qiagen).

Parasitological examination and anthelmintic treatment

S. stercoralis infection was diagnosed by the presence of IgG Abs to the recombinant NIE Ag as described previously (18, 19). This was further confirmed by stool microscopy using nutrient agar plates. Stool microscopy was performed to rule out the presence of other intestinal parasites. Filarial infection was excluded in all study participants by virtue of being negative in tests for circulating filarial Ag. All 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. Follow-up examination was done by stool microscopy, which was negative for *S. stercoralis* infection. In addition, serology showed a significant decrease in the IgG titers to NIE Ag, as described previously (20).

Hematology

Hematological parameters were measured from fresh venous EDTA blood samples on all individuals using an AcT 5diff hematology analyzer (Beckman Coulter, Brea, CA).

Isolation of PBMCs and in vitro culture

PBMCs were isolated from the whole blood samples using density gradient centrifugation method, as described previously (21). The buffy coat cells were washed with 1× PBS to remove platelet-rich plasma fraction. Then, the cells were incubated with ACK lysing solution (to remove excess erythrocytes). Finally, the isolated cells were washed and resuspended with 1× PBS and checked for viability, and the cells were suspended in freezing medium and stored at −80°C.

Immunophenotyping

PBMC were thawed and washed with PBS first and PBS/1% BSA later. The cultures were then stimulated with purified protein derivative (PPD), *M. tuberculosis* whole cell lysate (WCL), PMA/ionomycin (P/I), or media alone at 37°C for 6 h. After 6 h of stimulation, the cells were harvested and stained with surface Abs for 30–60 min. Surface Abs used were CD45-PerCP (BD Biosciences), CD56 PerCP eFluor710 (eBiosciences), CD14–Pacific Blue (BioLegend), HLA-DR–PE-Cy7 (BD Biosciences) and CD16-allophycocyanin-Cy7 (BD Biosciences), CD54 FITC (BD Biosciences), CD80 PerCP (BD Biosciences), CD86 PE (BD Biosciences), CCR2 PE (BD Biosciences), CX3CR1 allophycocyanin (BD Biosciences), CD69 FITC (BD Biosciences), and HLA-DR PerCP (BD Biosciences). The cells were washed and permeabilized with BD Perm/Wash buffer (BD Biosciences) and stained for intracellular cytokines, IL-1α FITC, IL-1β FITC, TNF-α PE, IL-6 PE, IL-10 allophycocyanin, and IL-12 allophycocyanin (all Abs from BioLegend and BD Biosciences) for an additional 30 min before washing and acquisition. T cell activation was assessed by examining $CD4^+$ and $CD8^+$ T cells for expression of CD69 and HLA-DR. Eight-color flow cytometry was performed on a FACSCanto II flow cytometer with FACSDiva software, version 6 (Becton Dickinson). The gating was set by forward and side scatter, and 100,000 gated events were acquired. Data were collected and analyzed using FlowJo software version 10.4 (Tree Star, Ashland, OR). Compensation and gating boundaries were adjusted using unstained, single stained, and fluorescence minus one controls. Fluorescence minus one controls for each marker were used to calculate fluorescence intensity for the population. Baseline values following stimulation with medium are depicted as baseline frequency, whereas frequencies following stimulation with Ags are depicted as net frequencies (with baseline values subtracted).

Monocyte phagocytosis assay

At 6 h after incubation with media alone or WCL or LPS, monocytes were assessed for phagocytic activity using the Phagocytosis Assay Kit (Cayman Chemical, Ann Arbor, MI). Briefly, latex beads with rabbit IgG-FITC conjugates (1:100) were incubated with cells for 6 h followed by a 1-min incubation with trypan blue to quench non-phagocytosed bead fluorescence. Cells were removed from the culture plate in which they were cultured by gentle scraping. The cells were transferred to tubes for staining with anti-human CD14, incubated on ice for 20 min, then washed once with assay buffer and read on a flow cytometer.

Monocyte respiratory burst assay

The monocyte respiratory burst was assessed using the Monocyte Respiratory burst assay kit according to the manufacturer's instructions (Cayman Chemical) in which 1×10^6 cells/ml were incubated with WCL, LPS, or PMA for 45 min at 37°C. Next, 20 μl of 1,2,3-dihydrorhodamine was added for a further 20 min at 37°C, and the oxidation to rhodamine within $CD14^+$ monocytes was measured by flow cytometry.

ELISA

PBMC culture supernatant levels of IL-1α, IL-1β, TNF-α, IL-6, IL-10, and IL-12 (R&D Systems, Minneapolis, MN) were measured using ELISA kits, according to the manufacturer's instructions.

Table I. Baseline hematological parameters of study population

Hematology Profile (Cells/ml)	*S. stercoralis*–positive (n = 25)	*S. stercoralis*–negative (n = 25)	p Value
RBC count, $\times 10^6$	4.06 (3.7–6.08)	4.08 (2.12–5.86)	NS
WBC count, $\times 10^3$	9,500 (5,700–16,900)	8,600 (5,400–15,500)	NS
Lymphocyte count	2,516 (1,554–3,712)	2,464 (1,487–4,412)	NS
Neutrophil count	4,804 (3,112–11,069)	4,998 (2,563–9,934)	NS
Monocyte count	465 (379–1,000)	658 (337–1,100)	$p = 0.0186$
Eosinophil count	822 (503–3,519)	265 (74–433)	$p = 0.0078$
Basophil count	89 (13–306)	86 (32–387)	NS

qPCR of arginase-1 and iNOS expression

PBMCs were lysed using the reagents of a commercial kit (QIAshredder; Qiagen, Valencia, CA). Total RNA was extracted according to the manufacturer's protocol (RNeasy mini kit; Qiagen), and RNA was dissolved in 50 μl of RNase-free water. The cDNA synthesis was performed with 1 μg RNA using the Prime Script first-strand cDNA synthesis kit according to the manufacturer's instructions (RNeasy Mini Kit; Qiagen). The cDNA obtained from cells were quantified by quantitative real-time PCR, and the levels of arginase-1*(ARG1)* and *iNOS* and an endogenous 18S rRNA control were measured using an ABI 7500 sequence detection system (Applied Biosystems, Fullerton, CA), using Applied Biosystems, TaqMan assays-on-demand reagents. The relative transcript numbers were calculated using the equation $0.5^{\text{CT of target} - \text{CT of control}}$, where CT is the threshold cycle during the exponential phase of amplification.

Statistical analysis

Data analyses were performed using GraphPad PRISM (GraphPad Software, San Diego, CA). Geometric means (GM) were used for measurements of central tendency. Statistically significant differences were assessed using the nonparametric Mann–Whitney *U* test or the Wilcoxon signed rank test for paired analyses. Multiple comparisons were corrected using the Holm

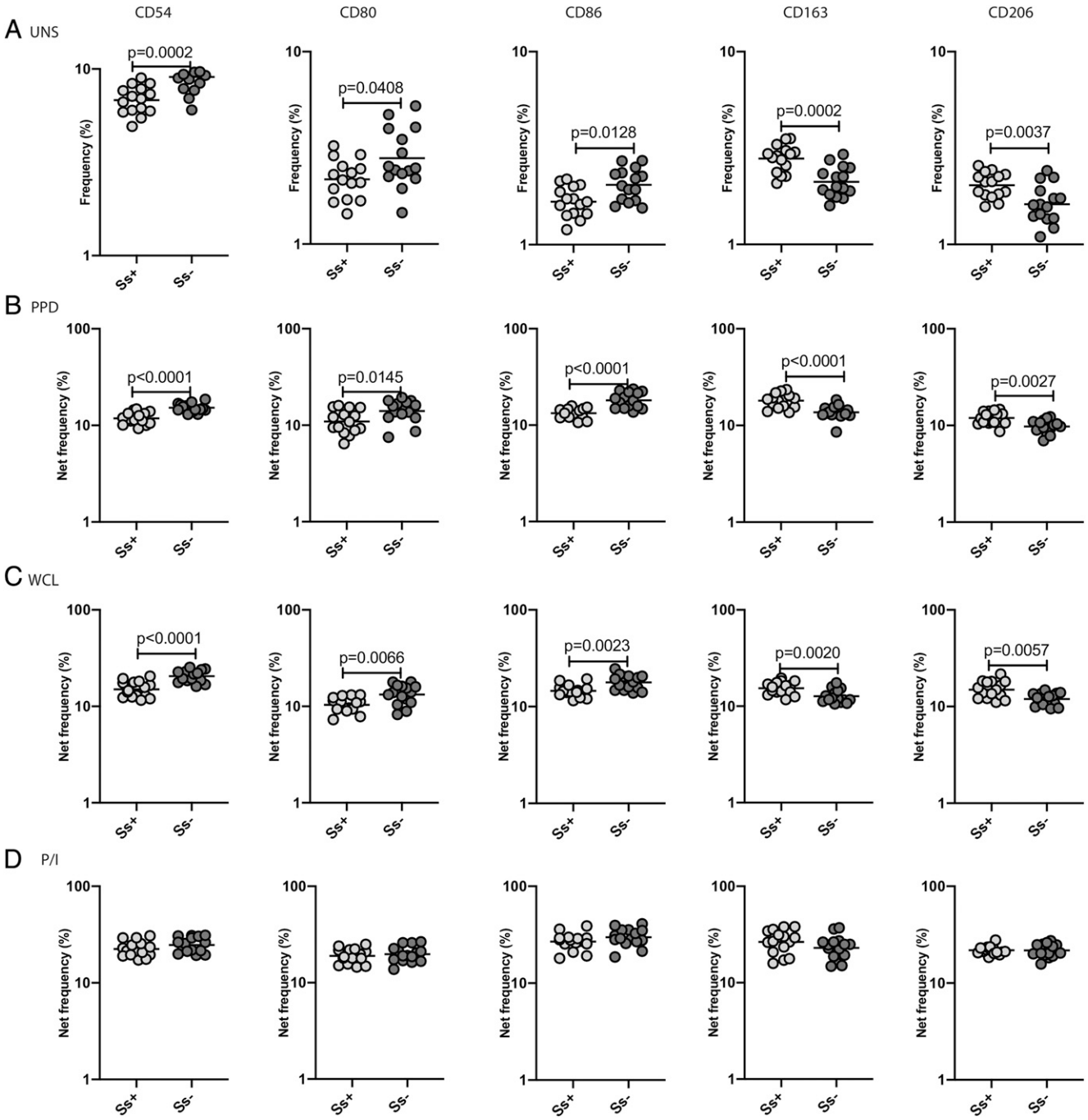

FIGURE 1. LTBI/*S. stercoralis* coinfection is associated with decreased monocyte activation and increased M2 polarization. Baseline and Ag-stimulated frequencies of monocytes expressing activation and M2 markers were determined by flow cytometry in *S. stercoralis*–positive (Ss+; light gray $n = 15$) and *S. stercoralis*–negative (Ss−; dark gray $n = 15$) individuals. The (**A**) baseline (unstimulated) as well as (**B**) PPD-, (**C**) WCL-, and (**D**) P/I-stimulated frequencies of monocytes expressing activation markers (CD54, CD80, CD86) and M2 markers (CD163 and CD206) are shown. Each circle represents a single individual and the bars represent the GM values. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The *p* values were calculated using the Mann–Whitney U test.

correction. Analyses were performed using GraphPad PRISM Version 8.1.2.0 (GraphPad).

Results

Study population characteristics

The baseline hematological characteristics of the study populations are shown in Table I. As can be seen, *S. stercoralis*–positive individuals had significantly higher absolute eosinophil counts and significantly lower monocyte counts than *S. stercoralis*–negative individuals. No significant differences in the other hematological parameters were observed. In addition, there was no correlation between total monocyte counts and the parameters measured in the study, indicating that variations in monocyte counts was not a confounding variable in the study results.

LTBI/S. stercoralis coinfection is associated with decreased monocyte activation and increased M2 polarization

To determine the baseline and Ag-stimulated monocyte activation and M2 polarization in helminth–LTBI coinfection, we measured the frequency of monocytes expressing CD54, CD80 and CD86 (known activation markers), and CD163 and CD206 (M2-specific markers). A representative dot plot showing the expression of activation and M2 markers in an *S. stercoralis*–positive and an *S. stercoralis*–negative individual is shown in Supplemental Fig. 1. As shown in Fig. 1, we demonstrate that the baseline frequency of $CD54^+$, $CD80^+$, or $CD86^+$ monocytes was significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals. Similarly, following stimulation with PPD and *M. tuberculosis* WCL, the net frequency of monocytes expressing CD54, CD80, and CD86 was significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals (Fig. 1B, 1C). There were no differences in the net frequency of monocytes expressing the above markers following P/I stimulation (Fig. 1D).

In contrast, we demonstrate that the baseline frequency of $CD163^+$ and $CD206^+$ monocytes was significantly higher in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals (Fig. 1). Similarly, following stimulation with PPD and WCL, the net frequency of monocytes expressing CD163 and CD206 was significantly higher in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals. Finally, there were no differences in the net frequency of monocytes expressing M2 markers upon P/I stimulation. To additionally verify the enhanced M2 polarization in monocytes in the *S. stercoralis*–positive group, we also performed qPCR analysis of Arginase-1 (M2 marker) and iNOS expression (M1 marker) in baseline PBMC samples from *S. stercoralis*–positive and *S. stercoralis*–negative individuals. As shown in Supplemental Fig. 4, Arginase-1 expression was 1.4-fold higher in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals, whereas iNOS expression was 1.4-fold lower. Thus, LTBI/*S. stercoralis* coinfection is associated with diminished monocyte activation and enhanced M2 polarization.

*LTBI/*S. stercoralis *coinfection is associated with diminished monocyte phagocytosis and respiratory burst activity*

To understand the influence of *S. stercoralis* infection on monocyte function in LTBI, we examined the frequency of

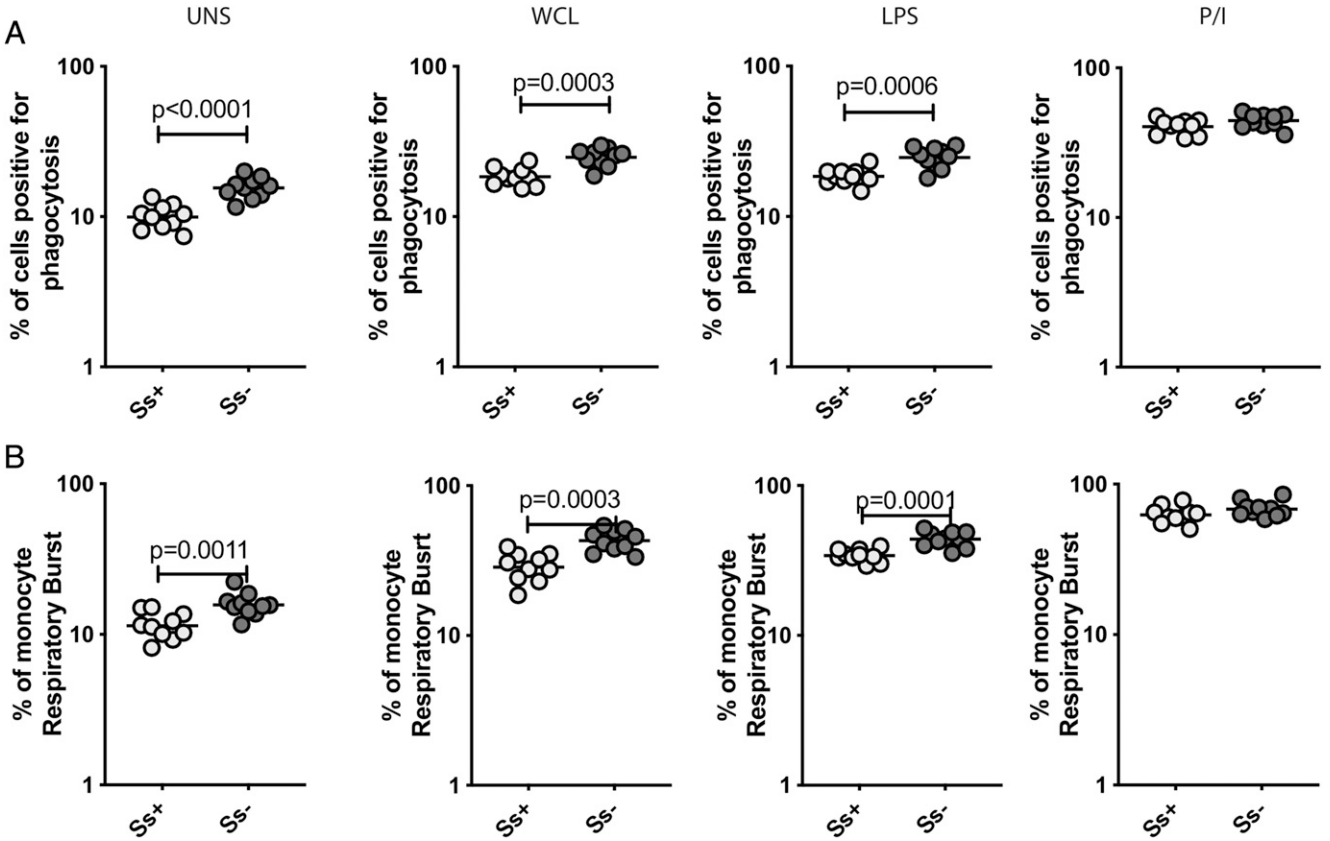

FIGURE 2. LTBI/*S. stercoralis* coinfection is associated with diminished monocyte phagocytosis and respiratory burst activity. Baseline and Ag-stimulated frequencies of monocytes were assessed for phagocytic activity and respiratory burst activity in *S. stercoralis*–positive (Ss+; light gray $n = 10$) and *S. stercoralis*–negative (Ss−; dark gray $n = 10$) individuals. (**A**) PBMC were stimulated with WCL, LPS, and P/I for 6 h and the frequencies of monocytes undergoing phagocytosis was measured. (**B**) PBMC were stimulated with WCL, LPS, and P/I and the frequencies of monocytes undergoing respiratory burst were measured. Each circle represents a single individual and the bars represent the GM values. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The *p* values were calculated using the Mann–Whitney *U* test.

monocytes undergoing phagocytosis or respiratory burst activity in *S. stercoralis*–positive and *S. stercoralis*–negative individuals with LTBI. A representative dot plot showing the gating strategy and the measurement of phagocytosis and respiratory burst activity at baseline and following Ag – stimulation in an *S. stercoralis*–positive individual is shown in Supplemental Fig. 2A and 2B, respectively. As shown in Fig. 2A, the baseline as well as WCL- or LPS- but not P/I-stimulated frequency of monocytes undergoing phagocytosis was significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals. Similarly, as shown in Fig. 2B, the baseline as well as WCL or LPS but not P/I stimulated frequency of monocytes undergoing respiratory burst activity was significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals. Thus, LTBI/*S. stercoralis* coinfection is associated with diminished respiratory burst activity in monocytes reduction in their ability to phagocytose.

*LTBI/*S. stercoralis *coinfection is associated with diminished monocyte cytokine production*

To characterize monocyte cytokine production in LTBI/*S. stercoralis* coinfection, we examined the frequency of monocytes expressing cytokines in *S. stercoralis*–positive and *S. stercoralis*–negative individuals with LTBI. A representative dot plot showing the gating strategy and expression of cytokines in monocytes in an

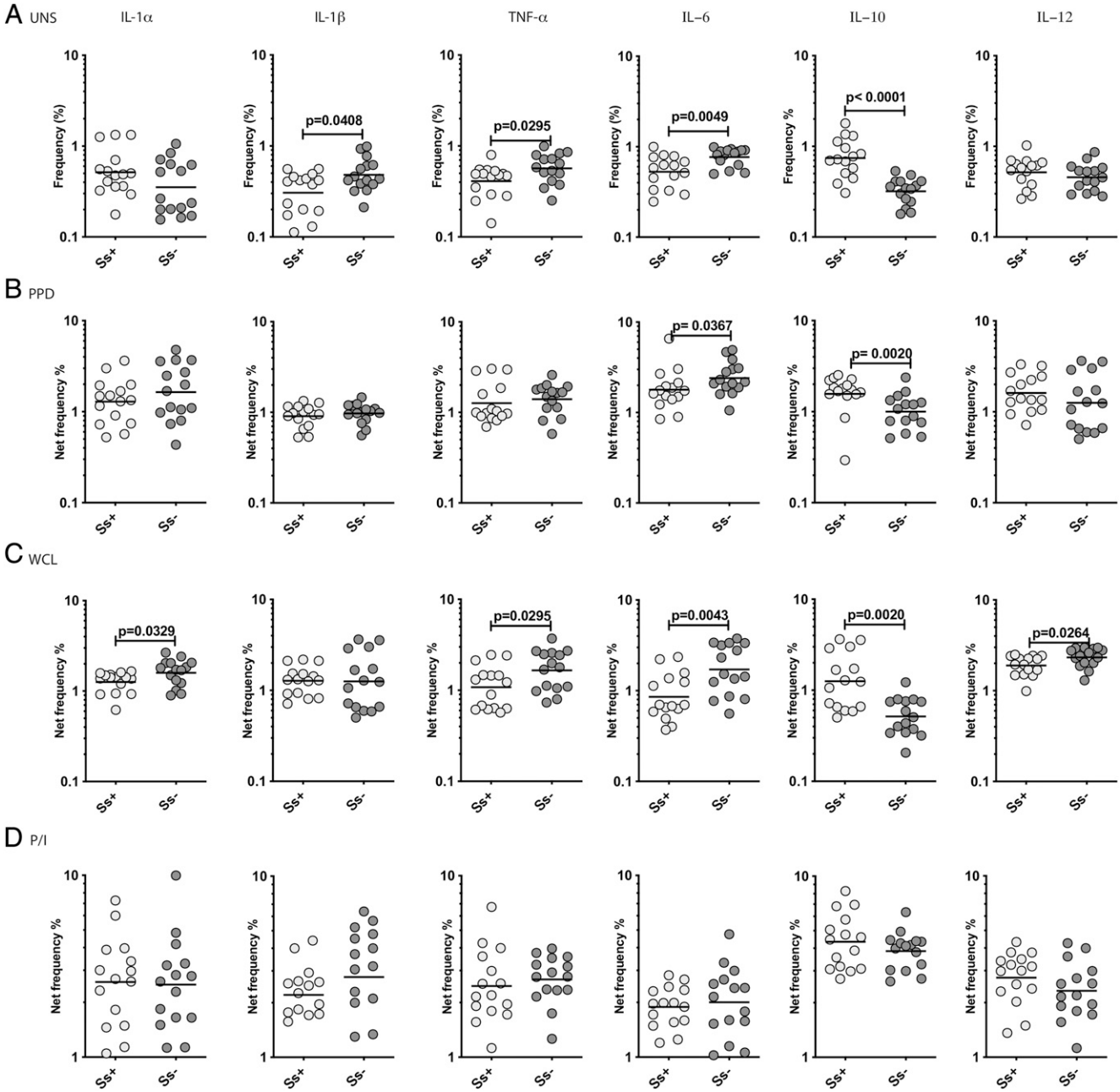

FIGURE 3. LTBI/*S. stercoralis* coinfection is associated with diminished monocyte cytokine production. Baseline and Ag-stimulated frequencies of monocytes expressing cytokines were determined by flow cytometry in *S. stercoralis*–positive (Ss+; light gray $n = 15$) and *S. stercoralis*–negative (Ss−; dark gray $n = 15$) individuals. The (**A**) baseline (unstimulated) as well as (**B**) PPD, (**C**) WCL, and (**D**) P/I stimulated frequencies of monocytes expressing cytokines (IL-1α, IL-1β, TNF-α, IL-6, IL-10, and IL-12) are shown. Each circle represents a single individual and the bars represent the GM values. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The p values were calculated using the Mann–Whitney U test.

S. stercoralis–positive individual is shown in Supplemental Fig. 3. As shown in Fig. 3A, the baseline frequencies of monocytes expressing IL-1β, TNF-α, and IL-6 were significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals. In contrast, the baseline frequencies of monocytes expressing IL-10 were significantly higher in *S. stercoralis*–positive individuals. In addition, following stimulation with PPD and WCL, the frequencies of monocytes expressing IL-6 (for PPD) and IL-1α, TNF-α, IL-6, and IL-12 (for WCL) were significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals (Fig. 3B, 3C). In contrast, the Ag-stimulated frequency of IL-10 expressing monocytes was significantly higher in *S. stercoralis*–positive individuals. Finally, as shown in Fig. 3D, there was no difference in the frequency of monocytes expressing the above cytokines following P/I stimulation. Thus, LTBI/*S. stercoralis* coinfection is associated with diminished monocyte cytokine production at baseline and following *M. tuberculosis* Ag stimulation.

*Diminished proinflammatory cytokine production and enhanced IL-10 production LTBI/*S. stercoralis *coinfection*

To additionally verify cytokine production in LTBI/*S. stercoralis* coinfection, we measured the levels of cytokines in baseline and stimulated PBMC culture supernatants of *S. stercoralis*–positive and *S. stercoralis*–negative individuals with LTBI. As shown in Fig. 4A, at baseline, IL-1α, IL-1β, TNF-α, and IL-6 levels were

FIGURE 4. Diminished proinflammatory cytokine production and enhanced IL-10 production in LTBI/*S. stercoralis* coinfection. Baseline and Ag-stimulated levels of proinflammatory cytokines were measured by ELISA using PBMC culture supernatants of *S. stercoralis*–positive (Ss+; light gray $n = 15$) and *S. stercoralis*–negative (Ss−; dark gray $n = 15$) individuals. The (**A**) baseline (unstimulated) as well as (**B**) PPD-, (**C**) WCL-, and (**D**) P/I-stimulated levels of cytokines (IL-1α, IL-1β, TNF-α, IL-6, IL-10, and IL-12) are shown. Each circle represents a single individual and the bars represent the GM values. Net cytokine levels were calculated by subtracting baseline cytokine levels from the Ag-stimulated cytokine levels for each individual. The *p* values were calculated using the Mann–Whitney *U* test.

significantly diminished in *S. stercoralis*–positive compared with *S. stercoralis*−negative individuals. In contrast, at baseline, IL-10 levels were significantly elevated in *S. stercoralis*–positive individuals. Upon PPD stimulation, the levels of IL-1α, IL-6, and IL-12 levels were significantly diminished, whereas following WCL stimulation, IL-1α, IL-1β, TNF-α, IL-6, and IL-12 levels were significantly diminished in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals (Fig. 4B, 4C). In contrast, PPD and WCL Ag-stimulated IL-10 levels were significantly elevated in *S. stercoralis*–positive individuals. Finally, as shown in Fig. 4D, there was no significant difference in the levels of the above cytokines following P/I stimulation. Thus, LTBI/*S. stercoralis* coinfection is associated with diminished cytokine production at baseline and following *M. tuberculosis* Ag stimulation.

*Anthelmintic therapy significantly increases monocyte activation and decreases M2 polarization in LTBI/*S. stercoralis *coinfection*

To examine whether the diminished monocyte activation and enhanced M2 polarization in LTBI/*S. stercoralis* coinfection is

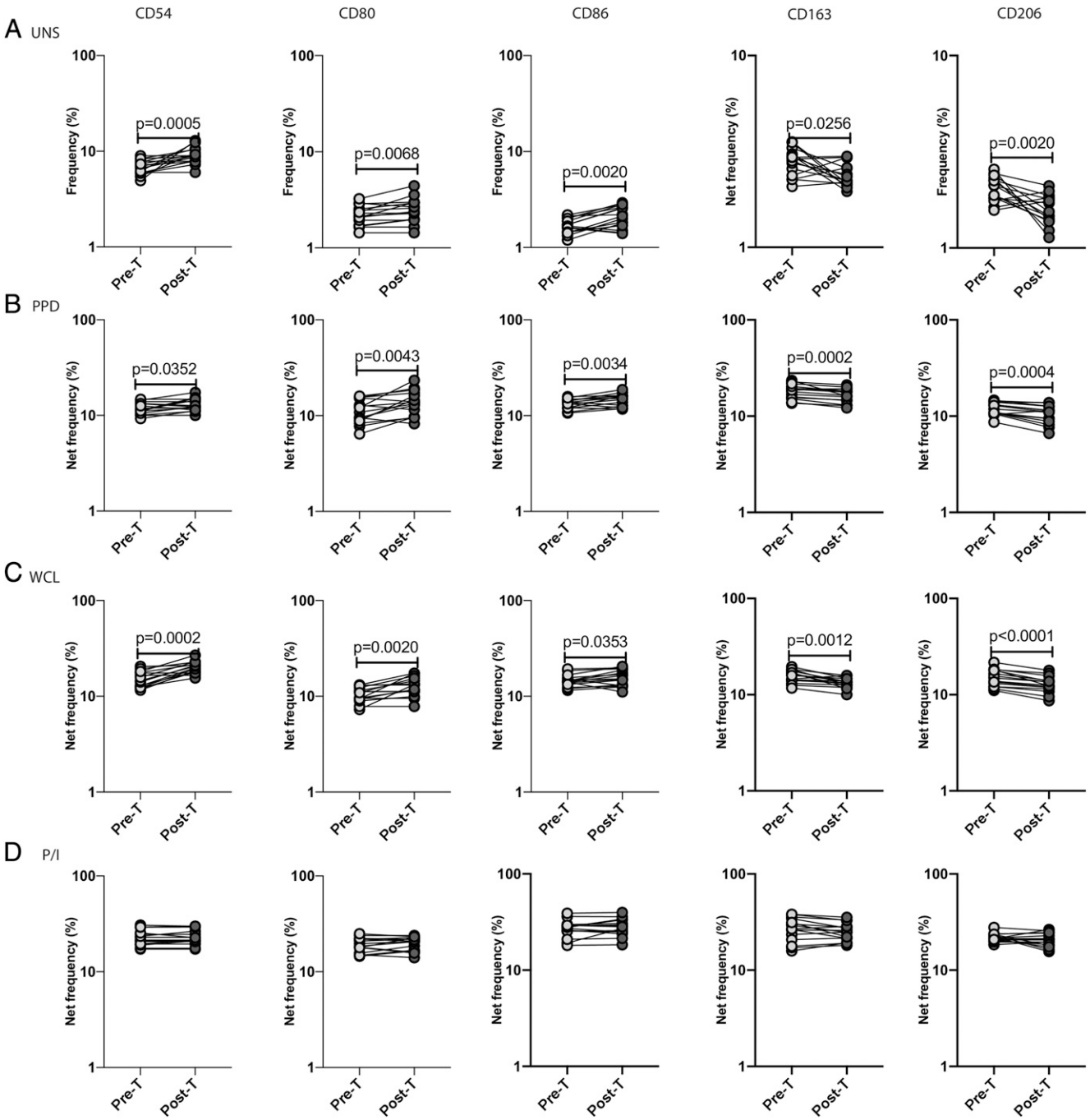

FIGURE 5. Anthelmintic therapy significantly increases monocyte activation and decreases M2 polarization in LTBI/*S. stercoralis* coinfection. Baseline and Ag-stimulated frequencies of monocytes expressing activation and M2 markers were determined by flow cytometry in *S. stercoralis*–positive (light gray $n = 15$) individuals before and after treatment with a standard dose of ivermectin and albendazole. The (**A**) baseline (unstimulated) as well as (**B**) PPD-, (**C**) WCL-, and (**D**) P/I-stimulated frequencies of monocyte expressing activation (CD54, CD80, CD86) and M2 (CCR2, CX3CR1) markers are shown. Each line represents a single individual. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The *p* values were calculated using the Wilcoxon signed rank test.

reversible following anthelmintic therapy, we examined the frequency of monocytes expressing activation markers (CD54, CD80, and CD86) and M2 markers (CD163 and CD206) in LTBI/*S. stercoralis* individuals 6 mo following anthelmintic treatment (Post-T). As shown in Fig. 5A, the baseline frequency of monocytes expressing CD54, CD80 and CD86 was significantly increased, whereas the frequency of monocytes expressing CD163 and CD206 was significantly decreased in *S. stercoralis*–positive individuals at Post-T compared with the pretreatment frequency (Pre-T). Similarly, as shown in Fig. 5B, 5C, the PPD and WCL stimulated net frequencies of monocytes expressing CD54, CD80, and CD86 were significantly increased, whereas the net frequencies of monocytes expressing CD163 and CD206 were significantly decreased at Post-T compared with Pre-T. Finally, as shown in Fig. 5D, no significant changes in the net frequency of monocytes expressing the above markers was observed upon P/I stimulation at Post-T. Thus, anthelmintic therapy significantly reverses the diminished monocyte activation and enhanced M2 polarization in LTBI/*S. stercoralis* coinfection.

*Anthelmintic therapy reverses the diminished phagocytosis or respiratory burst activity in monocytes in LTBI/*S. stercoralis *coinfection*

To examine whether the diminished monocyte function in LTBI/*S. stercoralis* coinfection is reversible following anthelmintic therapy, we examined the frequency of monocytes undergoing phagocytosis or respiratory burst activity in LTBI/*S. stercoralis* individuals Post-T. As shown in Fig. 6A, the baseline as well as WCL or LPS but not P/I stimulated frequencies of monocytes undergoing phagocytosis were significantly increased in *S. stercoralis*–positive individuals Post-T compared with the Pre-T. Similarly, as shown in Fig. 6B, the baseline as well as WCL or LPS but not P/I stimulated frequencies of monocytes undergoing respiratory burst activity were significantly increased at Post-T compared with Pre-T. Thus, anthelmintic therapy significantly reverses the diminished monocyte phagocytosis and respiratory burst function in LTBI/*S. stercoralis* coinfection.

*Elevated levels of monocytes producing cytokines in LTBI/*S. stercoralis *coinfection following anthelmintic therapy*

To examine whether the diminished monocyte expression of cytokines in LTBI/*S. stercoralis* coinfection is reversible following anthelmintic therapy, we examined the frequency of monocytes expressing cytokines in LTBI/*S. stercoralis* individuals Post-T. As shown in Fig. 7A, the baseline frequency of monocytes expressing IL-1β and IL-6 was significantly increased in *S. stercoralis*–positive individuals Post-T compared with the Pre-T. Similarly, as shown in Fig. 7B, 7C, the PPD- and WCL-stimulated net frequencies of monocytes expressing IL-1α (except PPD stimulation), IL-1β, TNF-α, IL-6, and IL-12 were significantly increased at Post-T compared with Pre-T. Finally, as shown in Fig. 7D, no significant changes in the net frequency of monocytes expressing the above cytokines was observed upon P/I stimulation at Post-T. Thus, anthelmintic therapy significantly reverses the diminished monocyte production of cytokines in LTBI/*S. stercoralis* coinfection.

*Anthelmintic therapy significantly increases proinflammatory cytokine production in LTBI/*S. stercoralis *coinfection*

To examine whether the diminished production of cytokines in LTBI/*S. stercoralis* coinfection is reversible following

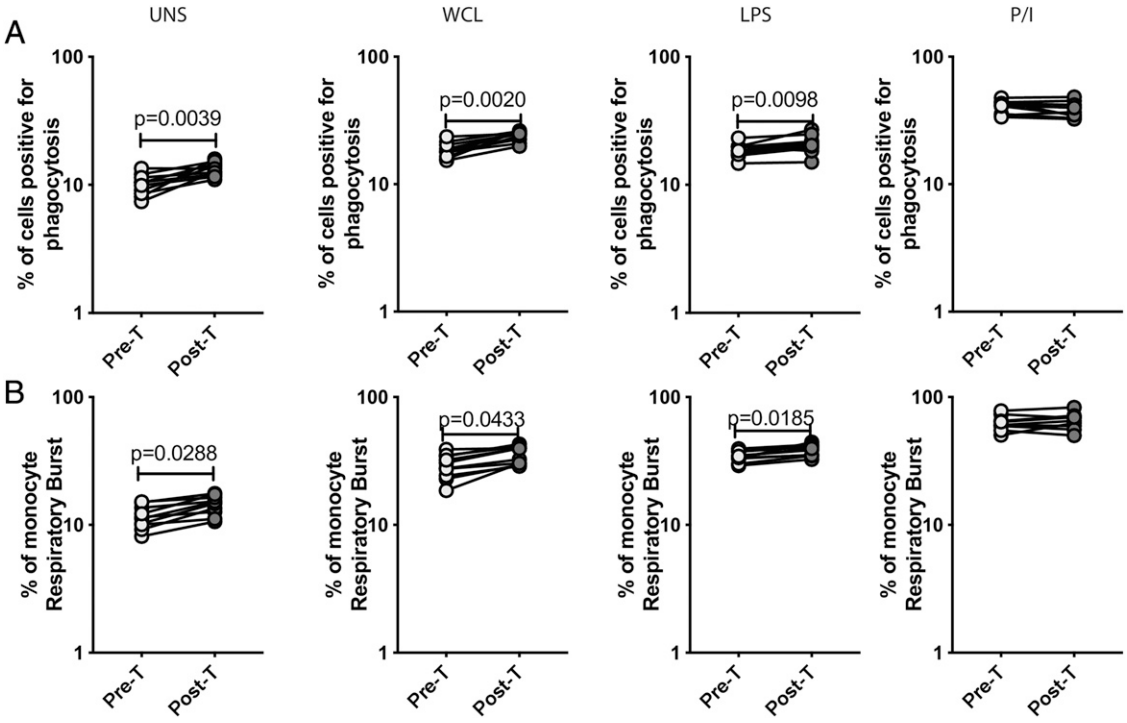

FIGURE 6. Anthelmintic therapy reverses the diminished phagocytosis or respiratory burst activity in monocytes in LTBI/*S. stercoralis* coinfection. Baseline and Ag-stimulated frequencies of monocytes were assessed for phagocytic activity and respiratory burst activity by flow cytometry in *S. stercoralis*–positive (light gray $n = 10$) individuals before and after treatment with a standard dose of ivermectin and albendazole (**A**) PBMC were stimulated at baseline (unstimulated), WCL, LPS, and P/I for 6 h, and the frequencies of monocytes undergoing phagocytosis were measured. (**B**) PBMC were stimulated at baseline (unstimulated), WCL, LPS, and P/I for 6 h and the frequencies of monocytes undergoing respiratory burst activity were measured. Each line represents a single individual. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The *p* values were calculated using the Wilcoxon signed rank test.

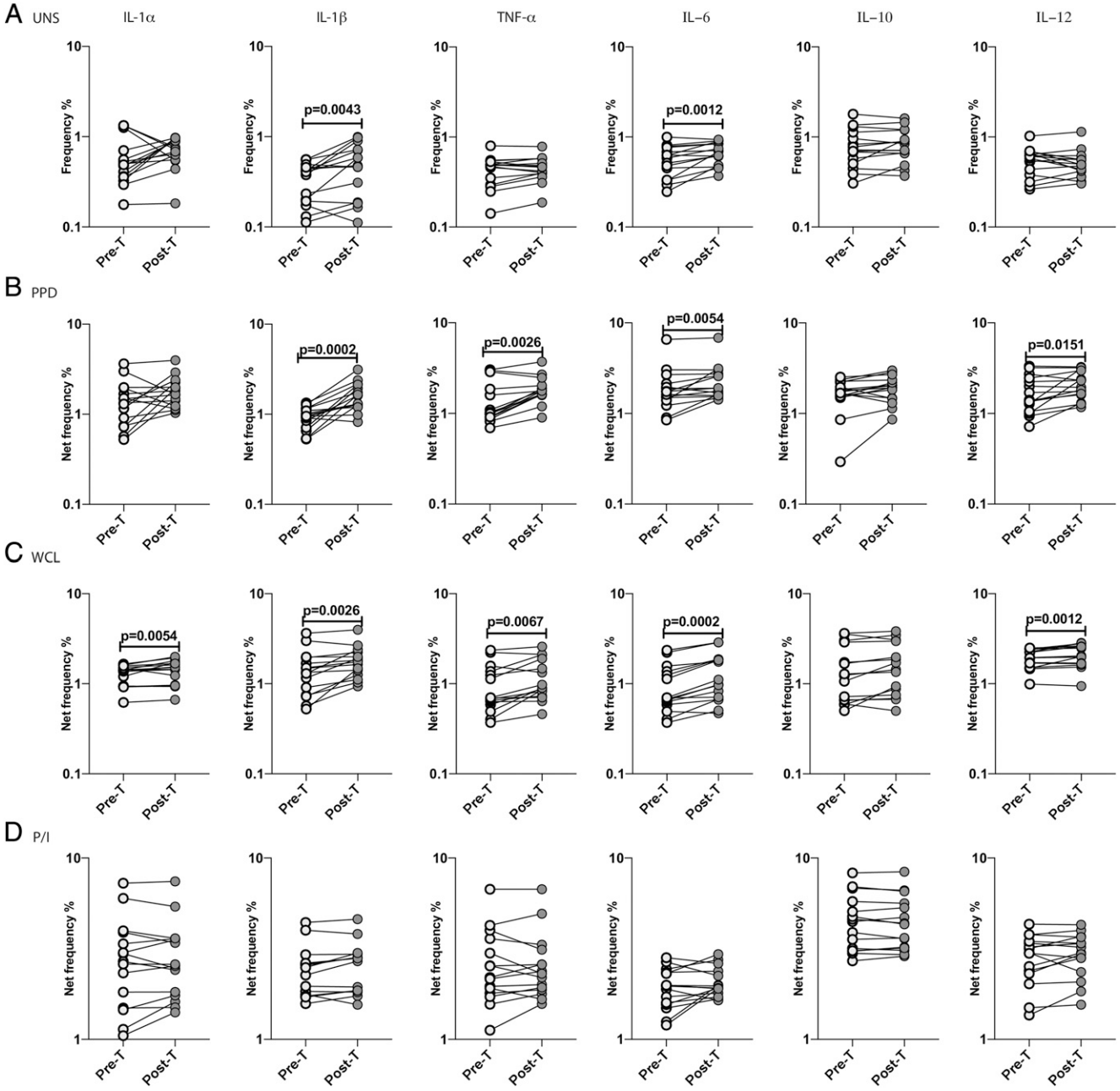

FIGURE 7. Elevated levels of monocytes producing cytokines in LTBI/*S. stercoralis* coinfection following anthelmintic therapy. Baseline and Ag-stimulated frequencies of monocytes expressing cytokines were determined by flow cytometry in *S. stercoralis*–positive (light gray $n = 15$) individuals before and after treatment with a standard dose of ivermectin and albendazole. The (**A**) baseline (unstimulated) as well as (**B**) PPD-, (**C**) WCL-, and (**D**) P/I-stimulated frequencies of monocytes expressing cytokines (IL-1α, IL-1β, TNF-α, IL-6, IL-10, and IL-12) were measured. Each line represents a single individual. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The *p* values were calculated using the Wilcoxon signed rank test.

anthelmintic therapy, we examined the levels of cytokines in LTBI/*S. stercoralis* individuals Post-T. As shown in Fig. 8A, at baseline, IL-1α, IL-1β, TNF-α, and IL-6 levels were significantly elevated in *S. stercoralis*–positive individuals Post-T compared with the Pre-T. Similarly, as shown in Fig. 8B and 8C, the PPD and WCL stimulated net cytokine levels of IL1α, IL-1β, TNF-α, IL-6, and IL-12 were significantly elevated at Post-T compared with Pre-T. Finally, as shown in Fig. 8D, no significant changes in the net cytokine levels of the above cytokines was observed upon P/I stimulation at Post-T. Thus, anthelmintic therapy significantly reverses the diminished production of cytokines in LTBI/*S. stercoralis* coinfection.

*LTBI/*S. stercoralis *coinfection is associated with decreased T cell activation*

To understand the implications of the *S. stercoralis*–induced monocyte dysfunction in LTBI/*S. stercoralis* coinfection, we measured the frequency of activated $CD4^+$ and $CD8^+$ T cells expressing CD69 and HLA-DR following stimulation with *M. tuberculosis* Ags. As shown in Fig. 9A, we demonstrate that in the absence of stimulation, or following stimulation with PPD and WCL, the frequencies of $CD4^+$ T cells expressing CD69 and HLA-DR were significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals. As shown in Fig. 9B, we also demonstrate that PPD- and WCL-stimulated

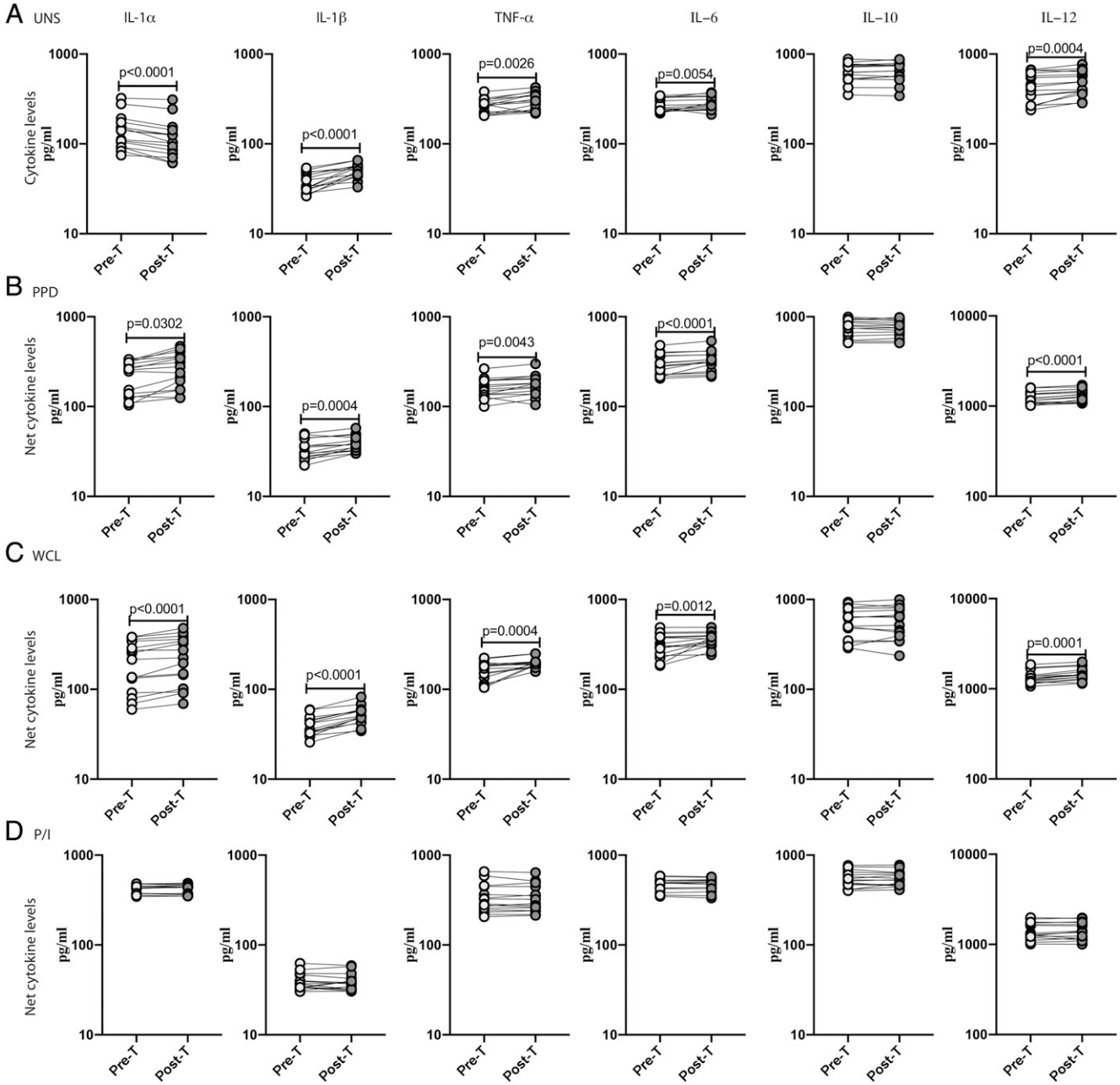

FIGURE 8. Anthelmintic therapy significantly increases proinflammatory cytokine production in LTBI/*S. stercoralis* coinfection. Baseline and Ag-stimulated levels of proinflammatory cytokines were determined by ELISA using PBMC culture supernatants of *S. stercoralis*–positive (light gray $n = 15$) individuals before and after treatment with a standard dose of ivermectin and albendazole. The (**A**) baseline (unstimulated) as well as (**B**) PPD-, (**C**) WCL-, and (**D**) P/I-stimulated levels of cytokines (IL-1α, IL-1β, TNF-α, IL-6, IL-10, and IL-12) were measured. Each line represents a single individual. Net cytokine levels were calculated by subtracting baseline cytokine levels from the Ag-induced cytokine levels for each individual. The *p* values were calculated using the Wilcoxon signed rank test.

but not baseline or P/I-stimulated frequency of activated $CD8^+$ T cells were significantly lower in *S. stercoralis*–positive compared with *S. stercoralis*–negative individuals.

Discussion

Monocytes/macrophages are central players in immunity to helminth infections (22). Helminth interactions with macrophages typically induces a population of cells preferentially expressing arginine instead of NO, and these macrophages are termed as alternatively activated macrophages (23). Certain helminth infections can also elicit an alternative activation phenotype in monocytes (24). Monocyte dysfunction in helminth infections is associated with decreased capacity to express costimulatory molecules and chemokine receptors, decreased functional activity including phagocytosis, and decreased production of proinflammatory or protective cytokines (25–27). Whether any or all of these mechanisms play a role in helminth-latent TB interaction is not known. Our study explores the role of four different aspects of monocyte activation and function in helminth–TB interactions. First, our study examines monocyte activation in terms of the ability to express activation markers in coinfected individuals. Second, our study examines the M2 polarization potential of monocytes in coinfected individuals. Third, our study examines the function of monocytes in terms of phagocytosis and respiratory burst activity.

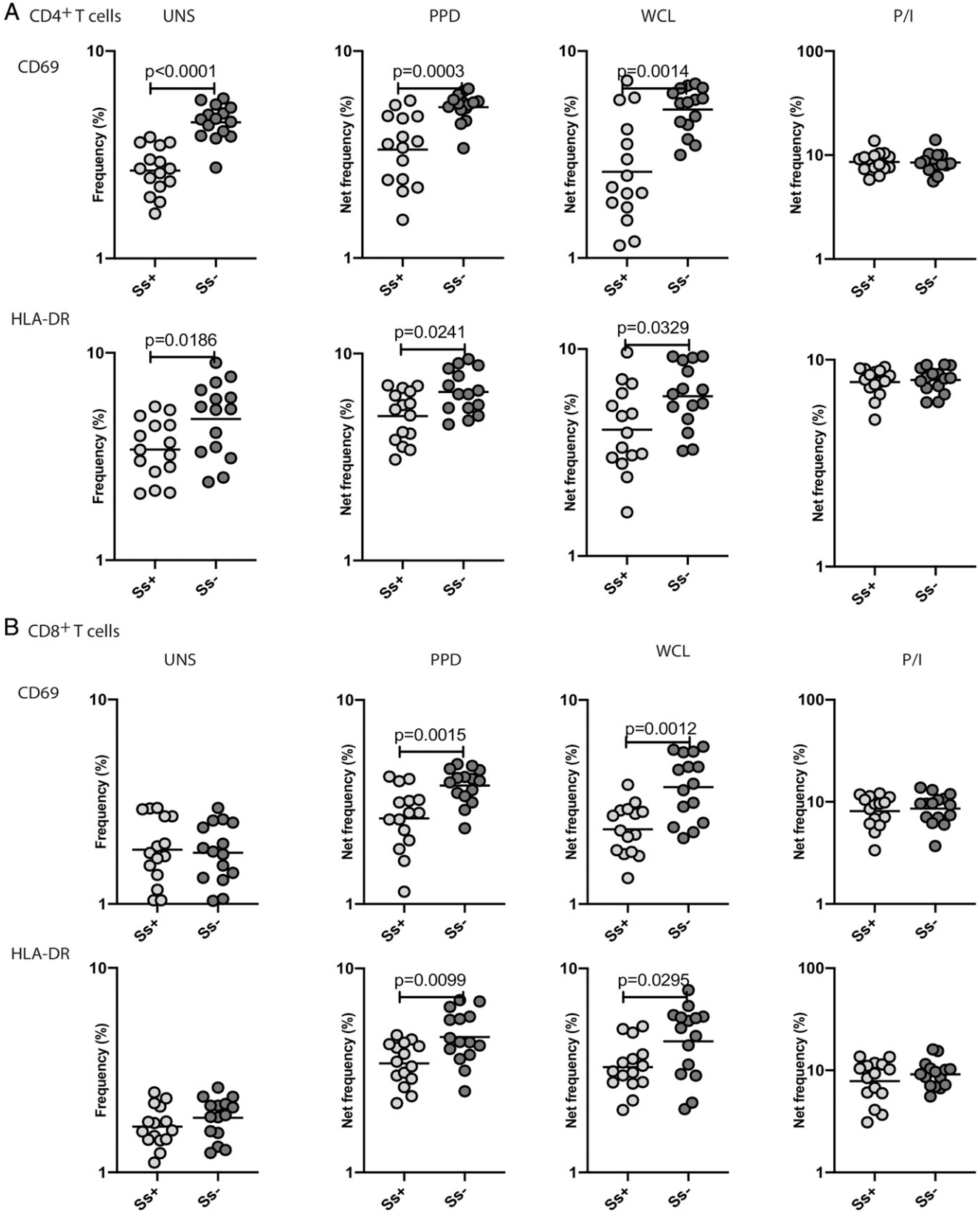

FIGURE 9. LTBI/*S. stercoralis* coinfection is associated with decreased T cell activation. Baseline and Ag-stimulated frequencies of T cell activation markers were determined by flow cytometry in *S. stercoralis*–positive (Ss+; light gray $n = 15$) and *S. stercoralis*–negative (Ss−; dark gray $n = 15$) individuals. (**A**) Baseline (unstimulated) and PPD-, WCL-, and P/I-stimulated frequencies of T cell activation markers (CD69 and HLA-DR) expressed by $CD4^+$ T cells are shown. (**B**) Baseline (unstimulated) and PPD-, WCL-, and P/I-stimulated frequencies of T cell activation markers (CD69 and HLA-DR) expressed by $CD8^+$ T cells are shown. Each circle represents a single individual and the bars represent the GM values. Net frequencies were calculated by subtracting baseline frequencies from the Ag-induced frequencies for each individual. The p values were calculated using the Mann–Whitney U test.

Finally, our study examines the capacity of monocytes to produce protective cytokines in the context of coinfection. Our study innovates by not only examining each of these parameters at baseline and following *M. tuberculosis* Ag stimulation but also by re-examining the same responses under the same conditions following anthelmintic treatment.

CD80 and CD86 are homologous costimulatory molecules expressed predominantly on Ag-presenting cells, including monocytes, and interaction of these molecules with CD28 and CTLA-4 on T cells is a critical step in T cell activation (28). Previous work has shown that both CD80 and CD86 are equally important in host immunity to TB (29, 30). Additionally, decreased expression of CD80/CD86 is associated with impaired T cell activation in TB (29). Therefore, our data on reduced frequencies of monocytes expressing both CD80 and CD86 both at baseline and following *M. tuberculosis* Ag stimulation imply a major negative effect on the activation of T cells in response to *M. tuberculosis* Ags. CD54 or ICAM-1 is an adhesion molecule that is critical for the arrest and transmigration of leukocytes out of blood vessels and into tissues (31). The LFA-1/CD54 pathway is important for T cell proliferation (32). CD54 functions as an activation marker on monocytes and its expression is upregulated upon TB infection (33). Thus, diminished expression of these receptors either at baseline or following *M. tuberculosis* Ag stimulation also implies a defect in the host innate immunity to TB in coinfected individuals. Indeed, we and others have previously reported compromised T cell activation and function in helminth-latent TB coinfection (11).

M2 polarization of monocytes/macrophages is associated with diminished resistance to mycobacterial infection (34–36). Because helminth infections are the predominant inducers of alternative activation (22), we examined and demonstrated that the expression of markers associated M2 polarization is significantly elevated in coinfected individuals. Interestingly, increased polarization is evident both at baseline and in response to *M. tuberculosis* Ags, indicating a trained immunity type of an immune response in coinfected individuals (37). It is also highly probable that this increased frequency of M2 monocytes potentially underlies the altered functional responses of monocytes to *M. tuberculosis* Ags. We have not addressed the mechanism underlying the shift in monocyte activation in *S. stercoralis* infection, and this will be examined in future studies.

Phagocytosis and respiratory burst activity are important components of host innate immunity to *M. tuberculosis* (38–40). Processes involved in phagocytosis include binding of the bacterium to the host cell, internalization, and finally growth inhibition or killing (39, 40). After pathogenic bacteria are engulfed into phagosomes, they are subject to killing via a variety of mechanisms, including phagosome-lysosome fusion, generation of reactive oxygen intermediates, and generation of reactive nitrogen intermediates, particularly NO (41). Understanding of these initial monocyte defenses may lead to important insights into the development of clinically active disease, as evasion of these monocyte defense mechanisms is likely a key step in promoting the active disease process (38). Indeed, our data clearly show that monocyte defense mechanisms are compromised in the setting of helminth coinfection. In particular, both baseline and *M. tuberculosis* Ag (and LPS) induced monocyte phagocytosis and respiratory burst activity (reflecting generation of superoxide and other radicals) is significantly reduced in coinfected individuals. This suggests that the ability of monocytes to engulf and kill *M. tuberculosis* might be inefficient in the presence of coinfection. Indeed, our laboratory and others have shown that helminth infection is associated with higher bacterial burden in active TB (42, 43).

Monocyte production of IL-1α, IL-1β, TNF-α, IL-6, and IL-12 also plays an important role in host defense against *M. tuberculosis* (38, 44, 45). Whereas IL-1α and IL-1β play a protective role in resistance, TNF-α contributes to host immunity by both promoting killing of *M. tuberculosis* and formation/maintenance of granulomas (46). IL-6 is an important for recruitment of immune cells, and IL-12 is the key cytokine in the induction of Th1 responses, which are critical against *M. tuberculosis* (46, 47). Our study demonstrates a deficiency in the production of these cytokines in the presence of coinfection, suggesting that one major mechanism by which helminth infection modulate immunity to TB is by impairment of monocyte cytokine production. In contrast, monocyte production of IL-10 is enhanced in coinfection and, because IL-10 is detrimental to host defense against *M. tuberculosis* (48), indicates another pathway for compromising host immunity. Our data on the ELISA measurements of culture supernatants, either unstimulated or TB–Ag-stimulated, additionally verify the alteration in the cytokine production in *S. stercoralis* infections and its reversibility following chemotherapy. Finally, our data also reveal that the impairment of monocyte activation and function appears to be associated with diminished T cell activation, suggesting that helminth infection impairs both the innate and adaptive arms of the immune system in the context of LTBI. This data are similar to our previous study demonstrating parasite–Ag-specific downmodulation of Th1 and Th17 responses in chronic *S. stercoralis* infection (49).

Our study also reveals an important facet of compromised immunity engendered by helminth infection in that most of these defects are at least partially reversed upon anthelmintic treatment. Thus, examination of coinfected individuals 6 mo following administration of a single dose of ivermectin and albendazole is sufficient to restore (at least partially) immune homeostasis and the ability of monocytes to respond optimally to TB antigenic stimuli. This is an important finding because it suggests that prior administration of anthelmintic treatment, especially in endemic areas, would be a prudent strategy to restore immune responsiveness before the conduct of TB vaccine trials or host directed therapies. In summary, our study identifies yet another mechanism of hyporesponsiveness or immune dysfunction in host immunity to helminth–TB coinfection and this time reveals a compromise in the immune response of monocytes as a major component of this deficit.

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Disclosures

The authors have no financial conflicts of interest.

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