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Microbial Translocation Associated with an Acute-Phase Response and Elevations in MMP-1, HO-1, and Proinflammatory Cytokines in *Strongyloides stercoralis* Infection

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ABSTRACT Microbial translocation, characterized by elevated levels of lipopolysaccharide (LPS) and related markers, is a common occurrence in HIV and some parasitic infections. This is usually associated with extensive inflammation and immune activation. To examine the occurrence of microbial translocation and the associated inflammatory response in asymptomatic *Strongyloides stercoralis* infection, we measured the plasma levels of LPS and other microbial translocation markers, acute-phase proteins, inflammatory markers, and proinflammatory cytokines in individuals with (infected [INF]) or without (uninfected [UN]) *S. stercoralis* infections. Finally, we also measured the levels of all of these markers in INF individuals following treatment of *S. stercoralis* infection. We show that INF individuals exhibit significantly higher plasma levels of microbial translocation markers (LPS, soluble CD14 [sCD14], intestinal fatty acid-binding protein [iFABP], and endotoxin core IgG antibody [EndoCAb]), acute-phase proteins (α-2 macroglobulin [α-2M], C-reactive protein [CRP], haptoglobin, and serum amyloid protein A [SAA]), inflammatory markers (matrix metalloproteinase 1 [MMP-1] and heme oxygenase 1 [HO-1]), and proinflammatory cytokines (interleukin-6 [IL-6], IL-8, monocyte chemoattractant protein 1 [MCP-1], and IL-1β) than do UN individuals. INF individuals exhibit significantly decreased levels of tissue inhibitor of metalloproteinases 4 (TIMP-4). Following treatment of *S. stercoralis* infection, the elevated levels of microbial translocation markers, acute-phase proteins, and inflammatory markers were all diminished. Our data thus show that *S. stercoralis* infection is characterized by microbial translocation and accompanying increases in levels of acute-phase proteins and markers of inflammation and provide data to suggest that microbial translocation is a feature of asymptomatic *S. stercoralis* infection and is associated with an inflammatory response.

KEYWORDS microbial translocation markers, *Strongyloides stercoralis*, acute-phase proteins, inflammatory markers, proinflammatory cytokines

Microbial translocation refers to the process by which the translocation of bacterial products results in elevated levels of lipopolysaccharide (LPS) in the circulation without overt bacteremia (1). LPS and 16S rRNA (common to most bacteria) are often used as indicators of bacterial translocation, and endotoxin core IgG antibody (EndoCAb) is also used as a surrogate marker for the measurement of circulating LPS levels (1, 2). iFABP may also reflect a breach in epithelial integrity associated with chronic intestinal infections (3). The prevalence of increased acute-phase protein concentrations during episodes of inflammation is used as a supporting prognostic and diag-

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nostic tool for inflammatory gastrointestinal diseases (4). Matrix metalloproteinases (MMPs) and their endogenous inhibitor, tissue inhibitor of metalloproteinases 4 (TIMP-4), also play an important role in the process of tissue remodeling and destruction and can be detected in the sera of individuals with a variety of inflammatory disorders (5, 6). Heme oxygenase 1 (HO-1), a cytoprotective enzyme with anti-inflammatory and antioxidant properties, has been shown to play a fundamental role in the control of systemic inflammation (7).

Microbial translocation is commonly observed under conditions associated with a disruption of the gastrointestinal (GI) epithelial barrier, such as inflammatory bowel disease, graft-versus-host disease, and chronic viral infections, including infections by human immunodeficiency virus (HIV) and hepatitis C virus (1, 2). Microbial translocation is also commonly associated with acute and chronic systemic immune activation and perturbations in T cell subset numbers (1–3, 8, 9).

Although bacterial translocation is known to occur in infections affecting the integrity of the gut epithelium (10, 11), very few studies have examined the occurrence of this phenomenon in intestinal helminth infections. Among the common helminth parasites known to establish chronic infections in humans, *Strongyloides stercoralis*, the causative agent of strongyloidiasis (12), is unique in its ability to exist in free-living and autoinfective cycles (13, 14), with the latter allowing persistent long-lived infection. Chronic *S. stercoralis* infection, although commonly clinically silent, can cause cutaneous, gastrointestinal, and/or pulmonary symptoms and, in the face of immune suppression, may present as hyperinfection syndrome or disseminated strongyloidiasis, conditions that are potentially fatal (15).

To examine whether microbial translocation is a feature of *S. stercoralis* infection, we measured the circulating levels of bacterial translocation markers, acute-phase proteins, inflammatory markers (MMP-1, TIMP-4, and HO-1), and proinflammatory cytokines (interleukin-6 [IL-6], IL-8, monocyte chemoattractant protein 1 [MCP-1], and macrophage inflammatory protein 1β [MIP-1β]) in *S. stercoralis*-infected (INF) (before and after treatment) and uninfected (UN) individuals. Our data confirm the presence of microbial translocation and an associated acute-phase and proinflammatory response in *S. stercoralis* infection and its reversal following therapy.

RESULTS

***S. stercoralis* infection is associated with elevated levels of LPS, sCD14, iFABP, and EndoCAb.** To determine the association of microbial translocation and related markers in *S. stercoralis* infection, we measured the plasma levels of LPS, soluble CD14 (sCD14), iFABP, lipid-binding protein (LBP), and EndoCAb in INF and UN individuals. As shown in Fig. 1A, INF individuals had significantly higher levels of LPS (geometric mean [GM] of 0.1141 endotoxin units [EU]/ml in INF versus 0.06623 EU/ml in UN individuals; $P = 0.0005$), sCD14 (GM of 21.30 ng/ml in INF versus 14.76 ng/ml in UN individuals; $P = 0.0204$), and iFABP (GM of 0.1258 ng/ml in INF versus 0.06474 ng/ml in UN individuals; $P = 0.0384$) than did UN individuals. Thus, *S. stercoralis* infection is associated with elevated circulating levels of microbial translocation markers.

To determine the effect of anthelmintic treatment on microbial translocation markers, we measured the circulating levels of LPS, sCD14, iFABP, LBP, and EndoCAb in INF individuals before treatment (pre-T) and after treatment (post-T). As shown in Fig. 1B, LPS (mean fold change of 0.751 post-T compared to pre-T; $P = 0.0005$), sCD14 (mean fold change of 0.788; $P = 0.0004$), iFABP (mean fold change of 0.819; $P = 0.0057$), and EndoCAb (mean fold change of 0.892; $P = 0.0266$) levels were significantly decreased from pretreatment levels 6 months following treatment. In contrast, LBP levels (mean fold change of 1.85; $P = 0.0132$) were significantly increased posttreatment compared to pretreatment levels.

***S. stercoralis* infection is associated with elevated levels of α-2M, CRP, haptoglobin, and SAA.** To assess the levels of acute-phase proteins in *S. stercoralis* infection, we measured the plasma levels of α-2 macroglobulin (α-2M), C-reactive protein (CRP), haptoglobin, and serum amyloid protein A (SAA) in INF and UN individuals. As shown

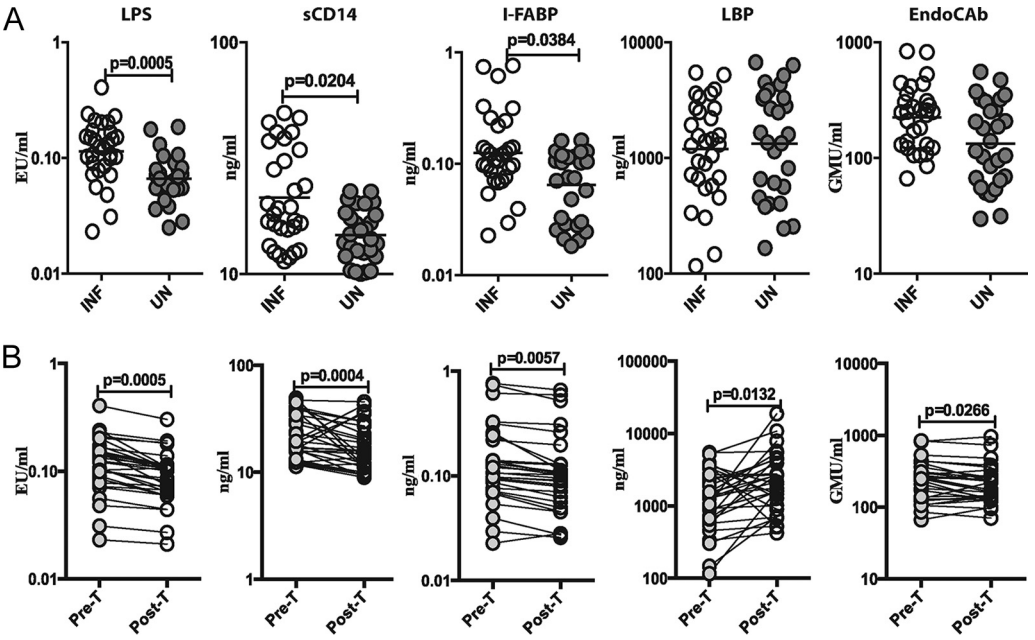

FIG 1 *S. stercoralis* infection is associated with elevated levels of LPS, sCD14, iFABP, and EndoCAb. (A) Plasma levels of LPS, sCD14, iFABP, LBP, and EndoCAb in *S. stercoralis*-infected ($n = 30$) or uninfected ($n = 28$) individuals were measured by ELISAs. Data are shown as scatter plots, with bars representing the geometric means. *P* values were calculated by using the Mann-Whitney test. (B) Plasma levels of LPS, sCD14, iFABP, LBP, and EndoCAb in *S. stercoralis*-infected individuals pre-T ($n = 30$) and 6 months following treatment (post-T) were measured by ELISAs. *P* values were calculated by using the Wilcoxon matched-pair test. GMU, IgG median units.

in Fig. 2A, INF individuals had significantly elevated levels of α-2M (GM of 5.482 ng/ml in INF versus 2.094 ng/ml in UN individuals; $P = 0.0116$), CRP (GM of 2.512 ng/ml in INF versus 1.087 ng/ml in UN individuals; $P = 0.0153$), haptoglobin (GM of 0.367 ng/ml in INF versus 0.2214 ng/ml in UN individuals; $P = 0.0256$), and SAA (GM of 0.2468 ng/ml in INF versus 0.1451 ng/ml in UN individuals; $P = 0.0290$) in comparison to those in UN individuals. Thus, *S. stercoralis* infection is associated with elevated levels of acute-phase proteins. Similarly, to determine the effect of treatment on acute-phase protein marker levels, we measured the circulating levels of α-2M, CRP, haptoglobin, and SAA in infected individuals before and after treatment. As shown in Fig. 2B, α-2M (mean fold change of 0.593 post-T compared to pre-T; $P = 0.0004$), CRP (mean fold change of 0.905; $P = 0.0003$), haptoglobin (mean fold change of 0.850; $P = 0.0002$), and SAA (mean fold change of 0.904; $P = 0.0310$) levels were significantly decreased from pretreatment levels 6 months following treatment.

***S. stercoralis* infection is associated with elevated levels of MMP-1 and HO-1 and decreased levels of TIMP-4.** To determine the association of inflammation with *S. stercoralis* infection, we measured the concentrations of the inflammatory markers MMP-1, TIMP-4, and HO-1 in INF and UN individuals. As shown in Fig. 3A, INF individuals had significantly elevated levels of MMP-1 (GM of 0.2306 ng/ml in INF versus 0.1272 ng/ml in UN individuals; $P = 0.0006$) and HO-1 (GM of 0.2576 ng/ml in INF versus 0.1579 ng/ml in UN individuals; $P = 0.0399$) in comparison to those in UN individuals. INF individuals had significantly decreased levels of TIMP-4 (GM of 0.3189 ng/ml in INF versus 0.4441 ng/ml in UN individuals; $P = 0.0018$) in comparison to those in UN individuals. Thus, *S. stercoralis* infection is associated with elevated levels of certain inflammatory markers. To determine the effect of treatment on the levels of inflammatory markers, we measured the circulating levels of MMP-1, TIMP-4, and HO-1 in INF individuals before and after treatment. As shown in Fig. 3B, MMP-1 (mean fold change of 0.590; $P = 0.0051$) levels were decreased posttreatment, while TIMP-4 (mean fold change of 0.893; $P = 0.0412$) levels were decreased posttreatment in comparison to pretreatment levels. HO-1 levels were not altered with treatment.

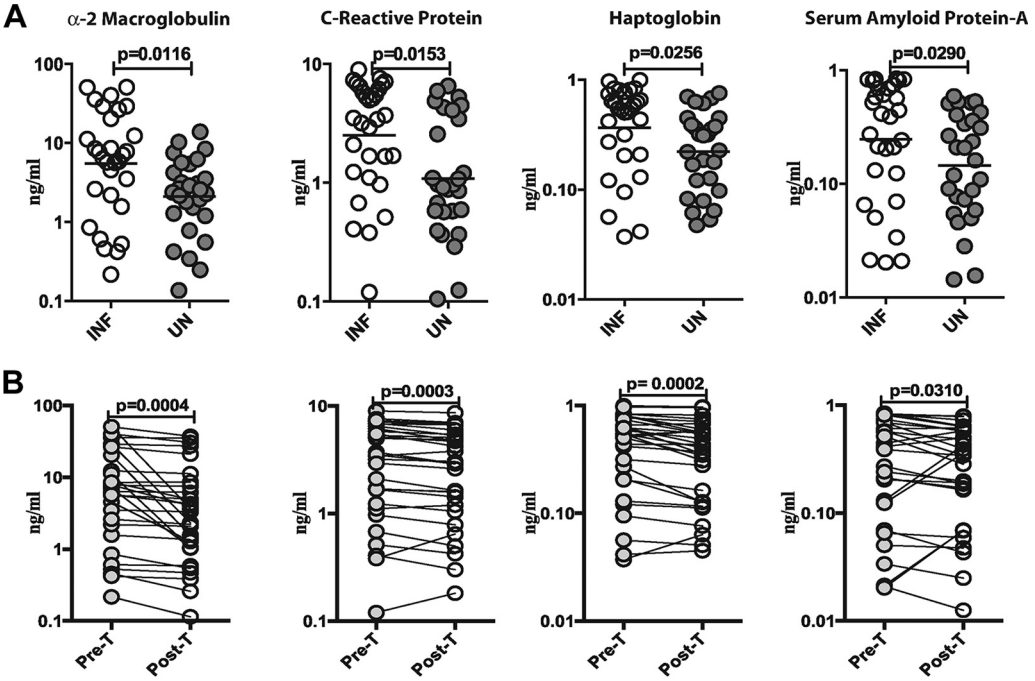

FIG 2 *S. stercoralis* infection is associated with elevated levels of α-2M, CRP, haptoglobin, and SAA. (A) Plasma levels of α-2 macroglobulin, CRP, haptoglobin, and SAA in *S. stercoralis*-infected ($n = 30$) or uninfected ($n = 28$) individuals were measured by ELISAs. Data are shown as scatter plots, with bars representing the geometric means. *P* values were calculated by using the Mann-Whitney test. (B) Plasma levels of α-2 macroglobulin, CRP, haptoglobin, and SAA in *S. stercoralis*-infected individuals pre-T ($n = 30$) and 6 months following treatment (post-T) were measured by ELISAs. *P* values were calculated by using the Wilcoxon matched-pair test.

***S. stercoralis* infection is associated with elevated levels of IL-6, IL-8, MCP-1, and MIP-1β.** To assess proinflammatory cytokines associated with *S. stercoralis* infection, we measured the plasma levels of IL-6, IL-8, MCP-1, and MIP-1β in INF and UN individuals. As shown in Fig. 4A, INF individuals had significantly elevated levels of IL-6 (GM of 68.92 pg/ml in INF versus 39.72 pg/ml in UN individuals; $P = 0.0004$), IL-8 (GM of 152.6 pg/ml in INF versus 66.78 pg/ml in UN individuals; $P = 0.0006$), MCP-1 (GM of 240.8 pg/ml in INF versus 142.3 pg/ml in UN individuals; $P = 0.0006$), and MIP-1β (GM of 188.1 pg/ml in INF versus 74.83 pg/ml in UN individuals; $P = 0.0007$) in comparison to those in UN individuals. However, there were no significant alterations in the levels of IL-6, IL-8, MCP-1, and MIP-1β 6 months following anthelmintic treatment compared to pretreatment levels (Fig. 4B).

Positive relationships between LPS levels and levels of microbial translocation markers, acute-phase proteins, MMP-1, and cytokines. The relationships among circulating levels of LPS and microbial translocation markers, acute-phase proteins, inflammatory markers, and proinflammatory cytokines were next assessed. As shown in Fig. 5A, the levels of LPS exhibited a highly significant positive correlation with the plasma levels of iFABP ($r = 0.4340$; $P = 0.0007$), LBP ($r = 0.4450$; $P = 0.0005$), and EndoCAb ($r = 0.4309$; $P = 0.0007$). Similarly, as shown in Fig. 5B, levels of LPS also exhibited a significantly positive correlation with the levels of α-2M ($r = 0.4704$; $P = 0.0002$), CRP ($r = 0.4261$; $P = 0.0009$), haptoglobin ($r = 0.4281$; $P = 0.0008$), and SAA ($r = 0.4795$; $P = 0.0001$). MMP-1 levels ($r = 0.3699$; $P = 0.0043$) also exhibited significantly positive correlations with circulating levels of LPS (Fig. 5C). Among cytokines, levels of IL-6 ($r = 0.3225$; $P = 0.0136$) and MCP-1 ($r = 0.3505$; $P = 0.0070$) were positively correlated with the levels of LPS (Fig. 5C).

DISCUSSION

Microbial translocation is characterized by the translocation of microorganisms, or microorganism products, from the lumen of the GI tract into the systemic circulation

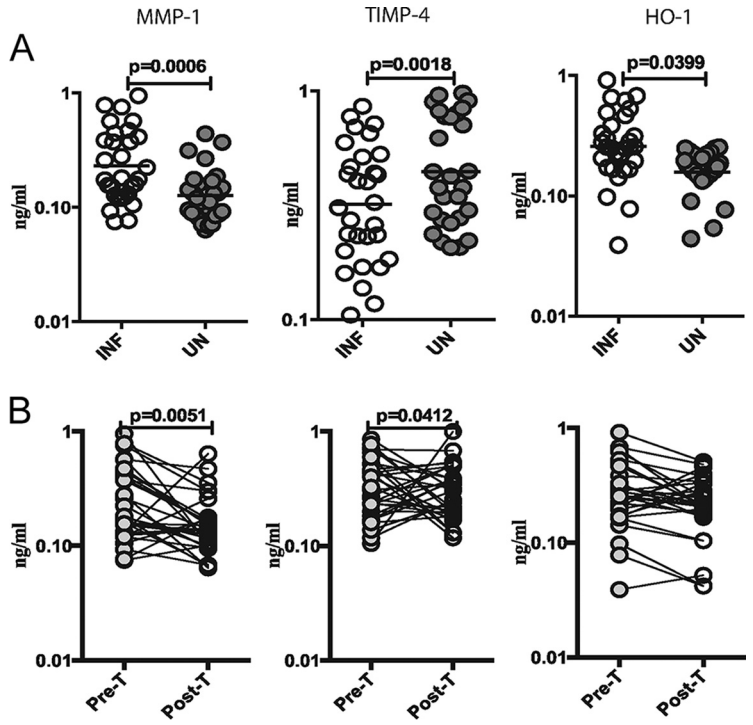

FIG 3 *S. stercoralis* infection is associated with elevated levels of MMP-1 and HO-1 and decreased levels of TIMP-4. (A) Plasma levels of MMP-1, TIMP-4, and HO-1 in *S. stercoralis*-infected ($n = 30$) or uninfected ($n = 28$) individuals were measured by ELISAs. Data are shown as scatter plots, with the bars representing the geometric means. *P* values were calculated by using the Mann-Whitney test. (B) Plasma levels of MMP-1, TIMP-4, and HO-1 in *S. stercoralis*-infected individuals pre-T ($n = 30$) and 6 months following treatment (post-T) were measured by ELISAs. *P* values were calculated by using the Wilcoxon matched-pair test.

and can have detrimental consequences, including disruption of the barrier function of the intestinal epithelium (16, 17). Previous reports have shown that infection with intestinal helminths is characterized by an enhanced leakiness of the intestinal epithelium, mediated by activated mast cells, which can lead to the movement of bacterial LPS into the portal circulation (10, 11). Studies using experimental animal models suggest that intestinal injury and systemic endotoxemia are two major factors leading to morbidity in helminth infections (11, 18). Infection with the enteric nematodes *Trichinella spiralis* and *Strongyloides venezuelensis* has been shown to be associated with an enhanced leakiness of the intestinal epithelium and the translocation of LPS into the circulation (10, 11). Even in nonintestinal helminth infections, such as infections by schistosomes that reside in the mesenteric veins, damage caused by worm eggs traversing the gastrointestinal epithelium can result in the systemic translocation of bacteria (18, 19). Our previous studies suggest that circulating microbial products, acute-phase proteins, and inflammatory markers are associated with the pathogenesis of disease in lymphatic filarial infection (20). We have also shown that hookworm infection also induced microbial translocation, associated with perturbations of the T cell and antigen-presenting arms of the immune system (3).

We examined five important circulating microbial or related products in our study. The level of LPS (a key indicator of microbial translocation) was found to be significantly elevated in INF individuals. This was accompanied by significant increases in the levels of sCD14 and iFABP. Although increased levels of LBP are a common feature of microbial translocation in other infections (16, 21, 22), we did not observe any significant alterations in LBP levels in *S. stercoralis* infections. This could be the result of the internalization of LBP by cells of the innate immune system. The level of LPS is commonly measured to assess the degree of microbial translocation quantitatively, and

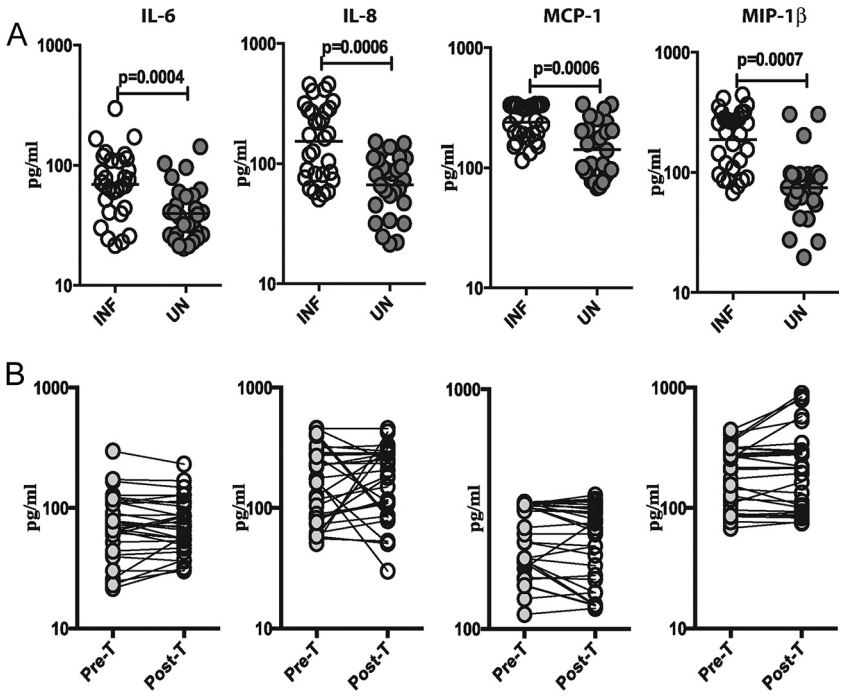

FIG 4 *S. stercoralis* infection is associated with elevated levels of IL-6, IL-8, MCP-1, and MIP-1β. (A) Plasma levels of IL-6, IL-8, MCP-1, and MIP-1β in *S. stercoralis*-infected ($n = 30$) or uninfected ($n = 28$) individuals were measured by ELISAs. Data are shown as scatter plots, with bars representing the geometric means. *P* values were calculated by using the Mann-Whitney test. (B) Plasma levels of IL-6, IL-8, MCP-1, and MIP-1β in *S. stercoralis*-infected individuals pre-T ($n = 30$) and 6 months following treatment (post-T) were measured by ELISAs. *P* values were calculated by using the Wilcoxon matched-pair test.

plasma levels are directly associated with the degree of intestinal permeability (23). sCD14 is produced primarily following the activation of monocytes and macrophages (24, 25). iFABP is an intracellular epithelial protein in the stomach and small and large intestinal mucosa and appears in the circulation after epithelial damage. Hence, plasma iFABP levels are considered useful and early markers of intestinal ischemia (26). A previous study on helminth infection showed that iFABP is a marker of microbial translocation (3), a finding that is further confirmed here. Interestingly, our data also clearly demonstrate a direct association of microbial translocation with active *S. stercoralis* infection, as treatment significantly reduced the elevated levels of microbial translocation markers in INF individuals.

Systemic inflammatory reactions are a prominent feature of many parasitic diseases and can reflect either acute or chronic infection (27). Acute-phase proteins are derived primarily from the liver, and plasma concentrations are believed to be a reflection of the response to proinflammatory cytokines (28). Measurement of the levels of acute-phase proteins is of clinical importance in determining the presence and extent of inflammatory tissue damage as well as in providing diagnostic and prognostic information (29, 30), Circulating microbial products are well-known inducers of acute-phase proteins, with the levels of SAA and haptoglobin being known to be markedly elevated following challenge with LPS (31). An elevated level of SAA was observed for *Nippostrongylus brasiliensis* infection (32). Elevated levels of CRP in individuals with lymphatic filarial disease have been reported (33), and we reported increased levels of acute-phase proteins such as α-2M, SAA, and haptoglobin in individuals with active filarial infection previously (20). In the present study, we measured plasma levels of the acute-phase proteins α-2M, CRP, haptoglobin, and SAA. We show that the levels of CRP, α-2M, SAA, and haptoglobin are also significantly elevated in individuals with *S. stercoralis* infection. Moreover, all the acute-phase proteins were observed to be present at significantly low levels at posttreatment time points compared to baseline levels, indicating

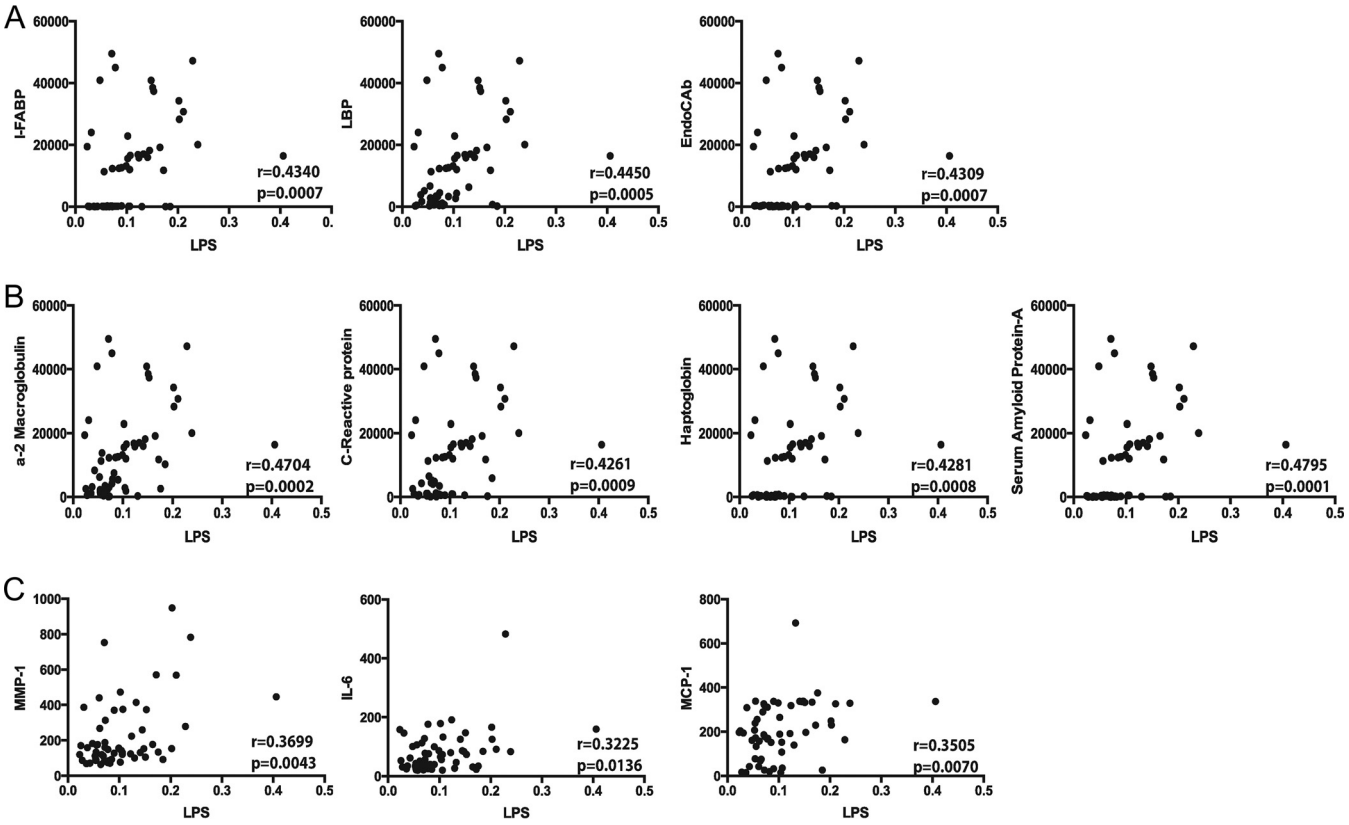

FIG 5 Positive relationships between LPS levels and levels of microbial translocation markers, acute-phase proteins, MMP-1, and cytokines. (A) Plasma levels of LPS were positively correlated with sCD14, iFABP, LBP, and EndoCAb levels in *S. stercoralis*-infected individuals ($n = 30$). (B) Plasma levels of LPS were positively correlated with α-2M, CRP, haptoglobin, and SAA levels in *S. stercoralis*-infected individuals ($n = 30$). (C) Plasma levels of LPS were positively correlated with MMP-1, IL-6, and MCP-1 levels. *P* and *r* values were calculated by using the Spearman rank correlation test at 95% confidence intervals.

that *S. stercoralis* infection is directly associated with an enhanced acute-phase response.

MMPs are zinc-dependent proteases associated with the breakdown of the extracellular matrix and tissue remodeling. TIMPs are specific inhibitors of MMPs and help control tissue pathology (34, 35). Thus, various MMPs have been shown to be upregulated and may play a role in mediating pathology in various parasitic infections such as malaria and neurocysticercosis (36, 37). While TIMPs are clearly known to bind and inhibit the function of MMPs, it is also becoming evident that TIMP binding to MMP can enhance the activity of certain MMPs (35). Helminth infections are known to modulate the expression pattern of MMPs and TIMPs (38) and, in the case of certain helminth infections, can act as inducers of these factors themselves (20). In the present study, *S. stercoralis*-infected individuals exhibited significantly elevated levels of MMP-1 and significantly lower levels of TIMP-4. Similarly, ulcerative colitis patients have been shown to exhibit elevated levels of MMP-1. Interestingly, when these patients were treated with metronidazole for 6 weeks, there was a significant decrease in MMP-1 levels, with a corresponding improvement of clinical symptoms and histological findings of irritable bowel disease (IBD) (39). Similarly, we have also shown that MMP-1 and TIMP-4 levels were decreased after treatment. Our data suggest that *S. stercoralis* infection plays a modulatory role with respect to the production of profibrotic factors and hence can have an impact on the degree of pathology. HO-1 is an enzyme that plays a critical role in defending the body against oxidant-induced injury during inflammatory processes (7, 40). Pathogen-associated molecular patterns (PAMPs) such as LPS, lipoteichoic acid, and peptidoglycan as well as several proinflammatory cytokines can induce HO-1 expression (41). The activation of HO-1 may thus act as an endogenous defensive mechanism to reduce inflammation and tissue injury in the

intestinal tract (42–44) and may also be protective against lethal endotoxemia (45). HO-1 levels were significantly elevated in the systemic circulation in *S. stercoralis*-infected individuals in the present study. Thus, HO-1 induction may be due to an endotoxin(s), and it might reflect systemic inflammation but also a compensatory response to combat subclinical endotoxemia in *S. stercoralis* infections.

Inflammatory cytokines are intricately linked to the induction of both circulating microbial products and acute-phase proteins. Previous reports suggested that IL-6 and IL-8 are morbidity markers in acute and chronic filarial diseases (46). Detection of microbial invasion by cells of the innate immune system usually results in the increased production of proinflammatory cytokines such as IL-6, IL-8, MCP-1, and MIP-1β. Our data also reveal an enhancement of the systemic levels of the proinflammatory cytokines IL-6, IL-8, MCP-1, and MIP-1β in individuals with *S. stercoralis* infection. However, we did not detect any significant alteration in the levels of cytokines after treatment.

Studies of HIV infection have demonstrated a direct association between levels of microbial translocation markers such as LPS and levels of proinflammatory cytokines (16, 21). Our previous study of individuals with filarial pathology also revealed a positive association of LPS with proinflammatory cytokines (20). In agreement with data from such studies, our examination of *S. stercoralis*-infected individuals also reveals a significantly positive relationship between LPS and iFABP, LBP, EndoCAb, acute-phase proteins, MMP-1, and proinflammatory cytokines. Our study clearly implicates an association of LPS, acute-phase proteins, and several of the proinflammatory cytokines with *S. stercoralis*-induced subclinical inflammation and/or pathology. Our study, to our knowledge, is the first to describe the process of microbial translocation in *S. stercoralis* infection. Our posttreatment data considerably strengthen the association of *S. stercoralis* infection with increased gut permeability and altered inflammatory responses and argue for a causal relationship. It is not known whether the natural history of infection is associated with the resolution of this inflammatory/translocation process. In addition, while treatment of hookworm infection has been associated with a reversal of the elevated levels of microbial translocation markers (3), HIV treatment is typically not associated with such a reversal (17). This might reflect differences in dysbiosis in different infectious settings.

In summary, our study describes a novel relationship between intestinal helminth infection and the process of microbial translocation, a process that might contribute to immune-mediated pathogenesis. A major potential significance of these findings is that hyperinfection or disseminated infection with *S. stercoralis* could be accompanied by an accelerated and exaggerated occurrence of microbial translocation and systemic inflammation, making it necessary to investigate novel therapies that restore intestinal barrier integrity and reduce microbial translocation and inflammation. These findings also suggest new targets for modulation of the pathology associated with *S. stercoralis* infection.

MATERIALS AND METHODS

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 (United States) and the National Institute for Research in Tuberculosis (India), and informed written consent was obtained from all participants.

Study population. We studied a total of 58 individuals comprising 30 clinically asymptomatic, *S. stercoralis*-infected (here INF) individuals and 28 uninfected, endemic, healthy (here UN) individuals in Tamil Nadu, South India (Table 1). These individuals were all recruited from a rural population by screening of individuals for helminth infection by stool microscopy and serology. None of these individuals had previous anthelmintic treatment, a history of helminth infections, or HIV. Follow-up was performed 6 months following recruitment and treatment.

S. stercoralis infection was diagnosed by the presence of IgG antibodies to the 31-kDa recombinant antigen (termed NIE), as described previously (47, 48). This was further confirmed by specialized stool examination with nutrient agar plate cultures (49). None of the individuals in the study population had lymphatic filariasis or infection by other intestinal helminths (based on stool microscopy). All INF individuals were treated with single doses of ivermectin and albendazole, and follow-up blood draws were performed 6 months later. Treated individuals were *S. stercoralis* infection negative by stool

TABLE 1 Demographic profile of the study population

Parameter	Value for study group	
	INF (n = 30)	UN (n = 28)
No. of males/no. of females	17/13	11/17
Median age (yr) (range)	42 (20–64)	40 (20–63)
NIE ELISA result	Positive	Negative

microscopy at 6 months posttreatment. All UN individuals were anti-*S. stercoralis* NIE negative and negative for filarial and other intestinal helminths.

Microbial translocation markers. To inactivate plasma proteins, plasma samples were heated to 75°C for 5 min. LPS levels were measured by using a limulus amebocyte lysate assay (Cell Sciences Hycult Biotech, Canton, MA, USA) according to the manufacturer's protocols. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to measure plasma levels of LBP, EndoCAb, iFABP (all from Cell Sciences Hycult Biotech), and sCD14 (R&D Systems, Minneapolis, MN, USA).

Acute-phase proteins. Plasma levels of α-2M, CRP, haptoglobin, and SAA were measured by using the Bioplex multiplex ELISA system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions.

Inflammatory markers (MMP-1, TIMP-4, and HO-1) and proinflammatory cytokines. Plasma levels of MMP-1, TIMP-4 (R&D Systems, Minneapolis, MN, USA), and HO-1 (Enzo Life Sciences, Inc.) were measured by using ELISA kits. Plasma levels of the cytokines IL-6, IL-8, MCP-1, and MIP-1β were measured by using the multiplex ELISA system.

Statistical analysis. Data analyses were performed by using GraphPad PRISM (GraphPad Software, Inc., San Diego, CA, USA). GMs were used for measurements of central tendency. Statistically significant differences were analyzed by using the nonparametric Mann-Whitney U test and the Wilcoxon matched-pair test. Multiple comparisons were corrected by using Holm's correction. Correlations were calculated by the Spearman rank correlation test.

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