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Systemic Cytokine Profiles in *Strongyloides stercoralis* Infection and Alterations following Treatment

Rajamanickam Anuradha,[a] Saravanan Munisankar,[a] Yukti Bhootra,[a] Jeeva Jagannathan,[a] Chandrakumar Dolla,[b] Paul Kumaran,[b] Kui Shen,[c] Thomas B. Nutman,[d] Subash Babu[a,d]

National Institutes of Health-NIRT-International Center for Excellence in Research, Chennai, India[a]; National Institute for Research in Tuberculosis, Chennai, India[b]; Bioinformatics and Computational Biosciences Branch, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA[c]; Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA[d]

***Strongyloides stercoralis* is a soil-transmitted helminth organism that infects ~50 to 100 million people worldwide. Despite its widespread prevalence, very little is known about the immune response that characterizes human *S. stercoralis* infection. To study the systemic cytokine profile characteristic of *Strongyloides* infection, we measured the circulating levels of a large panel of pro- and anti-inflammatory cytokines in asymptomatic, infected individuals ($n = 32$) and compared them to those in uninfected, controls ($n = 24$). Infected individuals exhibited significantly lower circulating levels of proinflammatory cytokines (gamma interferon [IFN-γ], tumor necrosis factor alpha [TNF-α], and interleukin-1β [IL-1β]) and significantly higher levels of anti-inflammatory cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37, and transforming growth factor β [TGF-β]). Moreover, treatment of *Strongyloides* infection resulted in a significant reversal of the cytokine profile, with increased levels of proinflammatory (IFN-γ, TNF-α, IL-2, IL-17A, IL-17F, IL-22, IL-23, and IL-1β) and decreased levels of anti-inflammatory (IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37, and TGF-β) cytokines following treatment. Thus, *S. stercoralis* infection is characterized by alterations in the levels of systemic cytokines, reflecting major alterations in the underlying immune response to this chronic helminth infection.**

Helminths are multicellular eukaryotic worms that reside for long periods of time in their hosts, eliciting type 2 and regulatory T cell immune responses. Among the common helminth parasites known to establish chronic infections in humans, *Strongyloides stercoralis*, the causative agent of strongyloidiasis, infects over 50 million people worldwide (1). *S. stercoralis* is unique in its ability to exist in a free-living and auto-infective cycle (2, 3). *Strongyloides* infection is often clinically asymptomatic and long lasting due, in large part, to the parasites' auto-infective life cycle and their ability to modulate or evade the host immune system (2, 3). Chronic *Strongyloides* infection can also cause cutaneous, gastrointestinal (GI), and/or pulmonary symptoms and, in the face of immune suppression, may present as hyperinfection syndrome or disseminated strongyloidiasis, conditions that are potentially fatal (4).

Animal models have suggested a role for both innate and adaptive immune mechanisms in mediating resistance to infection (5). The innate response is primarily mediated by eosinophils and interleukin-5 (IL-5), with neutrophils and macrophages playing accessory roles (6, 7). The adaptive immune system specifically involves type 2 responses, with Th2 cells secreting IL-4, IL-5, and IL-13, B cells producing IgG and IgE, and innate lymphoid cells secreting IL-5 and IL-13 (5). In contrast, regulatory T cells help blunt exuberant Th2 responses (8), and the interplay among Th1, Th2, and regulatory T cell responses appears to be crucial in the defense against this infection (4). Very little data are available on the role of these responses in human infection. It has been shown that Th2 responses are essential to protect against hyperinfection (9, 10) and that individuals with strongyloidiasis develop specific antibodies of the IgG, IgA, IgM, and IgE isotypes (11, 12).

To study the association of systemic cytokines (both pro- and anti-inflammatory) with asymptomatic infection, we compared circulating levels of these cytokines in *Strongyloides*-infected and -uninfected individuals. We also examined the effect of antihelminth treatment by comparing circulating levels of these cytokines before and after treatment. Our study shows that *Strongyloides* infection is associated with elevated anti-inflammatory and depressed proinflammatory plasma cytokines, a pattern that is reversible following treatment of 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 (USA) 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 34 clinically asymptomatic, *Strongyloides*-infected (here, infected) individuals and 24 uninfected, healthy (here, uninfected) individuals in Tamil Nadu, South India (Tables 1 and 2). These individuals were all recruited from a rural population by screening of individuals for helminth infection by stool microscopy and serology. Inclusion criteria were age of 18 to 65 years and willingness to give blood and stool samples for examination; exclusion criteria were past antihelminth treatment, other helminth infections, or HIV infection. Follow-up was performed at 6 months following recruitment and treatment. *Strongyloides* infection was diagnosed by the presence of IgG antibodies to the recombinant antigen, NIE, as de-

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Address correspondence to Subash Babu, sbabu@mail.nih.gov.

TABLE 1 Demographic profile of infected and uninfected individuals

Parameter	Value for the group[a]	
	Infected (n = 32)	Uninfected (n = 24)
No. of male subjects	19	16
No. of female subjects	13	8
Mean age (range [yr])	36 (20–61)	40 (20–60)
NIE ELISA result	Positive	Negative
Clinical status	Healthy	Healthy
Symptom(s)	None	None
Socioeconomic status	Rural workers	Rural workers
Result of stool examination for *S. stercoralis*	Positive (negative following treatment)	Negative
Presence of other helminth infections	Negative	Negative

[a] Differences in the values for gender and age between infected and uninfected groups were not significant.

scribed previously (13, 14). This was further confirmed by specialized stool examination with nutrient agar plate cultures (15). None of the study population had lymphatic filariasis or other intestinal helminths (based on stool microscopy). All infected individuals were treated with single doses of ivermectin and albendazole, and follow-up blood draws were obtained 6 months later (Table 3). All uninfected individuals were anti-*Strongyloides* NIE negative and negative for filarial and other intestinal helminths.

Hematological parameters. Leukocyte counts and differentials were performed on all individuals using an AcT 5Diff hematology analyzer (Beckman Coulter).

ELISA. Plasma levels of gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), IL-2, IL-4, IL-5, IL-10, IL-13, IL-1β, IL-17A, IL-17F, IL-18, IL-22, IL-23, IL-27, IL-37, transforming growth factor β (TGF-β) (all, R&D Systems) and IL-9 (eBioscience) were measured by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions. All samples were run in duplicates.

Statistical analysis. Data analyses were performed using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). Geometric means (GM) were used for measurements of central tendencies. Comparisons were made using either a Mann-Whitney U test for comparisons between two groups or a Wilcoxon signed-rank test for comparisons within groups. Corrections for multiple comparisons were performed by Holm's correction. Multidimensional scaling (MDS) analysis was performed on log2-transformed plasma cytokine levels for infected versus uninfected individuals using the R language.

TABLE 2 Hematological profile of infected and uninfected individuals

Factor[a]	GM (range) for the group		
	Infected (n = 32)	Uninfected (n = 24)	P value[b]
Hb (gm/dl)	12.48 (4.9–18.6)	11.14 (4.9–16.3)	0.0378
RBC (10^6/ml)	4.5 (3.5–6.06)	4.067 (2.11–5.84)	0.0391
WBC (10^3/ml)	8.83 (5.8–16.9)	8.81 (5.8–13.7)	NS
HCT (%)	36.85 (19.5–53)	33.55 (15–47.4)	NS
PLT (10^3/ml)	261.91 (140–417)	258.18 (198–363)	NS
Neutrophils (10^3/ml)	5.3 (3.3–7.2)	5.5 (4.2–6.89)	NS
Lymphocytes (10^3/ml)	2.63 (1.45–3.71)	3.04 (2.02–4.27)	0.0248
Monocytes (10^3/ml)	0.67 (0.38–1.2)	0.71 (0.43–1.09)	NS
Eosinophils (10^3/ml)	0.68 (0.11–3.45)	0.43 (0.18–1.1)	0.0354
Basophils (10^3/ml)	0.09 (0.02–0.33)	0.09 (0.05–0.35)	0.0028

[a] Hb, hemoglobin; RBC, red blood cells; WBC, white blood cells; HCT, hematocrit; PLT, platelets.
[b] NS, not significant.

TABLE 3 Hematological profile of infected individuals before and after treatment

Factor[a]	GM (range) for the group[b]	
	Pretreatment (n = 32)	Posttreatment (n = 32)
Hb (gm/dl)	12.48 (4.9–18.6)	12.59 (4.6–18.5)
RBC (10^6/ml)	4.5 (3.5–6.06)	4.54 (3.49–6.06)
WBC (10^3/ml)	8.83 (5.8–16.9)	8.03 (5.6–11.2)
HCT (%)	36.85 (19.5–53)	37.47 (15.9–53)
PLT (10^3/ml)	261.91 (140–417)	262.77 (134–417)
Neutrophils (10^3/ml)	5.3 (3.3–7.2)	5.1 (3.31–6.89)
Lymphocytes (10^3/ml)	2.63 (1.45–3.71)	2.84 (1.52–4.29)
Monocytes (10^3/ml)	0.67 (0.38–1.2)	0.67 (0.38–1.24)
Eosinophils (10^3/ml)	0.68 (0.11–3.45)	0.66 (0.11–3.45)
Basophils (10^3/ml)	0.09 (0.02–0.33)	0.08 (0.02–0.33)

[a] Hb, hemoglobin; RBC, red blood cells; WBC, white blood cells; HCT, hematocrit; PLT, platelets.
[b] For all parameters, differences between pretreatment and posttreatment values were not significant.

RESULTS

Study population characteristics. The baseline characteristics, including demographic and hematological features of the study population, are shown in Tables 1 and 2. As can be seen, infected individuals exhibited significantly higher levels of red blood cells, hemoglobin, and absolute eosinophil counts than uninfected individuals but significantly lower levels of absolute lymphocyte and basophil counts. In contrast, no significant differences in age, gender, socioeconomic status, or other hematological parameters were observed between the two groups. There were no significant differences in any of the hematological parameters following antihelminth treatment of infected individuals compared to pretreatment values (Table 3). However, NIE ELISA levels decreased significantly in all of the individuals following treatment (Fig. 1).

***Strongyloides* infection is associated with diminished systemic levels of proinflammatory cytokines.** To determine the systemic proinflammatory cytokine profile in *Strongyloides* infection, we measured the circulating levels of IFN-γ, TNF-α, IL-2, IL-17A, IL-17F, IL-18, IL-22, IL-23, and IL-1β in asymptomatic,

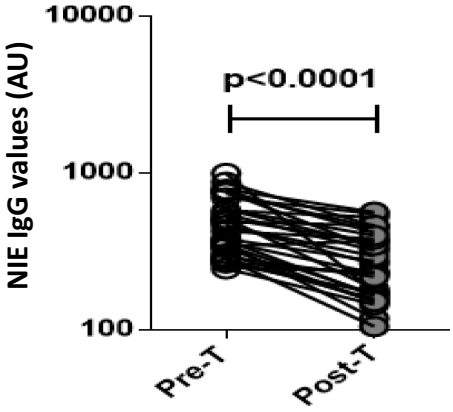

FIG 1 Treatment of *Strongyloides* infection is associated with decreased NIE-IgG ELISA values. The NIE-IgG levels (in arbitrary units, AU) were measured by ELISA in infected (n = 32) individuals before (Pre-T) and 6 months after (Post-T) antihelminth treatment. The results are shown as line diagrams with each line representing a single individual. The P value was calculated using a Wilcoxon signed-rank test.

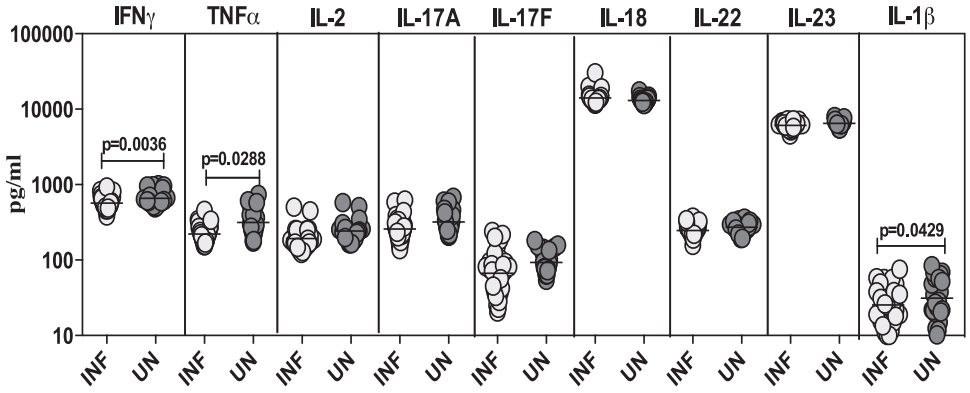

FIG 2 *Strongyloides* infections are associated with diminished plasma levels of proinflammatory cytokines at homeostasis. The plasma levels of proinflammatory cytokines (IFN-γ, TNF-α, IL-2, IL-17A, IL-17F, IL-18, IL-22, IL-23, and IL-1β) were measured by ELISA in infected (INF; $n = 32$) and uninfected (UN; $n = 24$) individuals. The results are shown as scatter plots with each circle representing a single individual and the bar representing the GM. *P* values were calculated using a Mann-Whitney test with Holm's correction for multiple comparisons.

infected and in control, uninfected individuals (Fig. 2). As shown in Fig. 2, the systemic levels of the proinflammatory cytokines IFN-γ (GM of 568.9 pg/ml in infected versus 656.1 pg/ml in uninfected subjects; $P = 0.0036$), TNF-α (GM of 222.1 pg/ml versus 315.2 pg/ml; $P = 0.0288$), and IL-1β (GM of 25.5 pg/ml versus 31.4 pg/ml; $P = 0.0429$) were significantly lower in infected than in uninfected individuals.

***Strongyloides* infection is associated with elevated levels of anti-inflammatory cytokines.** To determine the systemic anti-inflammatory cytokine profiles in *Strongyloides* infection, we measured the circulating levels of IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37, and TGF-β in infected and uninfected individuals (Fig. 3). As shown in Fig. 3, the systemic levels of the cytokines IL-4 (GM of 2,219.8 pg/ml in infected versus 1,746.1 pg/ml in uninfected; $P = 0.0008$), IL-5 (GM of 85.4 pg/ml versus 44.4 pg/ml; $P = 0.0007$), IL-9 (GM of 274.2 pg/ml versus 212.6 pg/ml; $P = 0.0006$), IL-10 (GM of 415.2 pg/ml versus 280.7 pg/ml; $P = 0.0035$), IL-13 (GM of 68.6 pg/ml versus 18.9 pg/ml; $P = 0.0412$), IL-27 (GM of 393.1 pg/ml versus 282.9 pg/ml; $P = 0.0429$), IL-37 (GM of 1,472.2 pg/ml versus 1,025.8 pg/ml; $P = 0.0454$), and TGF-β (GM of 3,416.9 pg/ml versus 2,809.2 pg/ml; $P = 0.0248$) were all significantly higher in infected than in uninfected individuals.

Alterations in systemic levels of pro- and anti-inflammatory cytokines following treatment of *Strongyloides* infection. To determine the effect of treatment on the systemic proinflammatory cytokine profile in *Strongyloides* infection, we measured the circulating levels of IFN-γ, TNF-α, IL-2, IL-17A, IL-17F, IL-18, IL-22, IL-23, and IL-1β in infected individuals before and after treatment (Fig. 4). As shown in Fig. 4, the systemic levels of the proinflammatory cytokines IFN-γ (GM of 568.9 pg/ml pretreatment versus 670.7 pg/ml posttreatment; $P = 0.0009$), TNF-α (GM of 222.1 pg/ml versus 256.3 pg/ml; $P = 0.0008$), IL-2 (GM of 192.7 pg/ml versus 234.3 pg/ml; $P = 0.0133$), IL-17A (GM of 258.8 pg/ml versus 335.5 pg/ml; $P = 0.0114$), IL-18 (GM of 14,031.3 pg/ml versus 14,052.2 pg/ml; $P = 0.0176$), IL-22 (GM of 247.3 pg/ml versus 266.4 pg/ml; $P = 0.0315$), IL-23 (GM of 6,092.3 pg/ml versus 6,424.3 pg/ml; $P = 0.0280$), and IL-1β (GM of 25.5 pg/ml versus 34.1 pg/ml; $P = 0.0475$) were significantly increased from pretreatment levels 6 months following treatment.

To determine the effect of treatment on the systemic anti-in-

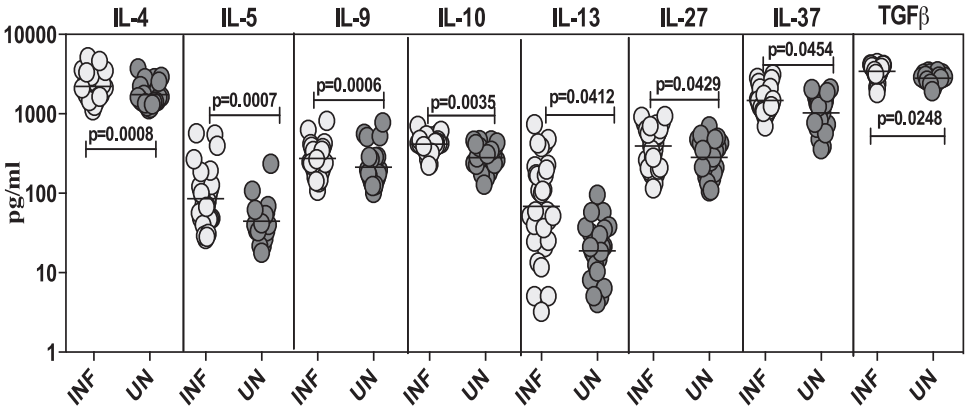

FIG 3 *Strongyloides* infections are associated with heightened plasma levels of anti-inflammatory cytokines at homeostasis. The plasma levels of anti-inflammatory cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37, and TGF-β) were measured by ELISA in infected (INF; $n = 32$) and uninfected (UN; $n = 24$) individuals. The results are shown as scatter plots with each circle representing a single individual and the bar representing the GM. *P* values were calculated using a Mann-Whitney test with Holm's correction for multiple comparisons.

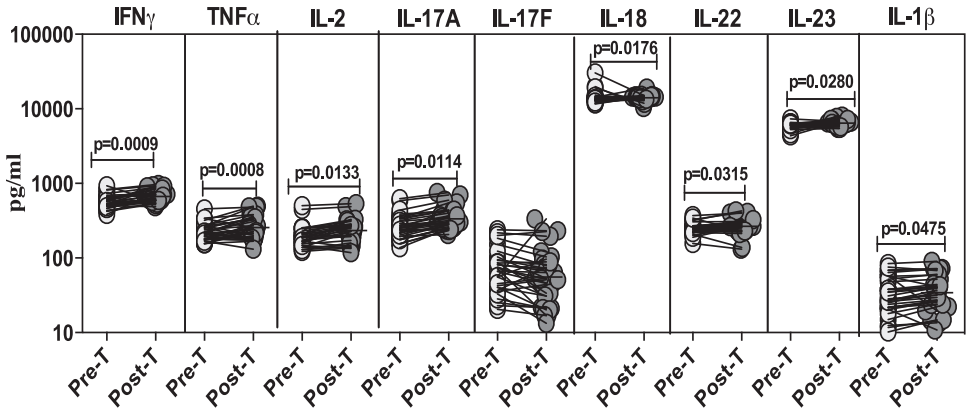

FIG 4 Treatment of *Strongyloides* infection is associated with heightened plasma levels of proinflammatory cytokines. The plasma levels of proinflammatory cytokines (IFN-γ, TNF-α, IL-2, IL-17A, IL-17F, IL-18, IL-22, IL-23, and IL-1β) were measured by ELISA in infected ($n = 32$) individuals before (Pre-T) and 6 months after (Post-T) antihelminth treatment. The results are shown as line diagrams with each line representing a single individual. *P* values were calculated using a Wilcoxon signed-rank test with Holm's correction for multiple comparisons.

flammatory cytokine profile in *Strongyloides* infection, we measured the circulating levels of IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37, and TGF-β in infected individuals before and after treatment (Fig. 5). As shown in Fig. 5, the systemic levels of the anti-inflammatory cytokines IL-4 (GM of 2,219.8 pg/ml pretreatment versus 1,581.7 pg/ml posttreatment; $P = 0.0008$), IL-5 (GM of 85.5 pg/ml versus 52.4 pg/ml; $P = 0.0007$), IL-9 (GM of 274.2 pg/ml versus 237.7 pg/ml; $P = 0.0006$), IL-10 (GM of 415.2 pg/ml versus 389.8 pg/ml; $P = 0.0010$), IL-13 (GM of 68.6 pg/ml versus 23.3 pg/ml; $P = 0.0008$), IL-27 (GM of 393.1 pg/ml versus 353.4 pg/ml; $P = 0.0021$), IL-37 (GM of 1,472.2 pg/ml versus 1,236.9 pg/ml; $P = 0.0088$), and TGF-β (GM of 3,416.9 pg/ml versus 3,103.1 pg/ml; $P = 0.0221$) were markedly lower than pretreatment levels 6 months following treatment.

MDS analysis reveals trends of cytokine modulation in *Strongyloides* infection. Because many of these cytokines are coordinately regulated, we sought to visualize the trends in the modulation of cytokines in *Strongyloides* infection by treating the cytokines as interdependent variables using MDS analysis on values for infected versus uninfected individuals (Fig. 6). MDS analysis enables the discrimination and visual clustering of two or more groups wherein individuals with similar patterns of cytokine expression are placed next to each other. As shown in Fig. 6, infected and uninfected individuals visually segregate on an MDS plot, based on their baseline plasma cytokine profiles. Thus, MDS analysis reveals clear trends in the modulation of systemic cytokines in *Strongyloides* infection.

DISCUSSION

Helminth infections are typically associated with a profound modulation of the immune response such that many arms of the immune system, including innate and adaptive immunity, as well as humoral and cellular responses, are affected (16, 17). This is classically exemplified in animal models of helminth infection, wherein proinflammatory responses, including type 1 and type 17 responses, are down-modulated and type 2 and anti-inflammatory responses are typically upregulated (16, 17). This has been shown to be true in human helminth (soil-transmitted and vector-borne) infections as well (18–20). However, scant data exist on the immune response engendered by the relatively common helminth parasite *S. stercoralis*. In this study, we sought to examine

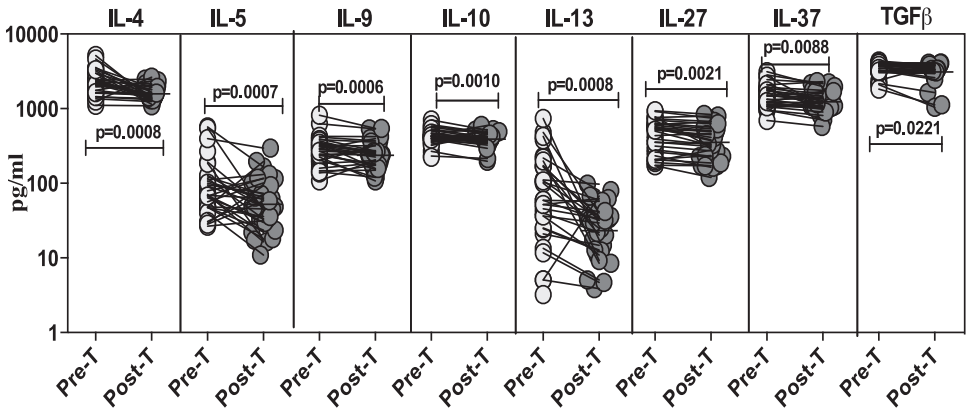

FIG 5 Treatment of *Strongyloides* infection is associated with diminished plasma levels of anti-inflammatory cytokines. The plasma levels of anti-inflammatory cytokines (IL-4, IL-5, IL-9, IL-10, IL-13, IL-27, IL-37, and TGF-β) were measured by ELISA in infected ($n = 32$) individuals before (Pre-T) and 6 months after (Post-T) antihelminth treatment. The results are shown as line diagrams with each line representing a single individual. *P* values were calculated using a Wilcoxon signed-rank test with Holm's correction for multiple comparisons.

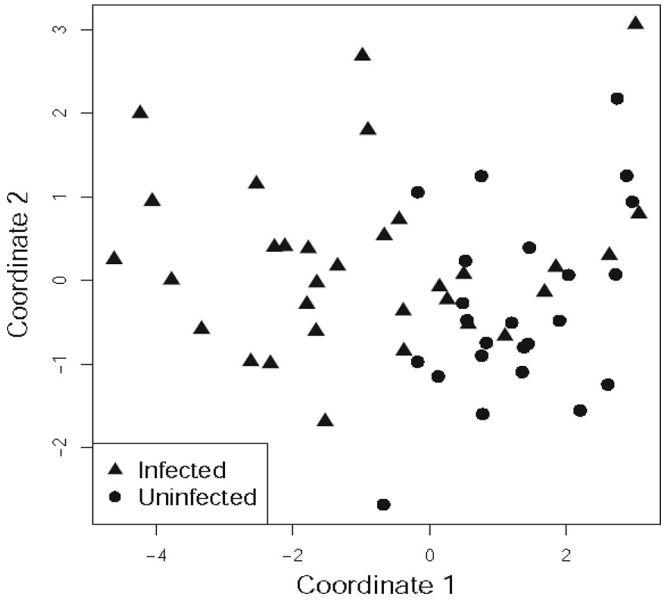

FIG 6 MDS analysis reveals clear trends in the systemic cytokine levels in *Strongyloides* infection. MDS plots for log2-transformed plasma cytokine levels were constructed to analyze the trends in the differences of systemic cytokine levels between infected and uninfected individuals. Each symbol represents one individual based on values of all of the cytokines studied. The percentage of variation is depicted on the two axes. The distance between each symbol represents the relatedness between each individual.

the systemic cytokine profile of *Strongyloides*-infected individuals and the effect of treatment on this cytokine profile.

Helminth infections have the propensity to control harmful inflammatory responses and promote homeostasis through systemic immune responses (21, 22). The induction of both type 2 and regulatory cytokine responses is postulated to contribute to the modulation of proinflammatory, type 1, and type 17 cytokine responses (21, 22). In this study, we examined the systemic cytokine levels of type 1, type 17, and other proinflammatory cytokines in *Strongyloides* infection and demonstrated that *Strongyloides* infection is associated with markedly diminished levels of some of these proinflammatory cytokines. Of greater interest perhaps are our data on the posttreatment responses that suggest that the presence of *Strongyloides* infection has a major influence on the systemic levels of other proinflammatory cytokines as well since the majority of proinflammatory cytokines examined exhibited enhanced levels posttreatment compared to their pretreatment levels. Type 1 cytokines, including IFN-γ, TNF-α, and IL-2, are typically associated with immunity to intracellular pathogens but could also contribute to the development of inflammatory and autoimmune disorders in a variety of settings (23, 24). On a similar note, type 17 cytokines, including IL-17A, IL-17F, IL-22, and IL-23, are associated with protective immunity against fungal pathogens but also contribute heavily to the pathogenesis of inflammatory and autoimmune disorders (25, 26). Finally, other proinflammatory cytokines, including IL-1β and IL-18, are major components driving harmful host inflammation (27). Thus, the fact that *Strongyloides* infection is associated with diminished levels (from normal) of most of the above-mentioned cytokines provides evidence that *Strongyloides* infection has the potential benefit of modulating harmful inflammation and autoimmunity in the host.

Helminth infections are typically characterized by increased production of type 2 cytokines, including IL-4, IL-5, IL-9, and IL-13 (16). In addition, helminth infections are also intricately associated with the increased expression of the regulatory cytokines IL-10 and TGF-β (17). Thus, the immune response induced by most helminth infections is composed of two compartments, a type 2 cytokine response and a regulatory cytokine response, both of which may contribute to the overall protective immune response to these infections (21). In addition, type 2 cytokine responses (particularly IL-13) also play an important role in fibrosis and wound healing, postulated to help heal the tissue damage induced by many tissue-invasive helminth parasites (28). Our study confirms data from both animal models and human infections for which predominant type 2 and regulatory cytokine responses in the setting of chronic, asymptomatic helminth infection have been reported. Both type 2 (IL-4, IL-5, IL-9, and IL-13) and regulatory (IL-10 and TGF-β) cytokines were present at markedly higher levels in infected individuals, and this was reversed following treatment. Of additional interest, we have also explored the role of two other anti-inflammatory cytokines in this infection and demonstrated that IL-27 and IL-37 are both present at elevated levels in infected individuals and that these levels are significantly diminished following therapy. IL-27 was initially described as a Th1-promoting factor, but subsequent studies have demonstrated its anti-inflammatory role (29–31). IL-27 has been shown to convert activated $CD4^+$ T cells into IL-10-producing Th1 cells or Tr1 cells, to suppress the production of IL-2, and to downregulate Th17 responses (32). IL-37 belongs to the IL-1 family of cytokines, but, unlike the other members of this family, its main role is the downregulation of inflammation (33–35). The anti-inflammatory activity of IL-37 appears to be IL-10 independent (35). Our study is the first to our knowledge to examine IL-27 and IL-37 in helminth infections and suggests that these cytokines could have immunoregulatory effects in *Strongyloides* infection.

Although *Strongyloides* is an intestinal helminth infection, it is clearly associated with profound alterations in the systemic cytokine response, a finding that may relate to the auto-infective cycle seen in *Strongyloides*. It has been postulated that this parasite can trigger a potent immune response in the gut that stimulates activated GI-associated dendritic cells to migrate through the lymphatic ducts to stimulate Th2 cell and regulatory T and B cell responses in the draining lymph nodes (21). In addition, excretory/secretory products might also disseminate and contribute to the development of systemic cytokine responses (36). Finally, since *S. stercoralis* migrates from the skin to the lungs through the circulation and then subsequently enters the intestine, it can also stimulate local immune responses at these various sites that can contribute to systemic cytokine responses, as has been reported for other intestinal helminths in animal models (37, 38). Thus, there are multiple mechanisms by which *Strongyloides* infection can exert its effects. Our study depends on both serological and microscopic confirmation of *Strongyloides* infection and illustrates the reliability of an NIE ELISA in diagnosing infection since ELISA values decreased significantly after treatment. However, a limitation of the study is that while statistically significant changes were observed in the different groups, the biological significance of these changes remains to be elucidated. Moreover, the sample size is another limitation of the study.

Helminths may protect humans against allergic and autoimmune disease, and, indeed, helminth-derived products have been shown to

prevent the development of such inflammatory diseases in mouse models and in experimental human trials (39). Here, we show that *Strongyloides* infection can profoundly modulate the systemic cytokine environment of the host by inducing strong anti-inflammatory responses and suppressing (possibly pathological) proinflammatory responses. In addition, independent analysis using MDS also confirms the trends observed in the modulation of systemic cytokines in *Strongyloides* infection and the separation of infected from uninfected individuals. Hence, it is possible that such immune modulation could also protect against exaggerated inflammatory responses associated with inflammatory and autoimmune diseases.

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