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Parasite Antigen-Specific Regulation of Th1, Th2, and Th17 Responses in *Strongyloides stercoralis* Infection

Rajamanickam Anuradha,* Saravanan Munisankar,* Chandrakumar Dolla,† Paul Kumaran,‡ Thomas B. Nutman,‡ and Subash Babu*,‡

Chronic helminth infections are known to be associated with modulation of Ag-specific CD4+ T responses. However, the role of CD4+ T cell responses in human infection with *Strongyloides stercoralis* is not well defined. To examine the role of CD4+ T cells expressing Th1, Th2, and Th17 cytokines in strongyloidiasis, we compared the frequency (Fo) of these subsets in infected (INF) individuals with Fo in *S. stercoralis*-uninfected (UN) individuals. INF individuals exhibited a significant decrease in the spontaneous and Ag-specific Fo of both monofunctional and dual-functional Th1 cells compared with UN. Similarly, INF individuals also exhibited significantly decreased Fo of monofunctional and dual-functional Th17 cells upon Ag stimulation compared with UN. In contrast, both the spontaneous and the Ag-induced Fo of monofunctional and dual-functional Th2 cells was significantly increased in INF compared with UN individuals. This differential T cell response was predominantly Ag specific because it was abrogated upon control Ag or mitogen stimulation. The regulation of Th1, Th2, and Th17 cells was predominantly dependent on IL-10, whereas the regulation of Th2, but not Th1 or Th17, cells was also dependent on TGF-β. In addition, treatment of *S. stercoralis* infection significantly increased the Ag-specific Fo of Th1 and Th17 cells and decreased the Fo of Th2 cells in INF individuals. Thus, *S. stercoralis* infection is characterized by a parasite Ag-dependent regulation of monofunctional and dual-functional Th1, Th2, and Th17 cells, a regulation also reversible by antihelminthic treatment. *The Journal of Immunology*, 2015, 195: 2241–2250.

CD4+ T cells can be classified into different subsets based on the pattern of cytokine expression (1). Thus, Th1 cells typically express IFN-γ, IL-2, and/or TNF-α, Th2 cells express IL-4, IL-5, and/or IL-13, whereas Th17 cells express IL-17, IL-22, and/or IFN-γ. Moreover, based on the expression of one or more cytokines within a single cell, CD4+ T cells can also be further classified into single cytokine-producing (hereafter monofunctional), dual cytokine-producing (dual-functional), and triple cytokine-producing (multifunctional) T cells (2). Multifunctional Th1 cells expressing IFN-γ, IL-2, and TNF-α are thought to play an important role in resistance to a variety of infections including viral, bacterial, and parasitic infections (2). Dual-functional or multifunctional T cells are also thought to produce more cytokines on a per-cell basis and for an extended period than monofunctional T cells (3, 4). Moreover, the presence of these multifunctional T cells is thought to be a better correlate of protective immunity to several infections and to vaccine-mediated immunity (5). Th2 cells expressing a dual-functional or multifunctional phenotype have been described in both allergic and parasitic infection (6). Th17 cells can also exhibit multifunctionality, and certain Th17 cell subsets are thought to play preferential roles in resistance to different infections (7). Although the concept of multifunctionality in cytokine production has been extensively investigated in intracellular infections, little is known about the role of multifunctional T cells in extracellular infections.

Human infections with *Strongyloides stercoralis* appear to be controlled through a Th2 response based on observations in patients with human T lymphotrophic virus 1 and *S. stercoralis* coinfection (8–10). Moreover, protective immunity to *S. stercoralis* larvae in mice is dependent on CD4+ T cells, and these cells typically produce IL-4 and IL-5 (11). Finally, primary infections of rats or mice with the rodent parasites, *S. ratti* and *S. venezuelensis*, respectively, result in a Th2 response, with production of IL-4, IL-5, and IL-13 and concomitant suppression of IFN-γ (12). The role of Th1 cells expressing other type 1 cytokines (IL-2 and TNF-α) has not been examined in animal models or human *S. stercoralis* infection; nor has the role played by Th17 cells been explored in any form of *S. stercoralis* infection. Finally, a detailed examination of the role of Th2 subsets in human *S. stercoralis* infection is lacking.

Therefore, we sought to determine the expression pattern of the major functional subsets of CD4+ Th1, Th2, and Th17 cells in *S. stercoralis* infections. Comparison of asymptomatic, infected (INF) individuals with uninfected control individuals reveals a distinct expression pattern of CD4+ T cell subset expression in *S. stercoralis* infection, a pattern that was associated with diminished frequency (Fo) of spontaneously expressed or Ag-induced monofunctional and dual-functional CD4+ Th1 and Th17 cells and an increased Fo of homeostatic and Ag-induced monofunctional and dual-functional Th2 cells. The changes associated with *S. stercoralis* infection were dependent on IL-10 and were reversible after chemotherapy. Thus, *S. stercoralis* infection is characterized by a complex repertoire of CD4+ T cells subsets whose interaction might determine both susceptibility and resistance to infection.

Abbreviations used in this article: Fo, frequency; GM, geometric mean; INF, infected; LIPS, Luciferase Immunoprecipitation System Assay; PII, PMA/ionomycin; PPD, purified protein derivative; SsAg, *S. stercoralis* somatic larval Ag; UN, uninfected.

*Correspondence and reprint requests to Dr. Subash Babu, National Institutes of Health, International Center for Excellence in Research, Chetpet, Chennai 600031, India; E-mail address: sbabu@mail.nih.gov

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Materials and Methods

Study population

We studied a total of 66 individuals: 43 clinically asymptomatic, S. stercoralis infection is associated with an increased Fo of total white cells, eosinophils, and basophils

As shown in Table I, INF individuals differed from UN individuals in having significantly higher levels of total white cell counts, absolute eosinophil, and basophil counts. Also, as shown in Table I, no significant differences in ages or sex were observed between the two groups.

S. stercoralis infection is associated with a spontaneous and an Ag-specific decrease in the Fo of monofunctional and dual-functional CD4+ Th1 cells

To examine the baseline (or steady-state) and Ag-stimulated expression pattern of Th1 cells in S. stercoralis infections, we cultured whole blood from INF and UN individuals with media alone or with SsAg, NIE, PPD, or P/I and measured the Fo of CD4+ T cells expressing each of the Th1 cytokines. A representative flow-cytometry plot is shown in Supplemental Fig. 1A. As shown in Fig. 1A, the baseline Fo of CD4+ T cells expressing IFN-γ or IL-2 alone (monofunctional Th1 cells) or the Fo of CD4+ T cells coexpressing TNF-α/IFN-γ or IFN-γ/IL-2 or TNF-α/IL-2 (dual-functional Th1 cells) was significantly reduced in INF individuals. Similarly, the Fo of monofunctional and dual-functional Th1 cells was also reduced significantly in response to SsAg and NIE stimulation (Fig. 1B, 1C). In contrast, neither PPD (Fig. 1D) nor P/I (Fig. 1E) induced any significant difference in the Fo of CD4+ Th1 cells. Thus, S. stercoralis infection is associated with profound alterations in the repertoire of monofunctional and dual-functional Th1 cells at steady-state and after parasite Ag stimulation.

S. stercoralis infection is associated with a spontaneous and an Ag-specific increase in the Fo of monofunctional and dual-functional CD4+ Th2 cells

To examine the baseline (or steady-state) and Ag-stimulated expression pattern of Th2 cells in S. stercoralis infections, we cultured whole blood from INF and UN individuals with media alone or with SsAg, NIE, PPD, or P/I and measured the Fo of CD4+ T cells expressing each of the Th2 cytokines. A representative flow-cytometry plot is shown in Supplemental Fig. 1B. As shown in Fig. 1B, the baseline Fo of CD4+ T cells expressing IL-4, IL-5, or IL-13 alone (monofunctional Th2 cells) or the Fo of CD4+ T cells coexpressing IL-4/IL-5 or IL-4/IL-13 or IL-5/IL-13 (dual-functional Th2 cells) was significantly increased in INF individuals. Similarly, the Fo of monofunctional and dual-functional Th2 cells was also increased significantly in response to SsAg and NIE stimulation (Fig. 2B, 2C). In contrast, neither PPD (Fig. 2D) nor P/I (Fig. 2E) induced any significant difference in the Fo of CD4+ Th2 cells. Thus, S. stercoralis infection is associated with profound alterations in the
reertoire of monofunctional and dual-functional Th2 cells at steady-state and after parasite Ag stimulation.

S. stercoralis infection is associated with an Ag-specific decrease in the Fo of monofunctional and dual-functional CD4+ Th17 cells

To examine the baseline (or steady-state) and Ag-stimulated expression pattern of Th17 cells in S. stercoralis infections, we cultured whole blood from INF and UN individuals with media alone or with SsAg, NIE, PPD, or P/I and measured the Fo of CD4+ T cells expressing each of the Th17 cytokines. A representative plot is shown in Supplemental Fig. 1C. As shown in Fig. 3A, the baseline Fo of CD4+ T cells expressing IL-17 or IL-22 alone (monofunctional Th17 cells) or the Fo of CD4+ T cells coexpressing IFN-γ/IL-17 or IL-17/IL-22 (dual-functional Th17 cells) was significantly increased in INF individuals. However, upon Ag stimulation, the profile was reversed, with the Fo of monofunctional and dual-functional Th17 cells being reduced significantly in response to SsAg and NIE stimulation (Fig. 3B, 3C). In contrast, neither PPD (Fig. 3D) nor P/I (Fig. 3E) induced any significant difference in the Fo of CD4+ Th17 cells. Thus, S. stercoralis infection is associated with profound alterations in the repertoire of monofunctional and dual-functional Th17 cells at steady-state and after parasite Ag stimulation.

IL-10 modulates the Fo of monofunctional and dual-functional Th1, Th2, and Th17 cells in S. stercoralis infections

To determine the role of IL-10 in the modulation of Th1, Th2, and Th17 subpopulations in INF, we measured the Fo of these major CD4+ T cell subsets after stimulation with the parasite Ag NIE in the presence or absence of anti–TGF-β1 neutralizing Ab in INF individuals (n = 15). As shown in Fig. 5A, TGF-β1 neutralization had no significant effect on the Fo of monofunctional and dual-functional Th1 cells in INF individuals. In contrast, as shown in Fig. 5B, TGF-β1 blockade resulted in significantly diminished Fo of monofunctional (IL-4 or IL-5 expressing) and dual-functional (IL-4/IL-5 or IL-4/IL-13 or IL-5/IL-13) Th2 cells in INF individuals. Finally, as shown in Fig. 5C, TGF-β1 blockade had minimal effect on the Fo of monofunctional and dual-functional Th17 cells in INF individuals, with the exception of IL-17 monofunctional cells, which were significantly decreased. Thus, TGF-β1 plays a major role in the modulation of Th2 cells Fo in S. stercoralis infections.

Anti–S. stercoralis therapy results in significantly increased Fo of Ag-stimulated Th1 and Th17 cells and significantly decreased Fo of Ag-stimulated Th2 cells

To determine the role of active infection in the regulation of monofunctional and dual-functional Th1, Th2, and Th17 cells in S. stercoralis infections, we measured the Fo of Th1, Th2, and Th17 cells in a subset of INF individuals (n = 15) who had been treated with anti–S. stercoralis chemotherapy 6 months earlier. As shown in Fig. 6A and Supplemental Fig. 2, treatment of S. stercoralis infection resulted in significantly increased Fo of monofunctional Th1 cells expressing IFN-γ, IL-2, or TNF-α alone or dual-functional Th1 cells expressing IFN-γ/IL-2 or IFN-γ/TNF-α or IL-2/TNF-α upon NIE or SsAg stimulation when compared with their pre-treatment levels. Interestingly, this effect was specific to the parasite Ag-stimulated Fo of CD4+ Th1 cells as neither PPD (Fig. 6B) nor P/I (Supplemental Fig. 2) significantly altered the Fo of these subsets after treatment. In contrast, as shown in Fig. 6A and Supplemental Fig. 2, treatment of S. stercoralis infection resulted in significantly increased Fo of monofunctional Th1 cells expressing IFN-γ, IL-2, or TNF-α alone or dual-functional Th1 cells expressing IFN-γ/IL-2 or IFN-γ/TNF-α or IL-2/TNF-α upon NIE or SsAg stimulation, but not in response to PPD or P/I stimulation (Fig. 6B, Supplemental Fig. 2). In addition, as shown in Fig. 6A and Supplemental Fig. 2, treatment of S. stercoralis infection resulted in significantly increased Fo of monofunctional Th17 cells expressing IL-17 or IL-22 alone or dual-functional Th17 cells expressing IL-4/IL-5 or IL-4/IL-13 or IL-5/IL-13 upon NIE or SsAg stimulation, but not in response to PPD or P/I stimulation (Fig. 6B, Supplemental Fig. 2). Thus, the Ag-driven modulation of CD4+ T cell subpopulations in S. stercoralis infection is reversible for the most part after treatment of infection.

Table I. Demographics of the study population and hematology data

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<tr>
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<th>INF (n = 43)</th>
<th>UN (n = 23)</th>
<th>p Value</th>
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<td>M/F, n</td>
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<td>10/13</td>
<td></td>
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<td>Age, y</td>
<td>40.18 (18–61)</td>
<td>38 (20–63)</td>
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<td>NIE and SsIR LIPS</td>
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<td>Negative</td>
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<td>Hemoglobin, g/dl, GM (range)</td>
<td>15.58 (6.9–17.2)</td>
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<td>RBC, 10^6/ml, GM (range)</td>
<td>5.5 (2.9–5.59)</td>
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<tr>
<td>WBC, 10^3/ml, GM (range)</td>
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<td>10.64 (6.1–12.9)</td>
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<tr>
<td>Hematocrit, %, GM (range)</td>
<td>36.44 (24.5–46.5)</td>
<td>40.10 (30.7–46.7)</td>
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<tr>
<td>Platelets, 10^12/ml, GM (range)</td>
<td>286.39 (171–742)</td>
<td>264.06 (124–378)</td>
<td>NS</td>
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<tr>
<td>Neutrophils, 10^3/ml, GM (range)</td>
<td>3.93 (2.15–8.17)</td>
<td>3.42 (1.92–6.14)</td>
<td>NS</td>
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<td>Lymphocytes, 10^3/ml, GM (range)</td>
<td>2.79 (1.72–3.71)</td>
<td>2.76 (1.55–4.41)</td>
<td>NS</td>
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<td>Monocytes, 10^3/ml, GM (range)</td>
<td>0.47 (0.11–0.87)</td>
<td>0.77 (0.27–2.32)</td>
<td>NS</td>
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<td>Eosinophils, 10^3/ml, GM (range)</td>
<td>1.68 (0.27–3.52)</td>
<td>0.54 (0.19–1.2)</td>
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<td>Basophils, 10^3/ml, GM (range)</td>
<td>0.44 (0.057–0.17)</td>
<td>0.17 (0.051–0.16)</td>
<td>0.0028</td>
</tr>
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</table>
S. stercoralis infection is associated with decreased spontaneously expressed and Ag-induced Fo of CD4+ monofunctional and dual-functional Th1 cells. Whole blood was cultured with media alone for 6 h and the baseline and Ag-stimulated Fo of Th1 cells determined. The baseline (A) as well as SsAg (B), NIE (C), PPD (D), and P/I (E) stimulated Fo of monofunctional and dual-functional CD4+ Th1 cells in INF (n = 28) and UN (n = 23) individuals are shown. Each circle represents a single individual and the bars represent the GM values. Net Fo were calculated by subtracting baseline Fo from the Ag-induced Fo for each individual. The p values were calculated using the Mann–Whitney U test.
UN and INF (before and after treatment) individuals, we plotted the Fo of each cell subset in response to different Ags. As shown in Supplemental Fig. 3, the data are plotted as pie charts with each piece of the pie depicting the percentages of each subset (monofunctional or dual-functional) in the total Th1, Th2, or Th17 cell population. Our data clearly reveal that although Th1 and Th2 cell responses to different Ags appear comparable in terms of these subsets, the percentages of Th17 cell subsets undergo dramatic alterations upon P/I stimulation compared with antigenic stimulation.

**Discussion**

*S. stercoralis*, an intestinal parasitic nematode, infects >100 million people worldwide (14). Infection with *S. stercoralis* can occur without any clinical symptoms whatsoever, with mild abdominal symptoms, or as a potentially fatal hyperinfection syndrome, or as a disseminated infection (14, 15). In addition, chronic infection is also associated with the autoinfection/tissue migration aspect of the life cycle (14, 15). The most common risk factors for these severe manifestations are immunosuppression caused by corticosteroids or by infection with human T lymphotrophic virus (16). Despite the
widespread global prevalence and its propensity to cause fatal disease, the immune responses to *S. stercoralis* infection in either humans or in animal models has been largely neglected (17). One of the major factors for this lacuna is the relative insensitive methods to diagnose asymptomatic infection. However, with recent advances in *S. stercoralis*–specific serology and/or PCR, diagnostic capabilities have been considerably enhanced (14, 15).

The canonical host immune response to helminth parasites in animal models is of the Th2 type and involves the production of cytokines—IL-4, IL-5, IL-9, IL-10, and IL-13, the Ab isotypes, IgG1, IgG4, and IgE—and expanded populations of eosinophils, basophils, mast cells, and alternatively activated macrophages (18). Over time, these responses are thought to be modulated in chronic infection by both adaptive and natural regulatory T cells.

**FIGURE 3.** *S. stercoralis* infection is associated with decreased spontaneously expressed and Ag-induced Fo of CD4+ monofunctional and dual-functional Th17 cells. Whole blood was cultured with media alone for 6 h and the baseline and Ag-stimulated Fo of Th17 cells determined. The baseline (A) as well as SsAg (B), NIE (C), PPD (D), and P/I (E) stimulated Fo of monofunctional and dual-functional CD4+ Th17 cells in INF (n = 28) and UN (n = 23) individuals are shown. Each circle represents a single individual and the bars represent the GM values. Net Fo were calculated by subtracting baseline Fo from the Ag-induced Fo for each individual. The p values were calculated using the Mann–Whitney U test.
alternatively activated macrophages, and other cell types (19).

Although Th1 cells are known to be downmodulated and Th2 responses are known to be required for resistance to infection (20, 21), the role of other subsets of CD4+ T cells, especially Th17 cells in helminth infections, is not well defined. Moreover, little is known about the regulation of these subsets in helminth infections in general, and in S. stercoralis in particular.

Our study expands the limited knowledge to date on the human CD4+ T cell response to S. stercoralis infection and offers certain major insights into the understanding of the regulation of CD4+ T cell responses in S. stercoralis infection. First, we established the downregulation of both monofunctional and dual-functional Th1 cells expressing the panel of cytokines: IFN-γ, TNF-α, and IL-2 in chronic S. stercoralis infection. More detailed analyses reveal that Th1 cells in S. stercoralis infection are downmodulated both at homeostasis (baseline) and after parasite Ag stimulation. Moreover, the basic downregulated response is remarkably similar in response to two very different sets of Ags: a somatic extract of larval Ags (SsAg) (22) and a defined recombinant Ag (NIE), shown serologically to be immunodominant (23). Interestingly, this regulation is highly parasite Ag specific because neither control Ag nor mitogenic stimulation could induce the downmodulation of Th1 cells in IN individuals. Thus, we show that T cells in S. stercoralis infections are not intrinsically defective in their ability to respond to antigenic stimulation but appear to exhibit a very active impairment only in response to S. stercoralis–specific parasitic Ags. Finally, we also provide data that this downmodulation of Ag-specific Th1 responses is reversible, because treatment with a standard regimen of anti-helminthic chemotherapy at least partially reverses this downregulation. Our studies using the pretreatment and posttreatment data from INF individuals clearly illustrate a pivotal role for active infection in the maintenance of impaired Th1 responses in chronic S. stercoralis infections. Th1 responses are typically associated with the development of pathology in helminth infections (18). Thus, in filarial infections (24), as well as in schistosomiasis (25), Th1 cells are closely involved in the development of pathology. Our data on the downregulation of Th1 responses in S. stercoralis infection would therefore appear to reflect an active ongoing regulatory process that might act to prevent tissue damage in this chronic infection.

The second major finding in our study is the induction of monofunctional and dual-functional Th2 cell responses in S. stercoralis infection. Different Th2 subsets have been previously

![Figure 4](http://www.jimmunol.org/Downloadedfrom)
shown to play different roles in infections and allergic disorders (6). Thus, IL-5+ Th2 cells have been found in greater Fo in patients with eosinophilic gastrointestinal disease, whereas peanut allergy was found to be associated with higher Fo of IL-5

2

Th2 cells (26). In addition, parasite Ag-driven Th2 subsets have been shown to be expanded in filarial infections, a process dependent on IL-10 and TGF-

b

(27). Moreover, whereas the IL-5

Th2 cells are predominantly involved in Ab class switching to IgG4 and IgE in filarial infection, IL-5

Th2 cells primarily regulate eosinophilia (27). Our data reveal that both monofunctional and dual-functional Th2 cells were expanded in the context of S. stercoralis infection at steady-state and that this expansion was further upregulated upon parasite Ag stimulation. Similar to the data from filarial infection (27), this regulation of Th2 subsets also appears to be dependent on IL-10 and TGF-

b

. Finally, the regulation of Th2 responses in S. stercoralis infection, similar to Th1 cells, also appears to be modulated by active infection because the Fo of these cells is considerably reduced after anthelmintic chemotherapy. Thus, chronic S. stercoralis infection is clearly characterized by modulation of Th2 cell subsets that probably play an effect role in the immune response to infection by either limiting the density of infection or by modulating pathology. Also, our data suggest that the mere expansion of Th2 cells in INF individuals is not sufficient to mediate protection and, therefore, the timing, intensity, and/or duration of expansion could potentially modulate immune resistance mechanisms.

The third major insight provided by our study is the identification of a modulated Th17 response in S. stercoralis infection. Our study provides a detailed examination of Th17 cell subsets in S. stercoralis infections and reveals that CD4

Th17 cells are decreased in Fo in an Ag-specific manner, similar to Th1 cells. Th17 cells are known to be predominantly involved in the immune responses in inflammatory and autoimmune diseases, but their role in infectious diseases is not very clear (28). Th17 cells are central to host protection against bacterial infections at barrier sites and play an important role in defense against extracellular infections (29). However, Th17 cells are also associated with pathogenesis of disease in a number of infectious diseases, including schistosomiasis and filariasis (24, 30). Our data suggest that Th17 cells are present at decreased Fo in S. stercoralis infections and, therefore, could reflect either a failed resistance mechanism against the
parasite or an active process of inhibiting excessive inflammation. This is further reinforced by the finding that treatment of active infection results in a significant increase in Th17 cells in an Ag-dependent manner.

The central players in regulating the immune response in parasitic infections are IL-10 and TGF-β (19). The role of IL-10 and TGF-β in the regulation of helminth infections, including schistosomiasis and filariasis, has been documented previously (31, 32). Our data reveal that IL-10 is the most important regulator of monofunctional and dual-functional Th1, Th2, and Th17 cells. TGF-β appears to play a minimal role in the regulation of Th1 and Th17 cells in S. stercoralis infection but does appear to regulate the expansion of Th2 cells. This dichotomy in regulation of immune responses by the central regulatory cytokines might reflect different sources of production or different modes of action of these cytokines, which deserves further study. Nevertheless, our data implicate S. stercoralis as yet another infectious model, where IL-10 appears to exert important immunomodulatory controls on the immune response.

Our study therefore highlights an important role for CD4+ T cell subsets in the regulation of immune responses in S. stercoralis infections. Our study also highlights the complexity of the T cell network underlying the susceptibility or resistance to infection and also suggests that the role of Th1, Th2, and Th17 cells in S. stercoralis infections warrants further investigation, perhaps using animal models of infection. Finally, our work emphasizes

FIGURE 6. Treatment of S. stercoralis infection is associated with increased Fo of Ag-specific Th1 and Th17 cells and decreased Fo of Ag-specific Th2 cells. (A) The Fo of Th1, Th2, and Th17 cells after stimulation with NIE before and after treatment with a standard dose of ivermectin and albendazole in a subset of INF individuals (n = 15). (B) The Fo of Th1, Th2, and Th17 cells after stimulation with PPD before and after treatment with a standard dose of ivermectin and albendazole in a subset of INF individuals (n = 15). Ag-stimulated Fo are shown as net Fo, with the baseline levels subtracted. Each line represents a single individual. The p values were calculated using the Wilcoxon signed rank test.
the growing importance of multifunctional CD4+ T cells in immunity to parasitic infections.

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Disclosures
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