Coinfections with *Schistosoma haematobium*, *Necator americanus*, and *Entamoeba histolytica/Entamoeba dispar* in Children: Chemokine and Cytokine Responses and Changes after Antiparasite Treatment

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The effect of polyparasite infections on cytokine and chemokine responses as well as the effect of antiparasite treatment was studied in children without parasite infection (the G0 group), in children singly infected with *Schistosoma haematobium* (the G1 group), and in children multiply infected with *S. haematobium/Schistosoma mansoni, Entamoeba histolytica/Entamoeba dispar*, and *Necator americanus* (the G3 group). Linear regression analysis disclosed a significant risk for coinfection with hookworm and *Schistosoma* species. Polyparasite infections detected in 23% of children before treatment were present in 5% at 15 months after treatment. Chemokine responses to *S. mansoni* adult worm antigen (SmAg) diminished after treatment for macrophage inflammatory chemokine (MIP)–1/chemokine (C-C motif) ligand (CCL)–3 (among G3 children, by a factor of 200 [95% confidence interval {CI}, 33–1111]) and for MIP-1β/CCL-4 (among G3 children, by a factor of 26 [95% CI, 6–117]) but were enhanced for thymus- and activation-regulated chemokine/CCL-17 (among G3 children, by a factor of 10 [95% CI, 3–32]) (*P* <.001 for all). In response to *E. histolytica* antigen, interleukin (IL)–13 levels increased after treatment among G1 children by a factor of 138 (95% CI, 12–1569) and among G3 children by a factor of 21 (95% CI, 7–64) (*P* <.001 for both). Cellular production of interferon (IFN)–γ in response to SmAg decreased 4 weeks after treatment among G3 children, whereas T helper cell type 2 (Th2) IL-13 production was enhanced among G1 and G3 children. In summary, polyparasite infections with *S. haematobium/S. mansoni, E. histolytica/E. dispar*, and *N. americanus* generated prominent proinflammatory cytokine and chemokine responses, and, after antihelminth treatment, the inflammatory chemokine response lessened as the Th2 responsiveness in coinfected children increased.

In large parts of sub-Saharan Africa, intestinal and intravascular helminth and protozoan infections often occur concurrently [1]. Up to 90% of schoolchildren may be found to be infected with helminths or protozoa [2], with every second child being coinfected with >1 parasite species [3, 4]. Although the prevalence and intensity of most parasite infections is extremely low, particularly during infancy, it reaches a peak at school age and may decline later in life. Gradually, the number of parasites and the parasite density will increase, multiple-parasite infections will generate a range of overlapping clinical symptoms [5], and chronic parasite persistence will impede the physical and cognitive development of children [6, 7]. The interaction between helminth infection and the development of clinical malaria has been investigated [8], and children coinfected with *Plasmodium falciparum* and *Schistosoma haematobium* were found to develop severe malaria more frequently than singly infected children; this finding was attributed to in-
creased levels of interferon (IFN)–γ and tumor necrosis factor–α [9]—2 cytokines considered to be critically important to the development of severe malarial disease [10]. Pro-inflammatory cytokines and chemokines—notably, macrophage inflammatory chemokine (MIP)–1α/chemokine (C-C motif) ligand (CCL)–3, MIP-1β/CCL-4, and RANTES (regulated on activation, normal T cell expressed and secreted)/CCL-5—are mediators of inflammation, cell recruitment, and activation [11] and may contribute to tissue and organ damage; it has been suggested that they aggravate the clinical condition in schistosomiasis [12], filariasis [13], malaria [14], and hepatitis [15]. Furthermore, regulatory monocyte-derived chemokine (MDC)/CCL-22 and thymus- and activation-regulated chemokine (TARC)/CCL-17 mediate recruitment of specific leukocyte subsets into pathogen-invaded or allergen-exposed tissues [16]. Despite the frequent occurrence of multiple-parasite infections and numerous antiparasite treatment campaigns and control programs [17], few studies have addressed the effect of helminth and intestinal protozoan coinfections in children. In adults with multiple-parasite infections, antiparasite treatment will change cytokine and chemokine responses [18, 19], but a single-drug intervention may not suffice to reestablish cytokine responses to control levels in those infected with multiple parasites [20]. In the present work, we addressed how polyparasite infections in children affect cytokine and chemokine responses and investigated to what extent antiparasite treatment changes their specific immune responses.

### METHODS

**Study population.** This study was conducted in the central region of Togo, Africa. All children examined (n = 729) were attending primary public schools (n = 11) not more than 5 km apart in suburban quarters of the town of Sokodé (Préfecture Tchaoudjo). For stool and urine examinations, a random sample of 25 pupils was selected from each class. Two 50-mL polypropylene tubes were distributed to the pupils, who collected samples the next morning; diagnostic procedures were performed by the laboratory staff at the Centre Hospitalier Régional (CHR), Sokodé. On the basis of the diagnostic results, 3 patient groups were formed (table 1): all children who did not have a parasite infection were considered to be control children, living as they did in the same area of endemicity under the same socioeconomic conditions (the G0 group). Parasite-negative children were asked to supply a second stool sample to confirm their status. Children singly infected with *S. haematobium* were assigned to the G1 group, and children multiply infected with *S. haematobium*/*Schistosoma mansoni*, *Entamoeba histolytica/Entamoeba dispar*, and *Necator americanus* (group G3+). Infections with *S. haematobium*, *S. mansoni*, *N. americanus*, and *E. histolytica/E. dispar* were determined by analysis of urine and stool samples before, 4 weeks after, and 15 months after antiparasite treatment: ND, not determined.

#### Table 1. Prevalence and intensity of single- and multiple-parasite infections in children before and after treatment.

<table>
<thead>
<tr>
<th>Pathogen, time point</th>
<th>N. americanus</th>
<th>S. haematobium</th>
<th>E. histolytica/E. dispar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G0</td>
<td>G1</td>
<td>G3+</td>
</tr>
<tr>
<td></td>
<td>Prevalence, %</td>
<td>Intensity*</td>
<td>Prevalence, %</td>
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</table>

**NOTE.** Groups are as follows: G0, children without parasite infection; G1, children singly infected with *Schistosoma haematobium*; and G3+, children multiply infected with *S. haematobium/Schistosoma mansoni*, *Entamoeba histolytica/Entamoeba dispar*, and *Necator americanus* (group G3+). Infections with *S. haematobium*, *S. mansoni*, *N. americanus*, and *E. histolytica/E. dispar* were determined by analysis of urine and stool samples before, 4 weeks after, and 15 months after antiparasite treatment; ND, not determined.

* Data are the median (minimum, maximum) level among positive children, given in eggs per gram of stool for *N. americanus* and in eggs per 10 mL of urine for *S. haematobium*.

**G0** group (in this group, n = 28).

**G1** group (in this group, n = 27 for G0, n = 15 for G1, and n = 45 for G3+).

**G3+** group (in this group, n = 12 for G0, n = 14 for G1, and n = 36 for G3+).

**G3+** group (in this group, n = 8 for G0, n = 7 for G1, and n = 29 for G3+).
Pathogens of the presence of *Plasmodium* showed signs of malaria (thick blood smears positive for *Plasmodium* species and fever), or who had diarrhea were excluded from the study. None of the children presented with *E. histolytica* trophozoites containing ingested red blood cells in stool samples, bloody stool, or clinical signs of invasive amoebiasis. Children belonging to the G0, G1, and G3+ groups (*n* = 87) were invited with their parents or legal guardians to the CHR, Sokodé, for blood sample collection. All children examined (*n* = 729) received a single dose of albendazole (400 mg), and those infected with *S. haematobium* or *S. mansoni* were treated with praziquantel according to the guidelines of the Togolese Ministry of Health. Diagnostic procedures to distinguish *E. histolytica* from *E. dispar* are not available at the laboratory facilities at the CHR, Sokodé. A previous study of *E. histolytica*/E. dispar in Togo found that noninvasive *E. dispar* was present in ~90% of study participants and that only 10% were infected with *E. histolytica* [21]. Treatment of *E. histolytica*/E. dispar is recommended on evidence of invasive amoebiasis (i.e., *E. histolytica* trophozoites containing ingested red blood cells in stool samples, bloody stool, or clinical signs of invasive amoebiasis). Four weeks after treatment, stool, urine, and blood samples from G0, G1, and G3+ children (*n* = 62) were reexamined in the same fashion as before treatment; 15 months after treatment, stool and urine samples from G0, G1, and G3+ children (*n* = 44) were reexamined as before.

Authorization and approval for the present was granted by the Togolese Ministry of Health (292/99/MS/CAB and 0407/2007/MMS/CAB/DGS), the regional ministry of education (MENR/SG/DRERC/13.06.2001), and the regional health authority (MS/DGS/DRS/RC/No.220 and MS/DGS/DRS/RC/No.261). Oral informed consent was given by all participating children, and written consent was provided by all parents or legal guardians after thorough explanation of the procedures, aims, and risks of the study; moreover, to ensure informed understanding, explanations were always given in the local language by the medical staff at the CHR, Sokodé.

**Parasitological analysis.** For determination of intestinal helminth and protozoan infections, fresh stool samples (0.5 g) were mixed with saline and dispersed on 2 microscope slides covered with a 24 × 48-mm slide; samples were examined by laboratory technicians. All stool samples were examined using the Kato-Katz technique for quantification of helminth eggs per gram of stool (helm-TEST; Labmaster). To detect *S. haematobium* infection, 10 mL of urine from each participant was filtered (polycarbonate membrane; pore size, 12 µm; Whatman); the filters were then examined under a microscope, and *S. haematobium* eggs were quantified. Thick blood smears were analyzed for the presence of *Plasmodium* species before and 4 weeks after antiparasite treatment of G0, G1, and G3+ children.

**Preparation of antigens.** Phytohaemagglutinin (PHA) and streptolysin O (SLO) were purchased from Difco. *E. histolytica* antigen (EhAg; trophozoites; strain HM-1 axenic culture) was a gift from B. Walderich (Institute for Tropical Medicine, University Clinics of Tübingen, Germany); *S. mansoni* adult worm antigen (SmAg) was made available by A. Deelder (Department of Parasitology, Leiden University Medical Center, Leiden, the Netherlands). *E. histolytica* trophozoites containing ingested red blood cells in stool samples, bloody stool, or clinical signs of invasive amoebiasis. Children belonging to the G0, G1, and G3+ groups (*n* = 87) were invited with their parents or legal guardians to the CHR, Sokodé, for blood sample collection. All children examined (*n* = 729) received a single dose of albendazole (400 mg), and those infected with *S. haematobium* or *S. mansoni* were treated with praziquantel according to the guidelines of the Togolese Ministry of Health. Diagnostic procedures to distinguish *E. histolytica* from *E. dispar* are not available at the laboratory facilities at the CHR, Sokodé. A previous study of *E. histolytica*/E. dispar in Togo found that noninvasive *E. dispar* was present in ~90% of study participants and that only 10% were infected with *E. histolytica* [21]. Treatment of *E. histolytica*/E. dispar is recommended on evidence of invasive amoebiasis (i.e., *E. histolytica* trophozoites containing ingested red blood cells in stool samples, bloody stool, or clinical signs of invasive amoebiasis). Four weeks after treatment, stool, urine, and blood samples from G0, G1, and G3+ children (*n* = 62) were reexamined in the same fashion as before treatment; 15 months after treatment, stool and urine samples from G0, G1, and G3+ children (*n* = 44) were reexamined as before.

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**Isolation of peripheral blood mononuclear cells (PBMCs) and cell culture experiments.** Venous blood (9 mL) was collected from each child in the present study before and 4 weeks after treatment. PBMCs were isolated by ficoll-hypaque density gradient centrifugation at 340 g for 35 min at room temperature. Plasma samples were collected and frozen at −20°C until further use. PBMCs were washed twice in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco) supplemented with 0.025 mol/L HEPES buffer, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B (Sigma), and the suspension was adjusted to 2.5 × 10^6 cells/mL of RPMI 1640 supplemented as described above plus 10% heat-inactivated fetal calf serum (Biochrom). PBMCs were cultured in 48-well plates at 37°C in 5% CO2 with saturated humidity in the presence of either PHA (1:100), EhAg (10 µg/mL), SmAg (10 µg/mL), or SLO (1:50) or were left unstimulated (baseline). Cell cultures were terminated after 48 or 72 h, and cell-free supernatants were collected and stored below −20°C until further use.

**Determination of cytokine and chemokine production in cell culture supernatants and plasma samples.** Cytokine and chemokine production was quantified by enzyme-linked immunosorbent assays (ELISAs) specific for interleukin (IL)–5 (BioSource), IL-12/IL-23p40, IL-13, and IFN-γ as well as for TARC/CCL-17, MDC/CCL-22, RANTES/CCL-5, MIP-1α/CCL-3, and MIP-1β/CCL-4 (R&D Systems). ELISAs were performed as recommended by the manufacturer and as described elsewhere [22]. The detection limit was 20 pg/mL for all assays (R&D Systems DuoSet) except for BioSource CytoSet (IL-5), for which the detection limit was 10 pg/mL. Optical densities (ODs) were transformed into concentrations (CON) according to the formula

\[
\text{CON} = \frac{K_{50}}{\left(\frac{A}{\text{OD}} - 1\right)^c}
\]

if OD ≠ 0 (if OD = 0, then CON = 0). Here, \(K_{50}\) is the concentration at which the OD is equal to half of the maximum OD (parameter A). The parameter \(c\) determines the slope at \(K_{50}\). The inverse of this function was fitted by least squares to the measured standard values.

**Statistical analysis.** JMP software (versions 5.1 and 7; SAS Institute) was used for statistical analysis of data. Because of multiple comparisons, *P* values were adjusted according to the Bonferroni-Holm method. Chemokine concentrations in plasma before and after treatment were analyzed by multifactorial analysis of variance, with the predictors infection group, time of observa-
tion, and the interaction between them as fixed factors and patients’ number nested under infection group as a random factor. Estimates were obtained by restricted maximum-likelihood analysis. To evaluate the effect of antiparasite treatment on chemokine and cytokine production by PBMCs before and after treatment, data were log transformed \( \log(\text{pg/mL} + 1) \), and for each patient cytokine/chemokine production after treatment was subtracted from cytokine/chemokine production before treatment \( \log(\text{cytokine/chemokine production in pg/mL after treatment} + 1) - \log(\text{cytokine/chemokine production in pg/mL before treatment} + 1) \). Changes in cytokine and chemokine production are shown using diamonds indicating means and 95% confidence intervals (CIs). To evaluate the effect of antiparasite treatment on cytokine and chemokine production, values from before and after treatment were analyzed by Student’s paired \( t \) test. Standardized log values of the cytokine and chemokine production by PBMCs (without subtraction of spontaneous cytokine and chemokine release [baseline]) were clustered by the Ward method for the 2 patient groups and the control group before and after treatment. Pairwise interactions between \( S. \) haematobium, \( S. \) mansoni, and \( N. \) americanus were described by odds ratios (ORs) together with their 95% CIs.

**RESULTS**

**Parasite coinfections in children.** Stool and urine samples collected from 729 children were examined. Of the samples, 49% were positive for \( N. \) americanus; 41% were positive for \( E. \) histolytica/E. dispar; 41% and 15% were positive for \( S. \) haematobium and \( S. \) mansoni, respectively; and 14% were free of parasites. Of the children, 32% were singly infected, and 54% were infected with 2 or more parasite species. The most common double infection was with \( N. \) americanus and \( E. \) histolytica/E. dispar; 41% and 15% were positive for \( S. \) haematobium and \( S. \) mansoni, respectively; and 14% were free of parasites. Of the children, 32% were singly infected, and 54% were infected with 2 or more parasite species. The most common double infection was with \( N. \) americanus and \( E. \) histolytica/E. dispers (7%); 5% had triple infections with \( N. \) americanus, \( E. \) histolytica/E. dispar, and \( S. \) haematobium. We observed significant pairwise interactions between hookworm and infection with Schistosoma species (OR for \( S. \) haematobium and \( S. \) mansoni, 4.73 [95% CI, 2.99–7.47] \( P < .001 \); OR for \( N. \) americanus and \( S. \) mansoni, 2.03 [95% CI, 1.32–3.11] \( P = .013 \); OR for \( N. \) americanus and \( S. \) haematobium, 1.74 [95% CI, 1.29–2.35] \( P = .005 \)). No interaction was observed for \( E. \) histolytica/E. dispar and any of the helminth infections.

**Infection groups and changes after treatment.** Children \( (n = 87) \) for whom cytokine and chemokine responses were investigated were grouped according to the presence or absence of infection (G0, G1, and G3+; see Methods). In the 3 study groups, more boys than girls were multiply infected, and more girls were free of infection. Even though children were repeatedly examined before being allocated into a study group, this finding could represent a sex bias between the study groups. Four weeks after treatment, the intensity of \( N. \) americanus infection was 204 eggs/g of stool among G3+ children, compared with 384 eggs/g of stool before treatment (table 1), and the \( S. \) haematobium infection intensity was 4 eggs/10 mL of urine, compared with 86 eggs/10 mL of urine before treatment. Among all children, 15 months after treatment, the prevalence of \( N. \) americanus infection was 44% (maximum intensity, 7584 eggs/g of stool); the prevalence of \( E. \) histolytica/E. dispar infection was 48%, and the prevalence of \( S. \) haematobium infection was 25% (maximum intensity, 30 eggs/10 mL of urine). Single infection with hookworm, \( S. \) haematobium, or \( E. \) histolytica/E. dispar was observed for 42% of the children; double and triple infection was observed for 30% and 5%, respectively, and 23% were negative for parasites.

**Cytokine and chemokine production in coinfected children after treatment.** The cellular production of cytokines and chemokines after activation of PBMCs with SmAg is shown in table 2, and posttreatment changes in cytokine and chemokine production for infected (G1 or G3+) children and noninfected control (G0) children (compared by Student’s paired \( t \) test) are shown in figure 1. Because of multiple testing, the Bonferroni-Holm adjustment was applied (number of tests, 36), and differences with \( P < .0016 \) were considered to be statistically significant. Four weeks after treatment, IL-13 production by PBMCs in response to EhAg enhanced after treatment for G1 and G3+ children by a factor of 138 (95% CI, 12–1570) and 21 (95% CI, 7–64), respectively (\( P < .001 \) for both). Also, SmAg-induced IL-13 responses increased after treatment in G1 and G3+ children (although the increase was not statistically significant \( P = .0026 \) and \( P = .002 \), respectively), whereas cellular IL-13 release remained similar for G0 children (\( P = .25 \)). Production of MIP-1\( \alpha/CCL-3 \) and MIP-1\( \beta/CCL-4 \) strongly diminished after treatment for G1 and G3+ children (for G3+ children, by a factor of 200 [95% CI, 33–1111] for MIP-1\( \alpha/CCL-3 \) and 26 [95% CI, 6–117] for MIP-1\( \beta/CCL-4 \); \( P < .001 \) for both), whereas TARC/CCL-17 production increased for G3+ children, by a factor of 10 (95% CI, 3–32) (\( P < .001 \)).

Production of RANTES/CCL-5 was enhanced for G0 children 4 weeks after treatment in response to EhAg and SmAg (statistically not significant \( P = .026 \) and \( P = .004 \), respectively)), whereas less RANTES/CCL-5 was produced in response to SmAg by PBMCs from G1 and G3+ children (statistically not significant \( P = .003 \) and \( P = .008 \), respectively) (figure 1). After treatment, the cellular production of IFN-\( \gamma \), IL-13, MIP-1\( \alpha/CCL-3 \), MIP-1\( \beta/CCL-4 \), and TARC/CCL-17 in responses to PHA did not change; also, no prominent changes in IL-12/IL-22p40 and IL-5 production were found in responses to any antigen.

**Plasma chemokine levels in children with polyparasite infections before and after treatment.** Before treatment, G3+ children had higher (\( P = .009 \)) levels of TARC/CCL-17 in plasma than did G0 children. Four weeks after treatment with albendazole and praziquantel, plasma chemokine levels decreased among G1 and G3+ children for TARC/CCL-17 (although the decrease was not statistically significant), and find-
ings were similar for MDC/CCL-22 (for G1, *P* = .004). Levels of TARC/CCL-17 and MDC/CCL-22 were increased in G0 children 4 weeks after treatment.

Cluster analyses of cytokine and chemokine production for infected and control children before and after treatment. Cluster analysis generated 3 groups, which matched T helper cell type 1 (Th1) or T helper cell type 2 (Th2) cytokine and proinflammatory chemokine profiles (figure 2A). All antigen and mitogen stimulations were merged for analysis, and distinct changes in cytokine and chemokine release were observed in clusters 2 and 3 after treatment. For cluster 2 (representing proinflammatory MIP-1α/CCL-3, MIP-1β/CCL-4, RANTES/CCL-5, and activation-associated TARC/CCL-17), chemokine levels were diminished among G1 children and were unaffected among G3+ children; however, levels of these chemokines strongly increased among G0 children. Levels of the Th2-associated cytokines of cluster 3 (IL-5 and IL-13) showed a clear increase after treatment among G1 and G3+ children but decreased among G0 children. For cluster 1 (representing IFN-γ, IL-12/IL-23p40, and MDC/CCL-22), cellular production did not change after antiparasite treatment in all groups.

**DISCUSSION**

In the present work, an increased risk for coinfections with *Schistosoma* species and hookworm has been identified for school-age children; furthermore, in those coinfected with hookworm, *Schistosoma* species, and *Entamoeba histolytica/Entamoeba dispar*, and *Necator americanus* (the G3+ group) in response to *S. mansoni* adult worm antigen (SmAg). PBMCs were activated with SmAg in vitro for 48 h, and cytokine and chemokine concentrations in cell culture supernatants were quantified as described in Methods. IQR, interquartile range.

<table>
<thead>
<tr>
<th>Group, cytokine or chemokine</th>
<th>Before treatment</th>
<th>4 weeks after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Level, median (IQR), pg/mL</td>
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<tr>
<td>IFN-γ</td>
<td>41</td>
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<tr>
<td>IL-13</td>
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</tbody>
</table>

**NOTE.** Cellular production of interferon (IFN)-γ, interleukin (IL)-13, macrophage inflammatory protein (MIP)-1α/chemokine (C-C motif) ligand (CCL)-3, MIP-1β/CCL-4, thymus- and activation-regulated chemokine (TARC)/CCL-17, and RANTES (regulated on activation, normal T cell expressed and secreted)/CCL-5 by peripheral blood mononuclear cells (PBMCs) from children without parasite infection (the G0 group), children singly infected with *Schistosoma haematobium* (the G1 group), and children multiply infected with *S. haematobium/Schistosoma mansoni, Entamoeba histolytica/Entamoeba dispar, and Necator americanus* (the G3+ group) in response to *S. mansoni* adult worm antigen (SmAg). PBMCs were activated with SmAg in vitro for 48 h, and cytokine and chemokine concentrations in cell culture supernatants were quantified as described in Methods. IQR, interquartile range.
Figure 1. Changes in cytokine (interferon [IFN]-γ and interleukin [IL]-13) and chemokine (macrophage inflammatory chemokine [MIP]-1α/chemokine [C-C motif] ligand [CCL]-3, MIP-1β/CCL-4, thymus- and activation-regulated chemokine [TARC]/CCL-17, and RANTES [regulated on activation, normal T cell expressed and secreted]/CCL-5) secretion by peripheral blood mononuclear cells (PBMCs) from children without parasite infection (the G0 group; \( n = 9 \) except for IL-13, for which \( n = 6 \)), children singly infected with *Schistosoma haematobium* (the G1 group; \( n = 12-14 \)), and children multiply infected with *S. haematobium*/*Schistosoma mansoni*, *Entamoeba histolytica*/Entamoeba dispar, and *Necator americanus* (the G3+ group; \( n = 29-33 \)) in response to antigens after antiparasite treatment. PBMCs were activated with *S. mansoni* adult worm antigen (SmAg) or *E. histolytica* antigen (EhAg) in vitro for 48 h, as described in Methods. The logs of the ratios for cytokine and chemokine production before and after treatment were calculated as described in Methods. Changes in cytokine and chemokine production are shown using diamonds indicating means and 95% confidence intervals (CIs). Data from before and after treatment were compared using Student’s paired \( t \) test. When the indicated 95% CIs did not intercept with 0, cytokine and chemokine production decreased or increased significantly after antiparasite treatment. \( \*P < .001 \) (significant change after \( \alpha \)-adjustment by the Bonferroni-Holm method).
by low infection intensities 15 months after treatment of children. A lack of IL-13 promotes chronic intestinal nematode infection in mice, whereas reconstitution of IL-13 expels *Trichuris muris* from their gut [23]. Such an amplified IL-13 production will enhance secretion of immunoglobulin (Ig) E, which plays an important role in the IgE-mediated cellular cytotoxicity that protects against helminth infections [24]. Furthermore, IL-13 is a potent inducer of TARC/CCL-17 secretion by PBMCs [25]; TARC/CCL-17 release by PBMCs increased significantly among G3+ children in response to SmAg, and TARC/CCL-17 is known for its capacity to induce the migration of Th2 cells into inflamed tissues. Such an augmentation of responsiveness after antiparasite chemotherapy in children with polyparasite infections suggests that cellular IL-13 as well as TARC/CCL-17 production may be related to the quantity of circulating parasite antigen present in an infected person. Chemotherapy and worm death will diminish the parasite antigen load and modulate the host cytokine and inflammatory chemokine responses, and this may accelerate the development of acquired immunity during schistosomiasis [26].

After antiparasite therapy, the cellular release of several chemokines strongly diminished, notably MIP-1α/CCL-3, MIP-1β/CCL-4, and RANTES/CCL-5. The CC chemokines MIP-1α/CCL-3 and MIP-1β/CCL-4 promote phagocyte migration and
activation and enhance inflammatory processes and granulocyte cytotoxicity, and their functional characteristics are observably not linked with an immune-modulating capacity [27]. In the present study, these chemokines were secreted in lower amounts 4 weeks after antihelminth therapy; therefore, inflammatory and granulomatous responses may have decreased in parasite-invaded tissues and organs. High MIP-1α/CCL-3 plasma levels have been found in the severe hepatosplenic form of schistosomiasis, with chronically infected individuals having higher MIP-1α/CCL-3 levels than uninfected control subjects [12]. In S. mansoni–infected mice, MIP-1α/CCL-3 and RANTES/CCL-5 were produced in cellular granulomas induced around S. mansoni eggs; chemokine neutralization diminished the size and changed the cellular composition of these granulomas [27]. Thus, the lowered levels of MIP-1α/CCL-3, MIP-1β/CCL-4, and RANTES/CCL-5 after treatment might signify diminished granulomatous responses in S. haematobium–infected children; also, given that these chemokines are associated with a Th1 immune response [28], proinflammatory monocyte–mediated and granulocyte-mediated activity may have decreased in strength as well.

Comparison of all cytokine and chemokine levels by cluster analyses [29] showed that levels of Th2 cytokines (cluster 3) increased in children with polyparasite infections after treatment, whereas levels of those that mediate inflammatory responses (cluster 2; e.g., MIP-1α/CCL-3 and MIP-1β/CCL-4) decreased after parasite elimination. The release of Th1 cytokines (cluster 1) did not change after treatment in any of the study groups; changes in clusters 2 and 3 were similar for the G1 and G3+ children and were clearly different from those for the G0 children.

Given that the common denominator between the G1 and G3+ groups is S. haematobium infection, this blood-dwelling helminth could have the predominant influence on PBMC responses. Similar changes in cytokine and chemokine responsiveness have been observed in adults coinfected with N. americanus, E. histolytica, and Mansonella perstans after parasite clearance by chemotherapy [18]. These and the present observations support the notion that, with diminished helminth and protozoan parasite loads, vigorous proinflammatory chemokine responses will decrease, and levels of cytokines and chemokines that favor Th2 responses will increase. Such changes in patients with polyparasite infections disclose a dynamic immune balance that may contribute to antihelminth immunity and regulate inflammatory processes and disease outcome.

In summary, coinfections with Schistosoma species, hookworm, and E. histolytica/E. dispar generated prominent proinflammatory cytokine and chemokine responses, and antihelminth treatment amplified Th2 responsiveness. However, a single intervention seemed to be insufficient to fully eliminate coinfections and was not able to generate immune responses in coinfected children that approached control levels.

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