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Differential Changes in Expression of Intestinal Antimicrobial Peptide Genes During Ascaris lumbricoides Infection in Zambian Adults Do Not Respond to Helminth Eradication

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Background. Intestinal helminthiasis modulates immune responses to vaccines and environmental allergens. To explore the impact on intestinal host defense, we assessed expression of antimicrobial peptide genes, together with T cell subset markers and cytokines, in patients with ascariasis before and after treatment.

Methods. Case patients (n = 27) and control subjects (n = 44) underwent enteroscopy for collection of jejunal biopsy specimens, which were used in quantitative, real-time reverse-transcription polymerase chain reaction for a range of host defense genes; blood samples were also analyzed simultaneously.

Results. The level of gene expression (mRNA) of HD5, hBD1, and LL-37 was lower in case patients than in control subjects, and the level of expression of HD6 was increased. However, after successful eradication, there was no trend to values seen in control subjects. Helminthiasis was associated with increased intestinal expression of the Th1 genes T-bet and interferon-γ. In peripheral blood mononuclear cells (PBMCs), a mixed profile of T cell markers and cytokines was increased. Ascaris-induced down-regulation of HD5 was observed in individuals with higher RORγt expression in PBMCs, but we found no evidence that this was mediated by circulating interleukin-22.

Conclusions. Human ascariasis was associated with changes in antimicrobial peptide gene expression and immunological markers. Such changes may have implications for susceptibility to infectious disease and responsiveness to oral vaccines in tropical populations.

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Helminth infections are common infectious diseases in tropical countries, where millions are infected with Ascaris species, hookworms, Schistosoma species, and Taenia species parasites [1]. Most infections have modest clinical significance, but there are probably effects on growth and development in children and on work capacity and well-being in adults. Some individuals with severe infections develop serious consequences [1, 2]. There is emerging evidence that immune dysregulation consequent upon helminth infection may alter responses to certain vaccines as well as to other infections [2–7]. Paradoxically, populations not exposed to the immunological effects of helminth infection may also be at risk of disease, including chronic inflammatory conditions, such as Crohn disease, ulcerative colitis, and asthma. There is some evidence that these disorders can be treated by reimpposition of the immunological restraints on inflammation that helminth infections confer [8–10].
In animal models—notably, murine infection models of *Trichuris muris* or *Schistosoma mansoni*—control and/or eradication of infection requires attack by eosinophils driven by a predominant Th2 response [11]. Hosts (such as the AKR strain of inbred mouse) that mount a predominantly Th1 response involving interferon (IFN)-γ go on to develop chronic infections [12], and this IFN-γ contributes towards a hyperplastic mucosal pathology [13]. A Th2-mediated immune response promotes the release of parasite-specific antibodies [7]. Interleukin (IL)–5 has been shown to be involved in eosinophilia and goblet cell hyperplasia, whereas IL-13 and alternatively activated macrophages are implicated in worm expulsion and anti-larval activity, with involvement of basophils in the innate immune response to infection [3, 14–16]. Mice with deletions in Toll-like receptor (TLR) 4 or MyD88 become resistant to *T. muris* infection, possibly because the expression of IFN-γ induced by TLR signaling mediates a Th1 response, which promotes subsequent pathology [17]. There is growing evidence of the activity of antimicrobial molecules in defense against other gut-tropic pathogens and in modulation of the intestinal microbiota [3, 18–21]. However, very little is known about the role of antimicrobial peptides (AMPs) in control of helminth infections or of the impact of colonisation by helminths on innate immune defenses. Lysozyme does not play a role, but the Paneth cell product intelectin is up-regulated in mice that go on to clear infection [22, 23].

With the growing evidence that AMP expression in the gut is important in determining susceptibility to intestinal infection and susceptibility to inflammatory disease [18–20, 24], we set out to define and characterize the AMP expression profile in chronic infection, *Ascaris lumbricoides* in human hosts. We analyzed the mRNA expression of the genes for α–defensins, β–defensins, and LL-37 in intestinal biopsy specimens, together with cytokine and T cell subset transcription factor marker genes both in the gut and in peripheral blood mononuclear cells (PBMCs) by means of real-time reverse transcription polymerase chain reaction (RT-PCR).

**MATERIALS AND METHODS**

**Study Setting**

Study participants were recruited from Misisi township in Lusaka, Zambia. This is an unplanned settlement south of Lusaka with poor sanitation and inadequate hygiene facilities. Adults (age, ≥18 years) were recruited for the study if they were resident in a defined sector of Misisi and gave informed consent. Potential participants were excluded if they were pregnant or lactating, had experienced diarrhea ≤1 month before planned participation, or had taken antibiotics or nonsteroidal anti-inflammatory drugs in the same period. Prior work in this population revealed that 17.4% of human immunodeficiency virus (HIV)–negative adults were infected with *A. lumbricoides*, compared with 13.1% of HIV-positive individuals [25]. Ethics approval was obtained from the University of Zambia Research Ethics Committee (007-10-07).

**Determination of Helminth Infection and Treatment**

All participants submitted 3 stool samples over 3–5-day periods that were screened for the presence of eggs using the Kato-Katz technique. If helminth infection was found, the persons were designated as case patients, and they were designated as control subjects if not. Depending on the parasite species detected, infected participants were treated with albendazole (Zentel; GSK; 400 mg twice daily for 3 days), praziquantel (Biltricide; Bayer Pharmaceuticals Corporation; 400 mg/kg in 3 divided doses on 1 day), and/or ivermectin (Stromectol; MSD; 9 mg [~200 µg/kg] as a single dose). Stool samples were collected after treatment and checked for infection to confirm eradication of worms. Ten milliliters of blood was collected prior to endoscopy to perform a complete blood cell count (including eosinophil count), to determine HIV status, and to perform a CD4 cell count if the subject was HIV seropositive, and serum was separated and stored at -80°C for cytokine enzyme-linked immunosorbent assay (ELISA).

**Biopsy Sample Collection and PBMC Isolation**

Participants underwent enteroscopy with a fiberoptic endoscope (Olympus SIF-10) under sedation to collect biopsy samples of the jejunum, followed immediately by treatment described above. An additional set of biopsies was performed either 7 or 14 days after initiation of treatment. Samples were immediately suspended in Tri Reagent (Sigma) and frozen at -80°C for RNA extraction. An additional 20 mL of venous blood was collected prior to endoscopy in EDTA tubes (BD Biosciences) before and after treatment. PBMCs were separated using Ficoll-Paque Premium (GE Healthcare) and washed once in phosphate-buffered saline (Sigma), and 1 × 10⁷ cells were resuspended in Tri Reagent (Sigma) and stored at -20°C ready for RNA extraction.

**RNA Extraction and cDNA Preparation**

RNA was extracted using the phenol-chloroform extraction method, as described elsewhere [26]. In brief, all gut biopsy samples were broken up using a mini-homogenizer and sterilized pestles and stored in fresh sterile molecular biology grade (MBG) water (Sigma). RNA was subjected to DNase treatment using RQ1 DNase (Promega, UK) in the presence of RNA ribonuclease inhibitor (Promega). DNase-treated RNA was then extracted using phenol-chloroform-isoamyl alcohol (ratio, 25:24:1; Sigma) and quantified by spectroscopy. cDNA was prepared using standard protocols.

**Quantitative Real-time RT-PCR**

Primers for the CD4⁺ T cell transcription factors RORγt, T-bet, GATA-3, and Foxp3 and the cytokines IFN-γ, transforming growth factor (TGF)–β, tumor necrosis factor (TNF)–α, IL-4,

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IL-5, and IL-10 were generated using Primer 3 software (http://frodo.wi.mit.edu/primer3/input.htm) and synthesized by Sigma. (For sequences, see http://www.icms.qmul.ac.uk/Profiles/DigestiveDiseases/KellyPaul.htm.) GAPDH was used as a reference standard for all PBMC cDNA, whereas CK-19 and GAPDH were used for gut cDNA. Quantitative real-time PCR was performed using a Corbett Rotor Gene thermal cycler and SYBR Green (Qiagen) detection over 45 cycles of 95°C, 60°C, and 72°C. cDNA from IL-1β–treated Caco-2 cells was used as a positive control for all the gut RT-PCRs, whereas MBG water was used as negative control (at least 3 in each run). HD5 and HD6 were expressed as transcripts/µg total RNA using plasmid standards, as previously described [20], but all other results were expressed relative to GAPDH and/or CK-19.

Cytokine ELISAs
Aliquots of serum from case patients and control subjects that had been stored at -80°C for ≤6 months were used for measurement of IFN-γ, IL-13, IL-22, and TGF-β by ELISA (R&D Systems) in accordance with the manufacturer’s instructions.

Data Analysis
Data analysis was performed using Stata software, version 10.1 (StataCorp), and GraphPad Prism, version 5.01 (GraphPad Software). Nonparametric statistical tests were used because of the nonnormal distribution of the data, which are summarized below as median and interquartile range (IQR). The Fisher exact test was used for proportions, the Kruskal-Wallis test was used to compare distributions of unpaired data (case patients and control subjects), and the Wilcoxon matched-pair rank sum test was used to compare pre- and posttreatment values for the same individual. Spearman’s rank correlation was used to determine the correlation of peripheral blood and intestinal cytokine and T cell subset markers.

RESULTS

Study Population in Misisi, Lusaka
We recruited 71 consenting individuals (age, 21–60 years). Case patients were defined as those participants found to have A. lumbricoides infection; coinfections with Strongyloides stercoralis, S. mansoni, and Taenia saginata were also found (Table 1). Case patients (n = 27) and control subjects (n = 44) were similar with respect to age and HIV status, although more women were found (P = .002) with helminthiasis (Table 1), and ownership of the house of residence showed clear evidence that case patients were of lower economic status (P = .03) (Table 1).

### Table 1. Characteristics of the Study Population and Helminthiasis Diagnosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Infected Group</th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Ratio of male to female subjects</td>
<td>3:24</td>
<td>20:24</td>
<td>.002*</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>38 ± 11.2</td>
<td>38.8 ± 11.1</td>
<td>NSb</td>
</tr>
<tr>
<td>Range</td>
<td>21–59</td>
<td>22–61</td>
<td></td>
</tr>
<tr>
<td>Ratio of HIV-positive to HIV-negative subjects (% of HIV-positive subjects)</td>
<td>10:17 (37)</td>
<td>15:29 (34.1)</td>
<td>NSb</td>
</tr>
<tr>
<td>CD4 cell count in HIV-infected subjects, cells/µL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>475 ± 128.8</td>
<td>502 ± 316</td>
<td>NSb</td>
</tr>
<tr>
<td>Range</td>
<td>247–671</td>
<td>242–1398</td>
<td></td>
</tr>
<tr>
<td>Eosinophil count, median (range)</td>
<td>0.2 (0.05–1×10^9/l)</td>
<td>0.08 (0.04–0.72)</td>
<td>.01×10^9/lbb</td>
</tr>
<tr>
<td>Hemoglobin concentration, median g/dL (range)</td>
<td>13.3 (6.9–18.9)</td>
<td>14.35 (11.3–15.8)</td>
<td>.02b</td>
</tr>
<tr>
<td>Infectious organism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris lumbricoides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of infected persons</td>
<td>27</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Median egg count (range)</td>
<td>22,096 (168–63,840)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Strongyloides stercoralis, no. of infected persons</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Schistosoma mansoni, no. of infected persons</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Taenia saginata, no. of infected persons</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Giardia intestinalis, no. of infected persons</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Secondary education, no. of subjects</td>
<td>4</td>
<td>7</td>
<td>NSb</td>
</tr>
<tr>
<td>Ownership of house, no (%) of subjects</td>
<td>9 (33)</td>
<td>27 (61)</td>
<td>.03</td>
</tr>
<tr>
<td>Estimated household income, kwacha</td>
<td>300,000</td>
<td>350,000</td>
<td>NSb</td>
</tr>
</tbody>
</table>

NOTE. HIV, human immunodeficiency virus; NS, not significant; SD, standard deviation.

* P value determined by the Fisher exact test.

b P value determined by the Kruskal-Wallis test.
Case patients also had eosinophilia ($P = .01$) and lower hemoglobin levels ($P = .02$). Stool samples examined after treatment showed successful helminth eradication in all case patients.

**Gastrointestinal Expression of AMPs Differed in Case Patients and Control Subjects**

We characterized the gastrointestinal expression profiles in jejunal biopsy samples from case patients and control subjects using RT-PCR for mRNA of the α-defensins (HD5 and HD6), the β-defensins hBD1 and hBD2, and the cathelicidin LL-37. Biopsy samples were taken before treatment and at 7 ($n = 6$) or 14 ($n = 21$) days after treatment; there was no difference in the changes in mRNA of HD5 and HD6 at 7 or 14 days, so these data are considered together (Figures 1A and 1B). After treatment, HD5 and HD6 expression did not change overall (Figure 1C and 1D), but some variation was evident between individuals: down-regulation of HD5 was observed in 60% of helminth-infected individuals during infection, compared with posttreatment values (Figure 1C), and in 48% of these subjects, we observed down-regulation of HD6 expression (Figure 1D). Expression of HD5 was lower in biopsy samples taken from case patients before (median, 1234; IQR, 286.6 - 6444) and after (median, 1030; IQR, 276.1 - 14,045) treatment than in control subjects (median, 8721; IQR, 646.7 - 56,384) (Figure 1E), but expression of HD6 before (median, 43,353; IQR, 3048-405,461) and after (median, 73,946; IQR, 8267-360,127) was higher than in control subjects (median, 9210; IQR, 6904 - 27,750) (Figure 1F). hBD1 and LL-37 expression was lower in case patients, and there was no detectable mRNA of hBD2 in any biopsy specimen obtained in this study (Table 2), even though mRNA from IL-1β-treated Caco-2 cells gave a clear positive amplification signal for hBD2 (data not shown). Expression of these antimicrobial peptide genes was completely unaffected by HIV status (data not shown).

**Gastrointestinal Expression of Th1 Phenotype Increased During Helminth Infection**

We measured mRNA expression of CD4+ T cell subset signature transcription factors and cytokines in biopsy samples from the infected group before and after treatment but from none of the control subjects, because mRNA from intestinal tissue was limited in quantity. There was a decrease in Th1 markers after treatment, as reflected in IFN-γ mRNA expression (observed in 72% of case patients; $P = .009$) and T-bet (in 88% of case patients; $P = .01$), suggesting that they were up-regulated during infection. In quantitative terms, IFN-γ mRNA relative to GAPDH decreased following treatment ($P = .009$) and T-bet mRNA decreased ($P = .01$, using the Wilcoxon rank sum test) after treatment (Figure 2A and 2B respectively). There was no change in TNF-α or RORγt expression (Figure 2C and 2D, respectively) nor in expression of GATA-3, Foxp3, TGF-β, IL-4, IL-10, or IL-5 (data not shown).

**Helminth Infection Was Associated with Up-Regulated Peripheral Blood Markers of All Major T cell Subsets and Some Cytokines**

To compare the changes in the intestinal mucosa with the systemic response, we looked at peripheral T cell signature transcription factor mRNA expression in PBMCs from case patients before and after treatment and from control subjects. There was up-regulation of Th1 ($P < .0001$), Th2 ($P = .04$), Th17 ($P < .0001$), and Treg ($P = .0002$) markers in pretreatment case patients, compared with control subjects (Figure 3A), and altered expression was observed in a range of cytokines: IFN-γ ($P = .03$) and IL-10 ($P = .002$) were down-regulated, whereas IL-5 ($P < .0001$) and TGF-β ($P < .0001$) were up-regulated (Figure 3B). Neither TNF-α nor IL-4 showed any changes (data not shown). There was no change in subset markers after treatment (Figure 3A) or in any of the cytokines measured (IFN-γ, IL-5, TGF-β, and IL-10 [Figure 3B]; data not shown for TNF-α and IL-4). Expression of these genes did not differ between HIV-infected and HIV-uninfected case patients or control subjects.

**Correlations Between Peripheral Blood T cell Subset Markers and Cytokines**

To explore the modulation of ascariasis on these T cell markers and cytokines, we looked for correlations between the markers measured. Although the number of case patients and control subjects is modest, some strong and significant positive correlations were observed. Several correlations were seen only in helminth infection and not in control subjects, some of which are expected (such that between GATA-3 and Foxp3) and some of which link apparently antagonistic subsets (such as T-bet and IL-10 or IFN-γ and IL-4) (see Supplementary Tables 1 and 2 at https://docs.google.com/document/pub?id=1k0m0blJbJZGpppLheHNSldwXLMSoXLAM4OtLksI7YjW1). Helminth infection appears to abolish the correlation between GATA-3 and TNF-α or between Foxp3 and IL-4. None of the correlations were seen exclusively as a consequence of HIV infection (see Supplementary Tables 3 and 4, also at https://docs.google.com/document/pub?id=1k0m0blJbJZGpppLheHNSldwXLMSoXLAM4OtLksI7YjW1).

**HDS Suppression During Helminth Infection Was Associated With Peripheral Blood RORγt Expression**

To explore the modulation of HD5 and HD6 expression during infection, we dichotomized the cases into those with up-regulation during expression (as inferred from changes following eradication) and those with suppression, and we correlated this change with mRNA of T cell subset markers in peripheral blood. We found that HD5 suppression was significantly associated with increased expression of RORγt in PBMCs (Figure 4). The expression of Th1, Th2, and Treg markers was not associated with this suppression, and changes in HD6 were not significantly associated with any of the markers used (Figure 4). There was no significant association with any cytokine mRNA and HD5 or HD6 suppression, nor was it associated with HIV status.
Figure 1. Differential expression of α-defensins in the intestinal mucosa during ascariasis. mRNA for HD5 and HD6 was quantified in jejunal biopsy samples obtained from control subjects and from case patients before and after helminth eradication and are expressed as transcripts/µg of total RNA extracted. A and B, expression of individual samples in multiple biopsy specimens taken after 7 or 14 days after treatment. C and D, changes following treatment expressed as –fold change, relative to pretreatment values, such that values >1 represent an increase in expression and <1 as a decrease during infection. E and F, absolute mRNA quantities shown in case patients versus control subjects (represented as box whisker plots of median, interquartile range, and range).
Serum Cytokines Representative of Major T cell Subsets Were All Down-Regulated in Helminth Infection and Did Not Explain HD5 Suppression

To explore further the peripheral blood T cell responses, we measured concentrations of 4 cytokines in serum that had been chosen to be representative of the 4 major T cell subsets: IFN-γ, IL-13, IL-22, and TGF-β. IFN-γ was not detected in most serum specimens (data not shown). IL-13 and IL-22 concentrations were lower in case patients than in control subjects and did not change after helminth eradication. IL-13 was detected in all samples measured (in 35 control subjects and in 25 case patients) but was lower in case patients (median, 91.4 pg/mL; IQR, 82-142 pg/mL) than in control subjects (median, 220.3 pg/mL; IQR, 146.7-289.2 pg/mL; P < .0001) (Figure 5). IL-22 was detected in 16 control subjects (out of 30 samples) and 12 case patients (out of 27 samples) and was also lower (median, 0.04 pg/mL; IQR, 0.0-18.4 pg/mL) in case patients than in control subjects (median, 22.4 pg/mL; IQR, 0.0-36.5 pg/mL; P = .001). TGF-β was detected in 15 control subjects (out of 35 samples) and in 13 case patients (out of 25 samples), but the concentration did not differ between case patients (median, 0.0 pg/mL; IQR, 0.0-8.1 pg/mL) and control subjects (median, 0.0 pg/mL; IQR, 0.0-62.8 pg/mL) (Figure 5). There was no increase

Table 2. Ascariasis was Associated With Attenuated Expression of hBD1 and LL-37

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>hBD1</th>
<th>hBD2</th>
<th>LL-37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>27</td>
<td>NE</td>
<td>NE</td>
<td>8</td>
</tr>
<tr>
<td>After treatment</td>
<td>27</td>
<td>NE</td>
<td>NE</td>
<td>7</td>
</tr>
<tr>
<td>Control group</td>
<td>24</td>
<td>13a</td>
<td>NE</td>
<td>21a</td>
</tr>
</tbody>
</table>

**NOTE.** hBD, human β-defensin; NE, no detectable mRNA expressed.

* P = .0001, determined by the Kruskal-Wallis test of differences between control and pretreatment groups.

Figure 2. Association between helminth infection and increased expression of gastrointestinal CD4+ Th1 phenotype markers. Jejunal biopsy samples obtained before and after treatment were used to quantify, by real-time reverse-transcription polymerase chain reaction, the expression of interferon (IFN)–γ (A), T-bet (B), tumor necrosis factor (TNF)–α (C), and RORγt (D) represented as box whisker plots of median, interquartile, and range. mRNA expression is shown relative to GAPDH.
DISCUSSION

We have previously shown that helminth infection (particularly ascariasis) is common in this population [25], but the effects of helminth infection on innate defense mechanisms in the intestinal mucosa are, to our knowledge, unknown. Our finding that helminthiasis is associated with lower expression of HD5, hBD1, and LL-37 might explain some of the previously noted reduction in HD5 expression in this population [26] but not the reduction in HD6. However, this would only be true if exposure to helminths causes the change in HD5 expression and not if low HD5 expression predisposes to helminthiasis. To our knowledge, there are no data on the effect of human antimicrobial peptides on nematode development. In general,

Figure 3. Association between helminth infection and altered peripheral expression of CD4\(^+\) T subset markers and cytokines. Peripheral blood mononuclear cells were isolated from blood from control subjects and case patients before and after treatment, and real time reverse-transcription polymerase chain reaction was performed; mRNA expression is shown relative to GAPDH for CD4\(^+\) T subset markers (Figure 3A) and cytokines (Figure 3B) and represented as box whisker plots of median, interquartile range, and range.

after treatment and no computed association between HD5 suppression and concentrations of IL-13, IL-22, or TGF-β.

Figure 4. Association between HD5 suppression and increased expression of RORγt during infection. The study group was dichotomized into those with up-regulation of HD5 or HD6 during helminth infection and those with down-regulation of HD5 or HD6. mRNA of CD4\(^+\) T cell subset markers, mean expression ratio to GAPDH, in peripheral blood mononuclear cells is shown in relation to the changes in gastrointestinal expression of HD5 and HD6 expression. Asterisks represent statistical significance. *\(P = .01\).
the lower antimicrobial peptide expression in case patients (higher expression in the case of HD6) did not change significantly after treatment, which could be explained either if lower defensin expression predisposes to helminthiasis or if 14 days is insufficient for such changes to occur. We did not examine antimicrobial peptide expression after 14 days, because intercurrent intestinal infections, to which adults in this community are exposed [25], might complicate interpretation of immunological changes during convalescence. Therefore, we cannot distinguish between these possibilities, and we propose that the only way to resolve the issue of causation is to study experimental helminth infection in naive volunteers.

Our data indicate that the dominant T helper phenotype in control subjects was Th2 but that, in case patients, a Th1 subset was more prominent during infection. In animal studies, a dominant Th2 response is associated with clearance of helminth infection, but a dominant Th1 response is associated with persistent infection [11]. Our data are therefore consistent with the hypothesis that persistent gut helminthiasis in humans is at least partly a failure of the Th2 response. This also correlates with observations that a predominant Th2 phenotype (with its associated cytokines) in humans confers immunity to reinfection and/or immunity [7, 27, 28]. Proinflammatory cytokines can suppress defensin expression [29], which would fit with Th1- or Th17-mediated defensin suppression in helminthiasis when the Th2 response is attenuated.

Recent work done by Salzman et al [21] suggests that HD5 expression regulates IL-17A production by Th17 cells in the small intestine. Helminth infection has been shown not to induce the expression of IL-22 in the liver [30]. However, IL-22 has been reported to be involved in the immune response to other infections in the gut mucosa, so it is interesting to speculate that helminth-mediated immunomodulation might alter host susceptibility to those bacterial infections against which IL-22 is protective [31]. Helminth infection was associated with reduced concentrations of IL-22 in serum, so clearly more work needs to be done to understand the effect of helminth infections on Th17 responses. These interactions are also complicated by

**Figure 5.** Serum cytokine concentrations in helminth infection. Serum concentrations of 4 cytokines, selected to represent major T cell subsets (interferon [IFN]–γ, interleukin [IL]–13, IL-22, and transforming growth factor [TGF]–β), were measured by enzyme-linked immunosorbent assay in samples from control subjects and in pretreatment or posttreatment samples from case patients (represented as box whisker plots of median, interquartile range, and range). Asterisks represent statistical significance for Kruskal-Wallis test of differences. ***P < .0001; **P = .001.
the fact that RORγt expression in the intestine is not entirely restricted to the CD4+ Th17 phenotype and that there are other immune cells that also express RORγt and possibly secrete IL-22 [32, 33].

It is not known how the presence of luminal helminths is sensed by the immune system. Ascarid worms occupy a purely luminal niche, and there is no attachment or invasion within the mucosa, unlike adult hookworms or Strongyloides species, which have a more obvious point of contact with host immune sensory elements. However, the jejunum is the primary site for larval localisation [34]. It is also likely that soluble antigens shed by the worms could be sensed by inductive sites in Peyer’s patches if there are receptors that can distinguish them from food- or microbiota-derived antigens. Helminth-derived glycans are involved in the activation of various antigen presenting cells (reviewed in [35]). The dissociation between posttreatment CD4+ T cell subset phenotypes and cytokine expression in gut and peripheral blood might suggest the presence of long-lasting circulating antigens that could possibly circulate for long periods of time [36].

We were surprised about the predominance of women in the case patients. We have previously noted an under-representation of younger men in the Missisa cohort but we would expect this to apply to case patients and control subjects equally. The rate of house ownership, one measure of economic status, was significantly lower among case patients. We found no influence of HIV infection on antimicrobial peptide expression, which is entirely consistent with our previous observations in this population [20, 37]. We were somewhat surprised that we could detect no effect of HIV on mRNA of T cell subsets or the cytokines we tested. In the case of mucosal expression of these genes, we did not have sufficient mRNA to quantify the mRNA in control subjects, so it may be that helminth infection overrides any effect of HIV. In PBMCs, there was clearly no effect of HIV in either case patients or control subjects, but the number of control subjects was small.

We believe that our data showing modulation of AMP gene expression are the first data of this kind in humans. There were surprisingly few changes in AMP expression following eradication, at least within the time scale of our study. The effect of Th17 cells that are implied by our observations about RORγt are intriguing and deserve future exploration. It would be useful in future studies to characterize T cell subsets by flow cytometry, but even very detailed characterization will not answer the fundamental question that our study raises, which is whether the changes we observed are a consequence of helminth infection or in some way predispose to it. This question can probably only be answered by challenge studies in human volunteers.

Supplementary Data

Supplementary data are available at http://jid.oxfordjournals.org online.
34. Sukhdeo MVK, O'Grady RT, Hsu SC. The site selected by the larvae of Heligmosomoides polygyrus. J Helminthol 1984; 58:19–23.