Changes in Plasma Cytokines after Treatment of *Ascaris lumbricoides* Infection in Individuals with HIV-1 Infection

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Albendazole treatment of individuals with human immunodeficiency virus type 1 (HIV-1) and *Ascaris lumbricoides* co-infection has led to significantly improved CD4+ cell counts and a trend for lower plasma HIV-1 RNA levels in a previous randomized placebo-controlled trial. To define mechanisms by which deworming contributed to changes in markers of HIV-1 disease progression, plasma cytokine levels were evaluated. Albendazole treatment, compared with placebo, was associated with significantly decreased plasma interleukin (IL) 10 levels \((P = .04)\) but was not associated with significant changes in levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12p70, IL-13, interferon \(\gamma\), tumor necrosis factor \(\alpha\), or thymic stromal lymphopoietin. Treatment of *A. lumbricoides* co-infection may delay HIV-1 disease progression by reducing helminth-induced, IL-10-mediated immunosuppression.

The human immunodeficiency virus type 1 (HIV-1) pandemic has disproportionately affected individuals in sub-Saharan Africa, where over one-half of the population may also be co-infected with helminths [1]. A systematic review of available trials suggests that treatment for infection due to some helminth infected individuals, compared with that of helminth-uninfected control subjects, these immune perturbations result in lower levels of the Th1 cytokines interleukin (IL) 2 and interferon (IFN) \(\gamma\), higher levels of the Th2 cytokines IL-4 and IL-5, and higher levels of both proinflammatory (IL-6) and immunosuppressive (IL-10) cytokines [3]. These immune alterations could compromise control of chronic HIV-1 infection by inhibiting effective Th1/cellular immune responses to HIV-1 [1, 4] and by generalized immune activation which may contribute to HIV-1 pathogenesis [5].

We recently reported results from a randomized, double-blind, placebo-controlled trial that demonstrated that albendazole treatment of *A. lumbricoides* infection in HIV-1 co-infected individuals resulted in significantly higher CD4+ cell counts after 12 weeks of follow-up (a mean difference of 109 cells/mm3; \(P = .003\)) and a trend for decreased viral load (0.54 log10 copies/mL HIV-1 RNA levels; \(P = .09\)), compared with placebo [6]. Samples from this study afforded the opportunity to evaluate changes in plasma cytokine levels in individuals with HIV-1 and *A. lumbricoides* co-infection after treatment with albendazole or placebo, to identify whether deworming was associated with significant changes in plasma cytokine levels.

**Methods.** Plasma samples for this study were collected as part of a randomized, double-blind, placebo-controlled trial, details of which have been published elsewhere [6]. In brief, individuals with evidence of co-infection with albendazole-treatable soil-transmitted helminths (*A. lumbricoides*, *Trichuris trichiura*, or hookworm species) were enrolled and randomized to albendazole or placebo and followed up for 12 weeks. CD4+ cell counts and plasma HIV RNA assays were performed at baseline and at the 12-week visit. The study was approved by the Kenya Medical Research Institute Ethical Review Board and the University of Washington Institutional Review Board (Seattle, Washington). All participants provided written informed consent. Patients were randomized to receive an oral tablet of albendazole \(400 \text{mg} \) or a placebo daily for 3 days, followed by a placebo tablet daily for 9 days. Plasma samples for this study were collected as part of the trial [6].

Intervention to delay HIV-1 disease progression. Elucidating the mechanisms by which treatment of some helminths delays HIV-1 disease progression may inform the development of interventions to slow HIV-1 disease progression and lead to better understanding of HIV-1 pathogenesis.

Alterations in immunity caused by helminth infections may contribute to HIV-1 disease progression in co-infected individuals. Helminth infections induce Th2 and immunosuppressive pathways while simultaneously contributing to generalized immune activation. In the serum of *Ascaris lumbricoides*-infected individuals, compared with that of helminth-uninfected control subjects, these immune perturbations result in lower levels of the Th1 cytokines interleukin (IL) 2 and interferon (IFN) \(\gamma\), higher levels of the Th2 cytokines IL-4 and IL-5, and higher levels of both proinflammatory (IL-6) and immunosuppressive (IL-10) cytokines [3]. These immune alterations could compromise control of chronic HIV-1 infection by inhibiting effective Th1/cellular immune responses to HIV-1 [1, 4] and by generalized immune activation which may contribute to HIV-1 pathogenesis [5].
Levels of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, and tumor necrosis factor (TNF) α in stored plasma samples were determined using Lincoplex 13-plex High Sensitivity Human Cytokine Panels (Lincos/Milipore) on a Luminex 200 (Luminex). The upper limit of cytokine detection was 2000 pg/mL, and the lower limit of detection was 0.3 pg/mL for each cytokine. Samples with a cytokine level that was less than the lower limit of detection were assigned a value of 0.15 pg/mL. Plasma levels of thymic stromal lymphopoietin (TSLP) were determined with enzyme-linked immunosorbent assay (R&D Systems) with a lower limit of detection of 15.6 pg/mL; samples with cytokine levels that were less than the lower limit of detection were assigned a level of 7.8 pg/mL.

Statistical analyses were performed using Stata software, version 9.2 (StataCorp). The effect of treatment on CD4+ cell counts and HIV-1 RNA levels was determined as described elsewhere [7]. To compare cytokine levels between treatment groups and between the initial and follow-up time points, the Wilcoxon rank-sum test was used. Median regression, controlling for baseline cytokine levels, was used to determine the effect of albendazole treatment on the change in plasma cytokine levels [8]. Holm’s method was used to correct for multiple testing [9]. Spearman’s rank correlation was used to determine the relationship between cytokine levels and the CD4+ cell count and viral load. Principal components analysis was used to assess the clustering of baseline cytokine levels and HIV-1 plasma viral load and CD4+ cell count.

Results. Data were collected from 38 individuals with *A. lumbricoides* co-infection with available plasma samples from both initial and 12-week follow-up visits (18 albendazole-treated and 20 placebo-treated subjects). Demographic and baseline laboratory data from this subset of individuals were similar to data previously described for the cohort enrolled in the randomized trial [6]. At enrollment, the mean CD4+ cell count was 524 cells/mm3 and the mean plasma HIV-1 RNA was 4.68 log10 copies/mL. There were no statistically significant differences in baseline demographic or laboratory variables between the randomization arms. Albendazole treatment was associated with significantly higher CD4+ cell counts (a mean difference of 119 cells/mm3; *P* = .01) and a trend towards decreased viral load (0.46 log10 lower HIV-1 RNA level; *P* = .08) in the subset of *A. lumbricoides* co-infected individuals included in this analysis. These differences were similar to those seen in the entire intent-to-treat cohort reported elsewhere [6].

At both the initial and 12-week follow-up appointments, a wide range of cytokine concentrations, from <0.3 pg/mL to >100 pg/mL were detected for the Th2 cytokines (IL-4, IL-5, IL-13; Figure 1A), the Th1 cytokines (IL-2, IFN-γ, and TNF-α; Figure 1B), the T cell differentiation and maintenance cytokines (IL-7, IL-12p70, and TSLP; Figure 1C), the proinflammatory cytokines (IL-1β, IL-6, and IL-8; Figure 1D), and the immunosuppressive cytokine IL-10 (Figure 1E). Only levels of IL-10, TNF-α, and IL-8 were above the limit of detection in all individuals at both time points (Figure 1). Levels of several cytokines were frequently below the limit of detection of 0.3 pg/mL; in particular, less than one-half of the subjects had either initial or follow-up plasma levels of IL-4, IL-12p70, and IL-1β above the limit of detection (Figure 1). In addition, only 6 individuals (3 each in the placebo-treated and albendazole-treated arms) had levels of TSLP above the limit of detection of 15.6 pg/mL (Figure 1C).

Baseline cytokine levels of IL-4, IL-5, IL-13, IL-2, IFN-γ, TNF-α, IL-7, IL-12p70, TSLP, IL-1β, and IL-8 at the initial clinic visit did not differ between the placebo and the albendazole groups (*P* > .2 for all comparisons). At the baseline visit, levels of IL-6 were significantly higher in the albendazole arm (median IL-6 level, 4.7 pg/mL) than they were in the placebo arm (median IL-6 level, 1.6 pg/mL) (*P* = .015). At baseline, IL-10 levels were also significantly higher in the albendazole-treated group (median IL-10 level, 15 pg/mL) than they were in the placebo group (median IL-10 level, 7.7 pg/mL) (*P* = .01). The baseline plasma levels of IL-10 (Spearman’s rho = 0.50; *P* = .001) and IL-5 (Spearman’s rho = 0.35; *P* = .03) correlated directly with the viral load. Plasma levels of IL-10 (Spearman’s rho = −0.44; *P* = .005) and IL-2 inversely correlated with the CD4+ cell count (Spearman’s rho = −0.41; *P* = .01). A principal components analysis of baseline cytokine levels and CD4+ cell count and viral load revealed strong clustering between the cytokines within the proinflammatory, Th1, and Th2 groups, although TNF-α clustered with the proinflammatory cytokines. Only baseline IL-10 and IL-12p70 levels clustered with the CD4+ cell count and viral load.

No significant changes in the levels of the Th2 cytokines IL-4, IL-5, or IL-13 (Figure 2A); the Th1 cytokines IL-2, IFN-γ, or TNF-α (Figure 2B); the T cell differentiation and maintenance cytokines IL-7, IL-12p70, or TSLP (Figure 2C), or the proinflammatory cytokines IL-1β, IL-6, or IL-8 (Figure 2D) were observed with albendazole treatment, compared with placebo, controlling for baseline cytokine level (*P* > .2 for all comparisons). Although the albendazole-treated group did not demonstrate significant changes in any of these cytokine levels, 3 individuals displayed marked reductions in levels of TNF-α, IL-6, and IL-8 (Figure 1B and 1D). These reductions imply that generalized activation decreased in these individuals, although these changes did not correlate with changes in CD4+ cell counts or viral load.

Albendazole treatment, compared with placebo, was associated with a significant decrease in plasma levels of the immunosuppressive cytokine IL-10 when controlling for baseline IL-10 levels (*P* = .04; Figure 2E). There was no direct corre-
Figure 1. Plasma cytokine levels in subjects coinfected with human immunodeficiency virus type 1 (HIV-1) and *Ascaris lumbricoides* at the initial and follow-up clinic visits. The concentration of the Th2 (A), Th1 (B), T cell differentiation and maintenance (C), proinflammatory (D), and immunosuppressive (E) cytokines are shown on the y-axis for both the initial (Initial) and 12-week follow-up (FU) visits, as shown on the x-axis. The specific cytokine is indicated at the top of each plot. Each point represents the mean of duplicate measures of cytokine levels in 1 subject; placebo-treated individuals (n = 20) are indicated by circles, whereas those that received albendazole (n = 18) are indicated by triangles. For all cytokines except thymic stromal lymphopoietin (TSLP), the limit of detection was 0.3 pg/mL; cytokine levels below the limit of detection were assigned a value of 0.15 pg/mL. For TSLP, the limit of detection was 15.6 pg/mL, and levels below the limit of detection were assigned a level of 7.8 pg/mL.
Figure 2. Differences in cytokine levels after treatment of *Ascaris lumbricoides* co-infection in individuals with human immunodeficiency virus type 1 (HIV-1) infection. The difference in the concentration of the Th2 (A), Th1 (B), T cell differentiation and maintenance (C), proinflammatory (D), and immunosuppressive (E) cytokines between the initial and follow-up visits is shown on the y-axis for both individuals who received placebo (Plac) and those who were treated with albendazole (Alb). The specific cytokine is indicated at the top of the plot. Negative numbers indicate that the level of the cytokine dropped during follow-up while positive numbers reflect an increase in cytokine concentration. The median values and 95% confidence intervals are indicated. Interleukin (IL) 10 levels were significantly reduced in albendazole-treated individuals, comparison with levels in individuals who received placebo \( P = .04 \); no statistically significant changes attributable to albendazole treatment were found for the other cytokines \( P > .2 \).
lation between the change in IL-10 level and the change in CD4+ T cell count in the entire cohort (Spearman’s rho = −0.12; P = .46), among only those individuals who received albendazole therapy (Spearman’s rho = .13; P = .60), or among only those individuals who received albendazole and remained uninfected with A. lumbricoides at follow-up (Spearman’s rho = 0.33; P = .29). Although there was a trend for a correlation between the reduction in IL-10 levels and the reduction in HIV-1 load in the entire cohort (Spearman’s rho = 0.25; P = .13), this correlation did not persist when evaluating only those individuals who received albendazole treatment (Spearman’s rho = −0.08; P = .75), or those individuals who received albendazole and remained uninfected with A. lumbricoides at follow-up (Spearman’s rho = 0.13; P = .70).

Discussion. These data suggest that albendazole treatment of A. lumbricoides in individuals with HIV-1 co-infection is associated with significant reductions in plasma levels of the immunosuppressive cytokine IL-10. Albendazole treatment was not associated with similar changes in other plasma cytokines, including the Th2, Th1, T cell differentiation and maintenance, or proinflammatory cytokines. Baseline levels of IL-10 were directly correlated with viral load and inversely correlated with CD4+ cell count. However, the reductions in plasma IL-10 levels were not directly correlated with changes in the CD4+ cell count or viral load in albendazole-treated individuals, suggesting either that there was insufficient power to detect an effect or that IL-10 indirectly affects the CD4+ cell count and viral load.

These data suggest that helmint treatment may reduce immunosuppression in HIV-1 coinfected individuals. IL-10 is strongly induced by helmint infections, mediates some immunosuppressive effects of helmints, results in higher worm burdens, and promotes the persistence of viral infections [1, 4, 7, 10]. During chronic HIV-1 infection, IL-10 levels correlate with HIV-1 load, suggesting that IL-10 may promote HIV-1 replication [11]. In individuals who are co-infected with schistosomiasis, IL-10 production has been associated with compromised HIV-1 cytolytic activity [12]. Albendazole treatment of A. lumbricoides may therefore decrease worm burden, leading to decreased IL-10 production, improved cellular immunity to HIV-1, and ultimately to improvements in CD4+ T cell counts.

In this study involving adults, no significant changes in the levels of Th1, Th2, T cell differentiation and maintenance, or proinflammatory cytokines were detected after helmint treatment. This is consistent with studies involving children in which no significant changes in cytokine levels were noted after deworming [13]. Potential explanations for the lack of observable changes in cytokine levels after deworming include the fact that (1) plasma cytokine levels do not necessarily reflect compartmentalized changes that occur in response to anti-helmint therapy, (2) aspects of immunity other than changes in cytokine production contribute to the beneficial effects of deworming, (3) individuals may have been reinfected prior to assessment of cytokines at follow-up, and (4) the timing of sample collection at 12 weeks after treatment was either too early or too late to capture changes in cytokine production.

Significant strengths of this study included the use of data from a randomized trial, which allowed a direct comparison of cytokine levels before and after albendazole treatment, and incorporation of methods to adjust for multiple comparisons. Two earlier cross-sectional studies failed to detect significant differences in IL-10 levels when comparing treated with untreated subjects infected with either hookworm or A. lumbricoides [14, 15]. These cross-sectional studies may have been limited by baseline differences in cytokine levels between individuals. Consistent with this hypothesis, we observed differences between our randomization arms in baseline levels.

Limitations of this analysis include the relatively small sample size, which may have limited the power of the study to detect differences and associations. In addition, this study was limited to evaluation of plasma cytokine levels, and we were unable to determine the cellular source of IL-10 production in this analysis. Systemic levels of immune cytokines may not reflect compartmentalized changes in these immunologic parameters, such as in the gut-associated lymphoid tissue, an area active in the pathogenesis of both HIV-1 and helminth disease.

These data suggest that treatment of A. lumbricoides co-infection may improve control of HIV-1 by reducing worm-associated, IL-10–mediated immunosuppression. Further work is necessary to elucidate the immunologic mechanisms that explain why treatment of some helminth species, such as A. lumbricoides, results in improvement in markers of HIV-1 disease progression in co-infected individuals.

Acknowledgments

We thank all of the participants and the clinics and organizations that cared for persons living with HIV infection and AIDS who participated in this study and Dr. Julie Overbaugh, for her support and guidance in the study. All active drug (albendazole) and placebo were provided at no cost by Glaxo-Smith-Kline, and all stool collection containers were provided by Alpha-Tec.

References