Filarial Infections Increase Susceptibility to Human Immunodeficiency Virus Infection in Peripheral Blood Mononuclear Cells In Vitro

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Because helminth infections and human immunodeficiency virus (HIV) coexist in areas where the spread of AIDS is most dramatic, their in vitro interaction was explored. Cryopreserved peripheral blood mononuclear cells (PBMC) from patients with filarial infections (n = 24) and from unexposed control subjects (n = 12) were depleted of CD8 T cells and were infected with macrophage (M)– and T cell–tropic viruses. A trend toward increased HIV replication in PBMC from filaria-infected patients was observed. Furthermore, PBMC from 6 filaria-infected patients before antifilarial treatment were significantly more susceptible to replication of M-tropic virus than their posttreatment PBMC (P = .03). No intergroup differences were found in the surface expression of HLA-DR, CD25, CCR5, CXCR4, CCR3 on CD4 T cells, or monocytes before infection. PBMC from filaria-infected patients produced less RANTES (P = .02) but more intracellular interleukin-4 than those of control subjects. Thus, PBMC from persons with filarial infections appear to have enhanced susceptibility to HIV-1 infection mediated by an undetermined mechanism.

Although it has been postulated that immune activation due to the high prevalence of infectious, especially parasitic, agents in the developing world plays a major role in the explosive spread of human immunodeficiency virus (HIV) infection and the seemingly accelerated progression to AIDS in these regions [1], there are few data on the impact of these agents. It is estimated that >1 billion people are infected with intestinal helminths and that an estimated 160 million harbor ≥ 1 of the 4 main pathogenic filarial parasites: Wuchereria bancrofti, Brugia malayi, Loa loa, and Onchocerca volvulus. Because the cytokine microenvironment in vivo is critical in the modulation of HIV expression, any pre-existing immune bias could potentially have a profound effect on HIV pathogenesis. The host immune response to helminths is characterized by the production of interleukin (IL)-4, IL-5, IL-10, serum IgE, and eosinophilia [2]. The roles of IL-4 [3, 4] and IL-10 [5, 6] in HIV replication are controversial with evidence for both enhancement and suppression of replication.

Residents of endemic areas of Central America, India, Southeast Asia, and Africa are exposed to filarial parasites from birth. Estimates of the infection rate in children between the ages of 1 and 5 years (i.e., before the usual age of acquisition of HIV via sexual contact or drug abuse) often are >25% [7]. Therefore, we were interested in modeling the initial interaction between filaria-infected persons and HIV. We compared the in vitro susceptibility of CD8 T cell–depleted peripheral blood mononuclear cells (PBMC) from filaria-infected persons, unexposed control subjects, and filaria-infected patients before and after antifilarial treatment, to HIV infection with T cell (T)–tropic and macrophage (M)–tropic strains. In addition, parameters of immune activation, chemokine coreceptor expression, and cytokine production were measured.

Materials and Methods

Study population. We studied 24 filaria-infected persons 16–61 years old. Eighteen were North Americans who had lived in or traveled to various parts of Africa. Eight were infected with L. loa, 5 with O. volvulus, and 5 with W. bancrofti. Of the other 6, 4 infected with W. bancrofti were from Guyana, Burkina Faso, and Sierra Leone, and 2 patients infected with L. loa were from Ghana and Nigeria. All filaria-infected patients had lived in North America for varying periods before presentation; were HIV negative, and were otherwise healthy. PBMC from 12 filaria-unexposed North American blood bank donors were studied as controls.
Tissue culture. Cryopreserved PBMC were thawed, washed, and resuspended in RPMI without phenol red, L-glutamine, gentamicin, HEPES, and 10% fetal calf serum (C-RPMI) and were counted with trypan blue staining. CD8 cells were depleted from the PBMC population by use of CD8 Dynal beads (Dynal, Lake Success, NY); the remaining cells were washed and recounted. Cells (10^6/mL) were plated in 48-well Costar plates (Corning, Corning, NY) without antigen or mitogen and were incubated overnight at 37°C in 5% CO2, M-tropic virus (HIV BAL) and T-tropic virus (HIV Iib) were added in separate wells at tissue culture-infecting doses (TCID) of 100 and 20 and 80 and 20 for the 2 viruses, respectively. The TCID50 of the respective virus isolates were determined elsewhere from infections of CD8-activated T cell blasts obtained from pooled donors. Supernatants were collected at days 4, 7, 10, and 14 after infection, to determine cytokine levels and reverse transcriptase (RT) activity.

RT assay: RT assays were performed as described elsewhere [8]. The dried filters were counted in a scintillation counter (Beckman LS6001C; Beckman, Palo Alto, CA).

Cytokine ELISA. RANTES, IL-6, and tumor necrosis factor (TNF)-α were measured in supernatants collected at time zero (before infection) and at days 4 and 7 after infection with HIV, using kits from R&D Systems (Minneapolis, MN). IL-10 and IL-4 were measured by cytokine-specific assays described in detail elsewhere [9].

Flow cytometry. Samples were prepared as described elsewhere [10], and were run on a flow cytometer (FACScalibur; Becton Dickinson). In total, 10,000 events were collected, and percentages and mean fluorescence intensities were calculated. Cells were stained with different combinations of anti-CD4 fluorescein isothiocyanate (FITC) and phycoerythrin (PE; cloneclone B9.11; Coulter, Miami, FL); anti-CD14 FITC (monoclonal antibody P9; Becton Dickinson); anti-CCR3 (gift of LeukoSite, Worcester, MA); and anti-CXCR4 PE (12G5), anti-CCR5 PE (3A9), anti-CD25 PE (M-A251), and anti-CD4 FITC (clone MA4; all from PharMingen, San Diego, CA).

Intracellular staining for cytokines. Intracellular cytokine staining was performed on PBMC from a subset of patients, as described elsewhere [11]. The antibodies used were anti-IL-4 PE (clone MP4-25D2), anti-IL-5 PE (TRFK5), anti-IL-10 PE (JES3-9D7), and anti-interferon-γ PE (4S.B3), with appropriate isotype controls (all from PharMingen).

Polymerase chain reaction for 32-hp CCR5 deletion. The CCR5 genotype was determined as described elsewhere [12]. All study participants were wild type for the CCR5 gene; thus, no one was homozygous or heterozygous for the 32-hp deletion in the CCR5 gene associated with resistance to HIV infection [12].

Statistical analysis. Results were analyzed by Mann-Whitney U or Wilcoxon nonparametric tests or by Spearman’s rank correlation, as appropriate. Error bars represent the 95th percentile confidence interval.

Results
Acute HIV infection assays were performed on CD8 T cell-depleted PBMC, without the addition of exogenous cytokines or mitogenic stimuli. Using an RT value of 200 cpm/mL as the cutoff, cells from 20 (88.3%) of 24 filaria-infected patients and 9 (75%) of 12 control subjects were susceptible to infection with TCID50 HIV BAL, whereas cells from 16 (66%) filaria-infected patients and 9 (75%) control subjects supported replication with HIV Iib (TCID50). Within the responder subset in each group, kinetic analysis of RT production revealed considerable individual variation in RT values over time. RT values in the filaria-infected group were higher than in the control group (figure 1A), but these differences were not statistically significant, even when nonresponders were excluded from the analysis. Seven (29%) of the 24 patients had maximal RT values >10,000 cpm/μL, whereas no control subjects achieved that degree of infection with HIV BAL, TCID50 (P < .07). The degree of productive infection with T-tropic virus (HIV Iib; figure 1A[iii]) was consistently lower than that obtained with M-tropic virus (HIV BAL; figure 1A[i]), despite similar input TCID for both viruses. Similar trends were obtained with the higher inputs of HIV Iib (TCID50) and HIV Iib (TCID50) data not shown.

Because the lack of a significant difference in RT values over time appeared to be related to the high degree of variability seen in both the patient and control groups, the infection experiments were repeated in PBMC of 6 patients before and after treatment of their filarial infection. Thus, each patient acted as his or her own control. All were North Americans; 4 were infected with L. loa alone, 1 with O. volvulus alone, and 1 was coinfected with both parasites. All had been treated with the accepted 3-week course of diethylcarbamazine (for L. loa) or single-dose ivermectin (for O. volvulus; both drugs are well tolerated and not associated with immunosuppression) 1–2 years before collection of the posttreatment cells. Relevant laboratory data are shown in figure 2A. In this group, there was a statistically significant decrease in HIV BAL and HIV Iib replication in CD8-depleted PBMC after treatment of filarial infections, compared with pretreatment PBMC (P = .03; figure 2B). RT values decreased in 5 of 6 posttreatment cells to levels comparable with those in uninfected control subjects.

Of importance, the viability of pre- and posttreatment cells, as assessed by trypan blue, was similar, and maximal stimulation with phytohemagglutinin or anti–CD3 antibody rendered pre- and posttreatment cells equally susceptible to HIV (data not shown), thereby largely excluding technical factors as a possible explanation for the differences seen. Cell surface expression or mean fluorescence intensities of HLA-DR or CD25 on CD4 T cells and of CXCR4, CCR5, or CCR3 on CD4 T cells or monocytes before infection did not differ between any groups. Protein levels and intracellular production of cytokine potentiators of HIV replication (IL-6 and TNF-α), and important immunomodulatory cytokines (IL-4 and IL-10) did not differ between groups (data not shown); however, the frequency of IL-4-producing CD4 T cells measured before infection correlated significantly with RT production at days 4 (P = .02) and 14 after infection with HIV BAL (data not shown). The chemokine RANTES, IL-6, and tumor necrosis factor (TNF)-α were measured in supernatants collected at time zero (before infection) and at days 4 and 7 after infection with HIV, using kits from R&D Systems (Minneapolis, MN). IL-10 and IL-4 were measured by cytokine-specific assays described in detail elsewhere [9].

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Statistical analysis. Results were analyzed by Mann-Whitney U or Wilcoxon nonparametric tests or by Spearman’s rank correlation, as appropriate. Error bars represent the 95th percentile confidence interval.
Figure 1.  A, Pronounced intragroup variability in reverse transcriptase (RT) values as a measure of human immunodeficiency virus (HIV) replication in peripheral blood mononuclear cells (PBMC) from filaria-infected patients and healthy control subjects. Histogram plots of geometric mean (GM) RT values as a function of days after infection (x axis) show no significant difference. B, GM RANTES production after infection with M-tropic virus (HIV BAL, TCID\textsubscript{50}, 20) is significantly greater (* P < .02) in PBMC from uninfected control patients (solid bars) than from filaria-infected patients (open bars).

TES was produced in significantly larger quantities after HIV infection by PBMC from control subjects than by PBMC from the filaria-infected patients (P = .02; figure 1B).

Discussion

In contrast to the evidence in support of a role for intracellular parasites or antigens in stimulating HIV replication from cells harboring latent HIV infection [13, 14], our model addresses the important converse question: does a preexisting extracellular parasitic disease, such as filariasis, affect susceptibility to HIV infection? Our data suggest that it does, especially to M-tropic virus. We believe that the use of unstimulated PBMC from persons actively infected with filarial infection, rather than uninfected donor PBMC stimulated with parasite or nonparasite antigens as others have done [15], more accurately mimics the in vivo situation. Rather than attempting to control for the marked splay in RT values in persons from the filaria-infected and control groups by expanding our study population, we chose to obviate the problem by studying PBMC from the same patients before and after antifilarial treatment. The decrease in HIV-1 replication in posttreatment PBMC versus pretreatment PBMC was striking and significant (figure 2B), especially with HIV\textsubscript{BAL} (P = .03), which provides strong evidence for the effect of preexisting filarial infections on increased susceptibility to HIV-1 infection.
Figure 2. Treatment of filarial infections renders peripheral blood mononuclear cells (PBMC) significantly less susceptible to human immunodeficiency virus (HIV) infection. A, Antifilarial IgG and IgG4 levels, IgE levels, and eosinophil counts in 6 pre- and posttreatment pairs demonstrate significant decline in all values after treatment in 4 patients. No posttreatment sample was available for patient 5, although she was considered to be cured, by clinical and other laboratory parameters. Patient 6 required retreatment of loiasis after this posttreatment sample. B, Box and whisker plot of geometric mean reverse transcriptase (RT) values (y axis) after infection with macrophage (M)–tropic virus (TCID₂₀, HIVBAL) in PBMC from actively infected patients (open bars), compared with PBMC from same patients after treatment of filarial infection (shaded bars) shows significant decrease (*P = .03) in RT values in posttreatment PBMC at days 10 and 14 after infection.

Our flow cytometry data demonstrate that the number of CD4 T cells, their level of activation, or the baseline availability of CXCR4 and CCR5, the G protein–coupled coreceptors known to be fundamental in mediating entry of HIV into CD4 T cells and macrophages, respectively, were not responsible for the differences in HIV replication in pre- and posttreatment cells. Recent in vitro data showing a clear up-regulation of CXCR4 on pretreatment CD4 T cells stimulated with microfilarial antigen for 7 days in culture versus posttreatment CD4 T cells indicates, however, that chemokine receptor dynamics over time may be of more importance than baseline expression (R.G., unpublished data).

The similarity in constitutive production of the cytokines studied between groups and the lack of correlation between TNF-α production and HIV replication stand in contrast to other in vitro studies [14]. These findings probably reflect the fact that our cells were unstimulated; production of cytokines, such as IL-4, IL-10, and TNF-α, was often undetectable by ELISA in culture supernatants. RANTES production was, however, significantly greater from PBMC of control subjects than from filaria-infected patients (figure 1B). The implications of this finding are uncertain, given the equivalent levels of CCR5 expression at baseline in CD4 T cells and monocytes in both groups.

The mechanisms therefore of the enhanced HIV replication in cells from patients before treatment of coexistent filarial infection remain unclear. It is likely that the kinetics of regulation of chemokine receptors on the cell surface, as well as their
differential triggering of signal transduction events, are important. The role of monocytes and macrophages in mediating the increased susceptibility to HIV infection warrants further study. In addition, the role of eosinophils, whose numbers in the peripheral circulation are often dramatically increased in helminth infections and which carry the CCR3 coreceptor known to be capable of mediating entry of HIV-1 into the cell, is unexplored. Our results have important implications for the necessity of aggressive treatment and control programs for filarial diseases and possibly other helminth infections in areas of Africa, India, and Southeast Asia where the HIV epidemic is rampant. For most people in those parts of the world, largely without access to highly active antiretroviral therapy, parasite infections should be considered potential cofactors in the immunopathogenesis of HIV infection and treated accordingly.

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References