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DOI

[10.1086/318701](https://doi.org/10.1086/318701)

Publication date

2001

Published in

Clinical infectious diseases

[Link to publication](#)

Citation for published version (APA):

Juffermans, N. P., Speelman, P., Verbon, A., Veenstra, J., Jie, C., van Deventer, S. J. H., & van der Poll, T. (2001). Patients with active tuberculosis have increased expression of HIV coreceptors CXCR4 and CCR5 on CD4(+) T cells. *Clinical infectious diseases*, 32(4), 650-652. <https://doi.org/10.1086/318701>

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Patients with Active Tuberculosis Have Increased Expression of HIV Coreceptors CXCR4 and CCR5 on CD4⁺ T Cells

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Expression of human immunodeficiency virus (HIV) coreceptors CXCR4 and CCR5 was found to be elevated on CD4⁺ T cells (1) in blood samples obtained from patients with tuberculosis and (2) in blood samples obtained from healthy subjects and stimulated with mycobacterial lipoarabinomannan in vitro. These data suggest that the increase in HIV viremia that occurs in association with tuberculosis may result from up-regulation of CXCR4 and CCR5 on CD4⁺ T cells, thereby causing acceleration of HIV infection.

HIV infection is the strongest known risk factor for the development of tuberculosis (TB), and for HIV-infected patients who have a positive tuberculin skin test result, the lifetime risk of developing TB is $\geq 30\%$ [1]. Concurrent infection with TB results in immune cells having enhanced susceptibility to HIV infection, which facilitates entry and replication of HIV [2, 3]. In vitro, monocytes of patients with TB are more susceptible to HIV infection [2]. Moreover, the level of virus replication is increased in HIV-infected patients who develop active TB, and it returns to a baseline level after treatment [3]. This finding is of clinical relevance, since patients with TB have an accelerated course of HIV infection. The chemokine receptors CXCR4 and CCR5 act as coreceptors for the entry of HIV into the CD4⁺ T cells [4]. HIV coreceptor expression correlates with

enhancement of HIV entry into cells and HIV replication [5, 6].

We hypothesized that TB stimulates HIV coreceptor expression, thereby enhancing both the entry of HIV into immune cells and HIV replication. To investigate HIV coreceptor expression in association with TB, we measured expression of CXCR4 and CCR5 by means of fluorescence-activated cell sorter (FACS) analysis (1) done after whole blood samples from healthy subjects were stimulated in vitro with lipoarabinomannan (LAM; a cell wall component of *Mycobacterium tuberculosis*); and (2) done on whole blood samples from patients with TB.

Methods. Blood samples were obtained from 6 healthy subjects by use of a sterile collection system that consisted of a butterfly needle connected to a syringe (Becton Dickinson), and they were incubated at 37°C for 8 h. Anticoagulation was achieved using heparin (Leo Pharmaceutical Products; final concentration, 10 U/mL blood). Whole blood was added to sterile polypropylene tubes and was mixed with RPMI 1640 medium (Bio Whittaker; dilution, 1:1) to which LAM (which was mannose capped, isolated, and prepared from *M. tuberculosis* strain H37R) was added at a concentration of 1 $\mu\text{g}/\text{mL}$ (LAM was kindly provided by Dr. J. T. Belisle, Colorado State University, Fort Collins, CO, under the provisions of National Institutes of Health contract NO1-A1-75320); it was stimulated at 37°C for 8 h. After this was done, FACS analysis was performed.

Blood samples were obtained from 8 patients (mean age \pm SE, 31.9 \pm 4.2 years) with active, culture-proven TB. The patients were receiving treatment at the Academic Medical Center (5 patients), the Sint Lucas Hospital (2), or the Municipal Health Center (1) in Amsterdam. Three patients with TB were HIV seropositive, and 2 were HIV seronegative; the HIV status of the remaining 3 patients was not determined. These latter 3 patients did not belong to a group with classic risk factors for HIV infection, and they had normal CD4 counts. They did not give permission for an HIV screening test to be done. Of the 8 patients, 4 had pulmonary tuberculosis and 4 had extrapulmonary tuberculosis. On each day that a patient was analyzed, 1 healthy HIV-seronegative control subject (i.e., a laboratory worker or a physician) was analyzed (8 subjects; mean age \pm SE, 28.7 \pm 2.0 years). After blood samples were obtained, they were immediately prepared for FACS analysis.

The blood samples were prepared for FACS analysis as follows. Erythrocytes were lysed with bicarbonate-buffered ammonium chloride solution (pH, 7.4). Leukocytes were recov-

Received 22 February 2000; revised 6 July 2000; electronically published 7 February 2001.

Financial support: Supported by grants from the "Mr. Willem Bakhuys Roozeboom" Foundation (to N.P.J.) and the Royal Dutch Academy of Arts and Sciences (to T.v.d.P.).

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Clinical Infectious Diseases 2001;32:650-2

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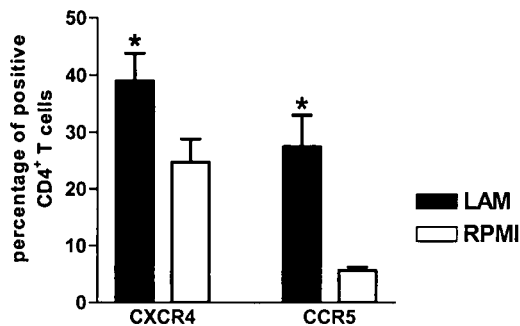


Figure 1. Expression of HIV coreceptors CXCR4 and CCR5 on CD4⁺ T cells after incubation of whole blood samples with lipoarabinomannan (LAM, 1 μg/mL) for 8 h. **P* < .05 versus incubation with RPMI 1640 medium.

ered after centrifugation at 600 g for 5 min and were counted. A total of 1×10^6 leukocytes were resuspended in cPBS, a PBS that contained EDTA, 100 mM; sodium azide, 0.1%; and bovine serum albumin, 5%. They were then placed on ice. Triple staining was done by means of incubation for 1 h with direct-labeled antibodies CD3-PE, CD4-Cy (both from Coulter Immunotech) and either CXCR4-fluorescein isothiocyanate (FITC) or CCR5-FITC (R&D Systems). Blood samples were also incubated with FITC-labeled CD25 (CLB) and phycoerythrin-labeled CD69 (Becton & Dickinson). We controlled for nonspecific staining by incubating cells with FITC-labeled mouse IgG2 (Coulter Immunotech). Cells then were washed twice in ice-cold cPBS and were resuspended for flow cytometric analysis (by use of Calibrite; Becton Dickinson Immunocytometry Systems). At least 10,000 lymphocytes were counted. Data on the number of positive cells were obtained by setting a quadrant marker for nonspecific staining.

Results were expressed as the mean \pm SE, unless otherwise stated. Data were analyzed using the Wilcoxon test; *P* < .05 was considered statistically significant.

Results. In comparison with incubation with medium alone, LAM induced up-regulation of the fraction of CD4⁺ T cells that were positive for CXCR4 (mean percentage \pm SE, 39.0% \pm 4.8% [for whole blood samples stimulated with LAM] vs. 24.7% \pm 4.1% [for whole blood samples incubated with medium only]) and for CCR5 (mean percentage \pm SE, 27.5% \pm 5.5% [for whole blood samples stimulated with LAM] vs. 4.3% \pm 1.7% [for whole blood samples incubated with medium only]; *P* < .05 for both; figure 1) after stimulation of samples of whole blood in vitro.

After having established that part of the cell wall of *M. tuberculosis* can up-regulate HIV coreceptor expression, we determined expression of CXCR4 and CCR5 in patients with active TB. The percentages of circulating CD4⁺ T cells and CD8⁺ T cells in patients did not differ from those in control subjects (mean percentage of CD4⁺ cells \pm SE, 41.3% \pm 4.7%

in patients with TB vs. 46.9% \pm 4.6% in control subjects; mean percentage of CD8⁺ cells \pm SE, 41.9% \pm 4.3% in patients with TB vs. 32.2% \pm 2.5% in control subjects; NS). The fraction of circulating CD4⁺ T cells that were positive for CXCR4 and CCR5 was higher in patients with TB than in healthy control subjects (figure 2; *P* < .005). The percentage of circulating CD4⁺ T cells that expressed lymphocyte activation markers CD25 or CD69 did not differ between patients and control subjects (mean percentage [range] of CD4⁺ cells that expressed CD25, 19.8% [1.0%–44.3%] in patients with TB vs. 25.3% [2.7%–54.4%] in control subjects, NS; mean percentage [range] of CD4⁺ cells that expressed CD69, 9.2% [0.4%–74.6%] in patients with TB vs. 7.2% [0.3%–54.4%] in control subjects, NS), which suggests that the observed up-regulation is due to specific receptor stimulation by antigens and that it is not due to an activated state of lymphocytes in patients with TB.

Discussion. The association of CXCR4 and CCR5 with HIV infection has been clearly demonstrated [4]; this makes knowledge of HIV coreceptor expression during concurrent infection a clinically important issue. This study is the first to report elevated expression of CXCR4 and CCR5 both in patients with TB and after in vitro stimulation with an antigen derived from *M. tuberculosis* in healthy subjects. It previously had been found that LAM can stimulate HIV expression in macrophages [7]. The observed increase in viremia in association with TB may occur as a result of up-regulation—which is, at least in part, mediated by LAM—of CXCR4 and CCR5 on CD4⁺ T cells in HIV-infected patients, thereby causing acceleration of HIV disease.

Three of the patients with TB who were studied were HIV positive. Of the remaining 5 patients, 2 were documented to be HIV negative, and 3 refused to undergo an HIV screening test. These latter 3 patients probably were HIV negative, since they did not belong to a group with classic risk factors, and since they had normal CD4 counts (data not shown). Although

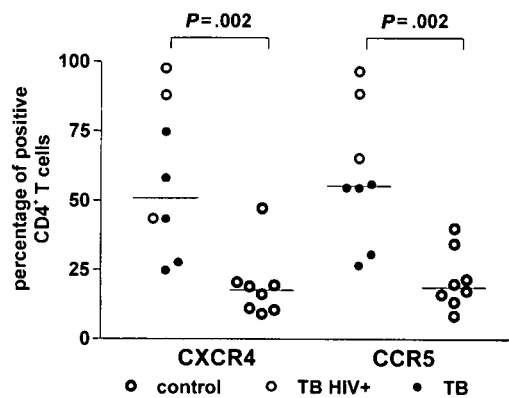


Figure 2. Expression of HIV coreceptors CXCR4 and CCR5 on the circulating CD4⁺ T cells of 8 patients with active tuberculosis (TB) and 8 healthy control subjects.

our study involved relatively few patients, there are several reasons why we consider it likely that TB, rather than HIV, caused the difference in HIV coreceptor expression between patients with TB and control subjects. First, in a previous study, HIV-positive subjects without coinfection were found to have reduced expression of CXCR4 and only modestly increased expression of CCR5 on CD4⁺ T cells, in comparison with HIV-negative control subjects [8]. Second, the difference in CXCR4 and CCR5 expression in patients with TB and control subjects remained significant when only patients with TB who had a documented or likely HIV-negative status were analyzed ($P < .05$ for both receptors). Third, LAM up-regulated HIV coreceptor expression in vitro.

HIV coreceptors are considered an area of focus for HIV therapy. This study contributes to the idea that blocking CXCR4 and CCR5 may slow progression of HIV infection during concurrent infection [4].

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