Lipopolysaccharide (LPS) can inhibit human immunodeficiency virus (HIV) infection in monocytes in vitro. To test the hypothesis that an LPS effect on CXC chemokine receptor 4 (CXCR4) and CC chemokine receptor 5 (CCR5), known coreceptors for HIV, contributes to this effect, 8 healthy men were intravenously injected with Escherichia coli LPS (4 ng/kg), and monocyte CXCR4 and CCR5 expression was monitored by fluorescence-activated cell sorter analysis. LPS induced a decrease in the fraction of peripheral blood monocytes expressing CXCR4 and CCR5, reaching a nadir after 2 h (both \( P < .001 \) vs. baseline). In whole blood in vitro, not only LPS but also lipoarabinomannan (a cell wall component of Mycobacterium tuberculosis) and lipoteichoic acid (a cell wall component of Staphylococcus aureus) down-regulated the expression of CXCR4 and CCR5 on monocytes (all \( P < .05 \)). Exposure of monocytes to (myco)bacterial agents may render them relatively resistant to infection with HIV by an effect on HIV coreceptors.

Monocytes and macrophages play an important role in the pathogenesis of human immunodeficiency virus (HIV) disease. These cells are susceptible to HIV infection but are relatively resistant to its cytopathic effects, enabling them to serve as virus reservoirs [1]. In addition, peripheral blood monocytes and macrophages play a key role in determining the course of HIV infection by influencing the host response of HIV-infected patients to invading microorganisms. Hence, monocytes and macrophages provide an attractive site for anti-HIV therapeutic strategies.

Chemokines are chemotactic proteins that direct leukocytes to the site of inflammation. Together with CD4, CXC chemokine receptor 4 (CXCR4) and CC chemokine receptor 5 (CCR5) function as HIV coreceptors and are essential for virus entry into cells [2]. Persons with a mutation in CCR5 receptors are resistant to HIV-1 infection, suggesting a key role for this receptor in HIV-1 pathogenesis [3].

It has been known for a decade that lipopolysaccharide (LPS), the principal stimulator of the innate immune response to gram-negative bacteria, inhibits HIV replication in macrophages [4–6]. LPS down-regulates CCR5 on monocytes and macrophages in vitro [7, 8], which is an important determining factor in the inhibitory effect of LPS on HIV infection in these cells [7]. The in vivo effect of LPS on monocyte HIV coreceptor expression is unknown. Therefore, in the present study we investigated CXCR4 and CCR5 expression on circulating monocytes after intravenous injection of LPS into healthy humans, and we compared the in vivo LPS effects with the effects of other (myco)bacterial antigens on monocytes in whole blood in vitro.

Materials and Methods

In vivo study. Eight healthy HIV-negative men (aged 23 ± 1 years [mean ± SE]) were admitted to the clinical research unit of the Academic Medical Center, Amsterdam, after documentation of good health by history, physical examination, hematologic and biochemical screening, chest radiographs, and electrocardiography. The participants did not smoke, used no medication, and had no febrile illness within 2 weeks of the start of the study. All volunteers received a bolus intravenous injection of LPS (from Escherichia coli, lot G; US Pharmacopeia Convention) at a dose of 4 ng/kg body weight. Venous blood samples were obtained immediately before the injection of LPS and at 1, 2, 4, 6, and 24 h thereafter. Blood was collected in heparin-containing vials and processed immediately for flow cytometry. LPS is frequently administered to healthy volunteers and is a safe model of human inflammation. As consistently documented by our laboratory and others, the standardized LPS preparation used in these investigations, given at doses of ≈ 4 ng/kg, elicits a reproducible clinical syndrome lasting for 2–3 h that resembles influenza...
and is not associated with clinically significant alterations in blood pressure or other vital parameters [9].

Flow cytometry. Heparinized whole blood was prepared for fluorescence activated cell sorter (FACS) analysis as follows. Erythrocytes in 4.5 mL of whole blood were lysed with bicarbonate-buffered ammonium chloride solution (pH 7.4). Leukocytes were recovered after centrifugation at 400 g for 5 min and counted. In total, 106 cells were resuspended in PBS, containing EDTA 100 mM, sodium azide 0.1%, and bovine serum albumin 5% (cPBS), and placed on ice. Staining was done by incubation for 1 h with directly labeled antibodies CXCR4–fluorescein isothiocyanate (FITC) or CCR5–FITC (R&D Systems). Nonspecific staining was controlled for by incubation of cells with FITC-labeled mouse IgG2 (Coulter Immunotech). Cells were then washed twice in ice-cold cPBS and resuspended for flow cytometry analysis (Calibrite; Becton Dickinson Immunocytometry Systems). At least 5000 monocytes, identified by forward and side-angle scatter, were counted. Data on the number of positive cells were obtained by setting a quadrant marker for nonspecific staining.

In vitro study. Blood was collected from 6 healthy subjects by use of a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson). Anticoagulation was done with heparin (Leo Pharmaceutical Products final concentration, 10 U/mL blood). Whole blood was added to sterile polypropylene tubes and diluted 1:2 with RPMI 1640 (BioWhittaker). LPS used in the time course experiments was from E. coli serotype O111:B4 (Sigma; 10 ng/mL). In a separate experiment, liposarabinomannan (LAM; mannosyl-capped, isolated, and prepared from Mycobacterium tuberculosis H37Rv, 1 mg/mL; provided by J. T. Belisle, Colorado State University, Fort Collins, under National Institutes of Health contract AI-75320), lipoteichoic acid (LTA), or staphylococcal enterotoxin B (SEB; 1 mg/mL; both antigens from Staphylococcus aureus; Sigma Chemicals) were added and incubated at 37°C for 8 h. FACS analysis was performed in whole blood as described previously. Incubation of whole blood with LPS does not lead to detectable monocyte death for at least 24 h (data not shown).

Statistical analysis. All values are given as mean ± SEM. In vivo data were analyzed by one-way analysis of variance. In vitro stimulation data were analyzed by Wilcoxon test. P < .05 was considered statistically significant.

Results

HIV coreceptor expression on circulating monocytes during human endotoxemia. Injection of LPS was associated with transient influenza-like symptoms, including headache, chills, vomiting, myalgia, and fever (peak temperatures, 38.6 ± 0.3°C). Injection of LPS induced a decrease in the number of monocytes. Monocyte counts at the time of FACS analysis were (106 cells/ L): 0.55 ± 0.4 (0 h), 0.05 ± 0.01 (2 h), 0.29 ± 0.06 (6 h), and 0.79 ± 0.07 (24 h). Intravenous LPS induced a decrease in the fraction of monocytes expressing CXCR4, reaching a nadir after 2 h (from 88.9% ± 1.9% at baseline to 40.7% ± 3.7%; P < .001) and returning to the initial level of expression after 6 h (figure 1). LPS administration also resulted in a down-regulation of CCR5 on circulating monocytes (from 72.5% ± 8.5% to 30.7% ± 4.2% at 2 h; P < .001), returning to baseline after 6 h (figure 1).

HIV coreceptor expression on monocytes after whole blood stimulation with (myco)bacterial agents. Having established that LPS down-regulates the expression of CXCR4 and CCR5 on monocytes in vivo, we studied the effect of other bacterial products on monocyte HIV coreceptors in vitro. To establish the optimal stimulation period, we incubated whole blood with LPS (10 ng/mL, an adequate dose to influence HIV coreceptors on T cells [10]) for different time periods. By 1 h, LPS induced a down-regulation of the percentage of monocytes positive for both HIV coreceptors. The most prominent difference was at 8 h (CXCR4, from 81.3% ± 3.0% at baseline to 32.0% ± 8.0% at t = 8; CCR5, from 57.7% ± 16.0% to 0.3% ± 0.1%; P < .05 for both). Further incubations were done with LAM (a cell wall component of M. tuberculosis), LTA (a cell wall component of S. aureus), or SEB (a superantigen produced by S. aureus), all for 8 h. LAM and LTA induced a down-regulation of CXCR4 and CCR5 on

![Figure 1](image-url)  
Figure 1. Down-regulation of expression of CXCR4 and CCR5 on monocytes after intravenous injection of lipopolysaccharide (LPS) in healthy humans. LPS (4 ng/kg) was injected in 8 subjects at t = 0. Data are mean ± SE percentage positive cells. P value indicates statistical significance for changes over time.
monocytes (figure 2). In contrast, the T cell stimulator SEB had no effect on monocyte HIV coreceptor expression.

Discussion

CXCR4 and CCR5 act as coreceptors for HIV. CXCR4, in particular, facilitates infection by T cell–tropic HIV strains, whereas CCR5 facilitates infection by macrophage (M)–tropic virus [2]. LPS inhibits HIV replication in monocytes/macrophages in vitro [4, 5]. Recently, it was shown that the mechanism of this protective effect of LPS was by down-regulation of CCR5 expression on macrophages in vitro, resulting in resistance to infection with an M-tropic HIV strain [7]. Here, we demonstrate that LPS injected into humans results in down-regulation of CXCR4 and CCR5 on circulating monocytes. In vitro, other bacterial products produced the same effect. Together, LPS-induced suppression of HIV replication in monocytes/macrophages may occur through down-regulation of HIV coreceptor expression, especially CCR5, thereby inducing resistance of these cells to HIV infection.

We reported elsewhere [10] that LPS injected into humans increased the expression of CXCR4 and CCR5 on circulating CD4 T cells and that expression of CXCR4 correlated with an increase in T-tropic HIV replication (i.e., strains that use CXCR4 as coreceptor). It is well documented that concurrent infections are accompanied by a higher plasma virus load [2]. We speculated that concurrent infections might induce a favorable environment for T-tropic viral strains, thereby modulating the course of an HIV infection.

In this study, we found that expression of CXCR4 and CCR5 on monocytes is reduced by LPS. This is consistent with a number of studies reporting a protective effect of LPS on HIV activity in monocytes/macrophages [5, 11]. However, one study found an up-regulation of CXCR4 on monocytes after 7 days of culture in the presence of LPS, in association with an increased susceptibility to T-tropic HIV [8]. Thus, the nature of the effect of LPS on HIV coreceptor expression, at least in part dependent on cell type and stimulation conditions, and HIV disease seems complex. Progression of HIV disease is thought to be associated with a viral transition of an M-tropic to a T-tropic HIV phenotype [2, 12]. Down-modulation of CCR5 on monocytes/macrophages during concurrent infections may suppress HIV infectability by M-tropic virus early in the course of HIV disease. However, at later stages, with predominance of T-tropic virus, up-regulation of CXCR4 on CD4 T cells may render these cells more susceptible to infection with HIV.

It has been proposed that LPS inhibits HIV infection through production of the natural ligand C-C chemokines that block HIV coreceptors [5]. In this context, it is of interest that LPS injection in healthy humans induces the release of CCR5 ligands [13], but there are conflicting data on this subject. In vitro, a correlation was found between the level of C-C chemokines and HIV replication [14]. In contrast, in several studies that used blood from HIV-positive donors to which neutralizing antibodies to C-C chemokines were added, no enhancement of HIV replication was observed, indicating that C-C chemokines alone cannot account for HIV suppression [15]. Recently, LPS-induced inhibition of HIV infection was found to be independent of the synthesis of the ligands macrophage inflammatory protein (MIP)–1α and MIP-1β, occurring through internalization of CCR5 [7].

Although placebo-treated control subjects were not included in the present study, we consider it unlikely that the changes induced by LPS are caused by the stress associated with injection of a substance that may induce a transient illness rather than by LPS itself. This notion is supported by the fact that the in vivo LPS effect could be reproduced in whole blood in vitro.

It is likely that the down-regulation of monocyte CXCR4 and CCR5 expression not only influences HIV infectability but also has implications for monocyte functions during sepsis. CXCR4 and CCR5 are, like other chemokine receptors, promiscuous regarding ligand specificity. Indeed, CXCR4 can bind stromal cell–derived growth factor (SDF)–1α and SDF-1β, whereas CCR5 can interact with MIP-1α, MIP-1β, RANTES, and monocyte chemo-
attractant protein (MCP)–2. Considering this and also that a certain chemokine can bind to more than one receptor (MCP-2 can bind to CCR1, CCR2B, CCR3, and CCR5), it is difficult to speculate on the net effect of down-modulation of these chemokine receptors on inflammatory processes. This issue warrants further investigation.

In summary, (myco)bacterial antigens decreased the expression of CXCR4 and CCR5 on circulating monocytes in human endotoxemia and in whole blood. Knowledge on the expression of HIV coreceptors may have important clinical implications for the development of novel anti-HIV strategies.

References