Evolution of the Cutaneous Immune Response to Experimental \textit{Haemophilus ducreyi} Infection and Its Relevance to HIV-1 Acquisition


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Evolution of the Cutaneous Immune Response to Experimental *Haemophilus ducreyi* Infection and Its Relevance to HIV-1 Acquisition


*Haemophilus ducreyi* causes the sexually transmitted disease chancroid, which facilitates HIV-1 transmission. Skin biopsies were obtained from subjects experimentally infected with *H. ducreyi* to study the evolution of the immune response and immunophenotypes relevant to transmission of HIV-1. Compared with peripheral blood, there was an enrichment of T cells and macrophages after 48 h of infection in the skin. Neutrophils became the predominant cell type by 7–9 days. By immunohistochemistry, macrophage-inflammatory protein-1α was not present early in infection, but was abundant at later stages. RANTES was present throughout the papular and pustular stages of experimental infection, but not present in uninfected control skin. Stromal cell-derived factor-1 was present at low levels in all samples examined. Macrophages in lesions had significantly increased expression of CCR5 and CXCR4 compared with peripheral blood cells, and CD4 T cells had significant up-regulation of CCR5. The magnitude of increased expression of these receptors was not replicated when PBMCs were incubated with *H. ducreyi* or *H. ducreyi* lipooligosaccharide in vitro. Together with the disruption of mucosal and skin barriers, the presence of cells with up-regulated HIV-1 coreceptors in *H. ducreyi*-infected lesions may provide an environment that facilitates the acquisition of R5 (CCR5), X4 (CXCR4), and dual-tropic HIV-1 strains. *The Journal of Immunology*, 2002, 169: 6316–6323.
HIV-1 is primarily sexually transmitted. HIV-1 uses CD4 as a receptor for entry into cells, along with the coreceptors CCR5 and CXCR4 (24, 25). HIV-1 has been classified according to coreceptor use. R5 viruses use CCR5 as a coreceptor for entry, while X4 viruses use CXCR4 as a coreceptor (26). Some primary isolates of HIV-1 can use both coreceptors, while many laboratory-adapted strains use CXCR4 only. Although the type of virus that is transmitted has not been directly studied, the majority of virus in semen are R5 (27). Shortly after seroconversion, most individuals have R5 virus circulating in their peripheral blood, although some individuals have X4 virus or both types (28). Individuals with a homozygous Δ32 mutation in CCR5 are resistant to infection with HIV-1, confirming the importance of R5 viruses in transmission (29–32). To our knowledge, no data exist on the type of virus acquired by persons who are infected with other sexually transmitted diseases.

GUDs such as chancroid are thought to facilitate HIV-1 acquisition by disrupting the mucosal integrity and by recruiting activated macrophages and CD4 T cells to the skin. Although recruitment of CD4-expressing cells has been demonstrated in both natural and experimental H. ducreyi infection, the hypothesis that these cells have up-regulated chemokine receptors that serve as HIV-1 coreceptors has not been tested.

In this study, we describe the evolution of recruitment of neutrophils, macrophages, T cells, and B cells to sites experimentally infected with H. ducreyi. Given the relationship between chancroid and HIV-1 acquisition, we also examined experimental lesions for infection. The hypothesis that HIV-1 coreceptors CCR5 and CXCR4 are enriched on macrophages and CD4 T cells has not been tested.

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## Materials and Methods

### Human subjects

Twenty-two healthy adult volunteers donated 37 tissue specimens for this study. Biopsies designated CS were obtained from seven subjects who participated in a previous human challenge trial (15, 18). Tissue specimens for FACS analysis were obtained from 10 volunteers (189 and 195–204) who were infected for the purposes of this study, 2 subjects who participated in a mutant parent trial (186 and 190) (33), and 2 subjects (164RR and 172RR) who participated in a reinfection trial (Table I). One uninfected volunteer donated normal skin (18). Informed consent was obtained from the subjects in accordance with the guidelines for human experimentation of the U.S. Department of Health and Human Services and the Institutional Review Board of Indiana University-Purdue University (Indianapolis, IN).

The enrollment procedures, exclusion criteria, preparation of the bacteria, and inoculation procedures for the human challenge experiments are described in detail elsewhere (13–15). Each volunteer was inoculated at two or three sites on the upper arm with live H. ducreyi 35000HP (a human passaged isolate of 35000) and at one site with heat-killed 35000HP. Up to three biopsy specimens were obtained from each subject (Table I). Some subjects were assigned to biopsy 1, 2, or 4 days after inoculation. Other subjects were biopsied when they developed painful pustules, which ranged from 6 to 10 days after inoculation. All 14 subjects who donated tissue for FACS analysis also donated peripheral blood for FACS analysis at the time of biopsy. After biopsy, infected subjects were treated with two doses of ciprofloxacin.

### Immunohistochemical analysis

Nine banked specimens (designated CS or control) of Formalin-fixed, paraffin-embedded tissue were deparaffinized and hydrated. Ag retrieval was performed with citrate buffer (DAKO Target Retrieval, pH 6.0; DAKO,

### Table I. Sources of tissue samples

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Inoculum</th>
<th>Day of Biopsy</th>
<th>No. of Biopsies Contributed</th>
<th>Reference or Source</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td>Live</td>
<td>Heat Killed</td>
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<td>44</td>
<td>7</td>
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<td>CS10</td>
<td>27</td>
<td>4</td>
<td>1</td>
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<tr>
<td>CS11</td>
<td>30</td>
<td>4</td>
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</tr>
<tr>
<td>172RR</td>
<td>56</td>
<td>7</td>
<td>1</td>
<td>C</td>
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</tbody>
</table>

Supplementary text:

- Estimated delivered dose of bacteria in CFU. All sites were inoculated with strain 35000HP or heat-killed 35000HP.
- Analyses for which the sample was used: I, immunohistochemistry; L, major cell lineages; C, coreceptor; L, C, both lineage and coreceptor.
- NA, not applicable; uninfected subject donated normal skin.
- Infected site used for both studies. Control biopsy used only for lineage study.
- Infected site used for coreceptor study. Control biopsy used for lineage study.
Carpenteria, CA) at 95°C for 20 min. Samples were cooled and rinsed with TBS (DAKO), then treated with 3% H₂O₂ (Fisher Scientific, Pittsburgh, PA) for 15 min. Nonspecific binding was blocked with normal donkey serum for 30 min. Polyclonal primary Abs directed against MIP-1α, RANTES, and SDF-1 (R&D Systems, Minneapolis, MN) were diluted 1/250 and were incubated overnight at 4°C. Biotinylated secondary Ab (donkey anti-goat IgG; Jackson ImmunoResearch Laboratories, West Grove, PA) was diluted 1/100 and incubated 30 min. Signal was detected with streptavidin-HRP (DAKO; 30 min), followed by diaminoenzidine (DAKO; 3 min). Tissues were counterstained with Mayer’s hematoxylin. As controls, normal skin and tonsil were probed with each Ab. The primary Ab was omitted. Samples were analyzed by a dermatopathologist and scored positive if at least three separate sections exhibited cytoplasmic staining. Samples were only included in the analysis if both positive and negative controls for that set of stained samples were appropriate.

**Immunophenotyping using four-color flow cytometry**

Fourteen subjects contributed 28 biopsies for flow cytometry. A total of 13 biopsies were used to determine major cell lineages, 11 were used to determine chemokine receptor density, and 4 were used for both analyses. We analyzed 17 biopsies (12 live, 5 heat killed) for major lineages and 15 biopsies (9 live, 6 heat killed) for chemokine receptor density. Entire 5-mm punch biopsies were minced in RPMI 1640 (Life Technologies, Rockville, MD) to release leukocytes from the tissue. To maximize cell yield, some samples were digested with collagenase and DNease (Sigma-Aldrich, St. Louis, MO) and others were digested with EDTA (Sigma-Aldrich). Enzyme and EDTA treatments did not affect major cell lineage markers. Enzyme treatment can affect chemokine receptors, so only EDTA treatment was used in those experiments. For the coreceptor expression experiments, skin biopsy specimens were obtained from H. ducreyi-naïve subjects and two volunteers who were participating in a reinfection trial. The level of coreceptor expression from reinfected subjects was similar to that of naive subjects. Therefore, we pooled data from both naive and reinfected subjects for this analysis.

**Abs used**

To determine the phenotype of the leukocytes recruited to infected sites (n = 18) and heat-killed control sites (n = 10), cells isolated from biopsy specimens were stained with fluorescent Abs (BD Biosciences, San Jose, CA). The following Abs were used, conjugated to FITC, allophycocyanin, PE, or PerCP: CD3 (clone SK7), CD4 (SK3), CD8 (SK1), CD14 (M5E2), CD19 (4G7), and CD45 (2D1).

Quantitative analysis of chemokine receptors was performed using the QuantIBRITE system (BD Biosciences), in which Abs are labeled in a 1:1 ratio with PE, Cy5, or PerCP. Calibrated beads were used to construct a standard curve from which Abs bound per cell were calculated from the geometric mean in the PE channel. Abs bound per cell for macrophages/monocytes and neutrophils were determined using quadrant statistics from dot plots of CD3 vs CD19.

**Staining and analysis**

Cells isolated from biopsies were pelleted, suspended in Ab solutions, and incubated 30 min on ice. RBCs were lysed with FACS lysing solution (BD Biosciences), washed once in PBS, fixed in PBS with 2% paraformaldehyde, and analyzed on a FACS Calibur flow cytometer (BD Biosciences). A threshold was set to eliminate debris, and the percentages and absolute numbers of lymphocytes, monocytes/macrophages, and neutrophils were determined utilizing a gating strategy using CD45 (pan-leukocyte marker) vs 90° FSC and SSC. To further analyze lymphocyte subpopulations, the lymphocyte population was gated using forward scatter (FSC) vs SSC, and then the percentages and absolute numbers of T and B lymphocytes were determined using quadrant statistics from dot plots of CD3 vs CD19.

To assess chemokine receptor density, cell types were isolated for analysis by gating on FSC vs SSC for either lymphocytes or monocytes/macrophages. These populations were further purified for analysis by using a second gating strategy whereby lymphocytes were anchor gated based on bright CD4 fluorescence and monocytes/macrophages were anchor gated based on bright CD14 fluorescence. These double-gated populations were then analyzed using a single-parameter histogram for the mean fluorescence intensity of either CCR5 or CXCR4. To account for the size difference between blood mononuclear cells and tissue macrophages, the FSC vs SSC gate was sized to include populations with a broad FSC, but to exclude populations with high SSC characteristics such as neutrophils.

Comparisons of the levels (Abs bound per cell) of chemokine receptors in 2-day vs endpoint lesions were made using a Student’s t test after a log transformation was applied to the data. Comparisons among site types (control, infected, and blood) were performed using a mixed model ANOVA. The model included a fixed site-type effect and a random effect for sub-samples to account for correlation of sites within the same subject. If a significant site-type effect was detected, pairwise comparisons among the three types were performed using a Tukey-Kramer adjustment for multiple comparisons.

**Preparation of lipooligosaccharide (LOS)**

LOS was purified from H. ducreyi, as previously described (34). Briefly, cells were harvested from overnight growth on chocolate agar plates and lysed with a French press. The lysate was treated with lysozyme, DNase, RNase, and proteinase K. LOS was extracted with hot phenol, then subjected to two rounds of low and high speed centrifugation. The LOS preparation was examined for protein and nucleic acid contamination. There were <2 μg/ml protein (Pierce, Rockford, IL; biocinchonic acid assay), and no protein bands were visible by silver staining. After agarose gel electrophoresis, there were no nucleic acids visible by ethidium bromide staining.

**In vitro stimulation of PBMCs**

For these studies, PBMCs were used from three infected subjects and two additional uninfected healthy volunteers. PBMCs were isolated from peripheral blood by Ficoll-Hypaque density gradient centrifugation. PBMCs were incubated with 1 μg/ml LOS or 10⁻¹⁰⁵ live H. ducreyi for 6 h at 37°C with 5% CO₂ (35). PBMCs were washed, stained for CCR5 and CXCR4, and examined by flow cytometry, as above. Comparisons of the levels of chemokine receptors on PBMCs treated with LOS or H. ducreyi vs untreated controls were made using a mixed model ANOVA after a log transformation. If a significant site-type effect was detected, pairwise comparisons were performed using a Tukey-Kramer adjustment for multiple comparisons.

**Results**

**Evolution of the cellular immune response to H. ducreyi**

We analyzed cell populations present at 17 sites inoculated with live or heat-killed H. ducreyi after 2 days of infection or at clinical endpoint (7.3 ± 1.3 days) (Table I). Using CD45 vs SSC gating and lineage-specific markers, we determined numbers of neutrophils, tissue macrophages (CD14⁺), T cells (CD3⁺), and B cells.
oculated with heat-killed biopsy specimens obtained. Control biopsies were obtained from sites in-
and all possible events were collected from each sample. The number of biopsy specimens obtained. Control biopsies were obtained from sites in-
oculated with heat-killed H. ducreyi 2 (n = 2) and 7 days (n = 2) after inoculation. The data from control sites were similar and were pooled.

We further characterized T cells as Th (CD4) or T cytotoxic (CD8). The skin biopsies were all 5 mm in diameter, and each sample was analyzed in its entirety by flow cytometry, with the number of events collected corresponding to the number of cells present in each sample.

There were ~20,000 total leukocytes present in control biopsy specimens obtained 2–7 days after inoculation with heat-killed bacteria (Fig. 1). After 2 days of infection, there were ~50,000 leukocytes per biopsy. Infected endpoint specimens contained ~200,000 leukocytes. There was an initial influx of T cells and macrophages into the lesions, with T cells accounting for nearly 50% and macrophages accounting for 20% of the total leukocytes present at day 2. Approximately 30% of cells were neutrophils after 2 days. By endpoint, ~75% of the cells in the lesion were neutrophils (Fig. 1). In contrast, the numbers of accumulated macrophages and T cells did not change greatly from 2 days to endpoint. B cells made up a minority of the cells at all time points investigated. The T cell populations in all samples were predominately CD4 cells, with a ratio of ~2:1 (CD4:CD8) in both peripheral blood and the biopsy specimens (Fig. 2). By endpoint, the ratio of CD4 to CD8 cells increased to ~3:1. The FACS analysis of the cellular infiltrate reported in this study closely resembles published immunohistologic data, although our earlier reports underestimated the number of macrophages present in the lesions (18).

**Immunohistochemical analysis of MIP-1α, RANTES, and SDF-1**

Because macrophages and T cells were the predominant mononuclear cells present in lesions, we analyzed banked Formalin-fixed, paraffin-embedded samples from eight additional lesions and one biopsy specimen of uninfected skin for representative chemokines associated with T cell and macrophage recruitment (Table II). MIP-1α was expressed by the mononuclear cells in the upper dermis, but not in cells deeper in the dermis (Fig. 3A, and data not shown). In addition, MIP-1α was not expressed in the early, papular stage of infection nor in the uninfected control, but was abundant at the pustular stage (Table II). Biopsies probed with anti-RANTES Ab showed positive staining in mononuclear cells (Fig. 3B). RANTES expression was evident at the earliest stages of infection examined, but not present in the uninfected control (Table II). Probing biopsy tissue with anti-SDF-1 Ab resulted in low levels of positive staining in mononuclear cells and occasionally keratinocytes, endothelial cells, or neutrophils (Fig. 3, C and D). Biopsies from different clinical stages (papule, pustule, and no infection) exhibited similar levels of SDF-1 expression.

**Table II. Expression of T cell- and monocyte-attractant chemokines in infected skin**

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Days of Infection</th>
<th>Clinical Appearance</th>
<th>Homeostatic</th>
<th>Inflammatory</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SDF-1</td>
<td>RANTES</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>Uninfected</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>CS16</td>
<td>1</td>
<td>Papule</td>
<td></td>
<td>−</td>
</tr>
<tr>
<td>CS12a</td>
<td>1</td>
<td>Papule</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CS12b</td>
<td>1</td>
<td>Papule</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CS10</td>
<td>4</td>
<td>Papule</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CS11</td>
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<td>Pustule</td>
<td>+</td>
<td>+</td>
</tr>
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<td>CS8</td>
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<td>Pustule</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CS7</td>
<td>9</td>
<td>Pustule</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Results are based on staining of at least three separate sections from each specimen.
Ndue to lack of specimen.
Biopsy specimens CS12a and CS12b were obtained from different sites from the same subject.
In vivo expression of CCR5 and CXCR4

The chemokines RANTES and MIP-1α bind to the HIV-1 coreceptor CCR5, and SDF-1 binds to CXCR4, another HIV-1 coreceptor. We examined T cells and macrophages from 15 additional biopsy specimens for cell surface expression of CCR5 and CXCR4. We compared the receptor densities on monocytes/macrophages and CD4 T cells from peripheral blood to control biopsy specimens, 2-day infected sites, and endpoint infected samples. Representative histograms are depicted in Fig. 4, and the absolute receptor density is shown in Fig. 5. Macrophages isolated from sites infected with heat-killed or live *H. ducreyi* had significantly increased levels of both CCR5 and CXCR4. CCR5 levels in tissue macrophages were increased (range, 16- to 36-fold) relative to levels of peripheral blood monocytes (*p* < 0.0001), and CXCR4 levels were increased (range, 7- to 10-fold) relative to that of blood monocytes (*p* < 0.0001). CD4 T cells from biopsy specimens had a more modest (range, 2.1- to 2.6-fold), but statistically significant increase in surface expression of CCR5 in the skin (*p* = 0.003). CXCR4 levels on CD4 T cells were not statistically different from peripheral blood (*p* = 0.43). Thus, macrophages present during experimental infection expressed increased levels of CXCR4 and CCR5, and CD4 T cells expressed increased levels of CCR5. There was no statistical difference in coreceptor density between macrophages and T cells isolated from heat-killed control sites and infected sites.

In vitro expression of CCR5 and CXCR4

Previous reports indicated that bacterial products such as LOS affect expression of CCR5 and CXCR4 in vitro. To determine whether *H. ducreyi* and/or LOS were responsible for the changes in coreceptor expression noted in vivo, we measured CCR5 and CXCR4 expression on PBMCs stimulated for 6 h with purified LOS or 10^6-10^7 CFU of live *H. ducreyi*. LOS was used at a concentration (1 μg/ml) that is equivalent to ~10^7 CFU of *H. ducreyi* (36). CD4 T cells stimulated with LOS in vitro did not exhibit any change in coreceptor expression relative to untreated controls (data not shown). CD4 T cells up-regulated CXCR4 1.3-fold in response to live *H. ducreyi*, a small, but statistically significant increase (*p* = 0.008). Levels of CCR5 on CD4 T cells incubated with live *H. ducreyi* were unchanged relative to controls. Monocytes exhibited significantly decreased levels of CCR5 (1.9-fold) when treated with LOS (*p* = 0.03), and increased CCR5 levels 2.2-fold in response to *H. ducreyi* (*p* = 0.01). Exposure of monocytes to LOS or *H. ducreyi* had no significant effect on CXCR4 levels relative to controls. Thus, the pattern and magnitude of the up-regulation of CCR5 and CXCR4 on macrophages and CCR5 on T cells in vivo were not replicated in vitro.

Discussion

In this study, we examined the kinetics of leukocyte infiltration in skin biopsy specimens from experimental *H. ducreyi* infection. After 2 days of infection, about one-half of the infiltrating leukocytes were T cells, followed in abundance by neutrophils and macrophages. Between day 2 and endpoint (7.3 ± 1.4 days), there was an influx of neutrophils into the infected site, and little change in the number of accumulated T cells and macrophages. We detected expression of the chemokines MIP-1α, RANTES, and SDF-1 in experimental lesions, as well as up-regulation of their respective receptors, CCR5 and CXCR4. In addition to the physical disruption of mucosal and skin barriers that occurs during chancroid, our data suggest that *H. ducreyi* infection provides an environment that may enhance the acquisition of X4, R5, or dual-tropic HIV-1.
MIP-1α and RANTES are inflammatory chemokines that serve as T cell and monocyte attractants (37). SDF-1 is a homeostatic chemokine responsible for normal lymphocyte and monocyte trafficking through tissues (38). As might be expected for inflammatory chemokines, MIP-1α and RANTES were highly expressed in the infected lesions, but not expressed in uninfected control tissue. SDF-1, in contrast, was expressed in all tissues examined. The receptors for these chemokines, CCR5 and CXCR4, serve as HIV-1 co-receptors. In vitro, the presence of SDF-1 prevents infection of transfected HeLa cells and PHA-stimulated PBMCs by HIV-1 (39, 40), but RANTES and MIP-1α do not suppress HIV-1 replication in monocytes/macrophages (41, 42). Thus, the up-regulation of MIP-1α and RANTES in chancroidal lesions may not block HIV-1 entry via CCR5.

We found a highly statistically significant increase in density of CCR5 and CXCR4 on tissue macrophages relative to peripheral blood monocytes in response to experimental infection with H. ducreyi. A statistically significant increase in density of CCR5 was also present on infiltrating T cells relative to peripheral blood T cells. Sites infected with heat-killed bacteria contained fewer leukocytes than sites infected with live bacteria, but these leukocytes also had increased surface expression of CCR5 and CXCR4 that was similar to levels present on leukocytes recruited to infected sites. Wounding of the skin and injection of heat-treated bacteria are probably sufficient to create a microenvironment that recruits T cells and macrophages with up-regulated receptors to the skin. Bacterial replication that occurs in the model (43) most likely increases the number of inflammatory cells that accumulate at infected sites compared with mock-infected sites.

We did not examine co-receptor expression levels in normal skin. A 5-mm punch biopsy of an experimental chancroid lesion is 0.19 cm², and contains approximately the same number of T cells as is found in 10 cm² of human facial skin (J. Campbell, personal communication). Thus, we would have needed to obtain 50 punch biopsies of normal skin to obtain sufficient cells to perform a comparison between inoculated and uninfected skin, which was impractical. To our knowledge, the level of CCR5 and CXCR4 expression on macrophages in normal skin has not been determined. However, the majority of CD4 cells found in normal skin express CCR5 (44). Thus, the CD4 cells recruited to experimental chancroid lesions differ from those that traffic to normal skin in numbers, but not in ability to express CCR5. This is consistent with the concept that CCR5 expression may be a common phenotype of tissue infiltrating T cells (44).

We detected a 2.1- to 2.6-fold increase in CCR5 expression on T cells recruited to infected sites vs those found in peripheral blood. The percentage of CD4 T cells that were CCR5+ in lesions was ~2.4-fold higher than those in peripheral blood. Similarly, there are no changes in the level of CCR5 expression on T cells obtained from the skin of volunteers injected with synthetic Treponema pallidum lipopeptides, although the percentage of T cells expressing CCR5 recruited to these sites is increased (45). Compared with peripheral blood, sites of inflammation are enriched for CCR5+ T cells (46, 47). In peripheral blood, CCR5 is expressed on a subset of memory/effector T cells, while CXCR4 is present on naive T cells (48, 49). The T cells present in experimental H. ducreyi lesions are predominantly memory/effector cells in that they are CD45RO+ (18). Similarly, T cells that infiltrate the skin following injection of T. pallidum lipopeptides are also predominantly memory/effector cells (45). In vitro stimulation of PBMCs with LOS or live H. ducreyi did not affect CCR5 levels on T cells. Therefore, for CCR5, the increased receptor expression reported for T cells most likely reflects selective migration of CCR5+/CD45RO+ memory/effector T cells into the skin rather than an up-regulation of receptor density on T cells, although acquisition of the phenotype at the site of infection cannot be excluded.

Although H. ducreyi infection leads to accumulation of tissue macrophages that had significant increases in receptor density for both CCR5 and CXCR4 relative to peripheral blood monocytes, in vitro stimulation of monocytes with H. ducreyi LOS decreased expression of CCR5, while stimulation with live H. ducreyi resulted in a small (2.2-fold), but statistically significant increase in CCR5 expression. Incubation of monocytes with LOS or H. ducreyi had no significant effect on levels of CXCR4. Thus, the up-regulation of co-receptors on monocytes by H. ducreyi in vivo is at least an order of magnitude greater than in vitro. In contrast to our findings during infection, the effects of purified bacterial products on CCR5 and CXCR4 expression on monocytes and macrophages have been variable (35, 45, 50–54). In general, an increase in one co-receptor is accompanied by a decrease or no change in expression of the other co-receptor. Changes in the levels of chemokine receptors caused by treatment of blood monocytes with bacterial products in vitro may not reflect changes that occur in vivo due to the complex microenvironment that promotes recruitment and accumulation of macrophages at sites of infection.

To our knowledge, this is the first demonstration that a bacterial infection leads to accumulation of macrophages with up-regulation of both CCR5 and CXCR4 and CD4 T cells that express CCR5 at the site of disease. We did not examine other skin infections and cannot exclude the possibility that the changes observed are not H. ducreyi specific. Nonetheless, the up-regulation of both CCR5 and CXCR4 by experimental H. ducreyi infection may have major implications for HIV-1 acquisition. First, R5 strains of HIV-1 are the most common sexually transmitted strains (55–57). The striking up-regulation of CCR5 on macrophages and the influx of macrophages and CCR5-expressing T cells into the lesion provide a population of R5-susceptible cells. The increased expression of CXCR4 also offers an environment that could allow for acquisition of X4 viruses. Finally, the infiltrating leukocytes could readily promote acquisition of dual-tropic strains. Thus, H. ducreyi infection not only disrupts epithelial barriers, but provides a microenvironment that could readily lead to acquisition of R5, X4, and dual-tropic HIV-1.

In the human infection model, volunteers are infected until they develop a painful pustule or for 2 wk. The average duration of infection for naturally occurring chancroid is estimated to be 5 wk (58). Due to a lack of readily available specimens, the expression levels of CCR5 and CXCR4 on macrophages and T cells in lesions of naturally occurring chancroid have not been determined. However, the histopathology of experimental and natural disease is nearly identical (16–18). We speculate that increased co-receptor expression may occur in natural disease. Together with disruption of mucosal barriers, recruitment of cells with increased co-receptor expression may be responsible for the relationship between chancroid and HIV-1 acquisition. In future studies, we plan to isolate T cells and macrophages from experimental lesions and test their susceptibility to primary isolates of R5, X4, and dual-tropic HIV-1.

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References


