HIV-1 Target Cells in Foreskins of African Men With Varying Histories of Sexually Transmitted Infections

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Abstract

Numerous epidemiologic studies have found significant associations between lack of circumcision and HIV-1 acquisition in men. To our knowledge, this is the first study of human foreskin tissue that examines biologic mechanisms that increase susceptibility of uncircumcised African men to HIV-1. Foreskin specimens from 20 men with and 19 men with no history of sexually transmitted infections were examined for HIV-1 target cells. Most Langerhans cells were found in the epithelium; most CD4+ T cells and macrophages were in the submucosa. There were no differences in HIV-1 target cells between men with and those without history of sexually transmitted infections. However, Langerhans cells and macrophages were more abundant in the group with a history of infection. The densities and positions of HIV-1 target cells in the foreskin tissue of these Kenyan men indicate that the inner mucosal surface of the human foreskin contains cells that make it highly susceptible to HIV infection.

According to the Joint United Nations Programme on HIV/AIDS, it is estimated that 39.4 million people are infected with HIV-1 globally, with the vast majority of these infections occurring in sub-Saharan Africa through heterosexual activity. An estimated 25.4 million people living in this region, which accounts for only 10% of the world’s population, are infected with HIV, with approximately 3.1 million new infections occurring in 2004. The prevalence of HIV in Africa varies by different regions, and these discrepancies cannot be explained completely by sexual behavior patterns or other factors known to influence HIV transmission.

Differences in male circumcision practices have been proposed to contribute to these variations in prevalence, with higher prevalence in societies in which circumcision is uncommon. Because it is estimated that 70% of HIV-infected men globally and more than 90% in Africa have acquired the virus through heterosexual intercourse, the portal of entry for most HIV infections in men must be the penis. Significant associations between lack of circumcision and HIV-1 acquisition have been reported in more than 40 observational epidemiologic studies. The relative risk of acquiring HIV-1 in uncircumcised men ranges from 1.8 to 8.2 times greater than in circumcised men. However, owing to many possible confounding factors, doubts remain as to whether there is a true biologic relationship between HIV acquisition and presence of the foreskin.

It is important, therefore, to understand the biologic mechanisms by which the presence of the foreskin may increase susceptibility to HIV acquisition. HIV-1 target cells, including CD4+ T cells, macrophages, and Langerhans cells, are present in the mucosal layer of the foreskin and provide a route of infection for HIV-1, and these target cells express the
Circumcision is known to reduce HIV incidence in men. However, the mechanisms by which this occurs are not fully understood. A recent study aimed to quantify the percentage of HIV-1 target cells in foreskin tissue from African populations.

**Materials and Methods**

**Patients**

Foreskin tissues were obtained through the Universities of Nairobi, Illinois, and Manitoba Collaborative Research Project, an ongoing randomized, controlled trial examining whether male circumcision reduces HIV incidence being conducted in Kisumu, Kenya. In this trial, men aged 18 to 24 years are recruited into an unblinded trial with 2 arms: the circumcision arm generally are circumcised the same day or within 2 weeks. Circumcisions are performed by trained clinicians in a fully equipped operating room under sterile conditions.

Foreskin tissues obtained for this study were held at 4°C, soaked in normal saline, and processed immediately in a laboratory at the study clinic. Within 2 hours of the procedure, tissue samples were preserved in Streck Tissue Fixative (Streck Laboratories, Omaha, NE). They then were transported to Chicago, IL, where they were sectioned and preserved in paraffin blocks. Later, they were cut into 5-µm sections and adhered to silanized slides.

Of the 105 consecutive samples, 40 were obtained at random for this study and stratified by STI history (presence or absence). Within the stratum with history of STI, 12 participants were selected with an STI that had been diagnosed and treated by study staff and 8 with a history of a treated STI reported by the participant. Informed consent was obtained from all subjects, and the study was approved by 4 independent institutional review boards (University of Nairobi, Nairobi, Kenya; University of Illinois at Chicago; University of Manitoba, Winnipeg, Canada; and RTI International, Research Triangle Park, NC).

**Immunohistochemical Analysis**

Tissue sections were deparaffinized through xylenes and graded alcohols. After peroxidase quenching and blocking with mouse serum in phosphate-buffered saline, pH 7.4, with 5% nonfat dry skim milk, immunohistochemical analysis was performed using the Enhanced V-Red Detection Kit (Ventana Medical Systems, Tucson, AZ) according to the manufacturer’s recommendations. Commercially available antibodies to CD1a (clone MTB1, BioCare Medical, Walnut Creek, CA), CD4 (clone BC/1F6, Cell Marque, Hot Springs, AR), and CD68 (clone KP1, DakoCytomation California, Carpinteria, CA) were used at optimized concentrations.

CD1a is expressed on Langerhans cells and on cortical thymocytes and dendritic cells; it is involved in the display of nonpeptide and glycolipid antigens for T-cell responses. CD4 is present on T-helper/inducer cell populations and is a receptor for HIV. CD68 is expressed on macrophages, monocytes, dendritic cells, neutrophils, basophils, mast cells, myeloid progenitor cells, a subset of CD34+ hematopoietic progenitor cells, activated T cells, and some peripheral-blood B cells; its function is not yet known. CD1a and CD4 slides were processed with a Benchmark automated immunohistochemical processor (Ventana Medical Systems), and CD68 slides were processed with Borg Decloaker (BioCare Medical) using a Nexes automated immunohistochemical processor (Ventana Medical Systems).

The stained tissues were examined with a LEICA DMR-X microscope (Leica, Wetzlar, Germany) equipped with a 3-chip charged coupled device color camera (DCX-750P, Sony, © American Society for Clinical Pathology

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Tokyo, Japan). Digital images were transferred to a computerized image analysis system (Quantimet Q5501 W, Leica, Cambridge, England). Percentages of cells were based on examination of the internal mucosal surface containing 50% epithelium and 50% submucosa within the field. The percentage of positive cellular area per total cellular area in the foreskin tissue that contained T cells, macrophages, and Langerhans cells was quantified. The mean of 5 readings from a magnetic field was calculated and reported.

Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS) software package, version 8.2 (SAS Institute, Cary, NC). Because data were not distributed normally, a nonparametric measure was used to compare the means of the proportions for each cell type. When comparing the percentage of positive cells in the epithelium vs the submucosa, the Wilcoxon signed rank test was used. The Wilcoxon rank sum test was used to compare the percentage of cells in the STI history with the no-STI history groups. Correlations between different cell types were calculated using the Kendall rank correlation coefficient.

Results

Quantification of HIV-1 Target Cells on the Mucosal Surface

One patient declined to participate in the study, leaving 11 instead of 12 samples from the recently treated STI group. The mean cell percentages were derived from the amount of cellular area staining positive per total cellular area in 5 fields covering the internal mucosal surface containing 50% epithelium and 50% submucosa within each field. In the 39 slides examined, Langerhans cells were most abundant, with a median of 1.5%. T cells were less plentiful, with a median of 0.16%, and there were even fewer macrophages, with a median of 0.06%.

To determine whether men with more of one type of target cell were likely to have more of another type, we calculated correlation coefficients between the different types of cells. The antigen-presenting cells, Langerhans cells, and macrophages were significantly positively correlated (τ = 0.336; P = .004). CD4+ T cells also were correlated positively with macrophages, but only marginally (τ = 0.213; P = .085). Langerhans cells and CD4+ T cells were not correlated (τ = 0.087; P = .454).

To better characterize the distribution of HIV-1 target cells in human foreskin, each cell type was quantified in the epithelium and the submucosa in the tissue samples from 39 men, and median values were compared. A greater proportion of Langerhans cells was found in the epithelium than in the submucosa (1.23% vs 0.30%; P < .001). The majority of macrophages (0.02% vs 0.04%; P = .025) were found in the submucosa. There was no difference in the median percentages of CD4+ T cells found in the epithelium vs the submucosa, although the difference was statistically significant (0.080% vs 0.075%; P = .002) using nonparametric methods.

Quantification of HIV-1 Target Cells According to STI History

To examine differences in the presence of HIV-1 target cells by STI history, the foreskins of 20 men with no history of STI and 19 men with a history of STI were analyzed. The proportions of cells were based on the internal mucosal surface within the magnetic field containing 50% epithelium and 50% submucosa. The group of 19 men with STI history included 8 men who reported an STI history and 11 who were recently observed and treated for an STI. One participant in the recently observed and treated category declined to participate. There were no statistically significant differences in Langerhans cells, CD4+ T cells, or macrophages (P = .598, P = .304, and P = .533 respectively) between the two groups with reported vs diagnosed STI history.

In addition, no significant differences were found in median HIV-1 target cell proportions between men with and without an STI history. In the group with an STI history, the median of Langerhans cells was 1.99% compared with 1.24% in the group with no STI history (P = .134). There were more macrophages in the group with a history of STI (median cell proportion, 0.10%) than in the group with no STI history (0.02%), but the difference was not significant (P = .172). There was no difference in the medians of CD4 + T
cells in the group with no STI history (0.33%) compared with the group with a history of STI (0.14%; \( P = .829 \)). Finally, no differences were found in the proportions of the 3 target cells in the group of participants with no history of STI compared with those who recently were observed and treated for an STI.

**Discussion**

In this study, we established that in foreskin tissue obtained from men ages 18 to 24 years enrolled in a randomized clinical trial of male circumcision in Kisumu, Kenya, HIV-1 target cells were present, accounting for 2.0% of all cells detected in the inner foreskin epithelium and submucosa. Most Langerhans cells were detected near the surface of the epithelium, whereas most CD4+ T cells and macrophages were found in the submucosa. These findings are consistent with those from studies of English, North American, and

**Image 1** Immunohistochemical staining of HIV-1 target cells in a man with a history of sexually transmitted infection. Cells staining alkaline phosphates red are positive for a specific HIV-1 target cell, with blue hematoxylin used as the counterstain. 

- **A**, A great proportion of Langerhans cells are present in the epithelium compared with the submucosa (1.1% vs 0.1%) (×100).
- **B**, The majority of macrophages were found in the submucosa (2.7%) rather than the epithelium (0.4%) (×100).
- **C**, Although there were no statistically significant differences seen in CD4+ T cells, they are in greater abundance in the submucosa (2.0%) than in the epithelium (0.5%) (×100).

**Figure 2** HIV-1 target cells in human foreskin categorized by a history of sexually transmitted infection (STI) and no history of STI. Medians are based on the percentage of positive cellular area per total cellular area of the internal mucosal surface within the magnetic field containing 50% epithelium and 50% submucosa within the field for Langerhans cells (\( P = .134 \)), CD4+ T cells (\( P = .829 \)), and macrophages (\( P = .172 \)).
Australian men, in which HIV-1 target cells were found in human foreskin at higher proportions than in other tissues. Langerhans cells were detected at high densities in the mucosal layer of the foreskin in infant and adult English patients, and they were at higher densities in foreskin than in cervical, rectal, or vaginal mucosa. In children and adults in the United States, the proportion of HIV-1 target cells in foreskin was 6 times higher than in adult cervical tissue. In studies specifying positions of cells, more Langerhans cells were found in the epithelium, whereas more CD4+ T cells were in the submucosa. Thus, evidence from studies in diverse settings and populations is consistent: many HIV target cells are present in human foreskin, and Langerhans cells are present near the mucosal surface of the inner foreskin. This suggests that because CD4+ T cells and macrophages are found deeper in the tissue, Langerhans cells are likely the first to be infected by HIV.

The internal mucosal surface of the foreskin is covered with no or minimal keratin. Although abundance and position of target cells are likely important, the degree of keratinization of the epithelium may be as significant a factor in determining susceptibility to HIV infection. The outer surface of the foreskin and the glans penis of the circumcised and uncircumcised penes have thick layers of keratin compared with the inner surface of the foreskin, where keratin is nearly absent. In the epithelium of the circumcised penis is keratinized similarly to the outer surface of the foreskin, the barrier presented to HIV by a thick layer of squamous epithelium on the circumcised penis could contribute to a greater risk of HIV acquisition in uncircumcised men. This should be verified through study of tissue from the circumcised penis.

The proportions of antigen-presenting cells, Langerhans cells, and macrophages were positively correlated. These cells recognize and process antigens by engulfing them, degrading them into peptides, and assembling them into major histocompatibility complex molecules that are displayed on the cell surface, where they are recognized by T cells. When an antigen is taken up by a cell, it migrates through the lymph system to the lymph node, where it infects T cells. This leads to the infection and depletion of the CD4 subset of T cells, which is characteristic of HIV disease.

The number of HIV-1 target cells in penile tissue may be a function of immune activation, with a higher proportion of HIV-1 target cells present in response to past infections. STIs increase risk of HIV infection, particularly ulcerative STIs, possibly through the presence of inflammatory exudate, consisting of CD4 cells, monocytes, and Langerhans cells at the site of infection. An increase in endocervical CD4 lymphocytes has been seen in women with nonulcerative STIs as well.

We had hypothesized that men with recent STIs would have elevated numbers of HIV target cells compared with those with no reported or diagnosed history of STI. For Langerhans cells and macrophages, we detected a larger percentage in the group with an STI history, but the results were marginal, and no difference was found between percentages of CD4 T cells.

There are several possible explanations for these findings. First, treatable STIs were diagnosed presumptively or through laboratory analyses and treated 2 weeks before circumcision. Treatment before circumcision may have reduced immune activation. Second, the sample was small. Based on the means and sample sizes used in this study, the power detected to see a significant difference in cell proportions between participants with a history of STI and those without at a .05 level of significance is low. The power corresponds to 0.20 for Langerhans cells, 0.13 for CD4+ T cells, and 0.41 for macrophages. It is possible that a lesion or abrasion in the foreskin tissue might have caused cellular recruitment to one specific area, reducing the difference in target cell proportions between men with and without an STI history. However, it is unlikely that lesions would have gone undetected because 5 magnetic field readings were taken from each tissue. Another limitation of this study is that one of the groups with STI history was self-reported. For this reason, a sample of participants whose STI history was diagnosed in the laboratory also was included, but results in these men also did not differ from those in men without an STI history.

To our knowledge, this study provides the first data establishing that HIV target cells are present at high densities in foreskin tissues from uncircumcised African men. Langerhans cells were present near the surface of the epithelium of the inner foreskin where there is no or minimal keratin barrier to obstruct uptake of HIV. In the light of existing evidence, it is plausible that circumcision reduces the risk of HIV acquisition through the penis by physically removing HIV-1 target cells positioned close to the mucosal surface of the inner foreskin. In vitro studies are needed to elucidate the precise processes by which HIV infiltrates target cells in penile tissue. Such studies should include examination of HIV acquisition with varying levels of immune activation as seen in secretion of cytokines, up-regulation of coreceptors CCR5 and CXCR4, and the number of cells expressing activation markers on their cell surface. Such research would increase understanding of the means by which circumcision might protect against HIV infection and also could provide the basis for development of effective penile microbicides.
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References


