

INTRODUCTION

Women constitute half of the world's population of HIV-infected individuals and heterosexual transmission is now the most common mode of HIV transmission. HIV infection occurs through sequential binding of the viral envelope to the CD4 receptor and CCR5 or CXCR4 chemokine receptors on activated T-cells. Specific sequences in the viral envelope determine which chemokine receptor is used during infection suggesting the existence of different sub-species of HIV in any given person. Many studies have documented this and current models of HIV transmission suggest that the inoculum is a quasispecies of CCR5-tropic and CXCR4-tropic viruses. Regardless, in an overwhelming majority of naïve hosts CCR5-tropic viruses are more prevalent soon after transmission. Therefore, there is great interest in utilizing CCR5 inhibitors initially developed as prophylactic interventions to prevent transmission of HIV. Specifically, a number of these inhibitors are being studied for use as vaginal/cervical microbicides. One potential concern about the use of these microbicides is how they might alter natural immunological responses in the cervix against other infecting agents to which the cervix is exposed. A better characterization of cervical CCR5 expression and immune activation will be important when evaluating the safety of CCR5 inhibitors as vaginal microbicides. Therefore, we have conducted studies examining CCR5 and CXCR4 expression of cervical lymphocytes and the related cytokine milieu of cervical secretions in healthy women.

Goals of this study:

- 1) To determine the levels of HIV target cells (CD4⁺CCR5⁺ T-cells) in the cervix of healthy women.
- 2) To evaluate whether the presence of RANTES, CCR5 ligand, in the cervix correlates with the amount of CCR5⁺ T-cells present in that compartment.
- 3) To measure immune activation in the cervix by examining the presence of IFN γ , TNF α , and IL-10.

METHODS

Female Participants

Participant:	5	7	8	9	11	12	14	17	22	23
Age	22	21	25	18	23	31	32	29	42	32
Pregnancy	N	N	N	N	N	N	N	N	N	N
Sperm	N	N	N	N	N	N	N	N	N	N
Syphilis	N	N	N	N	N	N	N	N	N	N
Gonorrhea	N	N	N	N	N	N	N	N	N	N
Chlamydia	N	N	N	N	N	N	N	N	N	N
HSV	N	N	N	N	N	N	N	N	N	N
HPV	N	N	N	N	N	N	N	N	N	N
Trichomonas	N	N	N	N	N	N	N	N	N	N
Bacterial Vaginosis	N	N	N	N	N	N	N	N	N	N
Vaginal Candidiasis	N	N	N	N	N	N	N	N	N	N
Vaginal Inflammation	N	N	N	N	N	N	N	N	N	N

All participants < 40 PMNs/hpf

All participants tested negative (N) for all of the items listed in the table. Venous blood and cervical cells were collected from each participant. All sample collection was performed at the Emory University Hope Clinic and all sample processing was done at the Emory Vaccine Center. Four other participants were additionally sampled but they were excluded from this study due to insufficient collection of cervical lymphocytes (< 2500/ml).

Cytobrush Collection of Cervical Lymphocytes

Cervical Lymphocytes were collected by inserting and rotating a nylon bristle cytology brush in the cervical canal. The brush was placed in sterile PBS containing antibiotics. Within 4 hours of collection, the samples were vigorously vortexed to dislodge the cells and washed prior to staining with anti-CD4, anti-CD8, anti-CCR5, and anti-CXCR4 antibodies. Cell surface phenotypic analysis was performed using multi-parameter flow cytometry. The significance of chemokine receptor expression on the different cell types was calculated using a standard t-test.

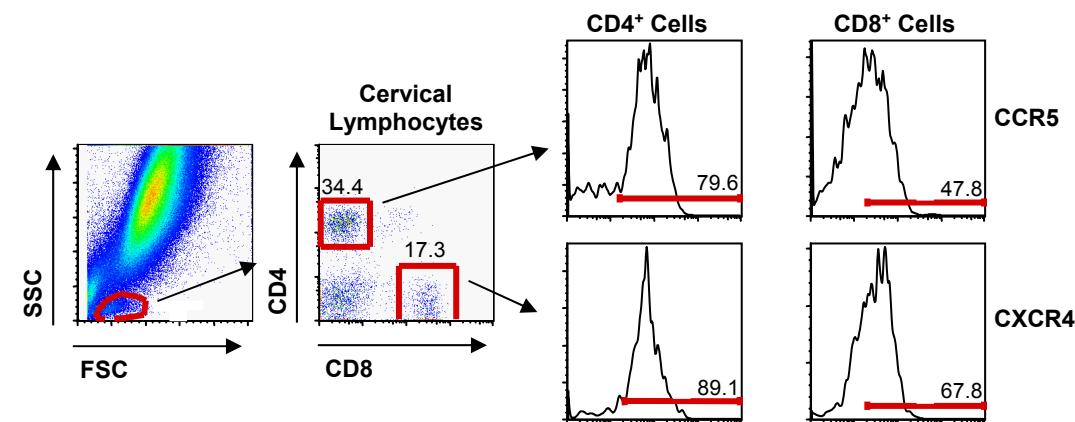
Collection of Cervical Lavage

The cervical lavage was collected by directing sterile PBS in the cervical canal and collecting all the secretions. ELISA assays were used to determine the relative levels of RANTES, IFN γ , TNF α , and IL-10.

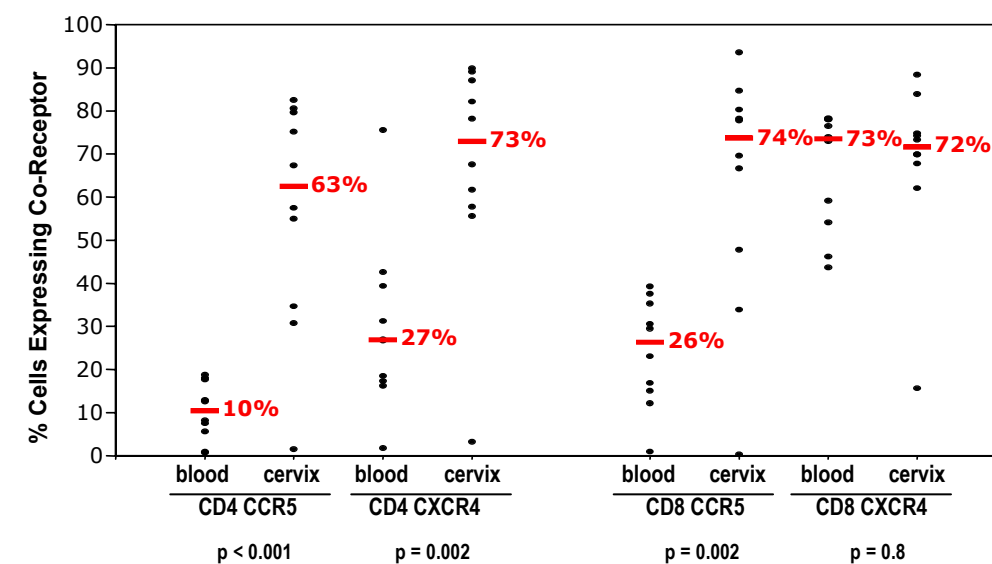
RESULTS

CHARACTERIZATION OF CCR5 AND CXCR4 ON CERVICAL LYMPHOCYTES

Gating Strategy of Cervical Lymphocytes



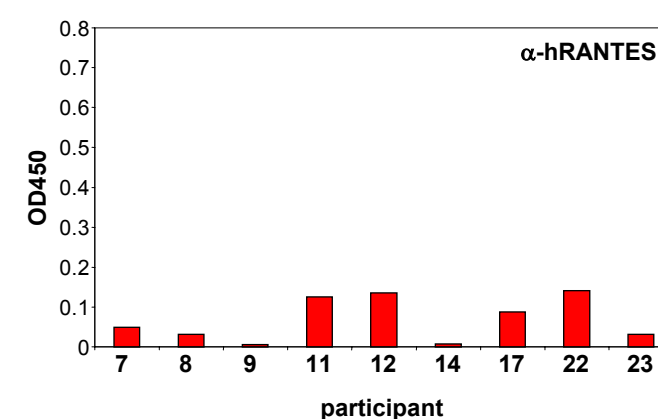
CCR5 and CXCR4 are Expressed at Significantly Higher Levels on Cervical CD4⁺ Lymphocytes than Peripheral Blood CD4⁺ Lymphocytes. CCR5 Expression on Cervical CD8⁺ Lymphocytes is Significantly Higher than on Peripheral Blood CD8⁺ Lymphocytes. There is No Difference in CXCR4 Expression on CD8⁺ Lymphocytes Between the Blood and Cervix.



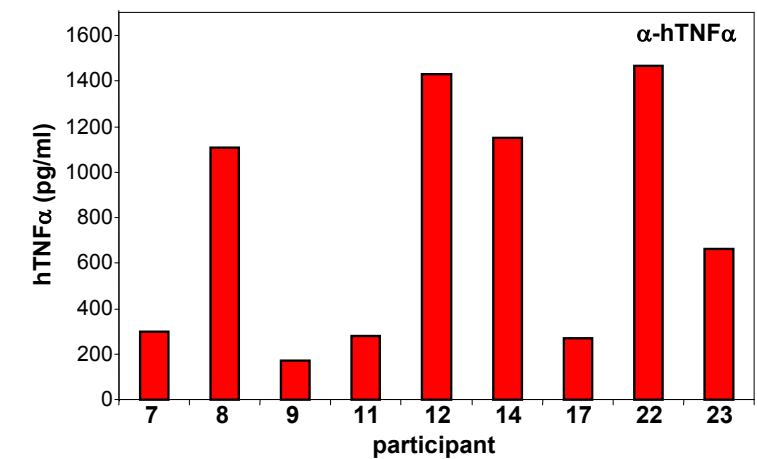
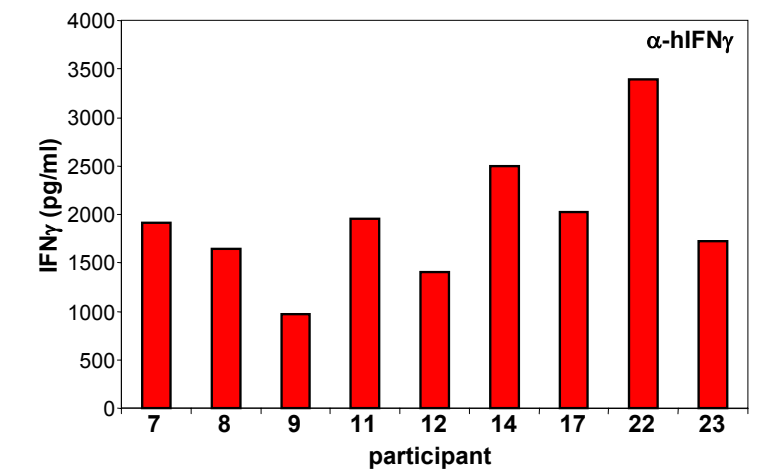
The percent of CCR5 or CXCR4 expressing CD4⁺ and CD8⁺ lymphocytes in peripheral blood and cervical samples was determined by flow cytometry. The gating strategy is shown above. The median values are indicated in red and the individual participant values are presented in black. The significance (p-value) between blood and cervical co-receptor expression is indicated on the figure.

CYTOKINE PROFILE IN THE CERVIX

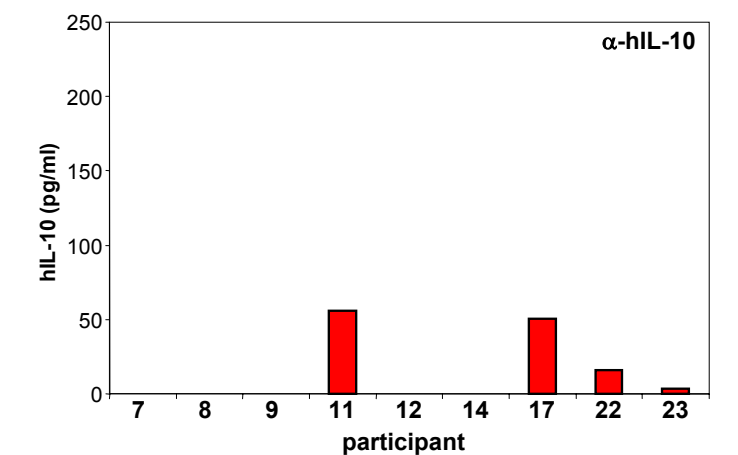
The CCR5 Ligand, RANTES, is Present in Cervical Secretions at Very Low Levels



Local Concentrations of IFN γ and TNF α Suggest Activation of Cellular Immunity in the Healthy Cervix



The Classically TH2 Cytokine, IL-10, is Present at Low Levels in the Cervix of Healthy Women



CONCLUSIONS

The cervixes of healthy women have a higher proportion of CD4⁺ and CD8⁺ lymphocytes that express CCR5 and/or CXCR4 as compared to the peripheral blood. Our data demonstrates that, in the absence of STDs or inflammation, there appears to be generalized immune activation (TNF α and IFN γ) in the cervix. These observations suggest that the cervixes of healthy women are rich in potential target cells for HIV infection. We also show that the CCR5 chemotactic ligand, RANTES, and immune suppressing cytokine, IL-10, are present at low levels in the cervix of healthy women. Together, these data suggest that blocking cervical CCR5 receptors may alter the heightened state of immunity in the cervix. Therefore, the effects on cervical immune activation and homing of lymphocytes and macrophages should be evaluated in women with and without STDs or inflammation during the development of CCR5 inhibitors as vaginal microbicides.

ACKNOWLEDGEMENTS

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