Increased interleukin-10 in the endocervical secretions of women with non-ulcerative sexually transmitted diseases: a mechanism for enhanced HIV-1 transmission?

Craig R. Cohen*‡, Francis A. Plummer†§, Nelly Mugo‡, Ian Maclean§, Caixia Shen§, Elizabeth A. Bukusi‡¶, Erastus Irungu†, Samuel Sinei‡, Job Bwayo† and Robert C. Brunham§

Objective: Although non-ulcerative sexually transmitted diseases (STD) and bacterial vaginosis are implicated as cofactors in heterosexual HIV-1 transmission, the mechanisms have not been defined. Recent *in vitro* data suggest that interleukin (IL)-10 may increase susceptibility of macrophages to HIV-1 infection. Therefore, we performed this study to assess whether non-ulcerative STD are associated with detection of IL-10 in the female genital tract.

Methods: Women with clinical pelvic inflammatory disease with or without cervicovaginal discharge were recruited from an STD clinic in Nairobi, Kenya. Endocervical and endometrial specimens were obtained for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* DNA detection, *Trichonomas vaginalis* culture, and CD4 and CD8 T-cell enumeration. Bacterial vaginosis was diagnosed by Gram stain. IL-10 was detected in endocervical specimens using enzyme-linked immunosorbent assay. Blood was obtained for HIV-1 serology.

Results: One hundred and seventy-two women were studied. *N. gonorrhoeae*, *C. trachomatis*, bacterial vaginosis, and *T. vaginalis* were detected in 38 (21%), 17 (9%), 71 (43%), and 22 (12%) women, respectively. Cervical IL-10 was detected more often in women with *N. gonorrhoeae* [adjusted odds ratio (AOR), 3.4; 95% confidence interval (CI), 1.4–8.4], *C. trachomatis* (AOR, 4.4; 95% CI, 1.2–15.6), and bacterial vaginosis (AOR, 3.1; 95% CI, 1.4–6.9) than in women without these infections.

Conclusions: The association of non-ulcerative STD and bacterial vaginosis with increased frequency of IL-10 detection in endocervical secretions suggests a potential mechanism through which these infections may alter susceptibility to HIV-1 infection in women.

AIDS 1999, **13**:327–332

Keywords: Bacterial vaginosis, gonorrhea, *Chlamydia*, immunity, mucosal, HIV-1

From the *Departments of Obstetrics and Gynecology, University of Washington, Seattle, Washington, USA, the †Department of Medical Microbiology, the ‡Departments of Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya, the §Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada, and the ¶Centre for Microbiological Research, Kenya Medical Research Institute, Nairobi, Kenya.

Sponsorship: Supported by a grant from the National Institutes of Health (AI31448) and the Medical Research Council of Canada (GR-13301).

Requests for reprints to: Dr Craig R. Cohen, Department of Obstetrics and Gynecology, University of Washington, Box 356460, Seattle, WA 98195-6460, USA. E-mail: crcohen@u.washington.edu.

Date of receipt: 18 August 1998; revised: 30 November 1998; accepted: 7 December 1998.

Introduction

Non-ulcerative sexually transmitted diseases (STD) appear to be cofactors in the acquisition of HIV-1 infection by women [1,2]. Bacterial vaginosis is the most common infection in the female genital tract, and in cross-sectional studies has been associated with HIV-1 infection [3,4]. Although these investigations could not determine whether bacterial vaginosis is a risk factor for HIV-1 acquisition, recently reported data suggests that bacterial vaginosis may be associated with an increased incidence of HIV-1 infection [5]. Because of the relatively high proportion of HIV-1 at-risk women with these infections in many areas of the world, non-ulcerative STD, and perhaps bacterial vaginosis, could account for a large percentage of attributable risk associated with female acquisition of HIV-1. Their overall importance is suggested by the finding that syndromic management of these and other STD led to a 40% reduction of HIV-1 incidence in rural Tanzania [6].

Potential mechanisms by which these STD may increase susceptibility to HIV-1 infection include (i) increased number or activation of HIV-1 target cells such as monocytes, dendritic cells or CD4 T-lymphocytes [7] in the genital tract, (ii) disruption of the normal epithelial barrier [8], and (iii) reduction of protective T-helper (TH)1 and cytotoxic T-lymphocyte function [9]. In addition, bacterial vaginosis may increase susceptibility to HIV-1 infection through loss of naturally occurring virucidal mechanisms (i.e., absence of hydrogen peroxide-producing lactobacilli, reduction in myeloperoxidase halide activity, and increased vaginal pH) [10,11].

Macrophage-tropic HIV-1 strains appear to be primarily responsible for sexual transmission [12]. In the SIV rhesus macaque sexual transmission model, the first SIV-infected cells were found in the lamina propria of the vagina and resembled dendritic cells [13]. Factors that expose, activate or increase the density of these cells could lead to increased susceptibility of the genital tract to HIV-1 infection. Although early studies reported that interleukin (IL)-10 inhibited HIV-1 replication [14], later experiments demonstrated that a lower concentration of IL-10 synergistically enhanced the induction of HIV-1 replication in macrophages elicited by tumor necrosis factor-α and IL-6 [15]. Chemokine receptors such as CC-chemokine receptor (CCR)-5 act as coreceptors for HIV-1 infection of dendritic and monocyte/macrophages [16]. Individuals homozygous for the Δ32 mutation of the *CCR-5* gene are significantly less susceptible to infection even after multiple exposures [17]. Recently, Sozanni *et al*. [18] presented *in vitro* evidence that IL-10 selectively upregulates CCR-5, and increases the efficiency of macrophage-tropic HIV-1 infection of macrophages. These data suggest that factors leading to increased IL-10 in the female genital tract may enhance HIV-1 acquisition. We performed this study to determine whether non-ulcerative STD and bacterial vaginosis are associated with increased IL-10 in the female genital tract.

Methods

Between June 1997 and December 1997, women aged 18–40 years from the Special Treatment Clinic in Nairobi, with a complaint of low abdominal pain/abnormal vaginal discharge for $\leq$ 14 days were eligible for recruitment. Women were excluded if they were pregnant or breastfeeding, gave a history of surgery, delivery, or abortion in the previous 6 weeks, or reported receipt of at least 2 days of antibiotics in the preceding 2 weeks. Written informed consent was obtained from each participant in the study. The protocol was approved by the Institutional Review Board for Human Subjects at the University of Manitoba, Canada, and by the Human Subjects Review Board at Kenyatta National Hospital, Nairobi, Kenya.

After written informed consent was obtained, a demographic and clinical questionnaire was administered and women underwent a standardized physical examination. Vaginal specimens were obtained for *Trichomonas vaginalis* culture, and Gram stain for diagnosis of bacterial vaginosis. Cervical specimens were obtained for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* DNA detection using a polymerase chain reaction (PCR) assay. An Aspirette (Unimar, Inc., Wilton, Connecticut, USA) was used to atraumatically collect endocervical secretions for IL-10 measurement, and a cytobrush was used to obtain endocervical specimens for CD4 and CD8 T-cell measurement. Endometrial biopsies for *N. gonorrhoeae* and *C. trachomatis* PCR, and CD4 and CD8 T-cell count were obtained using a Pipelle (Unimar) endometrial biopsy device. Blood was obtained for HIV-1 serology and CD4 and CD8 T-lymphocyte enumeration. Empirical antibiotic treatment, consisting of a single dose of ciprofloxacin 500 mg, and doxycycline 100 mg and metronidazole 500 mg twice daily for 10 days, was administered to all women.

T. vaginalis was cultured using the InPouch (BioMed Diagnostics, Inc., San Jose, California, USA). The diagnosis of bacterial vaginosis by Gram stain criteria used the scoring system of Nugent *et al*. [19]. *N. gonorrhoeae* and *C. trachomatis* were detected from the cervix and endometrium using PCR (Roche Diagnostic System, Inc., Somerville, New Jersey, USA). Serum was tested for HIV-1 antibodies using a synthetic peptide enzyme immunoassay (Detect HIV-1, Biochem Immuno-Systems, Inc., Montreal, Canada). Those positive on

the initial screening test had a second confirmatory test (Recombigen, Cambridge Biotech Ltd, Galway, Ireland) performed.

In preparation for flow cytometry, endocervical and endometrial specimens were placed in 5 ml RPMI solution. Endometrial tissue was dispersed using a fine wire mesh. Cell suspensions were processed within 2–6 h of specimen collection. Specimens were centrifuged at 1300 **g** for 5 min to pellet the cells. Supernatants were aspirated and cell pellets resuspended in 2 ml lysing reagent (1 : 10 dilution of FACS Lyse buffer; Becton Dickenson, San Jose, California, USA), and centrifuged. This step was repeated twice. Cell pellets were resuspended and washed twice in physiologic buffered saline. One hundred microliters of suspended samples were reacted with 10 µl fluorescein isothiocyanate-conjugated monoclonal antibodies to CD4 and 10 µl phycoerythrin-conjugated monoclonal antibodies to CD8 (Becton Dickenson, Sunnyvale, California, USA) for 15 min. The gate was set by forward and side-scatter to minimize the number of monocytes. The number of events inside the gate was designated as the number of monocytes. The number of lymphocytes reactive with each monoclonal antibody was measured using a FACScan (Becton Dickenson, Sunnyvale), and reported as events per 10 000 mononuclear cells.

An enzyme immunoassay was used to detect IL-10 in endocervical secretions (PharMingen, San Diego, California, USA). The concentration of IL-10 was determined by comparing sample values with a standard curve of recombinant IL-10 performed with each assay. All specimens and negative controls were measured in duplicate. We defined the lower limit of IL-10 detection (4.0 pg/ml) as the mean plus two times the SD of values for negative controls.

Data were analyzed using SPSS for windows 7.5 (SPSS, Inc., Chicago, Illinois, USA). Univariate analysis was performed using χ^2 and Fisher's exact tests for categorical variables, Mann–Whitney test for interval, and Student's t-test for continuous variables. Multiple logistic regression was used for multivariate analysis.

Results

We recruited 172 women into the study: 134 (78%) with clinical pelvic inflammatory disease and 38 (22%) with a chief complaint of vaginal discharge without lower abdominal pain. Thirty-seven (22%) and 16 (9%) were infected with *N. gonorrhoeae* and *C. trachomatis*, respectively. Bacterial vaginosis was detected in 71 (43%) of the 164 women for whom the vaginal Gram stain was adequate for analysis, and *T. vaginalis* was cultured in 21 (12%) women. Multiple infections (e.g., gonorrhea and bacterial vaginosis) were detected in 38 women. Women with these non-ulcerative STD were compared with the 65 women without these infections.

Fifty-six (33%) women were infected with HIV-1. As is well established, HIV-1-infected women had reduced CD4 T lymphocytes and elevated CD8 T-lymphocyte counts in peripheral blood (Table 1). Similar perturbations in CD4 and CD8 T-cell counts were also observed in endocervical and endometrial specimens. Although non-ulcerative STD did not appear to significantly affect CD4 or CD8 T-lymphocyte counts or CD4/CD8 ratios from blood, endocervical, or endometrial specimens, the median number of CD4 T lymphocytes measured in endocervical specimens was greater among women infected with *N. gonorrhoeae* than among controls (103 versus 77; $P < 0.05$; Table 2). Similar, although not statistically significant, increases in CD4 T cells in endocervical samples were also noted in women with *C. trachomatis* infections.

IL-10 was detected in endocervical secretions from 77 (44%) women. The median level of IL-10 detected was 7.8 pg/ml, and the 25% and 75% quartile levels were 5.9 and 10.7 pg/ml, respectively. Women without gonorrhea, *Chlamydia*, *Trichomonas*, or bacterial vaginosis served as controls. Infection with *N. gonorrhoeae* [54% versus 32%; odds ratio (OR), 2.5; 95% confidence interval (CI), 1.1–5.7], and bacterial vaginosis (54% versus 32%; OR, 2.4; 95% CI, 1.2–4.9), but not *C. trachomatis* (56% versus 32%; OR, 2.7; 95% CI, 0.9–8.2), nor *T. vaginalis* (48% versus 32%; OR, 1.9; 95% CI, 0.7–5.2) were associated with an increased prevalence of IL-10 measured in endocervical secretions in comparison with women without these infections (Fig. 1).

The prevalence of *N. gonorrhoeae* (38% versus 36%), *C. trachomatis* (20% versus 20%) and bacterial vaginosis (55% versus 50%) was similar amongst HIV-1-seropositive and seronegative women. In univariate

Table 1. CD4 and CD8 T-lymphocyte counts and CD4/CD8 T-cell ratio from blood, endocervical, and endometrial specimens from HIV-1-infected and uninfected women.

	Median (range)		
	HIV-1-positive (n = 56)	HIV-1-negative (n = 114)	*P*
Blood			
CD4 ($\times 10^6$/l)	320 (10–1510)	740 (200–1760)	0.001
CD8 ($\times 10^6$/l)	965 (280–4020)	690 (200–1740)	0.001
CD4/CD8 ratio	0.3 (0–1.0)	1.0 (0.2–2.6)	0.001
Endocervix			
CD4 ($\times 10^6$/l)	57 (4–831)	98 (8–1073)	0.002
CD8 ($\times 10^6$/l)	168 (52–1538)	103 (15–821)	0.001
CD4/CD8 ratio	0.4 (0–2.5)	1.0 (0.2–11.0)	0.001
Endometrial			
CD4 ($\times 10^6$/l)	189 (20–1015)	337 (36–1694)	0.007
CD8 ($\times 10^6$/l)	1570 (74–6428)	520 (20–3355)	0.001
CD4/CD8 ratio	0.2 (0–2.1)	0.7 (0.1–3.2)	0.001

Table 2. CD4 and CD8 T-lymphocyte counts and CD4/CD8 T-cell ratio from blood, endocervical, and endometrial specimens from women with gonorrhea, *Chlamydia*, or bacterial vaginosis compared with women without these infections and *Trichomonas**.

	Median (range)			
	No infection† (n = 59)	*N. gonorrhoeae* (n = 36)	*C. trachomatis* (n = 17)	Bacterial vaginosis (n = 66)
Blood				
CD4 ($\times 10^6$/l)	630 (10–1600)	580 (180–1760)	530 (150–1510)	550 (80–1510)
CD8 ($\times 10^6$/l)	750 (310–2450)	930 (200–4020)	580 (200–2400)	870 (200–2490)
CD4/CD8 ratio	0.8 (0–2.6)	0.8 (0.2–2.0)	1.0 (0.1–2.0)	0.8 (0.1–2.1)
Endocervix				
CD4 ($\times 10^6$/l)	77 (4–1073)	103 (8–814)‡	109 (10–357)	87 (8–789)
CD8 ($\times 10^6$/l)	133 (16–767)	120 (15–821)	180 (26–410)	109 (15–1538)
CD4/CD8 ratio	0.7 (0–4.2)	0.7 (0.1–11.0)	0.7 (0–2.5)	0.6 (0–11.0)
Endometrial				
CD4 ($\times 10^6$/l)	295 (41–1103)	315 (33–1195)	198 (20–1195)	244 (20–1694)
CD8 ($\times 10^6$/l)	619 (57–6428)	421 (47–5038)	583 (57–2613)	526 (20–5082)
CD4/CD8 ratio	0.5 (0–3.2)	0.7 (0–2.5)	0.8 (0–2.5)	0.5 (0–2.5)

*178 rather than 189 specimens were analyzed due to multiple infections (e.g., gonorrhea and bacterial vaginosis), and a lack of endocervical/endometrial T-cell data from 11 subjects. †Negative for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and bacterial vaginosis. ‡$P < 0.05$.

analysis, HIV-1-seropositive women were marginally less likely to have IL-10 detected in endocervical secretions than HIV-1-seronegative women (37% versus 48%; OR, 0.6; 95% CI, 0.3–1.2).

IL-10 can be produced by many cell types in the female genital tract [20]. To determine the association of IL-10 with the number of CD4 and CD8 T cells measured in the genital tract, we analyzed the data to ascertain whether the median number of CD4 and CD8 T lymphocytes differed in endocervical and endometrial specimens in women with and without detectable IL-10 in endocervical secretions. The median endometrial CD4/CD8 ratio was greater in women with detectable IL-10 (0.7 versus 0.5; $P < 0.005$), and this difference was related to both an increase in the endometrial CD4 T-lymphocyte count (315×10^6/l versus 265×10^6/l; $P = 0.35$) and a corresponding decrease in the endometrial CD8 T-cell count (526×10^6/l versus 613×10^6/l; $P = 0.07$). However, when stratified by HIV-1 infection, the presence of IL-10 in the endocervix was not associated with an altered CD4/CD8 T-lymphocyte count in endocervical or endometrial specimens. Whether CD4 or CD8 T cells measured in endometrial tissue affects the local production of IL-10, or are confounders, cannot be determined from these data.

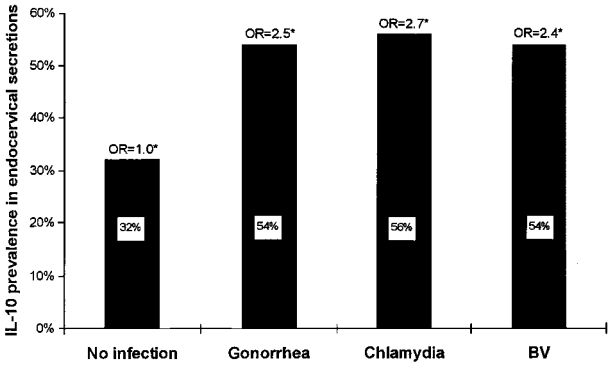

Fig. 1. Prevalence of IL-10 measured from the endocervical secretions of women with *N. gonorrhoeae*, *C. trachomatis*, or bacterial vaginosis (BV) infections in comparison with women without these infections. *Unadjusted OR.

Table 3. Multivariate analyses to assess for independent associations between *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and bacterial vaginosis with interleukin (IL)-10 in endocervical secretions, controlling for HIV-1 serostatus, and endometrial CD4 T-cell count.

	Adjusted OR (95% CI)
N. gonorrhoeae	3.4 (1.4–8.4)
C. trachomatis	4.4 (1.2–15.7)
Bacterial vaginosis	3.1 (1.4–6.9)

OR, Odds ratio; CI, confidence interval.

We performed multiple logistic regression to determine whether non-ulcerative STD were independently associated with an increased prevalence of IL-10 in endocervical secretions, adjusting for potential confounders. In separate multivariate analyses, after controlling for HIV-1 infection and endometrial CD4 T-cell count, gonorrhea, *Chlamydia* and bacterial vaginosis were significantly associated with IL-10 detection in endocervical secretions (Table 3).

Discussion

In our study, IL-10 was detected in endocervical secretions more frequently in women with gonorrhea, *Chlamydia* and bacterial vaginosis than in women without an identifiable infection. Whether these infections stimulate local upregulation of IL-10 in the female genital tract, systemic upregulation of IL-10 with transudation into endocervical secretions, or whether women

with increased IL-10 measured in the genital tract are more susceptible to infection by non-ulcerative STD and bacterial vaginosis, cannot be determined from these cross-sectional data. Previous studies have shown strong evidence that *N. gonorrhoeae* results in increased cytokine production at the site of infection, although the evidence that *C. trachomatis* and bacterial vaginosis induce cytokine production at the site of infection is weaker [21–23]. Why these three infections would cause similar increases of IL-10 is not clear.

The association between non-ulcerative STD and IL-10 was strengthened by the addition of endometrial CD4 T lymphocytes into the multivariate models. IL-10 can be produced by CD4 TH1 and TH2 T lymphocytes, macrophages, B lymphocytes as well as other cell types [20]. Since both endocervical and endometrial cellular components are likely to contribute to the detection of IL-10 in endocervical secretions, our data suggest that a proportion of IL-10 measured in endocervical secretions may derive from CD4 T lymphocytes residing in endometrial tissue. This may be particularly relevant in this cohort of women, most of whom had symptoms of acute upper genital tract inflammation.

The production of IL-10 in the female genital tract in response to non-ulcerative STD may elucidate part of one pathway that accounts for increased risk of female acquisition of HIV-1 associated with these infections [1,2]. In the vagina of the macaque, monocytic cells appear to be the target of SIV infection [13]. This finding is supported by studies of genotypic and phenotypic characterization of HIV-1 in patients with primary infection, which suggest that minor macrophage-tropic strains may be the viral variants responsible for sexual transmission of HIV-1 [12]. Although data are not equivocal [24], IL-10-stimulated monocytes may be more efficiently infected with macrophage-tropic HIV-1, possibly through enhancement of viral entry through CCR-5 [18]. Furthermore, resistance to HIV-1 infection has been associated with HIV-specific CD4 TH and CD8 cytotoxic lymphocytes [25,26]. Cell-mediated immunity and TH1 cytokines, observed in HIV-1-seronegative exposed individuals, have been correlated with resistance to HIV-1 infection [27]. IL-10 is a powerful downregulator of TH1 T-cell response, and thus IL-10 production induced by non-ulcerative STD could also alter susceptibility [23] to HIV-1 infection by inducing a shift to a TH2 response in the genital mucosa.

Non-ulcerative STD and bacterial vaginosis are common among women who are at risk of HIV-1 infection and have been associated with increased risk of HIV-1 acquisition in several studies. To date, little is understood about how these infections increase susceptibility to HIV-1. We have provided evidence that IL-10 is found in the female genital tract and is increased in women with gonorrhea and bacterial vaginosis, and also in women with *Chlamydia* when adjusted for potential confounding by HIV-1 serostatus, and CD4 T cells residing in endometrial tissue. Whether this increased IL-10 production in part accounts for increased HIV-1 susceptibility in women with STD remains to be established. If further studies support our findings, approaches that block IL-10 in the genital tract may prove useful strategies to prevent HIV-1 transmission.

Acknowledgments

The authors wish to thank Eunice Mwiti, Grace Njau, Josephine Wagombe, Oscar Lugalia, and the staff at the Special Treatment Clinic in Nairobi for providing excellent care and support for the study participants. The laboratory and support staff at the World Health Organization Collaborative Centre for Research and Training in Sexually Transmitted Diseases and HIV-1 at the University of Nairobi are also thanked for their support of the research.

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