

# Detection of HIV p17 antigen in lymphocytes but not epithelial cells from cervicovaginal secretions of women seropositive for HIV: implications for heterosexual transmission of the virus

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**SUMMARY** Human immunodeficiency virus (HIV) has been isolated from cervicovaginal secretions from infected women and is thought to be cell associated. To identify which cells harbour viral antigen, we used monoclonal antibodies to OKT4 and a monoclonal antibody directed against HIV p17 core antigen to perform indirect immunofluorescence assays of genital secretions from 17 HIV seropositive and 17 HIV seronegative women with leucorrhoea. OKT4 positive lymphocytes were detected in all tested samples. HIV p17 antigen was detected in the genital fluid lymphocytes in nine out of 14 seropositive subjects from whom lymphocytes were available. No viral antigen was detected in genital fluid lymphocytes of seronegative subjects, nor in any cervicovaginal epithelial cells. This study shows that lymphocytes are the major source of HIV in cervicovaginal secretions of infected women. Conditions that increase the lymphocyte population in the female genital tract, such as sexually transmitted disease (STD), chronic inflammation of the cervix, and menstruation, may facilitate the transmission of HIV during sexual intercourse.

Evidence for the heterosexual transmission of the human immunodeficiency virus (HIV), the retrovirus that causes the acquired immune deficiency syndrome (AIDS),<sup>1,2</sup> has been a subject of considerable debate. Although the transmission of HIV from men to women has been clearly shown by epidemiological studies,<sup>3,4</sup> transmission from women to men is still controversial. Some studies suggest its existence in both central Africa<sup>5,6</sup> and the Western world,<sup>7,8</sup> whereas others show little transmission even after repeated sexual contacts with virus carriers.<sup>9</sup> HIV has been isolated from the vaginal and cervical fluid of HIV carriers.<sup>10,11</sup> Those studies, however, did not elucidate which cells in the female genital secretions act as a target for or a reservoir of the retrovirus. The

present study was undertaken to attempt detection of HIV antigen on cervicovaginal epithelial cells and lymphocytes found in cervical secretions of HIV seropositive and seronegative women presenting with leucorrhoea.

## Patients and methods

### PATIENTS AND SEROLOGICAL METHODS

We studied 17 consecutive African women seropositive for HIV (by an immunoenzyme test) who attended the department of gynaecology of the Centre Hospitalier de Kigali, Kigali, Rwanda, because they had leucorrhoea. They were compared with 17 consecutive seronegative patients of the same geographic origin attending the same department for the same reason. Standard gynaecological examination and sampling was performed by one of us (AdeC). None of the women was examined while she was menstruating. A coded serum sample was sent to the laboratory to confirm the detection of antibodies to HIV by

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repeat immunoenzyme assays (EIA, Vironostika, Organon Teknika, Boxtel, Netherlands) and indirect immunofluorescence.<sup>1,2</sup> All results were further confirmed by either radioimmunoprecipitation (RIPA) or western blot, in which banding patterns were compared with those of serum known to be positive.<sup>1</sup> All laboratory investigations were blind.

#### VAGINAL AND EXOCERVICAL SECRETION SAMPLES

To obtain the maximum amount of fluid (more than 0.5 ml) vaginal and exocervical secretions were obtained by means of a wooden Ayre spatula and two or more sterile cotton swabs. The cotton swabs and spatula were placed in a sterile tube containing 3 ml phosphate buffered saline (PBS), coded, and sent immediately to the laboratory. Specimens were processed within 30 minutes after collection. Secretions were suspended by expressing the cotton swabs on the side of the tube and by shaking the wooden spatula in the solution. The cells were then pelleted by low

speed centrifugation, resuspended, and mononuclear cells were separated in Ficoll-Hipaque. This procedure yielded at least 600 lymphocytes a specimen in 27/34 genital fluid samples. Lymphocytes and vaginal and exocervical cells collected from the pellet were washed four times in PBS.

An aliquot of lymphocyte enriched suspension was used to count T helper (inducer) cells by indirect immunofluorescence using monoclonal antibodies (OKT4; Ortho Diagnostic System, Raritan, New Jersey, USA). Aliquots of lymphocyte enriched suspension and of the vaginal and exocervical epithelial cells were used to detect HIV p17 core antigen by indirect immunofluorescence. The monoclonal antibody used (8A6A7C5) was prepared and fully characterised by one of us (JCL). Briefly, cells (from the lymphocyte enriched fraction and from the pellet) were washed four times in PBS, prepared with glass slides (Bio Merieux), air dried, and then fixed in acetone for 10 minutes at  $-20^{\circ}\text{C}$  under agitation. Slides were then incubated for one hour at room temperature with the HIV p17 monoclonal antibody diluted 1:2. After washing, the slides were incubated for one hour at room temperature with a polyvalent goat anti-mouse IgG-FITC (fluorescein isothiocyanate) immunoglobulin (Tago, Burlingame, California, USA) diluted 1:40, washed, and air dried. The percentage of lymphocytes or cervicovaginal epithelial cells showing cytoplasmic fluorescence was then estimated with a fluorescence microscope (Laborlux 11 Leitz microscope). Control experiments were also performed on similar lymphocyte samples treated with PBS instead of HIV p17 monoclonal antibody.

Table 1 *Demographic data, gynaecological findings, and HIV antibody in 34 African patients presenting with leucorrhoea*

Case No	Age (years)	Gynaecological findings
<i>HIV seropositive patients</i>		
1	22	Chancroid
2	38	PID, SI
3	36	Relapsing genital herpes, PID
4	29	PID
5	26	Gonococcal cervicitis
6	21	PID, trichomonal cervicitis, SI
7	28	PID
8	36	PID
9	28	PID, SI
10	35	Relapsing genital herpes, PI, PID
11	21	Condyloma acuminata, pregnancy
12	26	Relapsing genital herpes
13	26	PID
14	30	Mixed gonococcal and trichomonal cervicitis
15	24	Vaginal candidiasis
16	28	Nil
17	28	Vaginal candidiasis
<i>HIV seronegative patients</i>		
18	30	Vaginal candidiasis, SI
19	35	Vaginal candidiasis
20	26	PID
21	34	Pregnancy
22	18	Nil
23	22	Genital herpes, gonococcal cervicitis
24	31	PID
25	23	PID
26	36	Nil
27	24	Nil
28	30	Relapsing genital herpes
29	28	Nil
30	32	Nil
31	27	PID, SI
32	38	Chancroid
33	29	Vaginal candidiasis
34	36	Nil

PID = pelvic inflammatory disease; PI = primary infertility; SI = secondary infertility.

## Results

### DEMOGRAPHIC, CLINICAL, AND SEROLOGICAL DATA

All 34 patients were African women presenting with leucorrhoea. Table 1 summarises the demographic data and clinical findings on gynaecological examination of the 34 women. In six (cases 5, 6, 10, 13, 17, and 22), scraping of the cervix provoked minor bleeding from erosions of the cervix, probably due to active cervicitis.

Of the 34 patients, 17 had detectable antibodies against HIV by repeated EIA, indirect immunofluorescence, and radioimmunoprecipitation or western blot. Sixteen of these women had active genital infections at the time the samples were collected. Of the 17 seropositive women, seven (cases 1, 7, 8, 9, 10, 13, and 16) had histories of repeated sexually transmitted disease (STD) in the previous two years, and one (case 3) had received a blood transfusion in Africa 18 months before the study began. This latter

Table 2 OKT4 and HIV p17 on lymphocytes and epithelial cells from cervicovaginal fluid samples of 34 women with or without antibodies to HIV

Patients (HIV antibody status)	Lymphocytes				Epithelial cells	
	No of patients OKT4 positive	Mean (SD) % OKT4 positivity	No of patients HIV p17 positive	% Lymphocytes HIV p17 positive	No of patients PBS positive (controls)	No of patients HIV p17 positive
Seropositive (n = 17)	17/17	22(11)*	9/14†	0.02-0.5	0	0/17
Seronegative (n = 17)	17/17	36(12)*	0/13†	0	0	0/17

\**t* test  $p = 0.01$ ; † $\chi^2$  4.4;  $p < 0.05$ .

patient and case 13 presented with severe weight loss and generalised lymphadenopathy. Of the 17 seronegative women, 10 had active genital infections and one was pregnant (in the third trimester). Gynaecological examination of the other six failed to detect any abnormalities or signs of pregnancy. One seronegative patient (case 28) had a history of multiple STDs in the previous two years and had experienced moderate weight loss and generalised lymphadenopathy.

#### OKT4 AND HIV P17 MARKERS ON LYMPHOCYTES AND EPITHELIAL CELLS FROM CERVICOVAGINAL FLUID

Table 2 shows the detection of OKT4 and HIV p17 on lymphocytes and epithelial cells from cervicovaginal fluid samples of the 34 patients. OKT4 positive lymphocytes were detected in genital secretions from all women tested. Higher proportions were, however, found in seronegative women (mean 36% (SD 12%), individual data not shown) than seropositive women (22% (11%)) (*t* test,  $p = 0.01$ ). Of 14 seropositive patients from whom vaginal fluid lymphocytes were available, nine had 0.02% to 0.5% of lymphocytes reactive with HIV p17 monoclonal antibodies. In contrast, of the 13 seronegative women from whom vaginal fluid lymphocytes were available, none showed staining for this antigen ( $\chi^2$  4.4;  $p < 0.05$ ). None of the cervicovaginal lymphocyte cell samples from either group of patients showed evidence of cytoplasmic fluorescence, and no fluorescence was detected in the samples treated with PBS instead of HIV p17 monoclonal antibody.

#### Discussion

The findings from this study provide laboratory data that support the epidemiological evidence for bidirectional heterosexual transmission of HIV.

One of the major properties of HIV is now known to be its tropism for T lymphocytes bearing the CD4 (T4) antigen. This particular lymphotropism has been shown to be based on the binding of envelope glycoproteins of the retrovirus with the T4 molecule itself.<sup>13 14</sup> The fact that OKT4 positive lymphocytes

can be found in the genital secretions of women with and without genital infection has considerable implications, as potential target cells of HIV in the female genital tract are essential to explain male to female sexual transmission of HIV.

HIV has been isolated in various body fluids, including vaginal and cervical secretions, from symptomatic and asymptomatic carriers of the virus.<sup>10 11</sup> Those studies, however, were of female carriers of HIV, only a few of whom experienced concomitant genital infections. HIV was recovered only after several transfers on fresh peripheral blood mononuclear cells, which indicated that little HIV was present. Filtering the vaginal fluid considerably reduced the rate of virus isolation, which suggested that most HIV particles are cell associated in vaginal secretions. Those studies did not, however, show which type of cell in the female genital tract could harbour the retrovirus.

The present study describes an indirect immunofluorescence technique to detect cell related HIV p17 antigen in cervicovaginal fluid. A similar technique has recently been shown to be effective in showing HIV p15 and p24 antigens on fresh corneal cells obtained from a touch preparation.<sup>15</sup> Monoclonal antibodies directed against the HIV *gag* precursor have also been shown, by an immunohistochemical method, to label dendritic reticular cells of infected patients (Chassagne J, *et al*, unpublished observation). The sensitivity of these techniques, however, remains to be evaluated in comparison with virus isolation.

Our results show that HIV p17 is present in the lymphocytes from genital secretions of seropositive, but not seronegative, patients. The percentage of lymphocytes in genital secretions that reacted with the monoclonal antibody to HIV p17 (from 0.02% to 0.5%) was higher than that in uncultured peripheral blood lymphocytes of infected patients (0.01%, S Sprecher-Goldberger, unpublished data). The reason for this difference is not known, but could be attributable to local chronic inflammation and lymphocyte activation resulting from the genital infection present in our subjects.

Two of the nine subjects with positive HIV p17

staining had minimal bleeding from the cervix at the time of scraping (a common event when the cervix is rendered friable by chronic cervicitis), thus increasing the number of lymphocytes in their genital secretions. The introduction of blood in these specimens cannot be thought to be disturbing when searching for infected cells, as it simulates menstruation and the traumatic (such as post-coital) bleeding often reported by patients with chronic cervicitis.

The lack of HIV p17 staining of cervicovaginal epithelial cells suggests that, though the integrity of the mucosa may be an important factor in HIV transmission, epithelial cells do not themselves act as targets or reservoirs for the retrovirus. Hybridisation assays using HIV deoxyribonucleic acid (DNA) probes should, however, be performed before excluding cervicovaginal epithelial cells as a potential reservoir for HIV.

Genital infections, contact bleeding from the cervix, and menstruation unquestionably increase the number of lymphocytes in the female genital tract. The fact that lymphocytes can behave as potential target cells and as reservoirs for HIV in female genital fluid has important ramifications for our understanding of transmission, both from men to women and from women to men. The strong association between past or present STDs and infection with HIV in prostitutes and their clients has been emphasised by epidemiological studies performed in eastern and central Africa.<sup>16 17</sup> Thus both laboratory and epidemiological studies support STDs as an enhancing factor in the heterosexual transmission of HIV. This could partly explain the relative lack of sexual transmission reported in the United States of America and Australia in non-promiscuous people.<sup>7 9</sup>

This study shows that lymphocytes are a target for and source of HIV in the female genital tract and suggest that STDs could be an enhancing factor in the bidirectional heterosexual transmission of AIDS.

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