Journal of Clinical Immunology, Vol. 16, No. 1, 1996

Comparison of Cervicovaginal Humoral Immunity in Clinically Asymptomatic (CDC A1 and A2 Category) Patients with HIV-1 and HIV-2 Infection

LAURENT BÉLEC,[1,2,6] CAROL TÉVI-BÉNISSAN,[2] THIÉRRY DUPRÉ,[2] ALI SI MOHAMED,[2] THIÉRRY PRAZUCK,[3] JACQUES GILQUIN,[5] JEAN-MARIE KANGA,[4] and JACQUES PILLOT[1]

Accepted: July 12, 1995

Paired sera and cervicovaginal secretions (CVS) from 11 HIV-1- and 11 HIV-2-infected women, all clinically asymptomatic (CDC A1 and A2 categories), were analyzed for total IgG, IgA, albumin (HSA), IgG, and IgA antibodies to *env*-encoded surface glycoproteins of HIV-1 (gp160) and of HIV-2 (gp105), by comparison to 15 age-matched healthy controls. Secretion rates of IgG and IgA into CVS were evaluated by calculation of their relative coefficients of excretion (RCE) by reference to HSA. Cervicovaginal production of anti-HIV antibodies was evaluated by comparison between specific antibody activities of IgG and of IgA to HIV in CVS and in sera. In HIV-1-infected women, total IgG and IgA in CVS were, respectively, 6- and 4-fold increased, whereas the secretion rate of total IgG was 2.1-fold increased and that of total IgA was 2.5-fold reduced. In contrast, total IgG and IgA as well as their secretion rates were normal in HIV-2-infected women. In HIV-1- but not in HIV-2-infected women, HSA levels in cervicovaginal washings were twofold increased, demonstrating alteration of the mucosal barrier in HIV-1 infection. In HIV-1-infected patients, IgG and IgA to gp160 were detected in all sera and CVS. In HIV-2-infected patients, IgG to gp105 was detected in all sera and CVS, whereas IgA to gp105 could be detected in only half of sera and one-third of CVS. Cross-reactivity by IgG and/or IgA to HIV-1 or HIV-2 against the surface glycoprotein of the other HIV type was observed in sera as well as in CVS, and more frequently in HIV-2- than in HIV-1-infected women. Finally, the mean specific activities of IgG and of IgA to gp160 or gp105 were higher in CVS than in sera, evidencing a possible local synthesis of both isotypes in HIV-1 as well as in HIV-2 infections. As early as the asymptomatic stages, HIV-1 affects the cervicovaginal mucosa more than HIV-2 does, suggesting higher viral replication within the female genital tract in HIV-1 infection than in HIV-2 infection.

KEY WORDS: HIV-1; HIV-2; cervicovaginal immunity.

INTRODUCTION

Like HIV-1, HIV-2 is associated with terminal AIDS. However, the pathogenic potential and the natural history of both infections appear to be quite different (1–3). Although HIV-1 and HIV-2 share similar modes of transmission, the spread of HIV-2 remains limited in West Africa. Epidemiologic reports from West African countries, including the Ivory Coast, Senegal, Guinea, and Guinea Bissau, have pointed to a faster increase in the prevalence of HIV-1 infection than that of HIV-2 infection, although HIV-2 was probably present earlier than HIV-1 in these countries (2, 4). Furthermore, the age-specific distribution of HIV-1 and HIV-2 infections in these West African populations differs in that HIV-2-infected individuals tend to be older than HIV-1-infected persons (2, 4). Taken together, these epidemiologic observations strongly suggest that heterosexual transmission of HIV-2 infection is less efficient than that of HIV-1 (2, 5, 6). At high CD4+ T-lymphocyte counts, the lower circulating viral load in HIV-2- than in HIV-1-infected persons (7) has been hypothesized to be responsible for the lower infectivity of HIV-2-infected patients (2).

Whether differences in viral load between HIV-1 and HIV-2 patients exist in genital secretions remains unknown. Previous studies focused on cervicovaginal immunity during HIV-1 infection have reported the usual detection of cervicovaginal antibodies to HIV-1 (8–12),

[1]Unité d'Immunologie Microbienne, Institut Pasteur, Paris, France.
[2]Laboratoire de Virologie, Centre Hospitalo-Universitaire (CHU) Broussais, Paris, France.
[3]Service des Maladies Infectieuses et Tropicales, Centre Hospitalier Intercommunal, Villeneuve St-Georges, France.
[4]Service de Dermatologie, CHU Treichville, Abidjan, Côte d'Ivoire.
[5]Service d'Immunologie Clinique, CHU Broussais, Paris, France.
[6]To whom correspondence should be addressed at Service de Microbiologie (Laboratoire de Virologie), Hôpital Broussais, 96, rue Didot, 75674 Paris Cedex 14, France.

0271-9142/96/0100-0012$09.50/0 © 1996 Plenum Publishing Corporation

mainly of the IgG isotype (11, 12), in association with increased levels of total IgG and IgA of undetermined specificities (11). HIV-1 infection elicits a local synthesis of specific IgG to HIV within the female genital tract (13). Alteration of the mucosal barrier, probably due to proinflammatory cytokines local overproduction (14), provides an increase in the physiological transudation of serum-borne immunoglobulins to the cervicovaginal mucosa (15). Detection of antibodies to HIV-2 by Western blot has also been reported in the cervicovaginal secretions of two HIV-2-infected African women living in Senegal (16).

The changes in cervicovaginal immunity during HIV-1 and HIV-2 infections can be considered as an indirect marker of local viral replication and could be relevant to heterosexual HIV transmission. Therefore, we compared the cervicovaginal humoral immunity in two homogeneous groups of women infected with HIV-1 or with HIV-2, all clinically asymptomatic and sexually active.

PATIENTS AND METHODS

Study Population

Eleven HIV-1-infected women (19–35 years), living in the Paris area, and 11 HIV-2-infected women (27–45 years), living in Abidjan (n = 5), Ivory-Coast, Dakar (n = 2), Senegal, and Paris (n = 4), France, were recruited after informed consent was obtained. All patients were Black, except one Moroccan HIV-2-infected woman living in Paris, and all were exclusively heterosexual. None of the women living in Africa were using any contraceptive method. Among the women living in Paris, the use of condoms was always recommended. Finally, a similar number of women (2/11 HIV-2-infected women and 4/11 HIV-1-infected women) was taking estroprogestative contraception. All patients were clinically asymptomatic; four HIV-1 and five HIV-2-infected women fulfilled the criteria of category A1 of the 1993 revised Centers for Disease Control classification for HIV infection, and the other patients the criteria of category A2. The mean ± standard error CD4 count was slightly higher in HIV-2-infected (578 ± 79/mm^3; range, 350–1300/mm^3) than in HIV-1-infected (433 ± 38/mm^3; range, 220–635/mm^3) women, but the difference did not reach statistical significance.

HIV-1 and HIV-2 infections were confirmed by Western blot (New Lav-Blot I and New Lav-Blot II, Sanofi-Diagnostics Pasteur, Paris, France), and results were considered positive if they met the World Health Organization criteria (17). To confirm HIV-2 infection and exclude dual HIV-1/HIV-2 reactivity, sera were also tested by dot-blot enzyme immunoassay containing synthetic peptides of HIV-1 gp41 and HIV-2 gp36 transmembrane glycoprotein regions (Peptilav 1-2, Sanofi-Diagnostics Pasteur).

Fifteen Black women living in Africa (18–39 years), all healthy and HIV seronegative, were recruited as controls.

Menstruating women, and subjects with vaginal discharge, genital bleeding, or evidence of sexually transmitted diseases at the genital examination, were excluded.

Collection, Treatment, and Storage of Samples

Cervicovaginal secretions were collected during the luteal phase of the menstrual cycle, by a nontraumatic, standardized 60-sec vaginal washing with 3 ml of phosphate-buffered saline (PBS), and immediately placed in thawing ice. Peripheral blood was simultaneously gathered in dry tubes. The paired samples were centrifuged within 1 hr at 4°C and 1500g for 10 min. The supernatants were aliquoted and kept frozen at −30°C until processing. The washing procedure was evaluated as corresponding to a 1:10 dilution of the native cervicovaginal secretions (18). This dilution factor was taken into account for estimations of immunoglobulin and human serum albumin (HSA) content and for calculations of anti-HIV antibody specific activities in cervicovaginal secretions.

Immunoglobulin G, IgA, and HSA Estimations

Concentrations of IgG and IgA in serum were quantified by a nephelometric method (Behring, Marburg, Germany). Concentrations of IgG and IgA in cervicovaginal secretions were measured by sandwich ELISA, as described previously (11), with normal serum (Standard-Human-Serum, Behringswerke AG, Marburg, Germany) serving as the standard. This sandwich ELISA permits similar recognition of all molecular forms of IgA, including monomeric IgA, polymeric IgA, and secretory IgA (11). The HSA concentration in cervicovaginal secretions was used as a transudation marker. Measurements of HSA in serum and in cervicovaginal secretions were carried out by a competition ELISA, as described previously (19).

Detection of IgG and IgA Antibodies to gp160 and to gp105

IgG and IgA to gp160 were detected as described previously (11). The gp160 antigen (1 μg/ml) consisted of a purified preparation of baculovirus-expressed recombinant gp160, derived from the envelope of the LAI strain of HIV-1 (Transgène, Strasbourg, France). IgA was detected after adsorption of IgG by protein G treatment, as described previously (20). Undiluted samples were then incubated at 37°C for 1 hr with 25% (vol/vol) of protein G–Sepharose (Sigma Chemical Co., St. Louis, MO), with constant gentle mixing. After a 5-min centrifugation at 1000*g* to pellet the agarose beads, the supernatant solution was used for testing.

The dilutions of serum were 1/2500 for IgG and 1/125 for IgA; the cervicovaginal washings were used diluted at 1/100 for IgG (final dilution of the cervicovaginal secretions: 1/1,000) and at 1/25 for IgA (final dilution: 1/250). PBS supplemented with 5% (wt/vol) skim powder milk was used as diluent. For the revelation step of IgG, horseradish peroxidase (HROP)-labeled polyclonal (sheep) antibodies to human Fcγ (Sanofi-Diagnostics Pasteur) were used. For IgA, the plates were incubated for 2 hr with biotinylated polyclonal (rabbit) anti-human Fcα (Dakopatts, Glostrup, Denmark); after five washings with PBS-Tween, HROP-labeled streptavidin (Sigma Chemical Co.), diluted at 1/40,000 in PBS, was added for 30 min. Absorbance was read at 492 nm. For each tested sample, a paired well without antigen, only coated with blocking solution, served as negative control. This control is indeed essential since some cervicovaginal components can nonspecifically bind immunoglobulins to the solid phase (11). Results are given as ΔOD, expressing the absorbance difference between the antigen-coated well and the antigen-free control well. For each assay, dilution, and isotype, the cutoffs (CO) of positivity for serum and cervicovaginal secretions were determined as the mean ΔOD plus 3 standard deviations (SD) obtained from the 15 HIV-seronegative controls.

IgG and IgA to gp105 were detected as above. The gp105 antigen (1 μg/ml) consisted of a purified preparation of baculovirus-expressed recombinant gp105, derived from the envelope of the ROD strain of HIV-2 (American Biotechnologies, Inc., Cambridge, MA).

Specific Anti-HIV Activities of IgG and IgA

In HIV-1-infected women, the specific activities (SA) of IgG and IgA to gp160 in serum and in cervicovaginal secretions were evaluated per microgram of serum or cervicovaginal total IgG or IgA, according to the following formula, where d is the reciprocal final dilution of serum or cervicovaginal secretions: $SA = \Delta OD \times d/[Ig]_{\mu g/ml}$.

In HIV-2-infected women, the specific activities of IgG and IgA to gp105 in serum and in cervicovaginal secretions were similarly evaluated.

Relative Coefficient of Excretion

To evaluate the secretion rate of immunoglobulins from serum (S) to cervicovaginal secretions (CVS), the relative coefficients of excretion (RCE) of cervicovaginal IgG and IgA were calculated using HSA as reference, according to the formula (21): RCE = ([HSA in S]/[HSA in CVS]) × [Ig in CVS]/[Ig in S]).

Statistics

Comparisons of IgG, IgA, and HSA levels and of specific activities were established by the Mann–Whitney U test for unpaired samples and by the Wilcoxon rank-order test for paired samples; comparisons of prevalences were obtained by the Fisher exact test. Results are given as mean ± standard error.

RESULTS

Total IgG and IgA Levels

In HIV-1-infected women, total IgG in serum and total IgG and IgA in cervicovaginal secretions were significantly higher than in controls and than in HIV-2-infected women (Table I); the total IgG and IgA levels in cervicovaginal levels were respectively about nine- and sixfold those of controls. In HIV-2-infected women, total IgG and IgA levels in serum and in cervicovaginal secretions did not differ from the levels of controls.

The mean CVS IgG/IgA ratios showed a tendency to be higher in HIV-1-infected women (7.11 ± 1.41; median, 4.92; range, 0.69–15.13) than in controls (4.07 ± 0.85; median, 3.93; range, 0.56–11.87) and in HIV-2-infected women (4.97 ± 2.05; median, 3.35; range, 0.64–24.62), but the difference did not reach statistical significance. However, the CVS IgG/IgA ratio was more frequently above 1 in HIV-1-infected women than in controls [10/11 (91%) versus 7/15 (47%); $P < 0.04$]; this ratio in HIV-2-infected women was above 1 in 5 of 11 (45%) of patients. The cervicovaginal level of IgG was higher than that of IgA, in healthy controls ($P < 0.002$), HIV-1-infected women ($P < 0.01$) and in HIV-2-infected women ($P < 0.05$).

Table I. Total IgG and IgA (Mean ± SE; mg/ml) in Serum and in Cervicovaginal Secretions of HIV-1- and HIV-2-Infected Women and of Healthy HIV-Negative Controls

		Serum		Cervicovaginal secretions[a]	
Women	*n*	IgG	IgA	IgG	IgA
HIV-1-infected	11	29.4 ± 1.8*	3.3 ± 0.7	1.10 ± 0.27**	0.17 ± 0.04***
		(29.3;18.9–37.9)[b]	(2.9;1.2–8.4)	(0.76;0.27–2.73)	(0.14;0.017–0.320)
HIV-2-infected	11	23.5 ± 1.1	1.8 ± 0.2	0.19 ± 0.10	0.044 ± 0.01
		(25.0;18.3–28.2)	(1.9;0.9–2.9)	(0.16;0.03–0.76)	(0.036;0.010–0.084)
Control	15	21.0 ± 1.5	2.3 ± 0.19	0.18 ± 0.06	0.040 ± 0.01
		(19.3;12.4–34.6)	(2.3;1.1–3.6)	(0.21;0.01–0.64)	(0.054;0.021–0.097)

[a]The 10^{-1}-dilution factor of cervicovaginal secretions is taken into account.
[b]Median and range.
*$P < 0.002$ versus controls and $P < 0.02$ versus HIV-2-infected women.
**$P < 0.005$ versus controls and HIV-2-infected women.
***$P < 0.01$ versus controls and HIV-2-infected women.

Albumin Levels

Mean HSA serum levels were similar in HIV-1- and HIV-2-infected women and in controls (Table II). HSA levels in cervicovaginal secretions of HIV-1-infected women were, respectively, 2- and 2.9-fold higher than those of controls and of HIV-2-infected women.

The mean serum/cervicovaginal secretion HSA ratio of HIV-1-infected women was, respectively, 2.6- and 4.1-fold lower than that of controls and that of HIV-2-infected women.

Relative Coefficients of Excretion

In cervicovaginal secretions of healthy controls, the mean RCE of total IgA was always above 1, i.e., above the RCE of HSA, indicating a predominant local secretion of the IgA isotype (Table III). The mean RCE of total IgG was also above 1 in most subjects (11/15), suggesting local production of IgG. The mean RCE of total IgA was higher than the mean RCE of total IgG ($P < 0.001$).

In cervicovaginal secretions of HIV-1-infected women, the RCE of total IgG were above 1 in all patients; their mean was 2.1-fold higher than that of healthy controls ($P < 0.04$). The mean RCE of total IgA was 2.5-fold decreased compared to normal controls ($P < 0.04$). Contrasting with normal values, the mean RCE of IgA in HIV-infected women did not differ significantly from the mean RCE of IgG. Three (27%) HIV-1-infected women had a RCE of IgA less than 1.

In cervicovaginal secretions of HIV-2-infected women, the RCE of total IgG and of total IgA were above 1 in, respectively, 8 (73%) and 11 (100%) of patients; their means were similar to those of controls. As in controls, the mean RCE of IgA in HIV-2-infected women was significantly higher than that of IgG ($P < 0.001$).

Taken altogether, these features indicate in HIV-1-infected women an increase in the cervicovaginal production of total IgG with a decrease in the cervicovaginal production of total IgA; in contrast, the local productions

Table II. Human Serum Albumin (Mean ± SE; mg/ml) in Serum and in Cervicovaginal Secretions of HIV-1- and HIV-2-Infected Women and of Healthy HIV-Negative Controls

Women	*n*	Serum	Cervicovaginal secretions[a]	Serum/cervicovaginal secretions
HIV-1-infected	11	35.1 ± 1.3	0.38 ± 0.08*	109 ± 16**
		(34.1;27.7–42.8)[b]	(0.35;0.19–0.75)	(88;49–200)
HIV-2-infected	11	38.2 ± 1.1	0.13 ± 0.04	450 ± 83
		(37.5;32.5–43.5)	(0.11;0.02–0.48)	(510;71–970)
Control	15	40.1 ± 2.4	0.19 ± 0.03	280 ± 51
		(39.4;22.8–45.4)	(0.15;0.05–0.47)	(200;78–700)

[a]The 10^{-1} dilution factor of cervicovaginal secretions is taken into account.
[b]Median and range.
*$P < 0.02$ versus controls and HIV-2-infected women.
**$P < 0.02$ versus controls and $P < 0.001$ versus HIV-2-infected women.

Table III. Relative Coefficients of Excretion (RCE) of Total IgG and of Total IgA (Mean ± SE) in Cervicovaginal Secretions of HIV-1- and HIV-2-Infected Women and of Healthy HIV-Negative Controls[a]

Women	*n*	RCE of IgG	RCE of IgA
HIV-1-infected	11	3.4 ± 0.8* (3.3; 1.1–8.8)[b]	3.5 ± 1.2 (3.6; 0.42–11.8)*, **
HIV-2-infected	11	1.9 ± 0.7 (1.6; 0.05–9.1)	10.2 ± 1.4 (8.8; 5.3–16.6)
Control	15	1.6 ± 0.4 (1.3; 0.06–4.1)	8.9 ± 1.9 (6.5; 1.1–10.8)

[a]The 10^{-1} dilution factor of cervicovaginal secretions is taken into account.
[b]Median and range.
*$P < 0.04$ versus controls.
**$P < 0.04$ versus controls and $P < 0.002$ versus HIV-2-infected women.

of both IgG and IgA appeared similar in HIV-2-infected women and in controls.

IgG and IgA Antibodies to gp160 and to gp105

In HIV-1-infected women, IgG and IgA antibodies to gp160 in serum as well as in cervicovaginal secretions were detected in all patients (Table IV). In HIV-2-infected women, IgG to gp105 in serum as well as in cervicovaginal secretions was also detected in all patients; in contrast, IgA to gp105 in serum and in cervicovaginal secretions could be detected, respectively, in only six (54%; $P < 0.04$) and four (34%; $P < 0.004$) women.

The cross-reactivity of anti-HIV IgG and IgA antibodies against *env*-encoded antigens of HIV-1 and HIV-2 was evaluated in serum and in cervicovaginal secretions. The proportion of IgG to gp105 reacting to gp160 antigen was significantly higher than the proportion of IgG to gp160 reacting to gp105 antigen, in serum [8/11 (73%) versus 2/11 (18%); $P = 0.03$] as well as in cervicovaginal secretions [8/11 (73%) versus 2/11 (18%); $P = 0.03$]. The proportion of IgA to gp105 reacting to gp160 antigen was significantly higher than the proportion of IgA to gp160 reacting to gp105 antigen, in serum [4/6 (67%) versus 1/11 (9%); $P < 0.03$] but not in cervicovaginal secretions [3/4 (75%) versus 2/11 (18%); $P = 0.076$].

Specific Activities of IgG and IgA to gp160 and to gp105

The ratio of antibody activity to the total amount of the same isotype was further compared in paired serum and cervicovaginal secretions. In HIV-1-infected women, the mean specific activities of IgG to gp160 and of IgA to gp160 were significantly higher in cervicovaginal secretions than in serum (Table V). Similarly, in HIV-2-infected women, the mean specific activities of IgG to gp105 and of IgA to gp105 were significantly higher in cervicovaginal secretions than in serum.

The specific activities of IgG to gp160 and those of IgA to gp160 in serum of HIV-1-infected women were respectively higher than the specific activities of IgG to gp105 ($P < 0.001$) and than those of IgA to gp160 ($P < 0.03$) in serum of HIV-2-infected women.

DISCUSSION

In the cervicovaginal secretions of healthy women, the highest immunoglobulin concentrations were found for the IgG isotype compared to the IgA isotype, as reported previously reported (22). For total IgA, the RCE values exceeded the value expected from molecular weight-affected seepage from plasma, confirming a primarily local synthesis. Similarly, RCE of total IgG exceeded slightly but significantly the RCE of HSA in most healthy women, indicating that diffusion cannot fully explain our results, since the low molecular weight of HSA renders it more diffusible than IgG. These observations are in keeping with genital elaboration of both IgG and IgA. Indeed, the cervicovaginal mucosa belongs to the mucosal immune system, with a predominance of IgA-producing cells and with more than 10% of plasma cells producing IgG (23). Furthermore, the uterine submucosa contains a high percentage of IgG-containing cells (24). The possibility of an active transepithelial transport for IgG, e.g., via Fcγ receptors, has also been

Table IV. Detection of Antibodies to gp160 and to gp105 of the IgG and IgA Isotypes, in Paired Serum and Cervicovaginal Secretions of 11 HIV-1- and 11 HIV-2-Infected Asymptomatic Women

	HIV-1-infected women				HIV-2-infected women			
	Serum		Vaginal secretions		Serum		Vaginal secretions	
Antibodies	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
To gp160	11 (100%)	11 (100%)	11 (100%)	11 (100%)	8 (73%)	4 (37%)	8 (73%)	3 (27%)
To gp105	2 (18%)	1 (9%)	2 (18%)	2 (18%)	11 (100%)	6 (54%)	11 (100%)	4 (36%)

Table V. Specific Activities (SA) of IgG and IgA to gp160 and to gp105 (Mean ± SE), in Paired Serum (S) and Cervicovaginal Secretions (CVS) of 11 HIV-1- and 11 HIV-2-Infected Women

HIV-1-infected women			
IgG to gp160		IgA to gp160	
SA in S	SA in CVS	SA in S	SA in CVS
2.6 ± 0.2* (2.3; 1.9–3.9)[a]	6.9 ± 0.6** (4.7; 1.8–12.5)	0.7 ± 0.2*** (0.6; 0.2–2.3)	4.2 ± 1.1**** (1.6; 0.2–13.5)
HIV-2-infected women			
IgG to gp105		IgA to gp105	
SA in S	SA in CVS	SA in S	SA in CVS
1.3 ± 0.2 (1.4; 0.15–2.55)	3.5 ± 0.8***** (3.3; 0.2–9.1)	0.2 ± 0.1 (0.1; 0.01–0.8)	1.1 ± 0.3****** (1.1; 0.03–2.5)

[a]Median and range.
*$P < 0.001$ versus SA in S of IgG to gp105.
**$P < 0.001$ versus SA in S of IgG to gp160.
***$P < 0.03$ versus SA in S of IgA to gp105.
****$P < 0.001$ versus SA in S of IgA to gp160.
*****$P < 0.02$ versus SA in S of IgG to gp105.
******$P < 0.005$ versus SA in S of IgA to gp105.

hypothesized (22). In HIV-1-infected asymptomatic women, the concentrations of total IgG and IgA in cervicovaginal secretions were, respectively, six- and fourfold higher than in controls; the mean RCE of total IgG and the one of total IgA were, respectively, higher and lower than those in healthy controls; and the preponderance of IgA over IgG excretion in normal subjects disappeared. These observations indicate a significant increase in total cervicovaginal IgG production in HIV-1-infected women, whereas the local production of total IgA appears to be reduced. Such a discrepancy between local overproduction of IgG and decreased local synthesis of IgA strongly suggests early impairment of the IgA class-specific immunoglobulin production in the cervicovaginal compartment. A reduction in the number and function of mucosal CD4+ T cells could contribute to the dysregulation of IgA-secreting cells in the intestinal mucosa during HIV infection (25, 26). In female rhesus macaques infected with the simian immunodeficiency virus, a marked IgA plasma cell deficiency was observed in the reproductive tissues (27). Similarly, it is possible that HIV-1-infected women may have a reduced number of T CD4+ lymphocytes and/or IgA-bearing plasma cells within their cervicovaginal mucosa, as early as the asymptomatic stage of the disease. In contrast, the levels and local production of total IgG and IgA in HIV-2-infected asymptomatic women were normal. Finally, the cervicovaginal immunity appears to be impaired early in the course of HIV-1 infection, whereas it remains normal in the early stages of HIV-2 infection.

In HIV-1- but not in HIV-2-, infected women, the levels of HSA in cervicovaginal secretions were significantly increased in comparison with those of normal controls. These findings indicate a compromised integrity of the mucosal barrier in HIV-1-, but not in HIV-2-, infected women at asymptomatic stages of the disease. Therefore, increased permeability of the cervicovaginal mucosa may occur, since HSA has exclusively a plasma origin. As a consequence, the physiological transudation of serum-borne immunoglobulin should be markedly increased in HIV-1-infected women, providing a higher delivery of IgG and possibly of monomeric IgA from plasma. This hypothesis is supported by the increase in total IgA in cervicovaginal secretions, although local IgA synthesis is unchanged or deficient. Finally, in HIV-1-infected women, increased levels of cervicovaginal total immunoglobulins appear to result from enhanced local IgG synthesis, in association with an increase in physiological IgG and monomeric IgA transudation.

In HIV-1-infected patients, we were able to detect IgG and IgA antibodies to gp160 in all tested sera and cervicovaginal secretions. Such a high prevalence of detectable IgG to HIV-1 in cervicovaginal secretions has been reported previously in HIV-1-infected women at asymptomatic or pre-AIDS stages (8, 9, 12) as well as at AIDS stage (9). The prevalence of detectable IgA to HIV-1 in cervicovaginal secretions after depletion of IgG appears higher than those previously reported, ranging from 28% (10), 62% (11), and 63% (9) to 66% (28). Differences in the expertise with which specimens were

collected by different clinicians, in the methods of sample processing, types of immunoassay (Western blot is less sensitive than ELISA), antigens and conjugates used, and in the immunological status and other characteristics of the patient populations probably account for some variations in the rate of detection of class-specific antibodies in cervicovaginal secretions between groups. In HIV-2-infected patients, we were able to detect IgG to gp105 in all tested sera and cervicovaginal secretions, whereas IgA to gp105 could be detected in only one-half of sera and in one-third of cervicovaginal secretions. A similar discrepancy between the IgG and the IgA reactivities to HIV-2 by Western blot was observed previously in the cervicovaginal secretions of two HIV-2-infected African women (16). These observations indicate that HIV-1 elicits a more important anti-HIV IgA response than HIV-2, at least at asymptomatic stages of the disease.

When the specific activities for antibodies to gp160 or to gp105 in paired sera and cervicovaginal secretions of HIV-1 as well as of HIV-2-infected women were compared, the relative proportions of specific IgG or IgA to HIV were higher in cervicovaginal secretions than in serum. These findings suggest that local synthesis of IgG and IgA to HIV generally occurs within the female genital tract during HIV-1 as well as HIV-2 infections. Intragenital synthesis of antibodies to HIV must involve the presence of the virus itself within the female reproductive tract, which contains antigen-presenting cells and lymphocytes (29). HIV-1 can be isolated by viral culture (30) or can be detected using the polymerase chain reaction (31) from female genital secretions. HIV-1 antigens have been evidenced in macrophages, monocytes, and lymphocytes within the submucosa of the uterine cervix (32, 33), and in lymphocytes from vaginal secretions (34), demonstrating local viral replication.

Cross-reactivity by IgG and IgA to HIV-1 or to HIV-2 against *env*-encoded glycoprotein of HIV-1 or of HIV-2 was observed in serum as well as in cervicovaginal secretions. The extensive serological cross-reactivity by HIV-1 and HIV-2 antibodies to heterologous antigens (4, 35, 36) is due to the high similarity of the amino acid sequences between HIV-1 and HIV-2 (37). In our patients, serum IgG and IgA to HIV-2 reacted more frequently against gp160/HIV-1 than did serum IgG and IgA to HIV-1 against gp105/HIV-2; in cervicovaginal secretions, IgG to HIV-2 were similarly more frequently reactive against gp160 than IgG to HIV-1 against gp105. The possibility exists that cervicovaginal IgG and IgA antibodies in an HIV-infected subject could somehow interfere in heterosexual transmission of the other type of HIV. Indeed, serum antibodies from HIV-2-infected individuals have been reported to cross-neutralize some HIV-1 strains (38), and analogous cross-neutralization could occur with cervicovaginal antibodies to HIV. In West Africa, mixed infections with HIV-1 and HIV-2 in the same individual are not rare, suggesting that infection with one HIV type does not necessarily prevent infection with the other type (39, 40). However, in a recent prospective study of a cohort of 1444 high-risk women in Senegal, the risk for subsequent HIV-1 transmission was 70% reduced in HIV-2-infected women in comparison with HIV-negative women (41). Conversely, in an HIV-infected individual, the sexual transmission of another type of HIV could be more efficient in the absence of cross-reacting genital antibody.

The specific activities of both IgG and IgA to HIV in cervicovaginal fluids were significantly higher in HIV-1 than in HIV-2 infections, suggesting indirectly higher local HIV replication in HIV-1 than in HIV-2 infections. Furthermore, as soon as these early stages, HIV-1 caused alterations of the cervicovaginal barrier, whereas HIV-2 did not. Such major differences observed between both infections could be related to a higher viral load within the female genital tract in HIV-1- than in HIV-2-infected women. At the systemic level, the isolation rate of HIV-2 from peripheral blood mononuclear cells of asymptomatic HIV-2-positive individuals is lower than the HIV-1 isolation rate from HIV-1-infected persons (7). Plasma viremia is also less frequently positive in HIV-2- than in HIV-1-infected patients (7). Finally, testing by the polymerase chain reaction more often yields negative results early in the course of infection with HIV-2 than with HIV-1 (7, 42, 43). Similar differences in viral load between HIV-1- and HIV-2-infected individuals should also exist at the level of the genital mucosae; they may account, at least in part, for the different patterns of the cervicovaginal humoral immunity associated with the two viruses.

In conclusion, at asymptomatic stages of HIV infection, HIV-1 affects the cervicovaginal mucosa more than HIV-2 does. Such feature could be relevant to the differential heterosexual transmission between both infections.

ACKNOWLEDGMENTS

This work was supported by a grant (94-130) from the Agence Nationale de Recherche sur le SIDA and from the Ministère de la Coopération, Paris, France. Dr. L. Bélec and Dr. C. Tévi-Bénissan were recipients of a fellowship from SIDACTION. We would like to thank the MRC AIDS Directed Programme Reagent Project,

National Institute for Biological Standards and Controls, United Kingdom, for providing the recombinant gp105.

REFERENCES

1. De Cock KM, Brun-Vézinet F, Soro B: HIV-1 and HIV-2 infections and AIDS in West Africa. AIDS 5 (Suppl 1):S21–S28, 1991
2. De Cock KM, Adjorlolo G, Ekpini E, Sibailly T, Kouadio J, Maran M, Brattegaard K, Vetter KM, Doorly R, Gayle HD: Epidemiology and transmission of HIV-2. Why there is no HIV-2 pandemic. JAMA 270:2083–2086, 1993
3. Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, Traore I, Hsieh C-C, Ciré Dia M, Gueye E-H, Hellinger J, Guèye-Ndiaye A, Sankalé J-L, Ndoye I, Mboup S, Essex M: Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science 265:1587–1590, 1994
4. De Cock KM, Brun-Vézinet F: Epidemiology of HIV-2 infection. AIDS 3 (Suppl 1):S89–S95, 1989
5. Kanki PJ, Travers KU, MBoup S, Hsieh C-C, Marlink RG, Gueye-Ndiaye A, Siby T, Thior I, Hernandez-Avila M, Sankalé J-L, NDoye I, Essex ME: Slower heterosexual spread of HIV-2 than HIV-1. Lancet 343:943–946, 1994
6. Möst J, Spötl L, Sarcletti M, Vogetseder W, Dierich MP, Zangerle R: Differences in heterosexual transmission of HIV-1 and HIV-2. Lancet 344:1572–1573, 1994
7. Simon F, Matheron S, Tamalet C, Loussert-Ajaka I, Bartczack S, Pépin JM, Dhiver C, Gamba E, Elbim C, Gastaut JA, Saimot AG, Brun-Vézinet F: Cellular and plasma viral load in patients infected with HIV-2. AIDS 7:1411–1417, 1993
8. Archibald DW, Witt DJ, Craven DE, Vogt MW, Hirsch MS, Essex M: Antibodies to human immunodeficiency virus in cervical secretions from women at risk for AIDS. J Infect Dis 156:240–241, 1987
9. Belec L, Georges AJ, Steenman G, Martin PMV: Antibodies to human immunodeficiency virus in vaginal secretion of heterosexual women. J Infect Dis 160:385–391, 1989
10. O'Shea S, Cordery M, Barrett WY, Richman DD, Bradbeer C, Banatvala JE: HIV excretion patterns and specific antibody responses in body fluids. J Med Virol 31:291–296, 1990
11. Lu X-S, Bélec L, Pillot J: Anti-gp160 IgG and IgA antibodies associated with a large increase in total IgG in cervicovaginal secretions from human immunodeficiency virus type-1 infected women. J Infect Dis 167:1189–1192, 1993
12. Bélec L, Grésenguet G, Dragon M-A, Meillet D, Pillot J: Detection of antibodies to human immunodeficiency virus in vaginal secretions by immunoglobulin G antibody capture enzyme-linked immunosorbent assay: Application to detection of seminal antibodies after sexual intercourse. J Clin Microbiol 32:1249–1255, 1994
13. Bélec L, Tévi-Bénissan C, Lu X-L, Prazuck T, Pillot J: Local synthesis of IgG to HIV within the female and male genital tracts during asymptomatic and pre-AIDS stages of HIV infection. AIDS Res Hum Retrovirus 11:719–729, 1995
14. Bélec L, Gherardi R, Payan C, Prazuck T, Tévi-Bénissan C, J-E Malkin, Pillot J: HIV-enhancing proinflammatory cytokines in cervicovaginal secretions from normal and HIV-infected women. Cytokine 7:568–574, 1995
15. Bélec L, Meillet D, Gaillard O, Prazück T, Michel E, Ngondi-Ekome J, Pillot J: Decreased cervicovaginal production of both IgA1 and IgA2 subclasses in women with AIDS. Clin Exp Immunol 101:100–106, 1995
16. Belec L, Peghini M, Georges AJ, Barabe P, Martin PMV: Antibodies to HIV-2 in genital secretions. Res Virol 140:15–21, 1989
17. World Health Organization: Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2 and HTLV-I/HTLV-II. Wkly Epidem Rec 37:281–283, 1990
18. Bélec L, Meillet D, Levy M, Georges AJ, Tevi-Benissan C, Pillot J: Dilution assessment of cervico-vaginal secretions obtained by vaginal washing for immunological assays. Clin Diag Lab Immunol 2:57–61, 1995
19. Lu X-S, Delfraissy JF, Grangeot-Keros L, Rannou MT, Pillot J: Rapid and constant detection of HIV antibody response in saliva of HIV infected patients; selective distribution of anti-HIV activity in the IgG isotype. Res Virol 145:369–377, 1994
20. Weiblen BJ, Schumacher RT, Hoff R: Detection of IgM and IgA antibodies after removal of IgG with recombinant protein G. J Immunol Methods 126:199–204, 1990
21. Jonard PP, Rambaud JC, Dive C, Vaerman JP, Gallan A, Delacroix DL: Secretion of immunoglobulins and plasma proteins from the jejunal mucosa. Transport rate and origin of polymeric immunoglobulin A. J Clin Invest 74:525–535, 1984
22. Bouvet J-P, Bélec L, Pirès R, Pillot J: Immunoglobulin G antibodies in human vaginal secretions after parenteral vaccination. Infect Immun 62:3957–3961, 1994
23. Kutteh WH, Mestecky J: Secretory immunity in the female reproductive tract. Am J Reprod Immunol 31:40–46, 1994
24. Brandtzaeg P, Christiansen E, Müller F, Purvis K: Humoral immune response pattern of human mucosae, including the reproductive tracts. *In* Local Immunity in Reproductive Tract Tissues, PD Griffin, PM Johnson (eds). Oxford University Press, Oxford, 1993, pp 97–130
25. Lim SG, Condez A, Lee CA, Johnson MA, Elia C, Poulter LW: Loss of mucosal CD4 lymphocytes in an early feature of HIV infection. Clin Exp Immunol 92:448–454, 1993
26. Janoff EN, Jackson S, Wahl SM, Thomas K, Peterman JH, Smith PD: Intestinal mucosal immunoglobulins during human immunodeficiency virus type 1 infection. J Infect Dis 170:299–307, 1994
27. Miller CJ, Kang DW, Marthas M, Moldoveanu Z, Kiyono H, Marx P, Eldridge JH, Mestecky J, McGhee JR: Genital secretory immune response to chronic simian immunodeficiency virus (SIV) infection: A comparison between intravenously and genitally inoculated rhesus macaques. Clin Exp Immunol 88:520–526, 1992
28. Archibald DW, Hebert CA, Alger LS, Johnson JP: Detection of HIV-specific antibodies in saliva and cervical secretions. Vaccine Res 1:215–219, 1992
29. Brandtzaeg P: Distribution and characteristics of mucosal immunoglobulin-producing cells. *In* Handbook of Mucosal Immunology, PL Ogra, W Strober, J Mestecky, JR McGhee, ME Lamm, J Bienenstock (eds). San Diego, CA, Academic Press, 1994, pp 251–262
30. Hénin Y, Mandelbrot L, Henrion R, Prainbaud R, Coulaud JP, Montagnier L: Virus excretion in the cervico-vaginal secretions of pregnant and non pregnant HIV-infected women. J AIDS 6:72–75, 1993
31. Clemetson DBA, Moss GB, Willerford DM, Hensel M, Emonyi W, Holmes KK, Plummer F, Ndinya-Achola J, Roberts PL, Hillier S, Kreiss JK: Detection of HIV DNA in cervical and vaginal secretions. Prevalence and correlates among women in Nairobi, Kenya. JAMA 269:2860–2864, 1993
32. Pomerantz RJ, De la Monte S, Donegan SP, Rota TR, Vogt MW, Craven DE, Hirsch MS: Human immunodeficiency virus (HIV) infection of the uterine cervix. Ann Intern Med 108:321–327, 1988

33. Nuovo GJ, Forde A, Mac Connell P, Fahrenwald R: In situ detection of PCR-amplified HIV-1 nucleic acids and tumor necrosis factor cDNA in cervical tissues. Am J Pathol 143:40–48, 1993
34. Van de Perre P, De Clercq A, Cogniaux-Leclerc J, Nzaramba D, Butzler J-P, Sprecher-Goldberger S: 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. Genitourin Med 64:30–33, 1988
35. Tedder RS, O'Connor T, Hughes A, N'Jie H, Corrah T, Whittle H: Envelope cross reactivity in Western blot for HIV-1 and HIV-2 may not indicate dual infection. Lancet 2:927–930, 1988
36. De Cock KM, Porter A, Kouadio J, Maran M, Lafontaine M-F, Gershy-Damet C-M, Hyeward WL, George R: Cross-reactivity on Western blots in HIV-1 and HIV-2 infections. AIDS 15:859–863, 1991
37. Guyader M, Emerman M, Sonigo P, Clavel F, Montagnier L, Alizon M: Genome organization and transactivation of the human immunodeficiency virus type 2. Nature 326:662–669, 1987
38. Weiss RA, Clapham PR, Weber JN, Whitby D, Tedder RS, O'Connor T, Chamaret S, Montagnier L: HIV-2 antisera cross-neutralize HIV-1. AIDS 2:95–100, 1988
39. Rayfield M, De Cock K, Heyward W, Golstein L, Krebs J, Kwok S, Lee S, McCormick J, Moreau JM, Odehouri K, Schochetman G, Sninsky J, Ou C-Y: Mixed human immunodeficiency virus (HIV) infection in an individual: Demonstration of both HIV type 1 and type 2 proviral sequences by using polymerase chain reaction. J Infect Dis 158:1170–1176, 1988
40. George JR, Ou C-Y, Parekh B, Brattegaard K, Brown V, Boateng E, De Cock KM: Prevalence of HIV-1 and HIV-2 mixed infections in Côte d'Ivoire. Lancet 340:337–339, 1992
41. Travers K, Mboup S, Marlink R, Guèye-Ndiaye A, Siby T, Thior I, Traore I, Dieng-Sarr A, Sankalé J-L, Mullins C, Ndoye I, Hseih C-C, Essex M, Kanki P: Natural protection against HIV-1 infection provided by HIV-2. Science 268:1612–1615, 1995
42. Grankvist O, Bredberg-Radèn U, Gustafsson A, Albert J, Albino P, Andreasson PA, Nauclér A, Biberfeld G, Wadell G: Improved detection of HIV-2 DNA in clinical samples using a nested primer-based polymerase chain reaction. J AIDS 5:286–293, 1992
43. Loussert-Ajaka I, Simon F, Farfara I, Houhou N, Couto-Fernandez J, Dazza MC, Brun-Vézinet F: Comparative study of single and nested PCR for the detection of proviral HIV2 DNA. Res Virol 145:337–342, 1994