JOURNAL OF CLINICAL MICROBIOLOGY, May 1994, p. 1249–1255
0095-1137/94/$04.00+0

Vol. 32, No. 5

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

LAURENT BÉLEC,[1,2]* GÉRARD GRÉSENGUET,[3] MARIE-AGNES DRAGON,[2] DOMINIQUE MEILLET,[4] AND JACQUES PILLOT[1]

Unité d'Immunologie Microbienne, Institut Pasteur,[1] Service de Microbiologie (Virologie), Hôpital Broussais,[2] and Service de Biochimie, Hôpital de la Salpêtrière,[4] Paris, France, and Centre National de Référence des Maladies Sexuellement Transmissibles, Bangui, Central African Republic[3]

Received 25 October 1993/Returned for modification 18 January 1994/Accepted 22 February 1994

In order to evaluate a commercial immunoglobulin G (IgG) antibody capture enzyme-linked immunoassay (ELISA) (Wellcozyme HIV1+2 Gacelisa; Murex Diagnostics Limited, Dartford, United Kingdom) for the detection of antibodies to human immunodeficiency virus (HIV) in vaginal secretion samples (VS) from HIV-seropositive and -seronegative women, serum samples (S) and VS were obtained from 129 African women living in the Central African Republic, a country of high HIV prevalence. Sera were tested for HIV by routine second-generation ELISA with confirmatory Western blot (immunoblot) (WB). By the Gacelisa IgG immunocapture assay, 45 VS were positive and 84 were negative, whereas by WB, 44 VS were confirmed positive and 85 were confirmed negative. Considering WB as a reference, the IgG immunocapture assay in VS was 97.7% sensitive (43 of 44 positive samples) and 97.6% specific (83 of 85 negative samples). Of 42 HIV-seropositive women, 41 (97.6%) had S and VS that both were HIV positive (S^+ VS^+), and of 87 HIV-seronegative women, 83 (95.4%) had S and VS that both were HIV negative (S^- VS^-). Five women had discordant results for S and VS. One (S^+ VS^-) possibly had a false-negative VS result. Two (S^- VS^+) had similar indeterminate patterns for S and VS in WB. Two (S^- VS^+) had a typical HIV-positive pattern on WB of VS, whereas S results in WB were indeterminate in one case and negative in the other case; for both women, detection of prostatic acid-phosphatase was positive in VS, strongly suggesting recent sexual intercourse with an HIV-positive man. HIV1+2 Gacelisa appears to be a useful, reliable assay to detect IgG antibodies to HIV in VS from HIV-infected women, in whom the anti-HIV IgG antibodies are largely predominant. This assay could also serve to detect IgG antibodies to HIV in VS from a non-HIV-infected woman who has had recent sexual intercourse with an HIV-infected man. Because all HIV-infected men have detectable IgG antibodies to HIV in the seminal fluid, an HIV-seronegative rape victim with HIV-positive VS (S^- VS^+) should receive short-term antiviral therapy to prevent possible HIV transmission.

In recent years, the problem of rape and sexual abuse has received increasing medical and societal attention (19, 20, 41). In North America, nearly 50% of reported rape victims are adolescents, and the majority of sexual assaults involve females (1). The risk of acquiring human immunodeficiency virus (HIV) infection at the time of a single sexual assault has been thought to be low (40). However, repetitive exposures, as might occur in a sexually abused child or adolescent; defloration (7); or the aggressor belonging to certain high-risk groups for HIV infection would likely represent a different set of circumstances with increased risk. In the literature, about 25 cases of documented HIV infection of children ostensibly acquired through sexual abuse have been reported (12, 14–17, 21, 24, 33, 36, 46). Although the American Academy of Pediatrics' Committee on Adolescence made no mention of HIV risk in its initial description of procedures and tests for evaluating adolescent victims of rape (1), the possibility of transmission of HIV at the time of a sexual assault has been subsequently emphasized and should now be investigated (40).

Genital secretions from female (2, 3, 5, 25, 26, 32) and male (4, 5, 32, 49) individuals infected by HIV have been demonstrated to contain immunoglobulin G (IgG) and IgA specific antibodies to HIV. In vaginal secretions of HIV-infected women, specific IgG antibodies to HIV have been found to be strongly predominant in comparison with specific IgA antibodies to HIV (25, 26). In the seminal fluid of all HIV type 1 (HIV-1)- as well as HIV-2-infected men, IgG antibodies to HIV can be easily detected by Western blot (immunoblot) (4, 5, 32, 49), with generally high titers, ranging from 400 to 40,000 (49), whereas IgA antibodies to HIV in the semen can be found in two-thirds of HIV-1-seropositive men (4). Such antibodies to HIV in vaginal secretions and in semen from HIV-positive individuals have been proposed to hamper HIV transmission via heterosexual intercourse. Furthermore, since all HIV-positive men have detectable IgG antibodies to HIV in the seminal fluid, the possibility of detecting specific antibodies in the vaginal lavage from a rape victim has important implications in forensic medicine.

Specific antibodies to HIV in genital secretions have been previously evidenced by techniques allowing qualitative detection of antibodies to HIV of the IgG and IgA isotypes, such as radioimmunoprecipitation assay (2) and Western blot (3–5, 32, 49), and recently with a noncommercial sandwich enzyme-linked immunosorbent assay ELISA permitting semiquantifi-

* Corresponding author. Mailing address: Service de Microbiologie (Virologie), Hôpital Broussais, 96 rue Didot, F-75 674 Paris Cedex 14, France.

cation of both IgG and IgA isotypes (26). However, this ELISA is not available for routine use and is expensive. Recently, commercial tests for detection of antibody to HIV based on the principle of antibody capture by an anti-human IgG antibody-coated solid phase have been proposed to detect antibody to HIV in body fluids with immunoglobulin levels lower than those in serum, such as saliva and urine (10, 11, 18, 23, 31, 34, 47).

The aim of this study was to assess the accuracy of the commercial IgG antibody capture ELISA Wellcozyme HIV1+2 Gacelisa (Murex Diagnostics Limited, Dartford, United Kingdom) to detect anti-HIV IgG antibodies in vaginal secretions from HIV-infected and -uninfected women. For this purpose, the vaginal fluids from 129 African women living in Bangui, the capital and largest city of the Central African Republic, have been tested by this assay. In Bangui, HIV infection is transmitted primarily heterosexually (42) and the HIV seroprevalence in the general adult population is very high, reaching 9.3% in 1989 (43). These epidemiologic characteristics of the HIV epidemic in this country facilitate the study of the local humoral immunity of vaginal secretions from HIV-infected women but also should permit testing of the presence of passively transmitted anti-HIV antibodies by recent sexual intercourse with an HIV-positive male partner.

(Preliminary results from this work have been presented at the 33nd Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 17 to 20 October 1993 [abstract 1261].)

MATERIALS AND METHODS

Study population. Serum and vaginal secretion samples were obtained from 129 African women from February to April 1993. Women were prospectively selected, on an unmatched volunteer basis, from the usual outpatients attending the National Reference Center for Sexually Transmitted Diseases of Bangui for sexually transmitted diseases, contraceptive counselling, or pregnancy surveillance. Ages ranged from 15 to 42 years. For each subject, routine HIV antibody screening of the serum specimens was performed by a conventional second-generation ELISA using recombinant gp160 of HIV-1 and synthetic peptides gp41 of HIV-1 and gp36 of HIV-2 as antigens (Genelavia mixt; Sanofi-Diagnostics Pasteur, Marnes-la-Coquette, France), and the positive sera were confirmed by Western blot (New LAV-blot 1; Sanofi-Diagnostics Pasteur). Eighty-seven women were found HIV negative at screening; the 42 remaining subjects were HIV-1 seropositive. The clinical statuses of the HIV-infected women, according to the 1986 Centers for Disease Control classification, were group I in 1 case, group II in 27 cases, group III in 6 cases, and subgroup IV_{C2} in 8 cases.

Collection and storage of samples. Nonmenses genital fluids were taken by vaginal washing with 3 ml of phosphate-buffered saline (PBS) and centrifuged, and the supernatant was aliquoted. The washing procedure was previously evaluated as corresponding approximately to a 1/10 dilution of the native genital secretions (3). Vaginal secretions and serum samples were kept frozen at −30°C until use and never thawed until the assays.

Screening for antibodies to HIV in vaginal secretions by capture assay. Fifty microliters of vaginal secretions, untreated and undiluted, was tested by HIV1+2 Gacelisa according to the manufacturer's instructions for urine or saliva. The optical density (OD) of each sample and controls was read spectrophotometrically at 492 nm. The cutoff value was calculated as the mean OD of three negative controls plus 0.2, as indicated by the manufacturer. ODs above the cutoff were considered positive. Although a nonnegligible background noise was observed, all vaginal secretions considered positive by Western blot (n = 44), except in one case (subject B1942), had ODs above the cutoff according to the kit procedure. Moreover, all these positive vaginal secretions by Western blot, except in three cases (including subjects B1942 and B2118), had ODs above the mean OD of the vaginal secretions from HIV-negative women plus 3 standard deviations.

Furthermore, all paired serum and vaginal secretion samples from patients in which both were found HIV positive (S^+ VS^+), 25 paired samples from randomly selected individuals in which both were found HIV negative (S^- VS^-), and all serum and vaginal secretion samples with discordant results (S^+ VS^- or S^- VS^+) were retested in parallel by the IgG immunocapture assay (50-μl volume per sample).

Confirmatory Western blot. The specificity of reactivities obtained by the IgG immunocapture assay was assessed by a confirmatory immunoblot. All vaginal secretions were tested for the presence of IgG antibodies to HIV-1 by Western blot (New LAV-Blot 1). Moreover, all paired serum and vaginal secretions giving discordant results (S^+ VS^- and S^- VS^+), as well as one of five paired samples found S^+ VS^+ or S^- VS^-, as controls, were tested in parallel by Western blot. Serum samples were tested at a 1/100 dilution in 1.5% (wt/vol) low-fat milk in PBS, as described in the kit procedure for detection of HIV-1 IgG antibodies. Vaginal secretions were diluted 1/5 (final dilution: approximately 1/50) in 3% (wt/vol) low-fat milk in PBS and incubated with nitrocellulose strips for 3 h at laboratory temperature (23°C) and then overnight at 4°C on a gyratory shaker; the nitrocellulose strips were revealed with peroxidase-conjugated sheep anti-human IgG (Sanofi-Diagnostics Pasteur).

The criteria recommended by the World Health Organization were used for interpretation of the immunoblot assay (50). Results obtained by Western blot were considered the reference for the determination of the sensitivity and the specificity of the HIV1+2 Gacelisa assay. Indeterminate patterns of Western blot, in the absence of reactivity to gp160, as frequently observed in Central Africa without association with HIV infection (13, 39), were considered negative.

Detection of antibodies to HIV in seminal fluid. To ascertain whether antibodies to HIV within the semen from an HIV-positive man could be detected by the HIV1+2 Gacelisa assay when introduced in vaginal secretions, 10^0 to 10^{-6} dilutions of seminal fluids from four HIV-1-seropositive African men were carried out using as a diluent four different vaginal fluid samples collected from HIV-seronegative African women, and these served as samples (50 μl) in the IgG immunocapture assay.

Statistical analysis. A correlation between antibodies against HIV in vaginal secretions and in serum has been searched for by Spearman's correlation test using the StatView statistical program (Abacus Concepts, Inc.).

RESULTS

Specific antibodies to HIV in vaginal secretions. Among the 129 vaginal secretion samples tested, 45 were positive and 84 were negative by the IgG immunocapture assay whereas 44 were positive and 85 were negative by Western blot (Table 1). Finally, considering the Western blot results as the reference, the HIV1+2 Gacelisa assay of vaginal secretions was 97.7% sensitive (43 of 44 positive results) and 97.6% specific (83 of 85 negative results).

Correspondence between antibodies to HIV in serum and

TABLE 1. Detection of IgG antibodies to HIV-1 in vaginal secretions from 129 African heterosexual women by the IgG immunocapture assay (HIV1+2 Gacelisa) and by Western blot

Result of Western blot (no. of samples)	No. of samples with IgG immunocapture assay[a] result of:	
	Positive	Negative
Positive (44)	43	1[b]
Negative (85)	2[c]	83

[a] Sensitivity of the HIV1+2 Gacelisa assay: (43/44) × 100 = 97.7%. Specificity of the HIV1+2 Gacelisa assay: (83/85) × 100 = 97.6%.
[b] This woman (B1942) had a weakly positive Western blot with vaginal secretions.
[c] Both women (B1941 and B1976) had indeterminate Western blots with vaginal secretions.

vaginal secretions. Results were concordant in serum and vaginal secretions for 124 women (96%) (Table 2).

According to the routine HIV serology and the results obtained by the IgG immunocapture assay of vaginal secretions, 41 of 42 (97.6%) HIV-seropositive women had serum and vaginal anti-HIV antibodies (S^+ VS^+) and 83 of 87 (95.4%) HIV-seronegative women did not have anti-HIV antibodies in serum and vaginal fluid (S^-, VS^-).

Western blot of the vaginal secretions of all HIV-positive women showed typical antibody-positive patterns for HIV-1, most samples containing antibodies to the majority of the *env*-, *pol*-, and *gag*-encoded proteins (Fig. 1). The antibody profiles on Western blot strips, including the strength of the bands, were similar for most serum and vaginal secretion samples. The vaginal secretions of 85 of the 87 HIV-seronegative women had negative (n = 52) or indeterminate (n = 33) Western blot patterns.

Five women had discordant results between the HIV serology by conventional second-generation ELISA with confirmatory Western blot and the IgG immunocapture assay of vaginal secretions: S^+ VS^- in one case and S^- VS^+ in four cases (Table 3). The antibody patterns on Western blot of serum and vaginal secretions from these women are shown in Fig. 1 and 2. An HIV-seropositive woman (B1942) had vaginal secretions that were HIV negative by the IgG immunocapture assay (S^+ VS^-); the Western blot yielded an HIV-positive pattern for serum, compatible with recent seroconversion, and the Western blot was indeterminate for vaginal secretions, with faint reactivities to gp160, p55, and p25 (Fig. 1), suggesting false-negative results by the IgG immunocapture assay. Two other HIV-seronegative women (B1976 and B1941) had vaginal

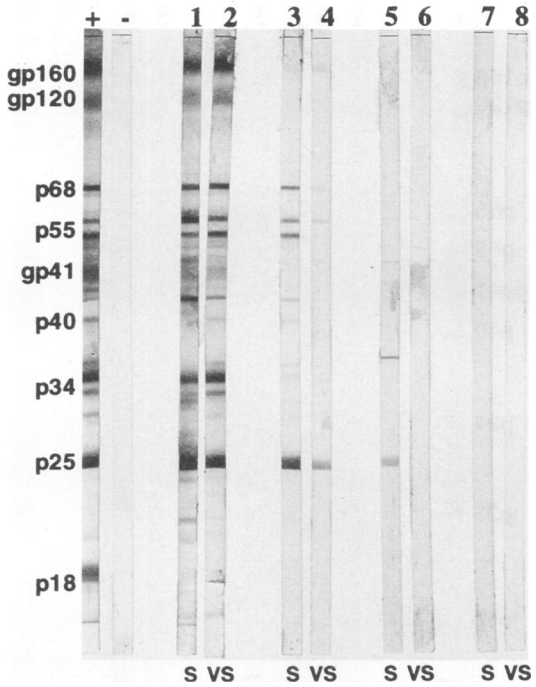

FIG. 1. Western blot analysis of serum samples (lanes 1, 3, 5, and 7) and vaginal secretions (lanes 2, 4, 6, and 8). B1834 (lanes 1 and 2) was an asymptomatic HIV-positive woman with a strongly positive Western blot pattern for both serum and vaginal secretions. B1942 (lanes 3 and 4) was an asymptomatic HIV-positive woman with an HIV-positive pattern for serum compatible with recent seroconversion and an indeterminate pattern for vaginal secretions, with faint IgG reactivities to the gp160, p55, and p25. B2069 (lanes 5 and 6) had an indeterminate pattern for serum and a negative pattern for vaginal secretions. B1451 (lanes 7 and 8) had negative Western blot patterns for both serum and vaginal secretions. Lanes + and −, positive and negative controls.

secretions weakly positive by the IgG immunocapture assay (S^- VS^+). They had indeterminate Western blot results for serum and vaginal secretions, with a close correspondence between the patterns on the nitrocellulose strips in serum and vaginal secretions. The significance of such weak reactivity remains unclear. Since the same specificity of the anti-HIV antibodies was found in serum and vaginal secretions, in the absence of antibodies directed to the surface HIV *env*-encoded

TABLE 2. Relationship between serum anti-HIV antibodies detected by conventional second-generation ELISA (Genelavia mixt) with confirmatory Western blot and vaginal anti-HIV IgG antibodies detected by the IgG immunocapture assay (HIV1+2 Gacelisa)

Result of second-generation ELISA (no. of serum samples)	No. of vaginal secretion samples with IgG immunocapture assay result of:	
	Positive	Negative
Positive (42)	41	1[a]
Negative (87)	4[b]	83

[a] This woman (B1942) had a weakly positive Western blot with vaginal secretions.
[b] Two women (B1941 and B1976) had indeterminate Western blots and two others (B1845 and B2118) had positive Western blots with their vaginal secretions.

TABLE 3. OD/cutoff ratios by routine second-generation ELISA (Genelavia mixt) and IgG immunocapture assay (HIV1+2 Gacelisa) with sera and vaginal secretions from the five women whose samples gave discrepant results[a]

Patient	OD/cutoff ratio[b] in:		
	Serum by:		Vaginal secretions by HIV1+2 Gacelisa
	Genelavia mixt	HIV1+2 Gacelisa	
B1942	14.1	7.2	0.67
B1845	0.45	1.25	6.07
B1941	0.18	0.68	1.13
B1976	0.14	0.56	1.02
B2118	0.11	0.19	1.24

[a] All results were concordant for the other 124 women.
[b] Discordant results are underscored.

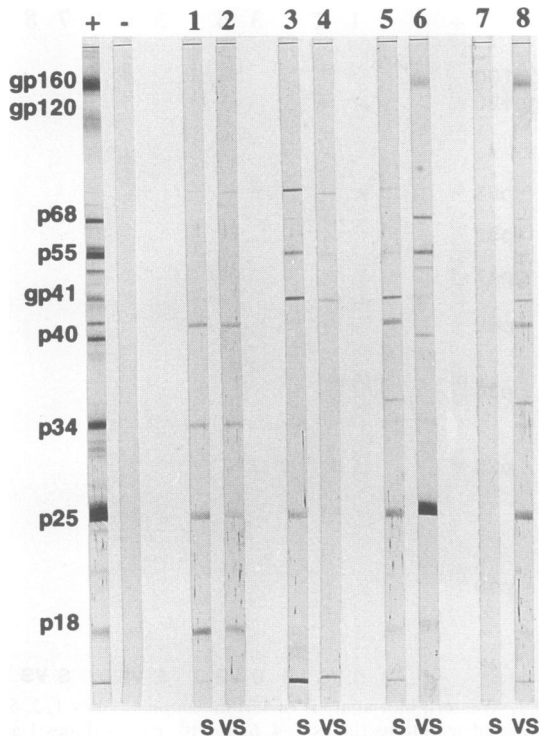

FIG. 2. Western blot analysis of serum samples (lanes 1, 3, 5, and 7) and vaginal secretions (lanes 2, 4, 6, and 8) from four women HIV negative by routine second-generation ELISA but who had positive results in vaginal secretions by the IgG immunocapture assay (HIV1+2 Gacelisa). B1976 (lanes 1 and 2) and B1941 (lanes 3 and 4) had indeterminate Western blot patterns for both serum and vaginal secretions. Note the close correspondence between the patterns on the nitrocellulose strips with serum and vaginal secretions for both women. B1845 (lanes 5 and 6) had an indeterminate pattern for serum but had a positive Western blot pattern, including anti-gp160 and anti-gp41 reactivities, for vaginal secretions, without correspondence between the patterns of Western blots for serum and vaginal secretions. B2118 (lanes 7 and 8) had a negative Western blot pattern for serum and had an HIV-positive pattern, including anti-gp160 and faint anti-gp41 reactivities, for vaginal secretions. For this woman, binding to gp120 in the vaginal washing was faint and is not seen on the lane 8, although it was visible on the original Western blot strip. Lanes + and −, positive and negative controls.

glycoproteins, false-positive reactivity was likely, as frequently encountered in the sera and vaginal secretions originating from the Central African Republic (3); however, for these women, a partial immune response to HIV could not be completely excluded. Two HIV-seronegative women (B1845 and B2118) had vaginal washings positive by the IgG immunocapture assay (S^- VS^+) and unexpectedly showed typical HIV-positive patterns on Western blot of vaginal secretions (Fig. 2). The serum of one of them (B1845) was indeterminate in Western blot, without anti-*env*-encoded glycoprotein reactivity and without correspondence between the patterns of Western blots with serum and with vaginal secretions. For this woman, the HIV serology was negative by ELISA but was weakly positive by the IgG immunocapture assay. In another woman (B2118), the Western blot with serum was completely negative. Attempts to find possible early seroconversion in these patients, particularly in the woman with the indeterminate Western blot with serum (B1845), were unsuccessful: no p24 antigen could be detected in serum either spontaneously or after acid dissociation of immune complexes, and retesting was impossible because the patients were discharged and not seen again. Interestingly, detection by enzyme immunoassay of prostatic specific antigens (PSA) (Tandem E PSA; Biotrol, Paris, France) as well as of prostatic acid-phosphatase (PAP) (Tandem E PAP; Biotrol) was positive in vaginal fluids for both (for B1845, PSA = 53 μg/liter and PAP = 20 μg/liter; for B2118, PSA = 69 μg/liter and PAP = 19 μg/liter), demonstrating recent vaginal sexual intercourse (37, 44).

Relationship between HIV antibody levels in serum and vaginal secretions. The distribution of the assay reactions in serum and vaginal secretions, expressed as test OD and OD/cutoff ratios, are given in Table 4 and shown in Fig. 3 for the 42 HIV-seropositive and 29 HIV-seronegative women, including those with discordant results between serum and vaginal secretions. A high correlation was observed between HIV antibody levels in serum and in vaginal secretions (Spearman's correlation coefficient, $Z = 7.33$; $P = 0.0001$).

Detection of antibodies to HIV in seminal fluid. Antibodies to HIV within the semen from HIV-positive men could be detected by the HIV1+2 Gacelisa assay when mixed with vaginal secretions from HIV-seronegative African women (Fig. 4). The greatest dilution of seminal fluid giving positive reactivity ranged from 10^{-3} to 10^{-6}.

DISCUSSION

Our data confirm and extend previous reports of HIV-1 antibody detection in vaginal fluid from HIV-1-infected women (2, 3, 25, 26, 32) and suggest that IgG antibodies to HIV can be easily detected by an antibody capture assay of vaginal secretions from most HIV-infected women as well as from women who have had recent vaginal intercourse with an HIV-positive man. The development of the antibody capture immunoassay, like the commercial HIV1+2 Gacelisa, involving an initial step of IgG concentration, has reliably permitted detection of antibodies to HIV in body fluids, particularly in saliva and urine (10, 11, 34, 47). In vaginal secretions, sensitivity and specificity of the IgG immunocapture assay to detect anti-HIV IgG antibodies were similar to those previously found for saliva or urine. In our series, vaginal fluids from one woman who recently seroconverted presented a false-negative reaction by the IgG immunocapture assay, and the vaginal

TABLE 4. ODs and OD/cutoff ratios by the HIV1+2 Gacelisa assay of serum and vaginal secretions from HIV-positive and HIV-negative African women

HIV status (no.) of women	Median ± SD value (range) in:			
	Serum		Vaginal secretions	
	OD	OD/cutoff	OD	OD/cutoff
Positive (42)	3.08 ± 0.76 (0.54–4.1)	7.39 ± 1.94 (1.03–10.7)	2.97 ± 0.85 (0.28–4.1)	7.17 ± 2.16 (0.67–10.31)
Negative (29)	0.39 ± 0.14 (0.03–0.75)	0.75 ± 0.23 (0.19–1.25)	0.46 ± 0.51 (0.22–2.54)	0.98 ± 1.25 (0.41–6.07)

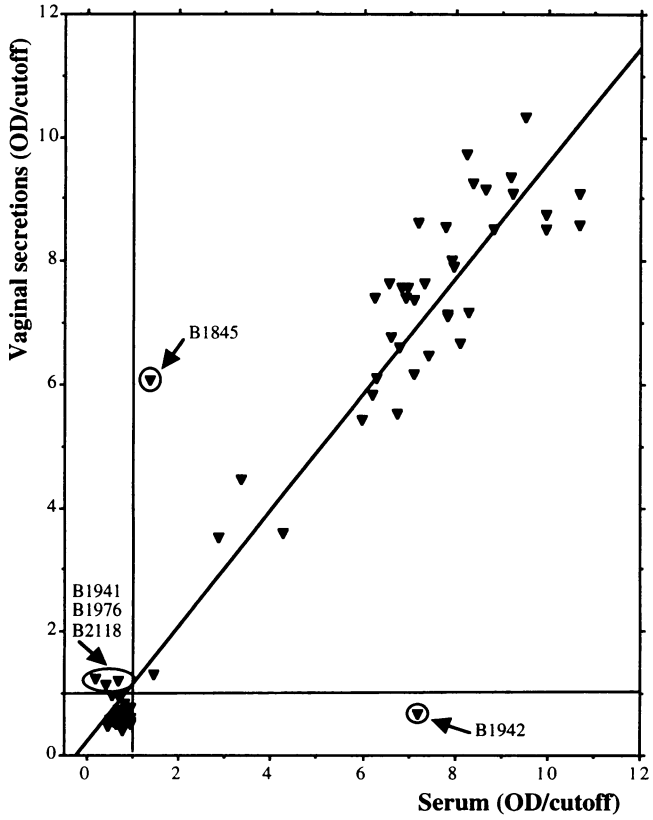

FIG. 3. Correlation between antibodies against HIV-1 in sera and vaginal secretions from 42 HIV-1-infected and 29 HIV-seronegative African women. Values are expressed as the OD/cutoff ratios of serum and vaginal secretions evaluated by the IgG immunocapture assay (HIV1+2 Gacelisa). The circled points correspond to the five women who gave discrepant results for serum and vaginal secretions. Vertical and horizontal lines represent limits of positivity.

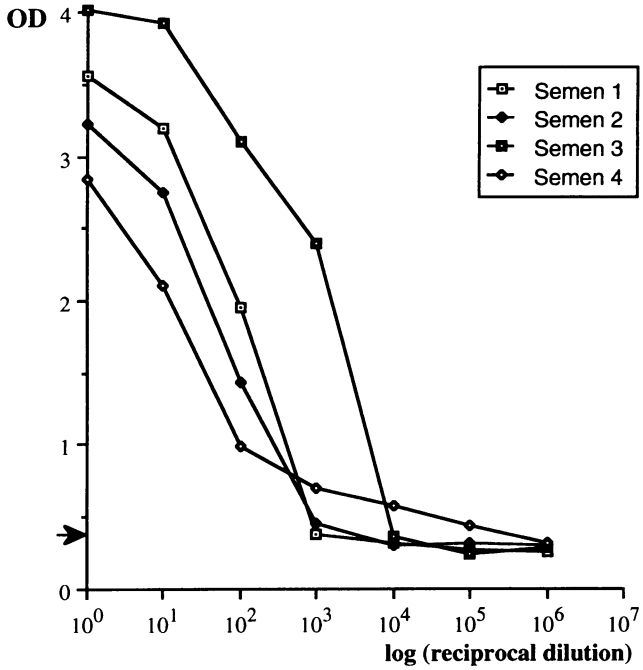

FIG. 4. OD of 10^{0} to 10^{-6} dilutions in vaginal fluids from HIV-seronegative African women of seminal fluid from four HIV-1-seropositive African men tested by the IgG immunocapture assay. The greatest dilutions of seminal fluid giving positive reactivity ranged from 10^{-3} to 10^{-6}. The arrow indicates the cutoff, the limit of positivity.

fluids from two HIV-negative women gave rise to possible weak false-positive results. In two HIV-seronegative women, vaginal secretions were reactive by the IgG immunocapture assay, and HIV-specific antibodies, including antibody to HIV *env*-encoded glycoproteins, were confirmed by Western blot. Both women had detectable PSA and PAP in their vaginal fluids, demonstrating recent vaginal sexual intercourse, most likely, in a country of high HIV prevalence, with HIV-infected men. Furthermore, we confirmed in vitro that heterologous antibodies to HIV from seminal fluid mixed with vaginal secretions can be detected in dilutions of 10^{-3} to 10^{-6} dilutions by the IgG immunocapture assay.

In the present series, the OD/cutoff ratios of antibodies to HIV in sera and vaginal fluids from HIV-positive women were strongly correlated, suggesting a proportional relationship between the concentrations of IgG to HIV in serum and in vaginal secretions. Previously, we have shown that anti-gp160 IgG antibody levels in vaginal secretions of HIV-1-infected African women without AIDS ranged from 10- to 100-fold that of anti-gp160 IgA (26). Physiologically, only a small quantity of serum-borne IgG usually crosses healthy mucosae into secretions (6, 28, 30). In HIV infection, the strong correlation between anti-HIV IgG antibodies levels in serum and vaginal secretions suggests a passive transudation of anti-HIV IgG antibodies from plasma to vaginal secretions. Indeed, serum IgG could be the main source of anti-HIV IgG in vaginal secretions. However, HIV antigens have been demonstrated in the female genital tract in relation to a local replication of HIV (35, 48), and there exists the possibility that IgG and IgA HIV-specific antibodies could also be locally produced within the female genital mucosa, as was previously observed in women with genital infections by herpes simplex virus type 2 (29) or *Chlamydia trachomatis* (8).

The Centers for Disease Control and Prevention recommend that victims of sexual abuse should receive prophylactic treatment against any sexually transmitted infection found in the assailant (9). Detection of specific antibodies to HIV in the vaginal washing of an HIV-negative woman victim of rape with intravaginal penile penetration could have major implications for the prophylactic treatment of sexual assault victims. Our study clearly shows that antigen-specific antibodies to HIV from an HIV-positive man can be detected within the vaginal fluid after vaginal intercourse with ejaculation. We suggest that laboratory procedures needed in the evaluation of a rape victim could include a test for antibodies to HIV in the vaginal washing in association with documentation of recent coitus by using wet mounts for the presence and motility of sperm and PSA or PAP determinations (20). If the vaginal washing contains HIV-specific antibodies with motile sperm or seminal fluid secretions, the screening for HIV infection of the assaulted woman, although controversial in a victim of sexual abuse (17, 19, 33), should be proposed in order to establish if the detected anti-HIV antibody comes from the aggressor or from the victim. In case of HIV seronegativity, rapid prophylaxis against sexual HIV transmission should then be proposed in the management of a rape victim, including zidovudine administration and, locally, application of a detergent spermicide, such as nonoxynol-9. Indeed, zidovudine can alter the

course of infection when administered a few hours after retrovirus inoculation (38, 45), and nonoxynol-9 inactivates in vitro enveloped viruses as well as their cellular vectors, including HIV, at concentrations easily achieved by vaginal barrier methods (22, 27). In the absence of HIV antibodies in vaginal washing, the assaulted woman should be reassured and counseled in an attempt to allay anxiety regarding potential risk for HIV infection.

ACKNOWLEDGMENTS

We thank Véronique Lorely and Laura Vernoux, Murex Laboratories, Châtillon, France, for valuable technical assistance and helpful suggestions. We thank S. Iskaki for reviewing the English of the manuscript. Murex Laboratories kindly provided reactive agents for this study.

This work was supported by a grant from the Agence Nationale de Recherche sur le Sida (93-032).

REFERENCES

1. **American Academy of Pediatrics Committee on Adolescence.** 1988. Rape and the adolescent. Pediatrics **81**:595–597.
2. **Archibald, D. W., D. J. Witt, D. E. Craven, M. W. Vogt, M. S. Hirsch, and M. Essex.** 1987. Antibodies to human immunodeficiency virus in cervical secretions from women at risk for AIDS. J. Infect. Dis. **156**:240–241.
3. **Belec, L., A. J. Georges, G. Steenman, and P. M. V. Martin.** 1989. Antibodies to human immunodeficiency virus in vaginal secretion of heterosexual women. J. Infect. Dis. **160**:385–391.
4. **Belec, L., A. J. Georges, G. Steenman, and P. M. V. Martin.** 1989. Antibodies to human immunodeficiency virus in the semen of heterosexual men. J. Infect. Dis. **159**:324–327.
5. **Belec, L., M. Peghini, A. J. Georges, P. Barabe, and P. M. V. Martin.** 1989. Antibodies to HIV-2 in genital secretions. Res. Virol. **140**:15–21.
6. **Bienenstock, J., and A. D. Befus.** 1980. Mucosal immunology. Immunology **41**:249–270.
7. **Bouvet, E., I. De Vincenzi, R. Ancelle, and F. Vachon.** 1989. Defloration as risk factor for heterosexual HIV transmission. Lancet **i**:615.
8. **Brunham, R. C., C. C. Kuo, L. Cles, and K. K. Holmes.** 1983. Correlation of host immune response with quantitative recovery of *Chlamydia trachomatis* from the human endocervix. Infect. Immun. **39**:1491–1494.
9. **Centers for Disease Control.** 1985. 1985 STD treatment guidelines. Morbid. Mortal. Weekly Rep. **34**(Suppl. 45):75S–108S.
10. **Connell, J. A., J. V. Parry, P. P. Mortimer, and R. J. S. Duncan.** 1993. Novel assay for the detection of immunoglobulin G antihuman immunodeficiency virus in untreated saliva and urine. J. Med. Virol. **41**:159–164.
11. **Connell, J. A., J. V. Parry, P. P. Mortimer, R. J. S. Duncan, K. A. McLean, A. M. Johnson, M. H. Hambling, J. Barbara, and C. P. Farrington.** 1990. Preliminary report: accurate assays for anti-HIV in urine. Lancet **335**:1366–1369.
12. **Datel, B. J., K. Coulter, M. Grossman, and L. B. Hauer.** 1988. Presence of HIV antibody in sexually abused children, abstr. 7219. IVth International Conference on AIDS, 12 to 16 June, Stockholm, Sweden.
13. **Delaporte, E., P. Peeters, F. Simon, A. Dupont, D. Schrijvers, D. Kerouedan, R. Josse, M. Merlin, A. Trebucq, M. Collet, L. Bedjabaga, C. Honore, B. Larouze, and F. Brun-Vezinet.** 1990. Interpretation of antibodies reacting solely with human retroviral core protein in western equatorial Africa. AIDS **3**:179–182.
14. **Fuller, A. K., and R. J. Bartucci.** 1988. HIV transmission and childhood sexual abuse. JAMA **259**:2235–2236.
15. **Gellert, G. A., and M. J. Durfee.** 1989. HIV infection and child abuse. N. Engl. J. Med. **321**:685.
16. **Gellert, G. A., M. J. Durfee, and C. D. Berkowitz.** 1990. Developing guidelines for HIV antibody testing of victims of pediatric sexual abuse. Child Abuse Neglect **14**:9–17.
17. **Gellert, G. A., and L. Mascola.** 1989. Rape and AIDS. In reply. Pediatrics **83**(Suppl.):644.
18. **Gershy-Damet, G. M., K. Koffi, L. Abouya, M. Sasson-Morokro, K. Brattegaard, J. V. Parry, K. M. De Cock, and P. P. Mortimer.** 1992. Salivary and urinary diagnosis of human immunodeficiency viruses 1 and 2 infection in Côte d'Ivoire, using two assays. Trans. R. Soc. Trop. Med. Hyg. **86**:670–671.
19. **Glaser, J. B., M. R. Hammerschlag, and W. M. McCormack.** 1987. Sexually transmitted diseases in victims of sexual assault. N. Engl. J. Med. **316**:1024–1025.
20. **Glaser, J. B., M. R. Hammerschlag, and W. M. McCormack.** 1989. Epidemiology of sexually transmitted diseases in rape victims. Rev. Infect. Dis. **11**:246–254.
21. **Gutman, L. T., K. K. St. Claire, C. Weedy, M. E. Herman-Giddens, B. A. Lane, J. G. Niemeyer, and R. E. McKinney.** 1991. Human immunodeficiency virus transmission by child sexual abuse. Am. J. Dis. Child. **145**:137–141.
22. **Hicks, D. R., L. S. Martin, J. P. Getchell, J. L. Heath, D. P. Francis, J. S. McDougal, J. W. Curran, and B. Voeller.** 1985. Inactivation of HTLV-III/LAV-infected cultures of normal human lymphocytes by nonoxynol-9 *in vitro*. Lancet **ii**:1422–1423.
23. **Klokke, A. H., D. Ocheng, S. E. Kalluvya, A. G. Nicoll, U. Laukamm-Josten, J. V. Parry, P. P. Mortimer, and J. A. Connell.** 1991. Field evaluation of immunoglobulin G antibody capture tests for HIV-1 and HIV-2 antibodies in African serum, saliva and urine. AIDS **5**:1391–1392.
24. **Leiderman, I. Z., and K. T. Grimm.** 1986. A child with HIV infection. JAMA **256**:3094.
25. **Lu, X.-S., L. Bélec, P. M. V. Martin, and J. Pillot.** 1991. Enhanced local immunity in vaginal secretions of HIV-infected women. Lancet **338**:323–324.
26. **Lu, X.-S., L. Bélec, and J. Pillot.** 1993. 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.
27. **Malkovsky, M., A. Newell, and A. G. Dalgleish.** 1988. Inactivation of HIV by nonoxynol-9. Lancet **i**:645.
28. **McDermott, M. R., A. D. Befus, and J. Bienenstock.** 1982. The structural basis for immunity in the respiratory tract. Int. Rev. Exp. Pathol. **23**:48–112.
29. **Mendis, L. N., J. M. Best, and J. G. Banatvala.** 1981. Class-specific antibodies (IgG and IgA) to membrane antigens of herpes simplex type-2 infected cells in patients with cervical dysplasia and neoplasia. Int. J. Cancer **27**:669–677.
30. **Mestecky, J., W. H. Kutteh, I. Ladjeva, and J. H. Peterman.** 1989. The common mucosal immune system in the human genital tract and mammary gland, p. 251–258. *In* L. Metter and W. D. Billington (ed.), Reproductive immunology. Elsevier, Amsterdam.
31. **Mortimer, P. P., and J. V. Parry.** 1991. Non-invasive virological diagnosis: are saliva and urine specimens adequate substitutes for blood? Rev. Med. Virol. **1**:73–78.
32. **O'Shea, S., M. Cordery, W. Y. Barrett, D. D. Richman, C. Bradbeer, and J. E. Batnavala.** 1990. HIV excretion patterns and specific antibody responses in body fluids. J. Med. Virol. **31**:291–296.
33. **Osterholm, M. T., K. L. MacDonald, R. N. Danila, and K. Henry.** 1987. Sexually transmitted diseases in victims of sexual assault. N. Engl. J. Med. **316**:1024.
34. **Parry, J. V., K. R. Perry, and P. P. Mortimer.** 1987. Sensitive assay for viral antibodies in saliva: an alternative to tests on serum. Lancet **ii**:72–75.
35. **Pomerantz, R. J., S. De la Monte, S. P. Donegan, T. R. Rota, M. W. Vogt, D. E. Craven, and M. S. Hirsch.** 1988. Human immunodeficiency virus (HIV) infection of the uterine cervix. Ann. Intern. Med. **108**:321–327.
36. **Rubinstein, A.** 1986. Pediatric AIDS. Curr. Probl. Pediatr. **16**:362–409.
37. **Rupp, J. C.** 1969. Sperm survival and prostatic acid phosphatase activity in victim of sexual assault. J. Forens. Sci. **14**:177–183.
38. **Ruprecht, R. M., L. G. O'Brien, L. D. Rossoni, and S. Nusinoff-Lehrman.** 1986. Suppression of mouse viraemia and retroviral disease by 3′-azido-3′-deoxythymidine. Nature (London) **323**:467–469.
39. **Schindzielorz, A. H., R. B. Belshe, and M. A. Mufson.** 1990. Occurrence, characteristics, and pattern of HIV-1 and HIV-2

Western blot indeterminate sera in low risk populations in West Virginia and pre-AIDS Africa. Am. J. Trop. Med. Hyg. **42:**460–464.

40. **Schonberg, S. K.** 1989. Rape and AIDS. In reply. Pediatrics **83**(Suppl.)**:**644–645.
41. **Schwartz, I. L.** 1991. Sexual violence against women: prevalence, consequences, societal factors, and prevention. Am. J. Prev. Med. **7:**363–373.
42. **Somsé, P., M. K. Chapko, and R. V. Hawkins.** 1989. Multiple sexual partners: result of a national HIV/AIDS survey in the Central African Republic. AIDS **7:**579–583.
43. **Somsé, P., J. Vohito, M. Massanga, K. Komba, J. Mbali, and I. Sow.** 1990. Surveillance de l'infection VIH-1 par sites sentinelles dans 4 groupes de population en République centrafricaine, abstr. TPE 29. Presented at the Vth International Conference on AIDS in Africa, 10 to 12 October 1990, Kinshasa, Zaire.
44. **Soules, M. R., A. A. Pollard, K. M. NBrown, and M. Verma.** 1978. The forensic laboratory evaluation of evidence in alleged rape. Am. J. Obstet. Gynecol. **130:**142–147.
45. **Tavares, L., C. Roneker, K. Johnston, S. Nusinoff-Lehrman, and F. De Noronha.** 1987. 3′-Azido-3′-dexoxythymidine in feline leukemia virus-infected cats: a model for therapy and prophylaxis of AIDS. Cancer Res. **47:**3190–3194.
46. **Thomas, P. A., K. Lubin, J. Milberg, R. Reiss, J. Getchell, and R. Enlow.** 1987. Cohort comparison study of children whose mothers have acquired immunodeficiency syndrome and children of well inner city mothers. Pediatr. Infect. Dis. J. **6:**247–251.
47. **Thongcharoen, P., C. Wasi, S. Louisirirotchanakul, J. Parry, J. Connell, and P. Mortimer.** 1992. Immunoglobulin G antibody capture enzyme-linked immunosorbent assay: a versatile assay for detection of anti-human immunodeficiency virus type 1 and 2 antibodies in body fluids. J. Clin. Microbiol. **30:**3288–3289.
48. **Van de Perre, P., A. De Clercq, J. Cogniaux-Leclerc, D. Nzaramba, J.-P. Butzler, and S. Sprecher-Goldberger.** 1988. 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.
49. **Wolff, H., K. Mayer, G. Seage, J. Politch, C. R. Horsburgh, and D. Anderson.** 1992. A comparison of HIV-1 antibody classes, titers, and specificities in paired semen and blood samples from HIV-1 seropositive men. J. Acquired Immun. Defic. Syndr. **5:**65–69.
50. **World Health Organization.** 1990. Acquired immunodeficiency syndrome (AIDS). Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2, and HTLV-I/HTVL-II. Weekly Epidemiol. Rec. **65:**281–288.