

Cervicovaginal Neutralizing Antibodies to Herpes Simplex Virus (HSV) in Women Seropositive for HSV Types 1 and 2

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Antibodies to herpes simplex virus type 1 (HSV-1) and HSV-2 of the immunoglobulin G (IgG) and IgA isotypes were detected in the cervicovaginal secretions (CVS) of 77 HSV-1- and HSV-2-seropositive but clinically asymptomatic African women by type-specific enhanced chemiluminescence Western blotting (ECL-WB). Of the 77 subjects, 34 were HIV negative, shedding HSV-2 DNA in their genital secretions; 20 were HIV positive, shedding HSV-2 DNA; and 23 were HIV negative, not shedding HSV-2 DNA. HSV-specific IgG was detected in CVS of nearly 70% of the women studied. HSV-specific IgA was found in CVS of 50% of the women studied. The distribution of CVS HSV-specific antibodies to each HSV type was highly heterogeneous, with a slight predominance of detectable IgG to HSV-1 (59%) over IgG to HSV-2 (41%), whereas the frequency of detectable IgA to HSV-1 (39%) was similar to that of IgA to HSV-2 (36%). The presence of detectable HSV-specific antibodies was inversely associated with HSV-2 DNA genital asymptomatic shedding but was not affected by HIV seropositivity. In addition, 13 of 77 (17%) CVS samples showed neutralizing activity against HSV-2, as assessed by an HSV-2 in vitro infectivity reduction assay. Neutralizing activity in CVS was associated with the presence of IgG and/or IgA antibodies to HSV-1 and/or to HSV-2 by ECL-WB. Among women whose CVS showed HSV-2-neutralizing activity, the specific activity of HSV-specific neutralizing antibodies was substantially (fivefold) higher in HSV-2 DNA shedders than in nonshedders. In conclusion, HSV-specific antibodies are frequently detected in CVS of asymptomatic African women seropositive for HSV-1 and HSV-2. A subset of these women had functional neutralizing activity against HSV-2 in their CVS. The origin of these antibodies and their role in HSV-2 disease of the female genital tract remain to be determined.

Herpes simplex virus type 2 (HSV-2) establishes latent infection in the dorsal root ganglion following infection at a genital or anal mucocutaneous surface. Intermittent HSV reactivation results in the production of infectious HSV with or without clinical symptoms. Asymptomatic or subclinical genital shedding of HSV-2 is detected in 55% of immunocompetent females and 81% of males according to daily sampling studies in the United States (32, 33). Cross-sectional studies with limited sampling have shown 20 to 40% HSV subclinical shedding rates in HIV-seronegative as well as HIV-infected women living in sub-Saharan Africa (19, 22).

The factors that affect whether recurrent HSV shedding from the genital mucosa is subclinical or symptomatic are not well understood. Specific immune responses against HSV occurring at the level of genital mucosa are likely to play a role (26). Both cell-mediated and humoral immune factors have been described in the genital tract during active genital herpes

episodes in humans (1, 2, 14, 16, 17, 21, 23) and in the mouse model of vaginal infection with an attenuated HSV-2 strain (20, 24). Women with symptomatic genital herpes have antibodies to HSV-2 of both the immunoglobulin A (IgA) and IgG isotypes in cervicovaginal secretions (CVS) (2, 21). The association of these antibodies with subclinical HSV excretion and their possible function remain poorly understood.

The aim of this study was to detect HSV antibodies and neutralizing activity in CVS of women seropositive for both HSV-1 and HSV-2 and to stratify the CVS HSV-2-specific antibody activity according to their HSV-2 DNA genital shedding status. We observed HSV-2-specific antibodies in the CVS of a high proportion of HSV-2-seropositive women. Antibody detection was inversely associated with viral shedding. Furthermore, in vitro neutralizing activity was detected in 17% of CVS samples and was associated with the presence of HSV-specific antibodies.

MATERIALS AND METHODS

Study population and sample processing. Women (mean age, 27 years; range, 15 to 48 years) attending the Centre National de Référence des Maladies Sexuellement Transmissibles et du SIDA (CNRMST/SIDA) of Bangui, the capital city of the Central African Republic, during the period July to October 1998 were recruited for a study of human immunodeficiency virus type 1 (HIV-1) RNA and HSV-2 DNA genital shedding, as previously reported (19). After verbal informed consent to participate was given, each of the 213 participants underwent general

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and genital examination. HIV testing and routine biological tests for the diagnosis of treatable sexually transmitted diseases (STD) were also carried out. A 7-day follow-up appointment was then arranged, and free appropriate STD treatment was provided. Women wishing to know their HIV serostatus received counseling at the voluntary counseling and testing unit of the CNRMST/SIDA. HIV-1-infected women belonged to the A1 ($n = 13$) and A2 ($n = 7$) categories of the Centers for Disease Control and Prevention classification for HIV infection. None received antiretroviral therapy, and none was pregnant at the time of sampling.

Ethical approval was given by the London School of Hygiene and Tropical Medicine, London, United Kingdom. Verbal informed consent was obtained from all participants.

Eighty-four women were selected because they had no sign of cervicitis or active STD infection at the time of enrollment and because their CVS samples were free of hemoglobin (Hb) traces and semen contamination. Of the 84 eligible women, 77 were selected because they had evidence of HSV-2 infection by either HSV-2 type-specific serum antibodies or detection of HSV-2 DNA in their CVS. All 77 women were found to be infected with both HSV-1 and HSV-2.

Samples for laboratory testing. Peripheral blood samples were taken, and aliquots of plasma separated from EDTA-anticoagulated blood were stored at -30°C before shipment to the United Kingdom for HSV serology. Serum IgG antibodies to HSV-1 and HSV-2 were detected by in-house competitive type-specific gG-based enzyme-linked immunosorbent assay (ELISA) at the Central Public Health Laboratory Services, Colindale, United Kingdom, as described previously (13).

During genital examination, CVS samples were collected by standardized nontraumatic 60-s vaginal washing with 3 ml of phosphate-buffered saline (PBS) (6). After centrifugation of the CVS at $1,000 \times g$ for 10 min, the cell supernatant and the cellular pellet were collected, aliquoted, and stored separately at -30°C . CVS samples were confirmed to be free of significant amounts of semen, as assessed by a negative test for prostatic specific antigen (PSA IMX system; Abbott Laboratories, Chicago, Ill.) (19). Traces of Hb in vaginal secretions were detected by spectrophotometry (threshold of sensitivity, 10 mg/ml) of the acellular fraction of genital fluids, as described previously (29).

Concentrations of CVS IgA and IgG. CVS levels of total Ig of unknown specificity of the IgA and IgG classes were measured by capture ELISA with goat anti-human Fc- α conjugated with peroxidase (Pierce, Rockford, Ill.) or goat anti-human Fc- γ conjugated with peroxidase (Pierce), as described previously (27), with a positivity threshold of 0.5 $\mu\text{g}/100 \mu\text{l}$.

Detection of CVS HSV-specific antibodies. Cell-free fractions of CVS were tested for IgA or IgG antibodies to HSV-1- or HSV-2-infected cell-protein mixtures by enhanced chemiluminescence Western blotting (ECL-WB) as described in detail in reference 10. In brief, proteins from diploid fibroblasts infected with HSV-1 (strain E115) or HSV-2 (strain 333) were separated by polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride (PVDF; Immobilon P; Millipore, Bedford, Mass.) as described previously (10). Strips were blocked with 0.5% Tween 20 in PBS for 1 h. CVS samples (25 μl) were diluted 1:40 in 4% goat serum (in PBS) and incubated overnight on strips. After washing, goat anti-human antibody conjugated to peroxidase (Boehringer Mannheim, Indianapolis, Ind.) was added. The ECL-WB detection system (Amersham, Arlington Heights, Ill.) was added per the manufacturer's instructions for 1 min. Treated strips were exposed to X-ray film (Hyperfilm-ECL; Amersham) for 30 s (IgG) or 2 min (IgA), and then the film was developed in a Kodak X-Omat processor.

Neutralizing activity of CVS HSV-specific antibodies. An HSV-2 infectivity inhibition assay was performed, as previously described (3), with slight modifications as follows. Duplicate portions of the acellular factor of each CVS were serially diluted twofold in 100 μl of Eagle's minimal essential medium (EMEM) with 2% fetal bovine serum (FBS) from 1:4 to 1:128 in 96-well plates (Falcon, Becton Dickinson Labware, Lincoln Park, N.J.). HSV-2 strain 333 was adjusted to 3.2×10^3 50% tissue culture infective doses (TCID₅₀)/ml, and 100 μl was added to each well. In addition, virus stock diluted twofold from 3.2×10^3 TCID₅₀/ml to 1×10^2 TCID₅₀/ml was added in duplicate to wells containing 100 μl of EMEM-2% FBS to generate a virus dilution curve. After 1 h of incubation at 37°C , the virus and CVS sample mixtures and the diluted virus were transferred to wells containing confluent monolayers of ELVIS cells (Diagnostics Hybrids, Inc., Athens, Ohio) (30). Plates were incubated overnight at 37°C with 5% CO₂. Cells were washed with PBS (pH 7.2) and then lysed with 100 μl of a buffered 1% NP-40 solution. Induced β -galactosidase activity secondary to HSV infection was detected by adding 135 μl of 3 mM ONPG (*o*-nitrophenyl α -D-galactopyranoside) (Sigma, St. Louis, Mo.) in a mixture of 0.1 M sodium phosphate (pH 7.5), 1 mM MgCl₂, and 45 mM β -mercaptoethanol. Absorbance was

read as the optical density at 415 nm (OD₄₁₅) on a Biotek EL340 plate reader (Winooski, Vt.). OD values were further processed with Biotek KC4 software.

To determine the extent of HSV-2 infectivity inhibition by the diluted secretions, OD values were first adjusted for background by subtracting the mean OD from six uninfected cell wells. Units of virus infectivity were interpolated from the virus dilution curve and expressed as neutralizing activity units (NAU).

The specific activities of HSV-specific neutralizing antibodies were calculated as the ratios of HSV-2 NAU to the CVS concentrations of total IgA plus total IgG in 100 μl of cell-free CVS. We used the assumption that the concentration of total IgM in CVS is much lower than those of IgA plus IgG, as previously observed in African women whatever their HIV serological status (5).

HSV DNA detection. HSV DNA was detected in the acellular fraction of CVS by in-house PCR for a conserved 290-bp segment of the DNA polymerase gene of HSV-1 and HSV-2 (12). Hybridization of PCR products was further carried out by DNA enzyme immunoassay (DEIA) according to the manufacturer's instructions (Gen-Eti-K; Sorin Biomedica, Saluggia, Italy) by using a single-stranded biotinylated HSV probe (5'-GTCTCACC GCCGAACTGAG-3'; positions 3109 to 3128) as previously described (19). The quantity of amplicons for HSV was assessed by the OD₄₅₀ of hybridized products as previously described (18). DEIA hybridization allowed semiquantitation of HSV DNA target. In this method, there was a linear association between ODs from the cutoff point (OD = 0.150) to 1.5 and the number of HSV DNA copies, ranging from 1 to 1,000 copies, respectively. ODs of 1.5 to 3 followed a nonlinear relationship. Strain differentiation was performed by restriction fragment length polymorphism as described previously (31).

Statistical analysis. Quantitative results are expressed as means \pm standard errors. The nonparametric Mann-Whitney U test was used to compare the specific activities of neutralizing antibodies between HSV-2 shedders versus nonshedders. Fisher's exact test was used to compare the frequencies of detectable HSV-specific antibodies among groups. Correlation between the distribution of the specific activities of CVS HSV-specific neutralizing antibodies and CVS levels of HSV-2 DNA was assessed with Spearman's rank order test.

RESULTS

Study subjects. Three groups of women were classified according to their HIV serostatus and their HSV-2 DNA genital shedding status as follows. Group I comprised 34 HSV-2-seropositive, HIV-seronegative women who all had HSV-2 DNA in their genital secretions. Group II comprised 20 HSV-2- and HIV-1-seropositive women who were shedding HSV-2 DNA. Group III comprised 23 HSV-2-seropositive, HIV-seronegative women without detectable HSV-2 DNA in their genital secretions. None of the study women had oral or genital signs or symptoms of herpes at the time of sampling.

CVS total IgG and IgA. Total IgG and IgA levels in CVS were measured to calculate the specific activities of HSV-neutralizing activity in CVS. Table 1 shows the higher concentrations of total IgG in CVS over those of total IgA regardless of HIV status or HSV-2 shedding. This is a well-established observation in HIV-positive and in HIV-seronegative women (5, 15). In the present series, the mean concentrations of IgG and IgA were not affected by either HIV infection or HSV-2 shedding.

CVS HSV-specific IgG and IgA. ECL-WB for IgG to HSV-1 and HSV-2 antibody was performed with samples from 75 of the 77 HSV-2-infected women; 68 subjects had sufficient CVS to perform ECL-WB testing for both IgG and IgA isotypes.

Fifty-two of 75 (69%) women tested had HSV-specific IgG in their CVS (Table 2). Seventeen of 34 (50%) of the HIV-seronegative women in group I (HSV-2 shedders) had IgG to HSV, mainly to HSV-1 (16 of 34 [47%]) and less frequently to HSV-2 (7 of 34 [21%]). Seventeen of 19 (89%) HIV-seropositive women in group II (HSV-2 shedders) had IgG. The frequencies of IgG to HSV-1 and IgG to HSV-2 were the same (13 of 19 [68%]). Finally, 18 of 22 (82%) of HIV-seronegative

TABLE 1. Levels of total IgG and IgA in CVS samples from 77 selected HSV-1- and HSV-2-seropositive African women living in Bangui, Central African Republic, classified according to HIV serostatus and shedding of HSV-2 DNA in CVS

Study group (<i>n</i>)	HSV-2 DNA shedding status	HIV status	Ig level ($\mu\text{g}/100 \mu\text{l}$) ^a	
			IgG	IgA
I (34)	Positive	Negative	16.1 \pm 7.2 (<0.5–227.5) ^f	1.4 \pm 0.5 (<0.5–13.0) ^c
II (20)	Positive	Positive	17.7 \pm 5.3 (<0.5–96.5)	2.2 \pm 0.5 (<0.5–7.6)
III (23)	Negative	Negative	13.1 \pm 0.6 (<0.5–59.0)	1.2 \pm 0.9 (<0.5–42.0)

^a Values are means \pm standard errors (with range in parentheses).

^b As assessed by qualitative PCR for HSV DNA polymerase gene.

^c Threshold of IgG or IgA detection, 0.5 $\mu\text{g}/100 \mu\text{l}$. Values below the threshold of measurement were arbitrarily taken as zero for calculations.

women in group III (HSV-2 nonshedders) had IgG to HSV-1 (15 of 22 [68%]) or to HSV-2 (11 of 22 [50%]).

Overall, 34 of 68 (50%) women tested had HSV-specific IgA in their CVS. Eleven of 31 (35%) of the group I women had IgA to HSV—mainly to HSV-1 (10 of 31 [32%]) and less frequently to HSV-2 (5 of 33 [16%]). Eleven of 16 (69%) of the group II women had IgA: 9 of 16 (56%) to HSV-1 and 10 of 16 (63%) to HSV-2. Twelve of 21 (57%) of the group III women had IgA: 8 of 21 (38%) to HSV-1 and 10 of 21 (48%) to HSV-2.

Only 20 of 53 (38%) HSV-2 shedders had HSV-2-specific IgG in their CVS compared with 11 of 22 (50%) nonshedders (not significant). Similarly, only 15 of 47 (32%) HSV-2 shedders had HSV-2-specific IgA in their CVS compared with 10 of 21 (48%) nonshedders (not significant). Thus, the presence versus absence of detectable IgG or IgA antibodies binding to HSV-2 did not appear to be closely linked to HSV-2 DNA shedding. However, if only HIV-negative women were compared, a significantly lower proportion of shedders (7 of 34 [21%]) than nonshedders (11 of 22 [50%]) had CVS IgG to HSV-2 ($P < 0.05$).

Fourteen of 53 (26%) HSV-2 shedders (10 in group I and 4 in group II) had only IgG to HSV-1 in their CVS; 7 of 49 (15%) HSV-2 shedders (6 in group I and 1 in group II) showed only IgA to HSV-1 in their CVS. Similar frequencies of the unique presence of detectable IgG to HSV-1 (7 of 22 [31%]) or IgA to HSV-1 (2 of 21 [10%]) were observed in women belonging to group III. As with CVS IgG to HSV-2, HIV-negative shedders had a significantly lower frequency of IgA to HSV-2 (5 of 31 [16%]) than did HIV-negative nonshedders (10 of 21 [48%]) ($P < 0.05$).

Overall, the frequency of detectable IgG to HSV-1 in CVS (44 of 75 [59%]) was slightly higher than that of detectable IgG to HSV-2 (31 of 75 [41%]) ($P < 0.05$), whereas the frequency

of detectable IgA to HSV-1 (27 of 68 [40%]) was similar to that of IgA to HSV-2 (25 of 68 [37%]). Furthermore, the frequency of detectable IgG to HSV-1 in CVS secretions of the study population (44 of 75 [59%]) was higher than that of detectable IgA to HSV-1 (27 of 68 [40%]) ($P = 0.02$). The frequency of detectable CVS IgG to HSV-2 was similar to that of detectable CVS IgA to HSV-2.

Finally, Table 3 depicts a wide variety in the distribution of CVS IgA and IgG antibodies to HSV-1 and HSV-2. The most obvious differences were in detection of antibodies to HSV-1 versus detection of antibodies to HSV-2. Several women had only IgG to HSV-1 ($n = 8$). Nine had both IgG and IgA to HSV-1 without antibodies to HSV-2, and 17 had IgG and IgA to both HSV-1 and HSV-2. Fifteen women had detectable HSV-specific IgG, but no IgA to either virus.

HSV-neutralizing activity. HSV-neutralizing activity was detected in the CVS of 13 women, including 10 of 54 (19%) HSV-2 DNA shedders and 3 of 23 (13%) HSV-2 nonshedders (Table 4). Twenty-five percent (5 of 20) of HIV-seropositive women shedding HSV-2 had detectable neutralizing activity in their CVS, compared to 14% (8 of 57) of HIV-seronegative women. Thus, the presence of neutralizing CVS activity against HSV was not greatly affected by HIV status or HSV-2 DNA shedding.

Neutralizing activity most often occurred in CVS that had detectable binding of antibody to HSV. Thus, 12 CVS samples with neutralizing activity had at least HSV IgG by ECL-WB, whereas only 1 sample lacked detectable IgG or IgA to HSV (Table 3). Ten of 12 CVS samples with neutralizing activity had both IgA and IgG to HSV-2, and 11 of 12 had IgG to HSV-2 (Table 3). These data provide suggestive evidence that at least a portion of HSV-2-neutralizing activity resides in the HSV-specific antibody fraction of CVS.

Among the women with positive tests for HSV-2 neutraliza-

TABLE 2. Detection of HSV-1-specific and HSV-2-specific IgG or IgA by ECL-WB in CVS samples from 75 selected African women living in Bangui, Central African Republic^a

Study group (<i>n</i>)	HSV-2 DNA shedding status	HIV status	No. with Ig detected/total ^b					
			CVS IgG to:			CVS IgA to:		
			HSV-1	HSV-2	HSV-1 and HSV-2	HSV-1	HSV-2	HSV-1 and HSV-2
I (34)	Positive	Negative	10/34	1/34	6/34	6/31	1/31	4/31
II (20)	Positive	Positive	4/19	4/19	9/19	1/16	2/16	8/16
III (23)	Negative	Negative	7/22	3/22	8/22	2/21	4/21	6/21

^a All women were seropositive for HSV-1 and HSV-2.

^b As assessed by ECL-WB. The denominator indicates the number of CVS lavage samples tested.

TABLE 3. Distribution of antibodies to HSV-1 and HSV-2 of the IgG and IgA isotypes as evidenced by type-specific ECL-WB and HSV-specific neutralizing activity in CVS lavage samples from 77 selected African women

IgA antibody type	No. of samples with indicated IgG antibody status ^a					Total no. of samples
	Negative	HSV-1 specific	HSV-2 specific	HSV-1 and HSV-2 specific	Not determined	
Negative	19 (1)	8	4	3 (1)	0	34
HSV-1 specific	0	9	0	0	0	9
HSV-2 specific	0	3	2 (2)	2	0	7
HSV-1 and HSV-2 specific	0	0	1 (1)	17 (7)	0	18
Not determined	4	1	0	2 (1)	2	9
Total	23	21	7	24	2	77

^a Numbers in parentheses represent numbers of CVS lavage samples harboring HSV-specific neutralizing activity.

tion, the specific activity of HSV neutralization in CVS was substantially higher in both HIV-seronegative and HIV-seropositive HSV-2 DNA shedders (mean ± standard error, 53.8 ± 17.5) than in HSV-2 DNA nonshedders (mean ± standard error, 9.6 ± 6.7) (Table 4) (*P* < 0.04). Moreover, the distribution of the specific activities of CVS HSV-specific neutralizing antibodies and CVS levels of HSV-2 DNA (as estimated from the OD₄₅₀ of hybridized HSV PCR products) in the 10 women shedding HSV-2 DNA in their CVS showed a highly significant correlation (*r*² = 0.91; *P* < 0.007). The higher the relative level of DNA, the higher the specific activity of neutralizing activity to HSV-2 (data not shown).

DISCUSSION

In this study, only African women infected with both HSV-1 and HSV-2 were selected for genital antibody studies. Rates of HSV-1 and HSV-2 seroprevalence are very high in Central Africa. HSV-1 is usually acquired in early childhood. HSV-2 is highly endemic in this population and is generally acquired after sexual debut (19). All study women were closely examined to exclude those with herpes-related lesions, and none was treated with antiherpetic or antiretroviral drugs. Subjects had no evidence of genital infection with other STDs or clinically apparent genital inflammation. Specimens with semen traces were excluded so that our data were not affected by vaginal contamination with HSV or antibodies from a male sexual partner. These inclusion criteria allowed for an evaluation of genital samples in women shedding or not shedding HSV-2 and with only HIV infection as a possible confounder of local antibody.

HSV-specific binding antibodies were detected in CVS by a highly sensitive Western blot (ECL-WB) method at rates of nearly 70% (IgG) and 51% (IgA). These observations confirm

previous reports of IgG and IgA to HSV in genital secretions (1, 2, 9, 14, 21, 25). Furthermore, as we have reported previously for women from the United States, HSV-1-specific antibodies, as well as HSV-2 antibodies, were readily detected in CVS (1).

The distribution of CVS HSV-specific antibodies by reference to the virus types was highly heterogeneous, with a slight but statistically insignificant predominance of IgG to HSV-1 over IgG to HSV-2, whereas the frequency of detectable IgA to HSV-1 was similar to that of IgA to HSV-2. These observations may reflect higher titers of transudated serum IgG to HSV-1 and higher levels of mucosal, dimeric IgA to HSV-2. By Western blotting, persons with dual positivity nearly always have a clear predominance of HSV-1 antibodies. Much of the IgG and monomeric IgA in CVS is likely from serum transudation, as has been suggested by studies of cervical antibodies appearing to parenteral HSV subunit vaccines (4) and by studies of antibody responses to other pathogens (5, 8). The relatively higher frequency of IgA to HSV-2 than to HSV-1 suggests that either (i) more monomeric IgA to HSV-2 is produced and transduced from sera than occurs with HSV-1 or (ii) IgA is produced locally in responses to HSV-2 shedding. Our studies did not discriminate monomeric from dimeric IgA in CVS, nor did we compare serum versus CVS profiles of protein targets of IgG or IgA. Previous studies have demonstrated identical IgG profiles in the two compartments and distinctly different IgA profiles, implying some level of local IgA production (2). It should be mentioned that we cannot rule out the possibility that HSV-2-specific IgG is also be produced locally at the genital level (7) in response to local replication of HSV-2.

HSV shedders were less likely than nonshedders to have either IgG or IgA to HSV-2 in CVS. This could be explained

TABLE 4. Distribution of CVS lavage samples harboring HSV-specific neutralizing activity and estimation of specific activities of HSV-specific neutralizing CVS antibodies in 77 selected African women living in Bangui, Central African Republic

Study group (n)	HSV-2 DNA shedding status	HIV status	No. (%) of CVS harboring HSV-neutralizing activity ^a	HSV-neutralizing activity (NAU range)	Sp act of HSV-neutralizing antibody (µg/100 µl) ^b
I (34)	Positive	Negative	5 (15)	252–1,935	17.3 (1.1–171.3)
II (20)	Positive	Positive	5 (25)	503–2,117	33.6 (17.9–100.4)
III (23)	Negative	Negative	3 (13)	435–522	3.1 (0.8–3.6)

^a As assessed by reduction of infectivity in BHKIP6LacZ-5 (ELVIS) cells.

^b Median and range (in parentheses) of specific activities calculated as NAU/(total IgG + total IgA). The medians were calculated for only CVS harboring HSV-neutralizing activity.

by in vivo binding between type-specific antibodies and virus being shed into genital secretions. Such an event would prevent antibody binding in our ECL-WB test but not affect DNA detection by PCR. As a group, HIV-infected women had the highest frequency of HSV IgG in CVS. This might reflect higher antibody titers or greater transudation of serum antibody (5).

Neutralizing activity against HSV was demonstrated in 17% of CVS samples: nearly always (92%) in the presence of detectable antibody binding to HSV-1 or HSV-2. The fact that most CVS with HSV IgG or IgA lacked neutralizing activity may be due to insufficient sensitivity of the neutralizing assay due to inherently low local antibody titers compounded by the dilution effect of CVS lavage (4, 27).

The single CVS with neutralizing activity that lacked binding antibodies may reflect the presence of nonimmune inhibitory factors in CVS. Alternatively, neutralization could be mediated by F(ab) fragments directed to HSV, since CVS fluid contains large amounts of F(ab) derived from locally produced secretory IgA or from IgG (15). Such fragments would not be bound efficiently by the secondary antibody in the ECL-WB assay.

The specific activity of HSV-specific neutralizing activity was fivefold higher in HSV-2 DNA shedders than in nonshedders, a finding that appears contradictory to the lower frequency of IgG or IgA to HSV-2 in HSV-2 shedders than nonshedders. Our data might reflect the fact that HSV-1 and HSV-2 antibodies are highly cross-reactive (11, 28). In our assay, HSV-1 antibodies are very effective at neutralizing HSV-2 (3). Most of the CVS (9 of 13 [69%]) with neutralizing activity also had CVS antibodies to HSV-1.

A direct relationship was observed between the specific activity of CVS neutralizing antibodies to HSV-2 and the level of HSV-2 DNA in the CVS. One interpretation of these data is that HSV-2 shedding in the genital tract induces production of HSV-specific neutralizing antibodies. Prospective studies that track CVS antibodies, neutralization of HSV shedding, and symptoms over time would be useful to prove a cause-effect hypothesis. Analyses to identify bound antibodies, to discriminate dimeric versus monomeric IgA, and to compare serum and CVS antibody profiles to individual viral proteins would be critical to interpreting such prospective data.

Our previous studies have demonstrated HSV-specific responses to genital infections (1, 2) and to vaccines (4). The in vitro and in vivo functions of the antibodies in genital secretions are ill defined. Further prospective evaluations of virus shedding, including genital, humoral, and cellular immune responses to HSV-2, and of the function of HSV-2-specific IgG and IgA (monomeric and dimeric) in CVS are needed to assess the possible role of local antibody in HSV-2 genital shedding in women.

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REFERENCES

- Ashley, R., A. Wald, and L. Corey. 1994. Cervical antibodies in patients with oral herpes simplex virus type 1 (HSV-1) infection: local anamnestic responses after genital HSV-2 infection. *J. Virol.* **85**:5284-5286.
- Ashley, R. L., L. Corey, J. Dalessio, P. Wilson, M. Remington, G. Barnum, and P. Trethewey. 1994. Protein-specific antibody responses to primary genital herpes simplex virus type 2 infections. *J. Infect. Dis.* **170**:20-26.
- Ashley, R. L., J. Dalessio, and R. E. Sekulovich. 1997. A novel method to assay herpes simplex virus neutralizing antibodies using BHKICP6LacZ-5 (ELVIS) cells. *Viral Immunol.* **4**:213-220.
- Ashley, R. L., F. M. Crisostomo, M. Doss, R. E. Sekulovich, R. L. Burke, M. Shaughnessy, L. Corey, N. L. Polissar, and A. G. Langenberg. 1998. Cervical antibody responses to a herpes simplex virus type 2 glycoprotein subunit vaccine. *J. Infect. Dis.* **178**:1-7.
- Belec, L., T. Dupré, T. Prazuck, C. Tévi-Bénissan, J. M. Kanga, O. Pathy, X. S. Lu, and J. Pillot. 1995. CVS overproduction of specific IgG to human immunodeficiency virus (HIV) contrasting with normal or impaired local response in HIV infection. *J. Infect. Dis.* **172**:691-697.
- Belec, L., D. Meillet, M. Levy, A. J. Georges, C. Tevi-Bénissan, and J. Pillot. 1995. Dilution assessment of CVS secretions obtained by vaginal washing for immunological assays. *Clin. Diagn. Lab. Immunol.* **2**:57-61.
- Berneman, A., L. Belec, V. A. Fischetti, and J. P. Bouvet. 1998. The specific patterns of human immunoglobulin G antibodies in serum differ from those in autologous secretions. *Infect. Immun.* **66**:4163-4168.
- Brandtzaeg, P. 1992. Humoral immune response patterns of human mucosae: induction and relation to bacterial respiratory tract infections. *J. Infect. Dis.* **165**(Suppl. 1):S167-S176.
- Coughlan, B. M., and G. R. Skinner. 1977. Antibody activity to type 1 and type 2 herpes simplex virus in human cervical mucus. *Br. J. Obstet. Gynaecol.* **84**:622-629.
- Dalessio, J., and R. L. Ashley. 1992. Highly sensitive enhanced chemiluminescence immunodetection method for herpes simplex virus type 2 Western immunoblot. *J. Clin. Microbiol.* **4**:1005-1007.
- Eberle, R., and R. J. Courteney. 1989. Topological distribution of virus-specific and cross-reactive antigenic determinants on the gB glycoprotein of herpes simplex viruses. *J. Med. Virol.* **27**:309-316.
- Espy, M. J., J. Aslanzadeh, and T. F. Smith. 1993. PCR detection of herpes simplex virus DNA sequences in cerebrospinal fluid, p. 332-336. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), *Diagnostic molecular microbiology: principles and applications*. American Society for Microbiology, Washington, D.C.
- Gopal, R., T. Gibbs, M. J. Slomka, J. Whitworth, L. M. Carpenter, A. Vyse, and D. W. Brown. 2000. A monoclonal blocking EIA for herpes simplex virus type 2 (HSV-2) antibody: validation for seroepidemiological studies in Africa. *J. Virol. Methods* **87**:71-80.
- Gronroos, M., and E. Honkonen. 1983. Cervical and serum IgA and serum IgG antibodies to *Chlamydia trachomatis* and herpes simplex virus in threatened abortion: a prospective study. *Br. J. Obstet. Gynecol.* **90**:167-170.
- Hocini, H., A. Barra, L. Belec, S. Iscaki, J. L. Preud'homme, J. Pillot, and J. P. Bouvet. 1995. Systemic and secretory humoral immunity in the normal human vaginal tract. *Scand. J. Immunol.* **42**:269-274.
- Koelle, D. M., M. Schomogyi, and L. Corey. 2000. Antigen-specific T cells localize to the uterine cervix in women with genital herpes simplex virus type 2 infection. *J. Infect. Dis.* **182**:662-670.
- Koelle, D. M., H. B. Chen, M. A. Gavin, A. Wald, W. W. Kwok, and L. Corey. 2001. CD8 CTL from genital herpes simplex lesions: recognition of viral tegument and immediate early proteins and lysis of infected cutaneous cells. *J. Immunol.* **166**:4049-4058.
- Mantero, G., A. Zonaro, A. Albertini, P. Bertolo, and D. Primi. 1991. DNA enzyme immunoassay: general method for detecting products of polymerase chain reaction. *Clin. Chem.* **37**:422-429.
- Mbopi-Keou, F. X., G. Gresenguet, P. Mayaud, H. A. Weiss, R. Gopal, M. Matta, J. L. Paul, D. W. Brown, R. J. Hayes, D. C. Mabey, and L. Belec. 2000. Interactions between herpes simplex virus type 2 and HIV infection in African women: opportunities for intervention. *J. Infect. Dis.* **182**:1090-1096.
- McDermott, M. R., L. J. Brais, and M. J. Eveleigh. 1990. Mucosal and systemic antiviral antibodies in mice inoculated intravaginally with herpes simplex virus type 2. *J. Gen. Virol.* **71**:1497-1504.
- Merriman, H., S. Woods, C. Winter, A. Fahnlander, and L. Corey. 1984.

- Secretory IgA antibodies in CVS secretions from women with genital infection due to herpes simplex virus. *J. Infect. Dis.* **149**:505–510.
22. **Mostad, S. B., J. K. Kreiss, A. J. Ryncarz, K. Mandalia, B. Chohan, J. Ndinya-Achola, J. J. Bwayo, and L. Corey.** 2000. Cervical shedding of herpes simplex virus in human immunodeficiency virus-infected women: effects of hormonal contraception, pregnancy, and vitamin A deficiency. *J. Infect. Dis.* **181**:58–63.
 23. **Murphy, J. F., D. F. Murphy, S. Barker, M. L. Mylotte, B. M. Coughlan, and G. R. B. Skinner.** 1985. Neutralizing antibody against type 1 and 2 herpes simplex virus in cervical mucus of women suffering with intra-epithelial neoplasia. *Med. Microbiol. Immunol.* **174**:73–80.
 24. **Parr, E. L., and M. B. Parr.** 1997. Immunoglobulin G is the main protective antibody in mouse vaginal secretions after vaginal immunization with attenuated herpes simplex virus type 2. *J. Virol.* **71**:8109–8115.
 25. **Persson, E., P. Eneroth, and S. Jeansson.** 1988. Secretory IgA against herpes simplex virus in cervical secretions. *Genitourin. Med.* **64**:373–377.
 26. **Posavad, C. M., D. M. Koelle, and L. Corey.** 1998. Tipping the scales of herpes simplex virus reactivation: the important responses are local. *Nat. Med.* **4**:381–382.
 27. **Quesnel, A., S. Cu-Uvin, D. Murphy, R. L. Ashley, T. Flanigan, and M. R. Neutra.** 1997. Comparative analysis of methods for collection and measurement of immunoglobulins in cervical and vaginal secretions of women. *J. Immunol. Methods* **202**:153–161.
 28. **Rouse, B. T., and M. Gierynska.** 2001. Immunity to herpes simplex virus: a hypothesis. *Herpes* **8**(Suppl. 1):2A–5A.
 29. **Si-Mohamed, A., M. D. Kazatchkine, I. Heard, C. Goujon, T. Prazuck, G. Aymard, G. Cessot, Y. H. Kuo, M. C. Bernard, B. Diquet, J. E. Malkin, L. Gutmann, and L. Belec.** 2000. Selection of drug-resistant variants in the female genital tract of human immunodeficiency virus type 1-infected women receiving antiretroviral therapy. *J. Infect. Dis.* **18**:112–122.
 30. **Stabell, E. C., and P. D. Olivo.** 1992. Isolation of a cell line for rapid and sensitive histochemical assay for the detection of herpes simplex virus. *J. Virol. Methods* **38**:195–202.
 31. **Vogel, J. U., B. Weber, and H. W. Doerr.** 1994. Typing and strain differentiation of clinical herpes simplex virus type 1 and 2 isolates by polymerase chain reaction and subsequent fragment length polymorphism analysis. *Zentbl. Bakteriol.* **281**:502–512.
 32. **Wald, A., J. Zeh, S. Selke, R. L. Ashley, and L. Corey.** 1995. Virological characteristics of subclinical and symptomatic genital herpes infections. *N. Engl. J. Med.* **333**:770–775.
 33. **Wald, A., J. Zeh, S. Selke, T. Warren, R. Ashley, and L. Corey.** 2002. Genital shedding of herpes simplex virus among men. *J. Infect. Dis.* **186**(Suppl. 1):S34–S39.