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Infection of vaginal and colonic epithelial cells by the human immunodeficiency virus type 1 is neutralized by antibodies raised against conserved epitopes in the envelope glycoprotein gp120

(virus receptors/glycosphingolipids/peptide antisera/epithelium/mucosa)

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ABSTRACT **The rectal and genital tract mucosae are considered to be major sites of entry for the human immunodeficiency virus (HIV) during sexual contact. We now demonstrate that vaginal epithelial cells can be infected by HIV type 1 (HIV-1) via a mechanism similar to that described for neuroglial cells and, more recently, for colorectal epithelial cells, involving initial interaction of the HIV-1 envelope glycoprotein gp120 with a cell-surface glycosphingolipid (sulfated lactosylceramide). A hyperimmune serum against gp120 was able to neutralize HIV-1 infection of vaginal epithelial cells. Site-directed immunization was employed to identify sites on gp120 recognized by antibodies neutralizing HIV-1 infection of vaginal and colonic epithelial cells. Hyperimmune sera were raised in monkeys against a series of 40 overlapping synthetic peptides covering the entire sequence of HIV-1 (HTLV-IIIB) gp120. Antisera raised against five synthetic peptides, corresponding to three relatively conserved regions and to the hypervariable region (V3 loop), efficiently neutralized HIV-1 infection of human vaginal epithelial cells *in vitro*. Similar results were obtained with the colonic cells. Hyperimmune sera to all five peptides have been shown earlier to neutralize HIV-1 infectivity in CD4+ T cells. These results have obvious implications for the design of mucosal subunit vaccines against sexually transmitted HIV-1 infections.**

Transmission of the human immunodeficiency virus type 1 (HIV-1) occurs most commonly in Europe and North America during homosexual intercourse by exposure of the rectal mucosa to infected seminal fluid (1, 2). Heterosexual transmission of HIV-1 through the genital mucosa by infected seminal fluid or vaginal secretions is less common in Europe but is the main route of infection in Africa and Asia (3–5). Since the conventional route of parenteral injection of vaccines is poorly efficient at inducing mucosal immune responses, an alternative approach is to prevent the virus from gaining entry at the mucosal site of infection by inducing mucosal immunity in the genital tract and in the rectum.

Increasing importance has been ascribed to the epithelium of the lower gastrointestinal tract as a site of entry for the HIV-1 during receptive anal intercourse (6). The ability of HIV-1 to infect human colorectal epithelial cells has been demonstrated both *in vitro* (7–11) and *in vivo* (12, 13) and appears to involve initial interaction of a cell-surface glycosphingolipid receptor (sulfatide and galactosylceramide) with the envelope glycoprotein gp120 (14, 15) by a mechanism similar to that described for HIV-1 infection of neuroglial cells (16, 17). However, the site(s) of HIV-1 gp120 engaged in initial interaction of HIV-1 with colorectal epithelial cells (9–11) and neuroglial cells (18) does not appear to involve the CD4 binding domain of the protein.

We now report that HIV-1 can infect human vaginal epithelial cells via initial attachment to a cell-surface glycosphingolipid receptor. Furthermore, antisera raised in monkeys against a series of 40 overlapping synthetic peptides covering the entire amino acid (aa) sequence of gp120 (19) were examined for their capacity to neutralize HIV-1 infection of human vaginal and colonic cells.

MATERIALS AND METHODS

Cells. The human colonic adenocarcinoma cell line HT-29 (ATCC no. HTB-38) and the two vaginal epithelial cell lines, Hs 760.T (transformed, ATCC no. CRL-7491) and Hs 769.Vg (nontransformed, no. CRL-7499), employed in this study were purchased from the American Type Culture Collection. Twelve subclones were established from the colonic cell line HT-29 by culture under limiting dilutions in glucose- or galactose-containing medium (20). The differentiation characteristics of resulting clones were assessed by morphology and by indirect immunofluorescence staining of cell-surface sucrose isomaltase and carcinoembryonic antigen. ACH-2 cell line, a HIV-1 latent T-cell clone (21), was obtained from Franciesca Chiodi (Karolinska Institute, Stockholm).

HIV-1 Infection of Human Colonic and Vaginal Epithelial Cells. A HIV-1 infectious stock of HTLV-IIIB-infected H-9 lymphoblastoid T cells (22) was prepared as described earlier (19). Clones of HT-29 cell lines and vaginal cell lines (Hs 769.Vg and Hs 760.T) were seeded at 5×10^5 cells per well in flat-bottomed 24-well plates (Costar). Next, serial 10-fold dilutions of virus, ranging from 1 to 10^{-5} tissue culture 50% infective doses ($TCID_{50}$) per cell, were added and incubation was carried out for 2 hr at 37°C. Cells were then washed five times with prewarmed medium and allowed to grow for 7 days in the respective growth medium. At that time, virus production in cell-free supernatants was tested by p24 antigen assay with a commercial ELISA kit (HIVAG-1; Abbott) and reverse transcription (RT) nested PCR as described below. Cells were then washed five times with medium, incubated with prewarmed phosphate-buffered saline containing trypsin (1 mg/ml) for 5 min at 37°C to remove possible residual virus, and washed with serum-free RPMI medium. HIV-1

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Abbreviations: HIV-1, human immunodeficiency virus type 1; gp120, glycoprotein 120; m.o.i, multiplicity(ies) of infection; aa, amino acid(s); $TCID_{50}$, tissue culture 50% infective dose(s); RT-PCR, reverse transcription polymerase chain reaction.

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infection was assessed by coculture with H-9 cells and PCR as described below.

Rescue of HIV-1 from Epithelial Cells by Coculture. H-9 cells (10^6) were added to each well and cocultured with epithelial cells for 24 hr. Then nonresident lymphoid H-9 cells were separated from the epithelial cells by transfer of the overlay medium to new 24-well Costar plates. These H-9 cell cultures were then examined daily for formation of syncytia and supernatants were harvested on day 7 and assayed for levels of p24 antigen as described above.

Detection of HIV-1 RNA in Cell-Free Supernatant. To detect cell-free virus, RNA was extracted by the RNAzol (Biotecx Laboratories, Houston) method from 0.5 ml of supernatants collected 7 days after exposure to HIV-1. RNA was treated for 1 hr at 37°C with 10 units of RNase-free DNase I (Boehringer Mannheim) and cDNA was transcribed by 200 units of Moloney murine leukemia virus reverse transcriptase (Promega) with 10 pmol of downstream PCR primer (*env* 122, see below). The synthesized cDNA was amplified by nested PCR using HIV-1 *env* gene-specific primers. The primers employed for the first PCR were *env* 106 (5′-GAAGAA-GAGATAGTAATTAGATCT-3′, corresponding to *env* gene positions 790–813 of the WMJ2 strain sequence, Los Alamos; ref. 23) and *env* 122 (5′-GGTGGGTGCTACTCCTAATGGT-TCAATTC-3′, WMJ2 *env* 1439–1467). One-tenth of the amplified product was reamplified by PCR using the *env* 106 primer and *env* 120 (5′-CCTCATATTTCCTCCTCCAG-GTCT-3′, WMJ2 *env* 1375–1398). PCR amplification was done in a 100-μl reaction mixture consisting of 10 μl of 10× PCR buffer (Promega), 1.5 mM $MgCl_2$, 0.125 mM of dNTP, 0.5 unit of *Taq* DNA polymerase (Promega), and 20 pmol of each primer. Reagents were cycled 40 times, each cycle consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Amplified products were detected after electrophoresis on 2% agarose gels and stained with ethidium bromide. RNA samples were also amplified by the same protocol but omitting reverse transcriptase to control for DNA contamination.

Detection of Proviral DNA by PCR. Total DNA from epithelial cells was prepared by phenol/chloroform extraction and ethanol precipitation and subjected to amplification by PCR. In each sample, 200 ng of DNA (equal to DNA from 3×10^4 cells) was amplified with a HIV-1 *env* gene-specific primer pair. They were *env* 111 (5′-GTAACGCACAGTTT-TAATTGTGGAGGGGAA-3′, WMJ2 *env* gene positions 1096–1125) and the *env* 120. PCR amplification was done as above for RT nested PCR except that reagents were cycled 35 times and 5 μCi of [α-^{32}P]dCTP (1 Ci = 37 GBq) was incorporated into the amplified fragment. One-tenth of the amplified product was subjected to electrophoresis on a 5% polyacrylamide gel. The gel was dried and exposed for 16 hr to x-ray film (X-Omat; Kodak) with intensifying screens. As an internal standard, reaction mixtures were amplified in parallel with a primer pair specific for the human β-actin gene.

To estimate the average of HIV-1 proviral DNA copy number in each cell type, serial dilutions of DNA extracted from ACH-2 cells, assumed to contain one proviral DNA copy per cell (24), were prepared. The total amount of DNA in each dilution was normalized to 200 ng using DNA from H-9 cells and PCR was done as above. After electrophoresis and exposure to x-ray film, radioactivity of the amplified bands was measured by a scintillation counter (LS2800; Beckman). For each experiment, a standard curve was prepared to estimate HIV-1 DNA copy number (see Fig. 1).

Detection of HIV-1 RNA Expression by RT-PCR. Total cellular RNA extracted from epithelial cells was digested by RNase-free DNase I and 500 ng of RNA was reverse-transcribed to cDNA with 10 pmol of downstream PCR primers. Amplification of the cDNA was done by PCR with primer pairs specific for HIV-1 *tat/rev* gene to detect HIV-1 regulatory RNA and for a major splice donor site to detect HIV-1 structural RNA (25). [^{32}P]dCTP was incorporated into the amplified fragments and 1/10th of the amplified product was subjected to electrophoresis on an 8% polyacrylamide gel. The gel was dried and exposed to x-ray film. Total RNA extracts from ACH-2 cells and HIV-1-infected H-9 cells were similarly treated as positive controls. RNA without RT was used as a negative control to ensure that there was no contamination with residual HIV-1 DNA.

Binding of HIV-1 gp120 to Lipids. Colonic HT-29 L20 cells and vaginal Hs 769.Vg and Hs 760.T cells were extracted with chloroform/methanol/water, 4:8:3 (by volume), as described (26). The lipid extract was separated by ion-exchange chromatography (27) into neutral and acidic lipids. The neutral lipids were saponified to degrade phospholipids, desalted, and then applied to silica gel chromatograms. Lipids were eluted with 20 bed volumes of chloroform/methanol, 19:1 (vol/vol); 5 bed volumes of chloroform/methanol, 4:1 (vol/vol); 10 bed volumes of chloroform/methanol/water, 65:25:4 (by volume); and 10 bed volumes of chloroform/methanol/water, 30:60:20 (by volume). The acidic lipid fraction was dialyzed and then chromatographed on silica gels as above.

The thin-layer chromatography (TLC)-ELISA procedure used is a modification of a previously described procedure (28, 29). The lipid fractions described above were chromatographed on high-performance TLC plates using chloroform/methanol/0.25% KCl, 50:40:10 (by volume), as developing solvent. Standards consisted of sulfatide, sulfated lactosylceramide, lactosylceramide, galactosylceramide, and the sulfated glucouronic acid-containing glycolipid LK1 and were chromatographed on the same plates. The plates were then treated with polyisobutylmethacrylate and preincubated, to block unspecific binding, with Tris/BSA (50 mM Tris·HCl, pH 7.8/15 nM NaCl/1% bovine serum albumin). HIV-1 recombinant gp120 (0.1 μg/ml; kindly provided by S. Olofsson, Department of Clinical Virology, Göthenburg, Sweden) in Tris/BSA was overlaid onto the TLC plate. After incubation at room temperature for 4 hr, a mouse monoclonal antibody to gp120 (30) (F58/H3; kind gift of L. Åkerblom, BMC, Uppsala), diluted in the same buffer, was applied to the plate. After overnight incubation at room temperature, bound antibody was detected by stepwise exposure of the plate to alkaline phosphatase-conjugated anti-mouse immunoglobulin antibody (Dakopatts, Glostrup, Denmark) and 5-bromo-4-chloro-3-indolyl phosphate (Boehringer Mannheim).

Neutralization Assays. Serial 5-fold dilutions of hyperimmune serum raised in guinea pig by recombinant gp120 (kindly provided by L. Åkerblom) were added at a final dilution of 1:40 to the virus and incubated for 2 hr at 37°C. Serially diluted heat-inactivated (56°C, 60 min) monkey antisera raised against 40 peptides [17–29 aa long, overlapping each other by ≈50% (19) and designated gp120-1 to gp120-40] covering the entire amino acid sequence of HIV-1 gp120 were assayed in the same way. Starting serum dilutions of 1:10 were employed in these assays. The serum/virus mixture was then added to culture wells seeded with the epithelial cells and incubated for 2 hr at 37°C. Dilutions of stock virus corresponding to 0.02 and 1 $TCID_{50}$ per cell were employed for HT-29 L20 and Hs 760.T cells, respectively. Culture wells were washed twice with the respective growth medium. Seven days after exposure, wells were washed five times and treated with trypsin as described above.

A serum was considered to have neutralizing capacity when it yielded a reduction by at least 90% in p24 antigen production and syncytium formation in the subsequent coculture of epithelial cells with H-9 cells. Neutralizing activity was further ascertained when the serum caused a reduction by at least 75% in proviral DNA copy numbers in epithelial

cell cultures. Preimmune sera from the guinea pig and monkeys were used as negative controls in all experiments.

RESULTS

HIV-1 Infection of Human Vaginal and Colonic Epithelial Cells. Twelve subclones were established from the colonic epithelial cell line HT-29. Three subclones from the parental cell line displaying low differentiation characteristics and nine with high differentiation characteristics, as well as the two vaginal cell lines, Hs 769.Vg (nontransformed) and Hs 760.T (transformed), were infected with HIV-1 strain HTLV-IIIB at different multiplicities of infection (m.o.i.). Virus in cell-free supernatants was undetectable in the colonic and vaginal epithelial cell cultures determined by p24 antigen assay. HIV-1 infection could be demonstrated in the transformed vaginal cell line Hs 760.T both by cocultivation with H-9 cells and by demonstration of proviral DNA in cultures infected at the m.o.i. of 1 $TCID_{50}$ per cell (Fig. 1*A* and Table 1). In cultures of the nontransformed vaginal cell line Hs 769.Vg, HIV-1 infection was not detected by the coculturing technique but the cells were found to contain proviral DNA by PCR. The PCR assay, which could amplify 37.5 copies of HIV-1 DNA in control studies using ACH-2 cells (Fig. 1*B*), thus seemed to be more sensitive than the coculture method for detection of HIV-1 infection. HIV-1 infection could also be demonstrated in six differentiated colonic cell clones but in none of three undifferentiated clones, after cocultivation with H-9 cells. Most permissive for HIV-1 infection were colonic epithelial subclones L10, L14, and L20. In these cells HIV-1 could be demonstrated after infection at an m.o.i. of 0.01 $TCID_{50}$ per cell by cocultivation with H-9 cells. Subclone L20 was chosen for further experiments. The copy number of HIV-1 DNA in infected Hs 769.Vg cells and HT-29 L20 clone gradually decreased in parallel with the reduction of the m.o.i. These results indicated that the semiquantitative assay of HIV-1 DNA was relatively accurate. Not all epithelial cells in a culture seemed to be infected by HIV-1 since the amount of proviral DNA ranged between 10 and 0.125 copies per 100 cells (Table 1).

Both regulatory and structural viral RNAs were detected in the HIV-1-infected colonic HT-29 L20 clone as well as in the two vaginal cell lines, though the level of transcription in the epithelial cells was lower than that in ACH-2 cells and HIV-1-infected H-9 cells (Fig. 2*A*). However, viral progeny was undetectable in the cell-free supernatants of the infected vaginal or colonic epithelial cells even by use of the RT nested PCR method (Fig. 2*B*). About 50 copies of genomic HIV-1 RNA could be detected by this method in control studies using *in vitro* transcribed HIV-1 RNA standard (data not shown).

Table 1. Susceptibility of vaginal and colonic epithelial cells to infection by HIV-1 (HTLV-IIIB)

Cell line	Method*	m.o.i., $TCID_{50}$ per cell			
		1	10^{-1}	10^{-2}	10^{-3}
Hs 760.T	Coculture	+	–	–	–
	PCR	10†	–	–	–
Hs 769.Vg	Coculture	–	–	–	–
	PCR	2.5	0.6	0.5	0.125
HT-29 L20	Coculture	+	+	+	–
	PCR	4	2	0.5	0.125

*Coculture with H-9 cells and subsequent p24 antigen detection or detection of proviral DNA by PCR.
†Copy number per 10^2 cells.

Glycosphingolipid Content in Vaginal and Colonic Epithelial Cells. In initial experiments, binding assays employing radio- or enzyme-labeled vaccinia virus-derived recombinant HIV-1 envelope proteins demonstrated that gp120 could bind to adherent monolayers of colonic and vaginal epithelial cells and to glycolipid but not to protein fractions of crude membrane extracts from the same cells. The ability of recombinant gp120 to bind to lipids separated by TLC was tested. The HIV-1 envelope protein was demonstrated to bind only to sulfated galactosylceramide (sulfatide) and sulfated lactosylceramide but not to the nonsulfated forms of these glycosphingolipids—i.e., galactosylceramide or lactosylceramide used as standards (Fig. 3). There was no binding to the sulfated glucuronic acid-containing glycolipid LK1, a finding supporting the notion that there was no unspecific binding to any sulfated glycolipid. The gp120 binding structures in lipid extracts of the colonic HT-29 L20 cells in the TLC-ELISA described above were identified as sulfatide and sulfated lactosylceramide. However, we were unable to demonstrate the presence of sulfatide in extracts from vaginal Hs 760.T and Hs 769.Vg cells. The only gp120 binding glycolipid in

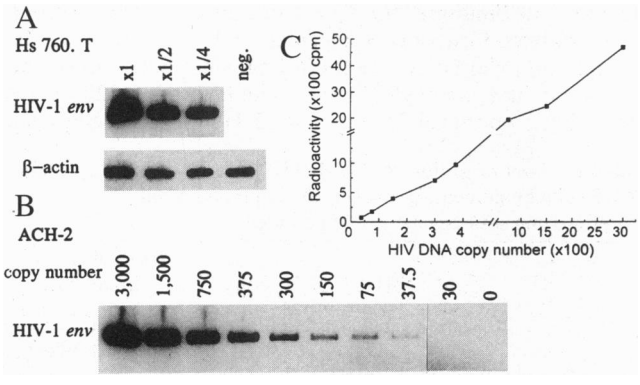

FIG. 1. Detection of HIV-1 DNA by PCR. (*A*) Serial dilutions (×1 to ×1/4) of DNA from HIV-1-infected vaginal epithelial cell line Hs 760.T (1 $TCID_{50}$ per cell) and DNA from H-9 cells (neg.) were amplified by PCR with HIV-1 *env*-specific primers. The total amount of DNA in each dilution was normalized to 200 ng (equal to DNA from 3×10^4 cells) using DNA from H-9 cells. Equal loading and quality of DNA were confirmed by PCR with β-actin primers. To estimate HIV-1 DNA copy number in infected epithelial cells, serial dilutions of DNA from ACH-2 cells, containing 0–3000 copies of HIV-1 DNA in 200 ng of cellular DNA, were amplified by PCR (*B*) and radioactivity of the amplified bands was plotted against HIV-1 DNA copy number (*C*).

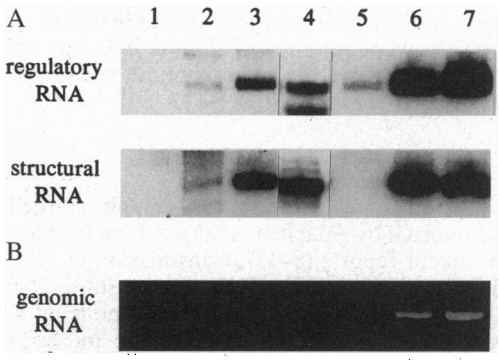

FIG. 2. (*A*) Detection of HIV-1 RNA expression by RT-PCR. Total cellular RNA was reverse-transcribed and amplified by primers specific for HIV-1 regulatory and structural genes. (*B*) Detection of genomic RNA in cell-free supernatants by RT nested PCR. Lane 1, H-9 cells (noninfected) as a negative control; lane 2, Hs 769.Vg cells (1 $TCID_{50}$ per cell); lane 3, Hs 760.T cells (1 $TCID_{50}$ per cell); lane 4, HT-29 L20 cells (1 $TCID_{50}$ per cell); lane 5, HT-29 L20 cells (0.1 $TCID_{50}$ per cell); lane 6, ACH-2 cells; lane 7, HIV-1-infected H-9 cells. Expression of HIV-1 structural RNA in HT-29 cells at an m.o.i. of 0.1 $TCID_{50}$ per cell (lane 5) is not seen in this figure but was detectable by a longer autoradiographic exposure.

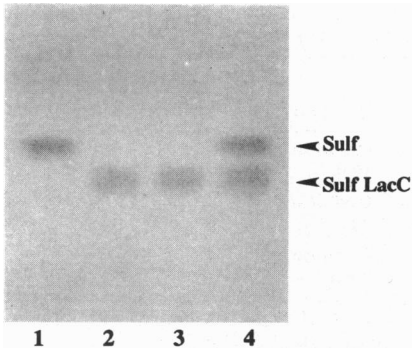

FIG. 3. TLC-ELISA showing binding of HIV-1 gp120 to glycosphingolipids. Purified sulfatide (Sulf) (lane 1) and sulfated lactosylceramide (Sulf LacC) (lane 2) served as standards. Chromatographed lipids were extracted from vaginal epithelial Hs 760.T cells (lane 3) and colonic epithelial HT-29 L20 cells (lane 4).

these vaginal epithelial cells was sulfated lactosylceramide (Fig. 3).

Cell-surface expression of CD4 as judged by flow cytometric analyses using CD4-reactive monoclonal antibodies could not be demonstrated on any of the epithelial cells used in the present study (data not shown).

Neutralization of HIV-1 Infection of Vaginal and Colonic Epithelial Cells by Antisera to Whole gp120 and gp120 Peptides. The guinea pig hyperimmune serum against recombinant gp120 neutralized HIV-1 infection of vaginal Hs 760.T and colonic HT-29 L20 subclone, determined by p24 antigen assay and inhibition of syncytium formation after cocultivation with H-9 cells and by PCR analysis of proviral DNA. To further analyze the sites on gp120 critically involved in antibody-mediated neutralization of HIV-1 in vaginal and colonic epithelial cells, 40 antisera from macaques immunized with overlapping peptides together covering the entire aa sequence of gp120 of HIV-1 were tested. Antiserum to one of the peptides (peptide gp120-19) was lost after being tested for neutralization of HIV-1 infection of vaginal Hs 760.T cells (see below) and therefore was not tested in neutralization assays using the HT-29 L20 cells. No reduction of infectivity was obtained with monkey preimmune sera. Four sera raised to peptides gp120-12 (aa 152–176), gp120-15 (aa 193–218), gp120-16 (aa 206–230), and gp120-24 (aa 307–330) were found to neutralize HIV-1 infection of the HT-29 L20 cells (Table 2). These four antisera also neutralized, and in a concentration-dependent manner (not shown), HIV-1 infection of vaginal Hs 760.T cells, as did the hyperimmune serum to peptide gp120-19 (248–269) (Table 3).

DISCUSSION

HIV-1 infection of cultured human colonic epithelial cells was first reported by Adachi *et al.* (7) and, on the basis of this and subsequent reports (8–11), transmission of HIV-1 from seminal fluid to colorectal epithelium with subsequent infection of $CD4^+$ lymphocytes by infected epithelial cells has been suggested to be an important route for homosexual transmission of HIV-1. In the present study, we show that, in addition to colonic epithelial cells, human vaginal epithelial cells can be infected by HIV-1 *in vitro*. We found both HIV-1 structural and regulatory RNA in vaginal as well as colonic epithelial cells but could not detect cell-free genomic RNA in the supernatants. Recently, Tan *et al.* (31) reported on productive HIV-1 infection of a cervix-derived epithelial cell line by cell-to-cell contact and on latent infection by cell-free virus. Although further investigation on HIV-1 infection in primary epithelial cultures of the female genital tract and analysis of epithelial cells from HIV-1 infected patients are needed, these findings indicate that HIV-1 transmission is not necessarily a blood-to-blood infection owing to coitus-induced traumatic microwounds in the mucosa and that epithelial cells of the female genital tract mucosa are implicated in heterosexual transmission of HIV-1.

Most of the epithelial cell lines studied that have been infected with HIV-1 have been shown to be either $CD4^-$ (9) or to express a truncated form of CD4 lacking the gp120 binding region (10, 11). Thus, it has not been possible to block HIV-1 infection of epithelial cells with antibodies to the CD4 molecule (9–11, 32). Galactosylceramide and/or sulfatide has been shown to be a probable alternative receptor for HIV-1 in both $CD4^-$ neural (16, 17) and colonic epithelial cells (14, 15). In keeping with this notion, a recent report indicates that HIV-1 gp120 can bind to the surface of $CD4^-$ lymphoblastoid cells having passively incorporated exogenous sulfated glycolipids (33). In the present study we were only able to demonstrate binding of the HIV-1 surface glycoprotein gp120 to the sulfated form of galactosylceramide—i.e., to sulfatide. In addition to sulfatide, we found that sulfated lactosylceramide—but not lactosylceramide—could bind gp120. Since the vaginal epithelial Hs 760.T and Hs 769.Vg cells were

Table 2. Neutralization of HIV-1 (HTLV-IIIB) infectivity in HT-29 L20 cells by guinea pig anti-gp120 serum and monkey hyperimmune sera against gp120 peptides

	Neutralization assay		
	By cocultivation		By PCR
Serum	Preimmune	Postimmune	Post-immune
Guinea pig anti-gp120	–	+	+
gp120-1 to gp120-11 (aa 1–164)	–	–	ND
gp120-12 (aa 152–176)	–	+	+
gp120-13 to gp120-14 (aa 165–205)	–	–	ND
gp120-15 (aa 193–218)	–	+	+
gp120-16 (aa 206–230)	–	+	+
gp120-17 to gp120-18 (aa 219–257)	–	–	ND
gp120-19 (aa 248–269)	ND	ND	ND
gp120-20 to gp120-23 (aa 258–320)	–	–	ND
gp120-24 (aa 307–330)	–	+	+
gp120-25 to gp120-40 (aa 321–511)	–	–	ND

ND, not done.

Table 3. Neutralization of HIV-1 (HTLV-IIIB) infectivity in Hs 760.T cells by guinea pig anti-gp120 serum and monkey hyperimmune sera against gp120 peptides

	HIV-1 DNA, copy per 3×10^4 cells		Neutralization assay by cocultivation	
Serum*	Pre-immune	Post-immune	Pre-immune	Post-immune
Guinea pig anti-gp120	375	<37.5	–	+
gp120-12 (aa 152–176)	375	<37.5	–	+
gp120-15 (aa 193–218)	375	<37.5	–	+
gp120-16 (aa 206–230)	300	75	–	+
gp120-19 (aa 248–269)	ND	<37.5	ND	+
gp120-24 (aa 307–330)	300	<37.5	–	+

ND, not done.

*Guinea pig anti-gp120 serum and monkey hyperimmune sera against gp120 peptides were tested at a final dilution of 1:40 and 1:10, respectively. The mixture of gp120 peptides antisera was tested at a final dilution of 1:20.

found not to contain galactosylceramide or sulfatide but sulfated lactosylceramide, this glycosphingolipid probably functions as a receptor for HIV-1 in these cells.

Since gp120 seems to mediate initial attachment of HIV-1 to epithelial cells, we examined whether antibody to gp120 could inhibit infection of vaginal and colonic epithelial cells. A hyperimmune serum raised in guinea pig to gp120 was able to neutralize HIV-1 infection of epithelial cells. Furthermore, site-directed immunization was employed to analyze sites on gp120 involved in antibody-mediated neutralization of HIV-1 in vaginal and colonic epithelial cells. We previously identified four distinct areas of gp120, including three relatively conserved regions, recognized by antibodies neutralizing HIV-1 infectivity in human $CD4^+$ lymphocytes (19). In the present study, the same antisera were shown also to neutralize HIV-1 infection in vaginal and colonic epithelial cells. Collectively, these observations indicate that antibody-mediated neutralization of HIV-1 infection can operate after attachment and possibly entry of antibody–virus complexes into the cytosol of both $CD4^+$ and $CD4^-$ permissive cells.

The glycosphingolipid binding region of gp120 has recently tentatively been mapped to a region defined by aa 206–275, within the second conserved domain of gp120 (34). Interestingly, three of the peptides (gp120-15, gp120-16, and gp120-19) in the present study found to induce antibodies in macaques that could neutralize HIV-1 infection of vaginal epithelial cells also map within this region. The two other peptides (gp120-12 and gp120-24) that induced neutralizing antibodies correspond to the discrete region between hypervariable regions one and two and to the hypervariable region three, respectively. In our preliminary study, the monkey antisera were not able to block binding of gp120 to sulfatide or sulfated lactosylceramide. Further, none of these antisera inhibited binding of gp120 to monolayers of vaginal and colonic epithelial cells, indicating that neutralizing antibodies interfere with a later state of the infectious process.

Taken together with earlier findings, our results provide support to the notion that HIV-1 can enter and replicate in an intact epithelium. Most importantly, we demonstrate that antibodies raised against synthetic peptides corresponding to four distinct, including three relatively conserved, areas of gp120 neutralize infection of epithelial cells by HIV-1. Whether antibodies endowed with the same specificities are present in rectal and/or cervico-vaginal secretions from HIV-1-infected persons should now be addressed. Although attempts at inducing production of antibodies with such properties at the very portal of HIV-1 entry during sexual contact remain to be evaluated, these regions of gp120 should be regarded as interesting components of a mucosal subunit vaccine against sexual transmission of HIV-1.

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1. Ho, D. D., Schooley, R. T., Rota, T. R., Kaplan, J. C., Flynn, T., Salahuddin, S. Z., Gonda, M. A. & Hirsch, M. S. (1984) *Science* **226,** 451–453.
2. Winkelstein, W., Jr., Lyman, D. M., Padian, N., Grant, R., Samuel, M., Wiley, J. A., Anderson, R. E., Lang, W., Riggs, J. & Levy, J. A. (1987) *J. Am. Med. Assoc.* **257,** 321–325.
3. Vogt, M. W., Witt, D. J., Craven, D. E., Byington, R., Crawford, D. F., Schooley, R. T. & Hirsch, M. S. (1986) *Lancet* **i,** 525–527.
4. Wofsy, C. B., Cohen, J. B., Hauer, L. B., Padian, N. S., Michaelis, B. A., Evans, L. A. & Levy, J. A. (1986) *Lancet* **i,** 527–529.
5. Forrest, B. D. (1991) *Lancet* **337,** 835–836.
6. Sabin, A. B. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8852–8855.
7. Adachi, A., Koenig, S., Gendelman, H. E., Daugherty, D., Gattoni-Celli, S., Fauci, A. S. & Martin, M. A. (1987) *J. Virol.* **61,** 209–213.
8. Moyer, M. P., Huot, R. I., Ramirez, A., Jr., Joe, S., Meltzer, M. S. & Gendelman, H. E. (1990) *AIDS Res. Hum. Retroviruses* **6,** 1409–1415.
9. Omary, M. B., Brenner, D. A., de Grandpre, L. Y., Roebuck, K. A., Richman, D. D. & Kagnoff, M. F. (1991) *AIDS* **5,** 275–281.
10. Fantini, J., Yahi, N. & Chermann, J.-C. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9297–9301.
11. Fantini, J., Yahi, N., Baghdiguian, S. & Chermann, J.-C. (1992) *J. Virol.* **66,** 580–585.
12. Nelson, J. A., Wiley, C. A., Reynolds-Kohler, C., Reese, C. E., Margaretten, W. & Levy, J. A. (1988) *Lancet* **i,** 259–262.
13. Mathijs, J. M., Hing, M., Grierson, J., Dwyer, D. E., Goldschmidt, C., Cooper, D. A. & Cunningham, A. L. (1988) *Lancet.* **i,** 1111.
14. Yahi, N., Baghdiguian, S., Moreau, H. & Fantini, J. (1992) *J. Virol.* **66,** 4848–4854.
15. Fantini, J., Cook, D. G., Nathanson, N., Spitalnik, S. L. & Gonzalez-Scarano, F. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2700–2704.
16. Harouse, J. M., Bhat, S., Spitalnik, S. L., Laughlin, M., Stefano, K., Silberberg, D. H. & Gonzalez-Scarano, F. (1991) *Science* **253,** 320–323.
17. Bhat, S., Spitalnik, S. L., Gonzalez-Scarano, F. & Silberberg, D. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7131–7134.
18. Harouse, J. M., Kunsch, C., Hartle, H. T., Laughlin, M. A., Hoxie, J. A., Wigdahl, B. & Gonzalez-Scarano, F. (1989) *J. Virol.* **63,** 2527–2533.
19. Vahlne, A., Horal, P., Eriksson, K., Jeansson, S., Rymo, L., Hedström, K.-G., Czerkinsky, C., Holmgren, J. & Svennerholm, B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10744-10748.
20. Pinto, M., Appay, M.-D., Simon-Assman, P., Chevalier, G., Dracopoli, N., Fogh, J. & Zweibaum, A. (1982) *Biol. Cell* **44,** 193–196.
21. Clouse, K. A., Powell, D., Washington, I., Poli, G., Strebel, K., Farrar, W., Barstad, P., Kovacs, J., Fauci, A. S. & Folks, T. M. (1989) *J. Immunol.* **142,** 431–438.
22. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. (1984) *Science* **224,** 497–500.
23. Myers, G., Korber, B., Wain-Hobson, S., Smith, R. F. & Pavlakis, G. N. (1993) *Human Retroviruses and AIDS 1993* (Los Alamos National Lab., Los Alamos, NM).
24. Besansky, N. J., Butera, S. T., Sinha, S. & Folks, T. M. (1991) *J. Virol.* **65,** 2695–2698.
25. Seshamma, T., Bagasra, O., Oakes, J. W. & Pomerantz, R. J. (1992) *J. Virol. Methods* **40,** 331–346.
26. Svennerholm, L. & Fredman, P. (1980) *Biochim. Biophys. Acta* **617,** 97–109.
27. Fredman, P., Nilsson, O., Tayot, J.-L. & Svennerholm, L. (1980) *Biochim. Biophys. Acta* **618,** 42–52.
28. Fredman, P., Mattsson, L., Andersson, K., Davidsson, P., Ishizuka, I., Jeansson, S., Månsson, J.-E. & Svennerholm, L. (1988) *Biochem J.* **251,** 17–22.
29. Fredman, P., Månsson, J.-E., Bigner, S. H., Wikstrand, C. J., Bigner, D. D. & Svennerholm, L. (1990) *Biochim. Biophys. Acta* **1045,** 239–244.
30. Åkerblom, L., Hinkula, J., Broliden, P.-A., Mäkitalo, B., Fridberger, T., Rosen, J., Villacres-Eriksson, M., Morein, B. & Wahren, B. (1990) *AIDS* **4,** 953–960.
31. Tan, X., Pearce-Pratt, R. & Phillips, D. M. (1993) *J. Virol* **67,** 6447–6452.
32. Phillips, D. M. & Bourinbaiar, A. S. (1992) *Virology* **186,** 261–273.
33. McAlarney, T., Apostolski, S., Lederman, S. & Latov, N. (1994) *J. Neurosci. Res.* **37,** 453–460.
34. Bhat, S., Mettus, R. V., Reddy, E. P., Ugen, K. E., Srikanthan, V., Williams, W. V. & Weiner, D. B. (1993) *AIDS Res. Hum. Retroviruses* **9,** 175–181.