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Synthetic Multimeric Peptides Derived from the Principal Neutralization Domain (V3 Loop) of Human Immunodeficiency Virus Type 1 (HIV-1) gp120 Bind to Galactosylceramide and Block HIV-1 Infection in a Human CD4-Negative Mucosal Epithelial Cell Line

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The glycosphingolipid galactosylceramide (GalCer), which binds gp120 with high affinity and specificity, is a potential alternative receptor for human immunodeficiency virus type 1 (HIV-1) in some CD4-negative neural and epithelial human cells, including the human colonic epithelial cell line HT-29. In the present study, we demonstrate that synthetic multibranched peptides derived from the consensus sequence of the HIV-1 V3 loop block HIV-1 infection in HT-29 cells. The most active peptide was an eight-branched multimer of the motif Gly-Pro-Gly-Arg-Ala-Phe which at a concentration of 1.8 μM induced a 50% inhibition of HIV-1 infection in competition experiments. This peptide was not toxic to HT-29 cells, and preincubation with HIV-1 did not affect viral infectivity, indicating that the antiviral activity was not due to a nonspecific virucidal effect. Using a high-performance thin-layer chromatography binding assay, we found that multibranched V3 peptides recognized GalCer and inhibited binding of recombinant gp120 to the glycosphingolipid. In addition, these peptides abolished the binding of an anti-GalCer monoclonal antibody to GalCer on the surface of live HT-29 cells. These data provide additional evidence that the V3 loop is involved in the binding of gp120 to the GalCer receptor and show that multibranched V3 peptides are potent inhibitors of the GalCer-dependent pathway of HIV-1 infection in CD4-negative mucosal epithelial cells.

Several studies have demonstrated a CD4-independent route of cell infection for human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS (15, 29). One putative non-CD4 HIV-1 receptor has been identified recently on $CD4^-$ brain-derived cells (2, 14) and in colonic epithelial cells (9, 31, 33). This receptor is a neutral glycosphingolipid, galactosylceramide (GalCer) or perhaps in some systems its sulfated congener sulfatide (3′ sulfogalactosyl ceramide) (19). HIV-1 infection of $CD4^-$ $GalCer^+$ cells in the brain and in the intestine may account for some of the HIV-associated disorders of these organs (10, 12). Moreover, the presence of GalCer on the apical side of some mucosal epithelial cells may facilitate the entry of the virus during sexual intercourse (30). Recently, Cook et al. (6) found that antibodies directed against the V3 loop of HIV-1 gp120 inhibit the interaction between GalCer and gp120 and blocked the infection of $CD4^-$ $GalCer^+$ intestinal HT-29 cells by HIV-1(IIIB). These data suggested that the V3 loop was involved in the binding between GalCer and gp120.

In the present study, we have used synthetic multimeric peptides based on the V3 loop consensus (clade B) motif and evaluated their activity as inhibitors of the GalCer-dependent pathway of HIV-1 infection. These peptides (synthetic polymeric constructions [SPCs]), consisting of an uncharged poly-Lys core matrix, were originally developed for use as synthetic immunogens (28). We recently reported that SPCs containing the GPGRAF consensus motif of the HIV-1 gp120 V3 loop were able to block HIV infection in lymphocytes and macrophages (32). In contrast, monomeric peptides had no such activity. We now demonstrate that multibranched V3 peptides are potent inhibitors of the GalCer-dependent pathway of HIV-1 infection in the CD4-negative mucosal epithelial line, HT-29.

MATERIALS AND METHODS

Materials. Rabbit anti-gp120 antiserum raised against denatured gp120 and recombinant gp120 (HIV-1 BH-10 clone) were gifts from R. Sweet (SmithKline Beecham, King of Prussia, Pa.). Culture media were obtained from Gibco-BRL (Cergy Pontoise, France), and fetal calf serum was obtained from Dutscher (Brumath, France). GalCer (with α-hydroxylated fatty acids) was obtained from Sigma. The anti-p24 monoclonal antibody (MAb) (9) was a gift from J. Hoxie (Department of Medicine, University of Pennsylvania). The anti-GalCer MAb (25) was purified from culture supernatants of hybridoma cells with a protein A-Sepharose column.

Peptide synthesis. Radially branched peptides using lysine skeletons in polymers (28) are referred to as SPCs. The nomenclature used in the present report for SPCs gives the peptide sequence in brackets, followed by the index of polymerization and the term SPC. For instance, $[GPGRAF]_8$-SPC means an SPC containing eight GPGRAF motifs, i.e., $(GPGRAF)_8$-$(K)_4$-$(K)_2$-K-βA (Fig. 1). $[PPPYVEPTTTQC]_4$-SPC was derived from the region of coordinates 124 to 135 of human T-cell leukemia virus type II Gag, and $[GKCMNRK]_8$-SPC was derived from the basic region of coordinates 27 to 33 of kaliotoxin. $[GPG(R)_DAF]_8$-SPC corresponds to a derivative of $[GPGRAF]_8$-SPC containing a D-Arg residue in the fourth position of the motif. The consensus sequence of the HIV-1 V3 loop was taken from the report of LaRosa et al. (18). Chemical synthesis of SPCs was performed by the solid-phase technique (20). The peptide

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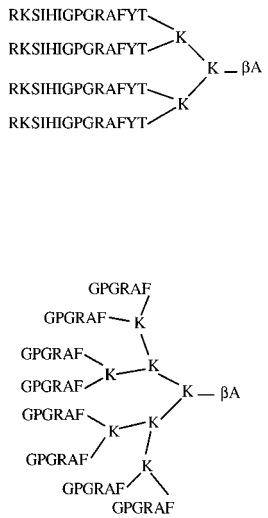

FIG. 1. Amino acid structure of two SPCs. The peptides shown correspond to $[RKSIHIGPGRAFYT]_4$-SPC (top) and $[GPGRAF]_8$-SPC (bottom).

chains were elongated stepwise on 4-(oxy-methyl)-phenylacetamidomethyl resin by optimized *t*-butyloxycarbonyl/benzyl chemistry, as previously described (26). Amino acid analysis of the purified SPCs agreed with the deduced amino acid ratios. $[GPGRAF]_8$-SPC was further characterized by electrospray mass spectrometry (experimental M_r = 5671.1; deduced M_r = 5671.6). Stock solutions of peptides (10^{-3} M) were filter sterilized on 0.22-μm-pore-size low-protein binding membranes (Costar) and stored in aliquots at −20°C.

HIV-1 infection of HT-29 cells. The human colonic adenocarcinoma cell line HT-29 (ATCC HTB 38) was routinely grown in Dulbecco's modified Eagle's medium-Ham's F12 medium (1:1, vol/vol) supplemented with 10% heat-inactivated fetal calf serum and 15 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.4). Cells were harvested from the culture flasks with trypsin-EDTA and subcultured every week. HT-29 cells were infected with HIV-1 as previously described (9). In brief, exponentially growing HT-29 cells (5×10^5 cells per well of a six-well plate) were infected for 2 h with 1,000 50% tissue culture infectious doses of either HIV-1(IIIB) or HIV-1(NDK) in the presence or absence of the appropriate peptide. After extensive washing, the cells were harvested with trypsin-EDTA and subcultured at a density of 5×10^4 cells per cm^2. After two more trypsinizations on days 2 and 4 postinfection, HIV-1 p24 was measured in the culture supernatant with an antigen capture enzyme-linked immunosorbent assay (Dupont).

Toxicity assay. Cellular toxicity was evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (27).

HPTLC overlay method. Purified GalCer (5 μg) was dissolved in chloroform-methanol (1:1), spotted on high-performance thin-layer chromatography (HPTLC) plates (silica gel 60; Merck, Darmstadt, Germany) and chromatographed in chloroform-methanol-water (60:35:8). The position of the chromatographed GalCer band was determined by orcinol staining of GalCer standards run in parallel on the same plate, and the lanes containing unstained GalCer were cut from the HPTLC plates, with the GalCer centered on segments of 2.5 by 1.0 cm as previously described (5). The HPTLC plates were treated for 2.5 min in 0.1% poly(isobutyl methacrylate) in *n*-hexane, and the dried plates were then immersed in 50 mM Tris-HCl–100 mM NaCl, pH 7.8, containing 1% gelatin for 2 h at room temperature to reduce nonspecific binding. The plates were further incubated with either recombinant HIV-1(IIIB) BH-10 clone gp120 (2.5 μg/ml) or the anti-GalCer MAb (1 μg/ml). Binding of gp120 was detected by incubating the plates first with a 1:200 dilution of rabbit anti-gp120 and then with ^{125}I-labeled goat anti-rabbit polyclonal antibodies (2×10^6 cpm/ml). Binding of anti-GalCer MAb was revealed by one incubation with a 1:100 dilution of rabbit anti-mouse immunoglobulin G (IgG) antibodies followed by another with the ^{125}I-labeled goat anti-rabbit antibodies. The plates were thoroughly washed in phosphate-buffered saline (PBS), dried, and exposed to Kodak X-ray film. When appropriate, the autoradiograms were scanned with a Biocom (Les Ulis, France) densitometer.

Transmission electron microscopy. Infected HT-29 cells were fixed in situ with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 1 h, washed for 10 min in the same buffer with 6.84% sucrose, postfixed in 1% osmium tetroxide, and then dehydrated in ethanol and embedded in Epon. Sections were cut perpendicularly to the plane of the cell monolayer and observed with a JEOL 1,200× electron microscope.

Immunofluorescence. HT-29 cells cultured on glass coverslips were incubated with the appropriate peptide (100 μM) for 45 min at 37°C. The cells were then incubated with either the anti-GalCer MAb or control mouse IgG3 (10 μg/ml) for 1 h at 4°C. The labeling was revealed with anti-mouse IgG-fluorescein isothiocyanate (Sigma) used at a dilution of 1:75, and the cells were observed under a Zeiss fluorescence microscope.

RESULTS

Characteristics of HIV-1 infection in HT-29 cells. Exposure of HT-29 cells to various HIV-1 isolates (including IIIB and NDK) leads to a stable and productive infection (9, 10, 31, 33). The culture supernatants recovered from HIV-1-infected HT-29 cells contain detectable levels of both HIV-1 p24 antigen [100 to 300 ng/ml/24 h, at 3 months postinfection with HIV-1(NDK)] and reverse transcriptase activity. Electron microscopy demonstrated the presence of mature virions (Fig. 2) that are infectious for peripheral blood lymphocytes as well as $CD4^+$ continuous cell lines (data not shown). Infection of HT-29 cells is sensitive to zidovudine treatment, with a 50% inhibitory concentration of 0.04 μM for HIV-1(NDK). Yahi et al. (31) have previously shown that HIV-1 entry into HT-29 cells is not blocked by anti-CD4 MAbs that can inhibit HIV-1 infection in $CD4^+$ cells, whereas treatment with an anti-GalCer MAb resulted in marked inhibition of infection. The infectibility of HT-29 cell clones was also related to the level of GalCer expression (9). Taken together, these data show that HT-29 can be readily infected by HIV-1, providing a unique model for studying the CD4-independent GalCer-dependent pathway of HIV-1 infection in vitro.

Multibranched peptides based on the HIV-1 V3 loop consensus sequence inhibit HIV-1 infection in HT-29 cells. In a recent report, Cook et al. (6) demonstrated that antibodies against the V3 loop inhibited binding between gp120 and GalCer. Furthermore, anti-V3 MAbs were also able to block the infection of HT-29 cells by HIV-1(IIIB) (6, 33). These data implicated the V3 loop in the recognition of GalCer by gp120 and prompted us to synthesize V3 loop-derived peptides to evaluate their activity in the GalCer-dependent pathway of HIV-1 infection. The peptides were prepared as multibranched polymers radially attached to a neutral core matrix (SPCs), a concept originally developed by Tam to enhance peptide immunogenicity (28). In the first set of experiments, V3-derived SPCs were incubated with HT-29 cells at the time of infection with HIV-1. The results indicated that SPCs containing the consensus sequence GPGRAF were able to block infection of HT-29 cells by both HIV-1(NDK) (Table 1) and HIV-1(IIIB) (data not shown). The effect was specific, since (i) monomeric V3 peptides as well as SPCs with an irrelevant motif were inactive, (ii) replacement of the fourth residue in the GPGRAF motif (i.e., Arg) by its D isomer resulted in a significant loss of activity, and (iii) SPCs that inhibited HIV-1 infection did not affect the infection of HT-29 cells by the La Crosse bunyavirus (data not shown). Moreover, the antiviral activity of $[GPGRAF]_8$-SPC was dose dependent, with a 50% inhibitory concentration of 1.8 μM (Fig. 3).

In order to assess whether $[GPGRAF]_8$-SPC was able to interfere with the entry of HIV-1 in HT-29 cells, the peptide was evaluated in a virus internalization assay (16). In these experiments, 100,000 HT-29 cells were incubated for 2 h with 2,000 50% tissue culture infectious doses of HIV-1(NDK) in the presence or absence of 15 μM $[GPGRAF]_8$-SPC. At the end of the incubation, the cells were thoroughly washed and the virus bound to the cells was removed from the cell surface by trypsin treatment. The internalized virus was detected in 1% Triton X-100 extracts by measurements of p24. Under these conditions, $[GPGRAF]_8$-SPC induced a 52% inhibition of cell-associated p24. These data are consistent with the hypoth-

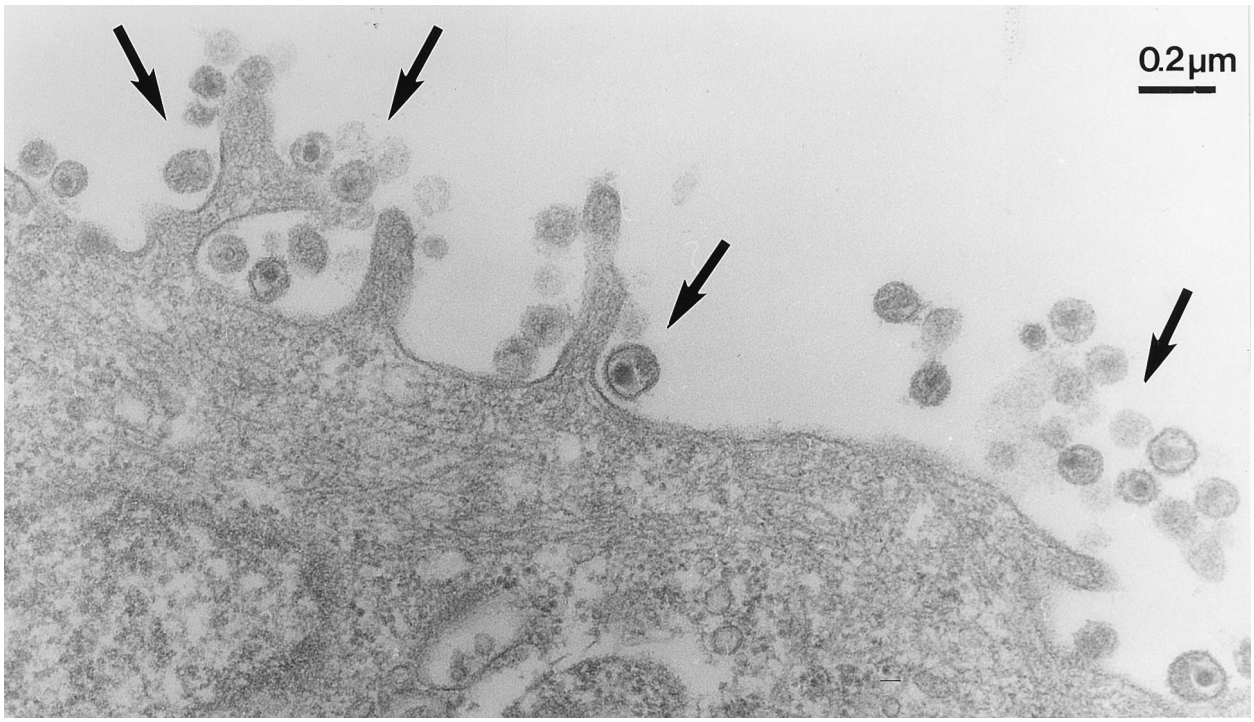

FIG. 2. Transmission electron micrograph of HT-29 cells infected with HIV-1. HT-29 cells infected with HIV-1(NDK) as described in Materials and Methods were fixed with glutaraldehyde, postfixed with osmium tetroxide, and then examined with an electron microscope 3 weeks postinfection. Numerous viral particles with typical lentiviral morphology (arrows) were produced by the cells.

esis that $[GPGRAF]_8$-SPC acts as a competitive inhibitor of HIV-1 entry in $CD4^-$ $GalCer^+$ cells.

$[GPGRAF]_8$-SPC does not decrease HIV-1 infectivity. Over the range of concentration from 0.1 to 100 μM, the $[GPGRAF]_8$-SPC peptide was not toxic to HT-29 cells as assessed by the MTT assay. Since $[GPGRAF]_8$-SPC was used in competition with the virus in our infection assays, it was also important to check that the peptide did not affect the infectious properties of the virus. HIV-1(NDK) was preincubated for 1 h at 37°C with $[GPGRAF]_8$-SPC (up to 100 μM) before being ultracentrifuged, and the infectivity of the virus was tested on HT-29 cells. Under these conditions, the infectious titer of the virus treated with the peptide was not decreased (data not shown). These data allowed us to rule out a potential virucidal effect of the peptide.

$[GPGRAF]_8$-SPC does not affect HIV-1 production in chronically infected cells. In order to further characterize the mechanism of action of $[GPGRAF]_8$-SPC, we next evaluated the effect of the peptide on the production of HIV-1 in HT-29

TABLE 1. Effect of multibranched peptides on infection of HT-29 cells by HIV-1[a]

Peptide	% Inhibition
None	0
GPGRAF monomer	0
$[GPGRAF]_8$-SPC	100
$[GPGRAF]_{16}$-SPC	100
$[GPG(R)_DAF]_8$-SPC	39
$[GKCMNRK]_8$-SPC	0
$[RKSIHIGPGRAFYT]_4$-SPC	100
$[PPPYVEPTTTQC]_4$-SPC	13

[a] HT-29 cells were exposed to 1,000 50% tissue culture infectious doses of HIV-1(NDK) in the presence of a 5 μM concentration of the indicated peptides. Following a 2-h incubation, the cells were trypsinized and cultured as indicated in Materials and Methods. HIV-1 infection was measured by determining the level of HIV-1 p24 antigen in cell-free supernatants on day 10 postinfection. The percent inhibition was calculated according to the following formula: 100 − [(p24 produced in presence of peptide/p24 produced in absence of peptide) × 100].

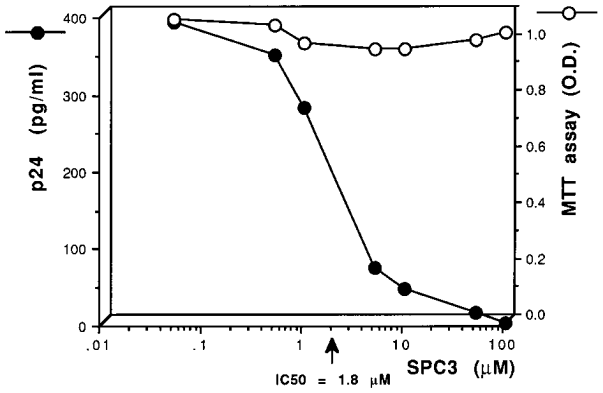

FIG. 3. Dose-dependent inhibition of HIV-1 infection by $[GPGRAF]_8$-SPC. HT-29 cells were exposed to 1,000 50% tissue culture infectious doses of HIV-1(NDK) in the presence of various concentrations of $[GPGRAF]_8$-SPC. After 16 h of incubation, the cells were trypsinized and subcultured three times before analysis at day 10 postinfection. HIV-1 production was monitored by the level of p24 antigen in cell-free culture supernatants. To evaluate any potential toxic effects associated with peptide treatment, exponentially growing HT-29 cells were treated with various concentrations of $[GPGRAF]_8$-SPC for 48 h and toxicity was assessed by the MTT assay. The results correspond to the optical density (O.D.) at a wavelength of 540 nm. These results are representative of three separate experiments. IC50, 50% inhibitory concentration.

TABLE 2. Failure of $[GPGRAF]_8$-SPC to inhibit HIV-1 production by chronically infected HT-29 cells[a]

No. of days of treatment	p24 production (pg/ml) with $[GPGRAF]_8$-SPC μM concn of:		
	0	10	50
2	32,847	25,336	41,500
4	473,140	603,305	524,793

[a] HT-29 cells chronically infected with HIV-1(NDK) were seeded in six-well plates. The infected cells were then treated for 2 or 4 days with the indicated concentrations of $[GPGRAF]_8$-SPC, and p24 production (in picograms per milliliter) was measured.

cells chronically infected by HIV-1(NDK). The infected cells were cultured in the presence of various concentrations of peptide (up to 50 μM), and the level of HIV-1 replication was determined by assay of supernatant p24 (Table 2). Under these conditions, treatment with the peptide was not associated with a decrease in p24 levels, indicating that the peptide did not interfere with later steps in the viral life cycle.

Multibranched peptides containing the GPGRAF motif bind to GalCer. The interaction between recombinant gp120 and the GalCer receptor can be visualized with an HPTLC binding assay (Fig. 4), as previously documented. When the viral glycoprotein was preincubated with an MAb directed against the V3 loop (0.5β), binding to GalCer was virtually abolished (Fig. 4). Antibodies raised against other domains of gp120 did not affect GalCer recognition (data not shown), suggesting that the V3 loop, or at least a closely related domain, was involved in GalCer binding (6). When we added multibranched V3 peptides as competitive inhibitors in the assay, there was a marked inhibition in binding (Fig. 4). Quantitative scanning of the autoradiograms showed that $[GPGRAF]_8$-SPC, $[GPGRAF]_{16}$-SPC, and $[RKSIHIGPGRAFYT]_4$-SPC inhibited the interaction by 76, 96, and 95%, respectively. In contrast, $[GPG(R)_DAF]_8$-SPC had little inhibitory activity (17% inhibition), and monomeric peptides GPGRAF and RKSIHIGPG RAFYT as well as control SPCs with an irrelevant sequence were totally inactive. In another experiment (Fig. 5), the peptides were first preincubated with GalCer, after which the plates were thoroughly washed and incubated with gp120 without peptide. Under these conditions, $[GPGRAF]_8$-SPC, $[GPGRAF]_{16}$-SPC, and $[RKSIHIGPGRAFYT]_4$-SPC were able to inhibit the binding of both gp120 and the anti-GalCer MAb, whereas the $[GPG(R)_DAF]_8$-SPC did not. These data suggested that multibranched V3 peptides bind to GalCer on HPTLC plates. Live-cell immunofluorescence labeling was used to verify that these peptides could also bind to GalCer on the surface of HT-29 cells. In these experiments, the cells were first incubated at 37°C with the peptides, washed, and probed with the anti-GalCer MAb. Pretreatment of HT-29 cells with $[GPGRAF]_{16}$-SPC (Fig. 6) or $[RKSIHIGPGRAFYT]_4$-SPC (data not shown) completely abrogated the binding of anti-GalCer MAb on the surface of these cells, in agreement with the HPTLC assay data. Irrelevant SPCs did not alter the immunofluorescence labeling pattern of the anti-GalCer MAb (Fig. 6).

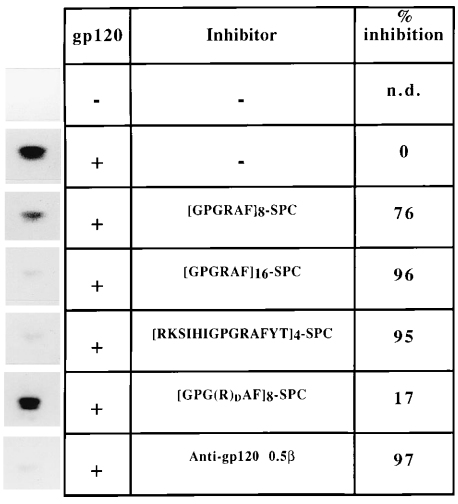

FIG. 4. Multibranched peptides containing the GPGRAF motif inhibit binding of gp120 to GalCer. GalCer on HPTLC plates was serially incubated with gp120 (2.5 μg/ml), rabbit anti-gp120, and ^{125}I-labeled goat anti-rabbit IgG prior to autoradiography. The indicated SPCs were present at a concentration of 100 μM during the incubation with gp120. The anti-V3 MAb 0.5β (50 μg/ml) was preincubated for 1 h with gp120 prior to binding to GalCer. The percent inhibition was calculated by quantitative scanning of the autoradiograms. The results shown are representative of three separate experiments. n.d., not done; —, no inhibitor.

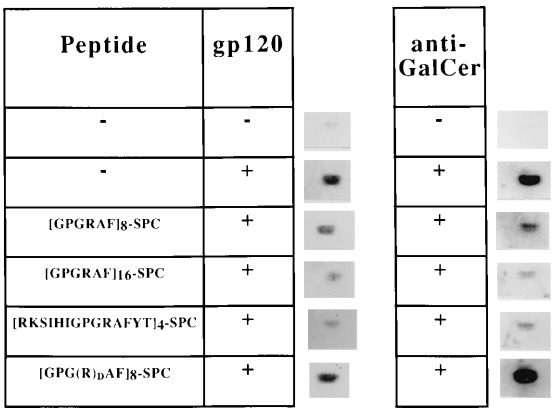

FIG. 5. Multibranched peptides containing the GPGRAF motif recognize GalCer. GalCer was first incubated with the indicated SPC, after which the HPTLC plates were extensively rinsed and serially incubated with gp120, rabbit anti-gp120, and ^{125}I-labeled goat anti-rabbit IgG (left panel) or anti-GalCer MAb, rabbit anti-mouse IgG, and ^{125}I-labeled goat anti-rabbit IgG (right panel) in the absence of peptide (—). Preincubation of the HPTLC plates with V3 peptides resulted in a marked inhibition of gp120 binding. The same peptides also inhibited the recognition of GalCer by the anti-GalCer MAb. The results shown are representative of three separate experiments.

DISCUSSION

The third variable region of the HIV-1 surface glycoprotein gp120 (V3 loop) plays an important role in HIV-1 infection and pathogenesis (21). First, the V3 loop (often referred to as the principal neutralization domain) is the major immunodominant epitope for the generation of type-specific neutralizing antibodies (3, 13). Second, mutations in the V3 loop may affect the infectivity and tropism of HIV-1 (4, 7). Third, the V3 loop could mediate binding of gp120 to accessory molecules working in conjunction with CD4 to allow HIV-1 entry into $CD4^+$ cells (17, 22). This would explain the anti-HIV effect of some V3 loop synthetic peptides in these cells (23, 32). Recently, it was found that antibodies against the V3 loop inhibited both binding between gp120 and GalCer and infection of $CD4^-$ $GalCer^+$ colonic epithelial cells (6, 33). Although these data argue in favor of a physical interaction between gp120 and this glycolipid receptor, one cannot rule out the possibility that binding to GalCer was in fact mediated by a domain of gp120

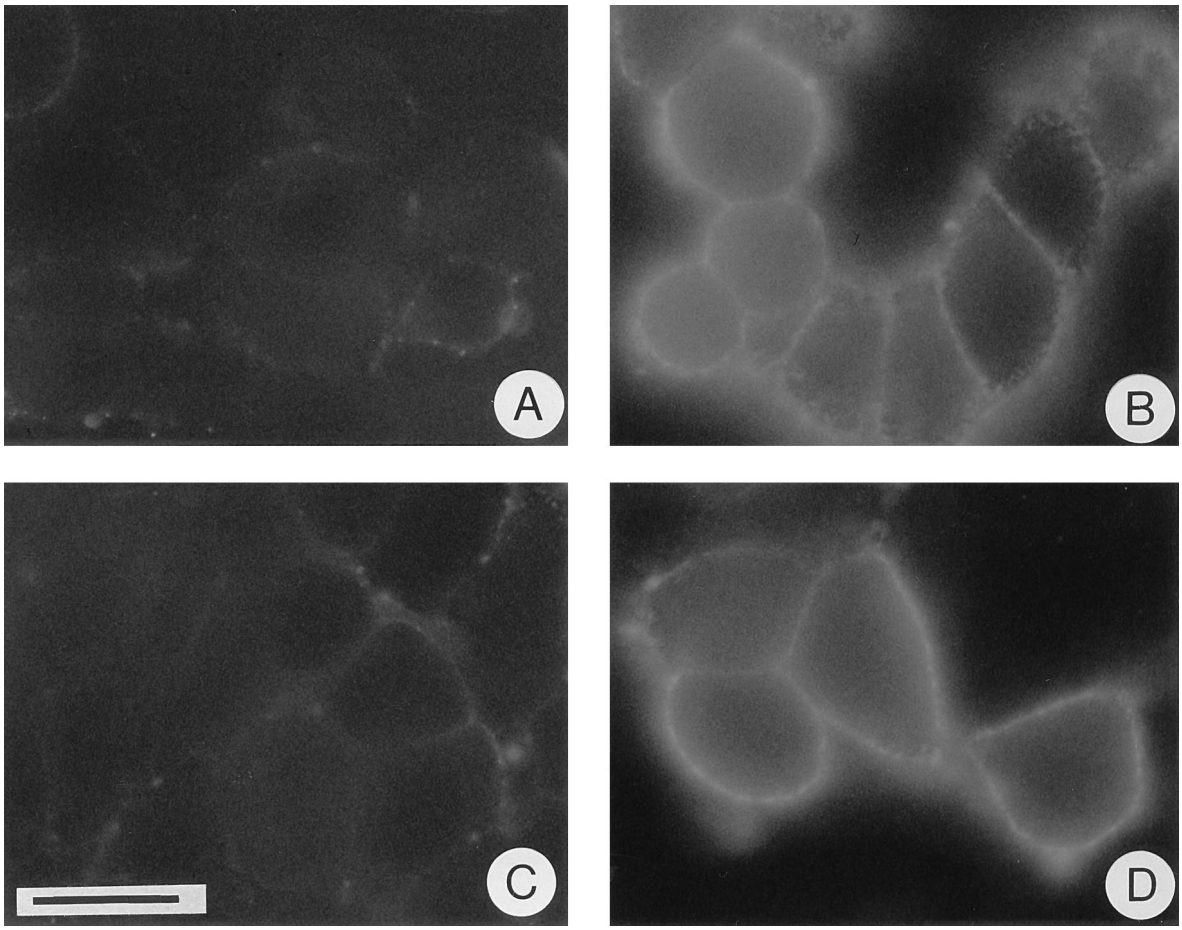

FIG. 6. Inhibition of binding of anti-GalCer MAb to HT-29 cells by live-cell immunofluorescence assay. Exponentially growing HT-29 cells cultured on glass coverslips were preincubated with PBS (A and B) or 100 μM either $[GPGRAF]_{16}$-SPC (C) or $[PPPYVEPTTTQC]_4$-SPC (D) for 45 min at 37°C. The cells were then rinsed and incubated for 1 h at 4°C with mouse IgG3 (A) or anti-GalCer MAb (B to D). The labeling was revealed with anti-mouse IgG-fluorescein isothiocyanate as described in Materials and Methods. Bar, 20 μm.

different from the V3 loop (1) and masked by anti-V3 MAbs because of steric hindrance.

In the present study, we found that multibranched peptides containing the consensus sequence from the crown of the V3 loop were able to bind specifically to GalCer and to induce a dose-dependent inhibition of HIV-1 infection in HT-29 cells (with a 50% inhibitory concentration of 1.8 μM). This effect was specific for HIV-1, since these peptides did not interfere with the infection of HT-29 cells by an unrelated virus (La Crosse bunyavirus). Moreover, a clear relationship could be drawn between the antiviral activity of a given peptide and its ability to inhibit the binding of gp120 to purified GalCer. The minimal sequence that was required to obtain a biological effect was the hexapeptide GPGRAF, in an 8- or 16-branched multimer. In contrast, the motif RKSIHIGPGRAFYT, which represents a greater portion of the V3 loop, was fully active in the four-branched multimer. These results suggest that (i) GalCer binds at least partly to GPGRAF and (ii) amino acid residues upstream and downstream from the GPGRAF sequence may determine the conformation of the hexapeptide motif. This is not surprising, since it has been suggested that the V3 domain from HIV-1 isolates containing the GPGRAF motif assumes two distinct conformations determining the isolates' preferential tropism for lymphocytes or macrophages (8). The lateral chain of the arginine residue in the fourth position of the motif may be involved in GalCer recognition, since replacement of this residue by its D isomer (i.e., $[GPG(R)_DAF]_8$-SPC) leads to a loss of biological activity.

Binding of gp120 to GalCer through the GPGRAF motif is consistent with the neutralizing activity of the anti-V3 MAb 0.5β, which recognizes the epitope TIGPGRAFVTIGKIG (8). However, this antibody does not bind to the V3 domain of HIV-1(NDK), which displays a widely divergent sequence (GLRQSL at the crown). Yet the infection of HT-29 cells by HIV-1(NDK) is efficiently blocked by both anti-GalCer antibodies (9, 31) and $[GPGRAF]_8$-SPC, suggesting that this isolate uses GalCer to gain entry to HT-29 cells. Moreover, we have recently found that rabbit polyclonal antibodies raised against a peptide corresponding to 19 amino acid residues of the NDK V3 loop (RTSIGLRQSLYTITGKKKT) can completely block the infection of HT-29 cells by HIV-1(NDK) (data not shown). Therefore, providing that there is no other alternative pathway of HIV-1 infection in these cells, it is likely that the V3 loop from the NDK isolate can interact with GalCer. As a consequence, this would mean that the overall conformation of the V3 loop, rather than its primary structure, is the important determinant for its interaction with GalCer. This hypothesis is supported by recent crystallographic studies suggesting that, despite its sequence variability, the tip of the V3 loop adopts a double-turn conformation which would be

necessary for its activity (11). In any case, since V3-derived SPCs are targeted to cellular and not viral determinants, they may be active against various HIV-1 isolates, in contrast with neutralizing anti-V3 antibodies that are generally type specific (24).

In conclusion, our data provide additional evidence that the V3 loop is involved in the binding of gp120 to the GalCer receptor. Since the V3 loop is a primary determinant of tropism, these results are consistent with biological data that suggest that only some HIV-1 strains can infect HT-29 cells (8a). Multibranched peptides containing the consensus hexapeptide motif GPGRAF bind to GalCer and inhibit the GalCer-dependent pathway of HIV-1 infection in CD4-negative mucosal epithelial cells. The use of such peptides as anti-HIV agents in mucosal tissues should be evaluated. In this respect, it is noteworthy that $[GPGRAF]_8$-SPC is not immunogenic, since rabbits and mice repeatedly injected with this peptide did not produce significant titers of anti-GPGRAF antibodies. The lack of immunogenicity of this peptide is presumably related to the shortness of its motif (six residues). Therefore, antibody-mediated clearance of such peptides during clinical trials would not be expected.

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