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SPC3, a synthetic peptide derived from the V3 domain of human immunodeficiency virus type 1 (HIV-1) gp120, inhibits HIV-1 entry into CD4$^+$ and CD4$^-$ cells by two distinct mechanisms

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ABSTRACT **The third variable region (V3 loop) of gp120, the HIV-1 surface envelope glycoprotein, plays a key role in HIV-1 infection and pathogenesis. Recently, we reported that a synthetic multibranched peptide (SPC3) containing eight V3-loop consensus motifs (GPGRAF) inhibited HIV-1 infection in both CD4$^+$ and CD4$^-$ susceptible cells. In the present study, we investigated the mechanisms of action of SPC3 in these cell types—i.e., CD4$^+$ lymphocytes and CD4$^-$ epithelial cells expressing galactosylceramide (GalCer), an alternative receptor for HIV-1 gp120. We found that SPC3 was a potent inhibitor of HIV-1 infection in CD4$^+$ lymphocytes when added 1 h after initial exposure of the cells to HIV-1, whereas it had no inhibitory effect when present only before and/or during the incubation with HIV-1. These data suggested that SPC3 did not inhibit the binding of HIV-1 to CD4$^+$ lymphocytes but interfered with a post-binding step necessary for virus entry. In agreement with this hypothesis, SPC3 treatment after HIV-1 exposure dramatically reduced the number of infected cells without altering gp120–CD4 interaction or viral gene expression. In contrast, SPC3 blocked HIV-1 entry into CD4$^-$/GalCer$^+$ human colon epithelial cells when present in competition with HIV-1 but had no effect when added after infection. Accordingly, SPC3 was found to inhibit the binding of gp120 to the GalCer receptor. Thus, the data suggest that SPC3 affects HIV-1 infection by two distinct mechanisms: (*i*) prevention of GalCer-mediated HIV-1 attachment to the surface of CD4$^-$/GalCer$^+$ cells and (*ii*) post-binding inhibition of HIV-1 entry into CD4$^+$ lymphocytes.**

The hypervariable V3 domain of HIV-1 surface envelope glycoprotein gp120 is an important determinant of HIV-1 tissue tropism and infectivity (1, 2). This region contains the principal neutralization domain of HIV-1 gp120, and anti-V3 antibodies block HIV-1-induced cell fusion (3). These antibodies are active after attachment of the virus to the CD4 receptor, which supports the concept that the V3 loop is involved in the post-binding events necessary for viral entry into CD4$^+$ cells (4, 5). In addition, HIV-1 can infect some CD4$^-$ cells, suggesting the existence of alternative receptors (6, 7). One such receptor, the glycosphingolipid galactosylceramide (GalCer), has been identified in neural and intestinal epithelial cells (8, 9). Interestingly, the infection of CD4$^-$/GalCer$^+$ colonic HT-29 cells can be blocked by anti-V3 antibodies, which also inhibit binding of HIV-1 gp120 to GalCer (10, 11). This suggests that the V3 loop is directly involved in the binding of HIV-1 to the GalCer receptor.

Due to the key role of the V3 loop in the HIV-1 infection process, this region has been extensively studied with the aim of developing vaccinal and/or therapeutical strategies. However, its high variability has rendered this task particularly difficult. For instance, neutralizing anti-V3 antibodies are generally isolate-specific (12). As a potential therapeutic approach, several attempts have been made to inhibit HIV-1 infection by V3-loop-related peptides, but controversial results have been reported (13, 14).

To develop another strategy, we have synthesized a multibranched polymer (SPC3) of a particularly conserved V3 loop sequence (15). This synthetic polymeric construction displays eight GPGRAF motifs radially branched on an uncharged polylysine core matrix. Such multibranched peptides have been originally developed to enhance peptide immunogenicity (16, 17). The rationale for using SPC3 instead of monomeric V3-loop-derived peptides as the anti-HIV compound is a presumed enhancement of ligand avidity due to multivalence. In addition, SPC3 was not immunogenic in animal models, probably because of the short size (6 residues) of the motif (18). This peptide is a potent inhibitor of HIV-1 infection in human lymphocytes and macrophages and in CD4$^-$ human colon epithelial cells (15). The broad inhibitory activity of SPC3 could not be explained by nonspecific virucidal effects or by cellular toxicity on HIV-1 target cells (18). The concentration of SPC3 that inhibited the infection by 50% (i.e., the 50% inhibitory concentration or IC_{50}) was different in lymphocytes and in HT-29 cells (50 nM and 1.8 μM, respectively). Thus, SPC3 exerts specific antiviral effects on different HIV-susceptible cell types. In the present report, we have studied the mechanisms of action of SPC3 in CD4$^+$ lymphocytes and in CD4$^-$/GalCer$^+$ epithelial cells.

MATERIALS AND METHODS

Peptide Synthesis. Chemical synthesis of SPC3 [$(GPGRAF)_8$-$(K)_4$-$(K)_2$-K-βA] was performed by the solid-phase technique (19) as described (20). Analytical C_{18} reversed-phase HPLC of the purified SPC3 showed a homogeneity >98%. Amino acid analysis of the purified SPC3 agreed with the deduced amino acid ratios. SPC3 was further characterized by electrospray mass spectrometry (experimental M_r, 5671.1; deduced M_r, 5671.6) and automated sequencing. Optical rotation of SPC3 was $[\alpha]_D = -81.1 \pm 1.1$ ($c = 1$ in H_2O at 23°C).

Cell Culture. Human peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors, activated with phytohemagglutinin, and cultured in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum and interleukin 2 as

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Abbreviations: GalCer, galactosylceramide; HIV, human immunodeficiency virus; RT, reverse transcriptase; $TCID_{50}$, tissue culture 50% infectious dose; PBMC, peripheral blood mononuclear cell; MOI, multiplicity of infection; mAb, monoclonal antibody; AZT, 3′-azido-3′-deoxythymidine.

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described (9). The human T-lymphoblastoid CEM cell line was grown in RPMI 1640 medium supplemented with 10% fetal calf serum. The human colon adenocarcinoma cell line HT-29 was cultured in Dulbecco's modified Eagle's medium/F-12/10% fetal calf serum (9).

HIV-1 Infection Assay. Laboratory strains HIV-1(LAI) (21) and HIV-1(NDK) (22) were produced in CEM cells. Culture supernatants from infected cells were harvested at the peak of viral production, filtered through 0.22-μm (pore size) filters, and stored at −80°C. CEM cells were exposed to HIV-1(LAI) at a multiplicity of infection (MOI) of 0.001 tissue culture 50% infective dose ($TCID_{50}$) per cell for 1 h at 37°C. The cells were then washed and cultured as indicated. HT-29 cells were exposed to HIV-1(NDK) at a MOI of 0.005 $TCID_{50}$ per cell. After extensive washings, the cells were harvested with trypsin/EDTA and subcultured two more times at days 2 and 4 after infection. The state of infection of CEM and HT-29 cells was assessed by measuring HIV-1 p24 in cell-free supernatants with a DuPont kit and/or determining the reverse transcriptase (RT) activity, as described (9). The percentage of HIV-1-infected cells at day 7 after infection was determined by an infectious center end-point assay (23). Briefly, serial dilutions of CEM cells were mixed with indicator human T-lymphoblastoid MT-2 cells and the presence of infected CEM cells was revealed by syncytium formation.

Metabolic Labeling and Radioimmunoprecipitation Assay. HIV-1-infected cells were preincubated in Cys/Met-free RPMI 1640 medium for 90 min. The cells were labeled for 16 h with [^{35}S]Cys/Met (Amersham, 100 μCi/ml; 1 Ci = 37 GBq). Radiolabeled cells were resuspended in 50 mM Tris·HCl; pH 8/100 mM NaCl/1 mM $MgCl_2$/1% Triton X-100/0.15 mM phenylmethylsulfonyl fluoride/2 mM benzamidine/leupeptin (2 μg/ml). Cellular extracts were centrifuged at 12,000 × g for 10 min. Viral extracts were prepared after ultracentrifugation (Beckman TL100, 5 min at 95,000 rpm) of culture supernatants from infected CEM cells. The extracts were immunoprecipitated with a polyspecific anti-HIV-1 serum from a seropositive patient or with monoclonal antibodies (mAbs) against p24, gp120, or gp160, as described (24). After separation by SDS/PAGE, gels were treated for 1 h with Amplify (Amersham), dried, and exposed for autoradiography.

Morphological Studies. The cells were fixed with 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium phosphate (pH 7.3) for 1 h, post-fixed in 1% osmium tetroxide, block-stained in uranyl acetate, then dehydrated in ethanol, and embedded in Epon. Semi-thin sections were observed under a Zeiss optical microscope and ultrathin sections were observed with a Jeol 1200X transmission electron microscope.

Binding of gp120 to CD4 and GalCer. Quantitation of gp120 binding to CD4-coated plates was done with a gp120 capture ELISA kit (Intracel, London). The binding of gp120 to GalCer was performed by using a high-performance thin-layer chromatography overlay method (11).

RESULTS

Anti-HIV Activity of SPC3 in CD4$^+$ Lymphocytes. CEM cells exposed to HIV-1(LAI) for 1 h were treated before, during, and/or after viral exposure with 10 μM SPC3 (Table 1). Pretreatment of the cells with SPC3 did not result in an inhibition of infection. Similar data were obtained when the peptide was present during the infection phase in competition with the virus. In contrast, a marked inhibition of HIV-1 production was observed when the cells were first exposed to HIV-1 for 1 h and subsequently cultured in the presence of SPC3 for 7 days. This SPC3-induced antiviral effect could reflect an inhibition of infection and/or a blockade of HIV-1 expression. The latter is, however, unlikely since SPC3 did not affect the production of p24 by HIV-1-infected CEM-8E5 cells (data not shown). Moreover, by using an infectious center end point assay, we could estimate that <1 cell per 100,000 cells was infected by HIV-1 in SPC3-treated cultures vs. 1–5 cells per 10 cells in untreated cultures. Thus, the antiviral activity of SPC3 corresponds to an inhibition of HIV-1 infection. Interestingly, it was not necessary to keep SPC3 in the culture medium throughout the experiment, since a minimal 2-h treatment after HIV-1 exposure was sufficient to inhibit infection.

Table 1. Effect of SPC3 on HIV-1 infection in CEM cells

Experimental design	Incubation time with SPC3, h	Inhibition, %
Pretreatment	1	0
Competition	1	0
Post-binding treatment	1	0
	2	99.7
	4	99.3
	24	99.6
	168	100
Competition + post-binding treatment	169	99.3

SPC3 (10 μM) was added before, during, and/or after the incubation of CEM cells with HIV-1(LAI) at a MOI of 0.001 $TCID_{50}$ per cell. The state of infection was assessed by p24 measurement after 7 days of culture. The results are expressed as the percentage of inhibition of p24 production in SPC3-treated cells compared with untreated control cells. The mean production of p24 by HIV-1-infected CEM cells was 10 ng/ml after 7 days of culture.

Metabolic Labeling and Radioimmunoprecipitation Assay Analysis of SPC3-Treated and Untreated Cells. CEM cells were exposed to HIV-1 for 1 h and cultured with or without SPC3 (10 μM) for 6 days, and then the cells were metabolically labeled for 16 h with a mixture of [^{35}S]Cys/Met. As shown in Figs. 1 and 2*A*, the overall pattern of newly synthesized proteins by HIV-1-exposed CEM cells was similar for SPC3-treated and untreated cells, with only a minor difference for a protein with an apparent molecular mass of 24 kDa that appeared to be specifically shut down in SPC3-treated cells (asterisks). This protein was associated with viral material pelleted from culture supernatants and thus is likely to correspond to the HIV-1 capsid p24 protein (Fig. 1). In addition, immunoprecipitation of cellular lysates with an anti-p24 mAb

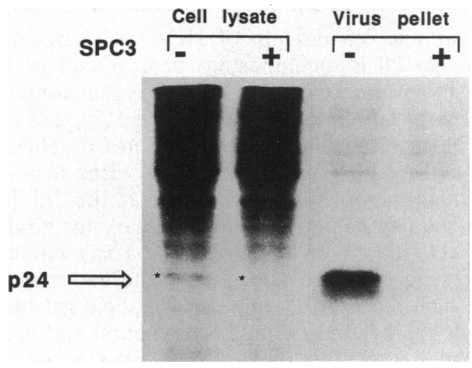

FIG. 1. Effect of SPC3 on the biosynthesis of proteins from HIV-1-exposed CEM cells. CEM cells were exposed to HIV-1(LAI) for 1 h and then treated (+) or not treated (−) for 7 days with 10 μM SPC3. At day 6 after infection, the cells were metabolically labeled. The overall pattern of protein biosynthesis was analyzed in Triton X-100 cell lysates. The culture supernatants were also analyzed after ultracentrifugation and lysis of virus-associated material (virus pellet). The proteins were separated by SDS/PAGE on 12.5% gels. The migration of a protein with an apparent molecular mass of 24 kDa is indicated (p24). Note the disappearance of this band in lysates from SPC3-treated infected cells (asterisks).

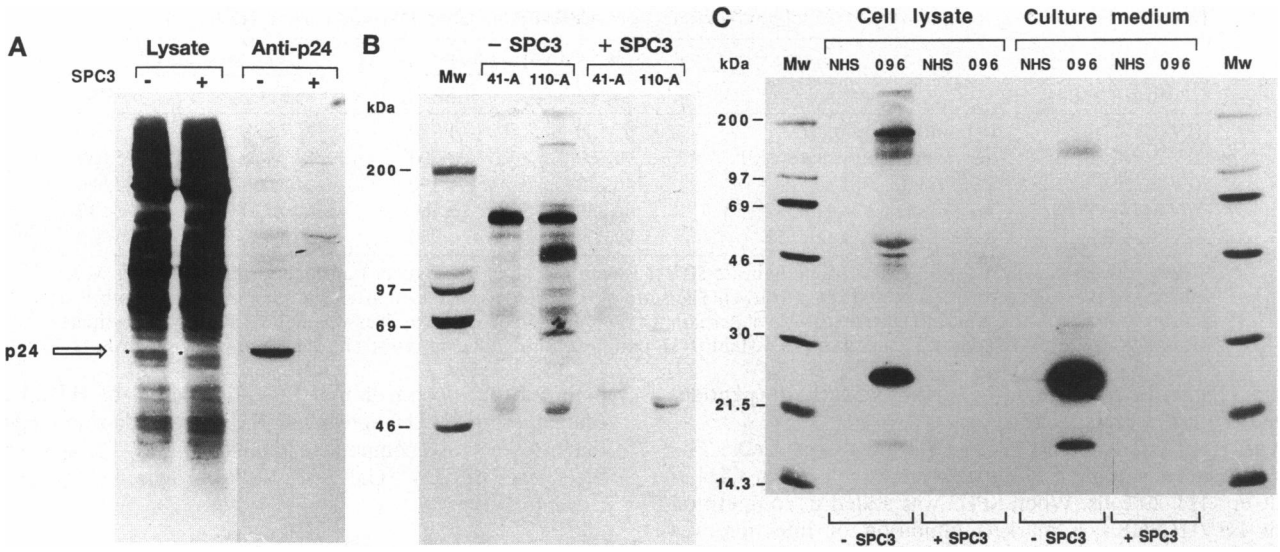

FIG. 2. Detection of HIV-1 proteins by radioimmunoprecipitation assay. (*A*) CEM cells exposed to HIV-1(LAI) were labeled as described in Fig. 1. The whole cell lysates and the material immunoprecipitated with an anti-p24 mAb were separated by SDS/PAGE on a 10% gel. The migration of p24 is indicated (arrow and asterisks). (*B*) Immunoprecipitation of the cell lysates with an anti-gp160 mAb (41-A) recognizing gp160 or an anti-gp120 mAb (110-A) recognizing both gp160 and gp120. The purified material was separated by SDS/PAGE on 7% gels. (*B* and *C*) Lanes Mw contains molecular mass markers. (*C*) Immunoprecipitation of HIV-1(LAI) proteins. CEM cells exposed to HIV-1(LAI) were treated or not treated by SPC3 and metabolically labeled. Both cell lysates and culture supernatants were immunoprecipitated with normal human serum (NHS) or a polyspecific anti-HIV-1 serum (096) from an infected patient. The material was analyzed by SDS/PAGE on 10% gels. In cell lysates, serum 096 immunoprecipitated gp160, gp120, p55, p24, and p17. In the culture medium ultracentrifuged to obtain virus-associated material, this serum mainly recognized the core proteins p24 and p17 but also detected gp120 and p66 to a lesser extent. None of these HIV-1 proteins were detected in the material purified from cells exposed to HIV-1 and then treated with SPC3.

showed that p24 could not be detected in SPC3-treated cells, in contrast with untreated infected cells (Fig. 2*A*). Similarly, the viral envelope glycoproteins gp160 and gp120 could not be detected in cell lysates from SPC3-treated cells (Fig. 2*B*). Finally, the overall expression of viral proteins was analyzed with a human polyspecific anti-HIV-1 serum (Fig. 2*C*). The HIV-1 gene products gp160, gp120, p55, gp41, p24, and p17 were easily detected in infected CEM cells, whereas none of these could be detected in SPC3-treated cells. Thus, these results confirmed that SPC3 treatment after exposure of CEM cells to HIV-1 resulted in a marked inhibition of infection.

Morphological Studies. The antiviral activity of SPC3 could not be related to cellular toxicity as assessed by the MTT assay (18). Moreover, the ultrastructural analysis of CEM cells showed that chronic treatment with 20 μM SPC3 did not alter the morphology of these cells (Fig. 3 *a* and *b*). In contrast, ≈20% of CEM cells treated with either 10 μM dideoxyinosine (Fig. 3 *c* and *d*) or 5 μM 3′-azido-3′-deoxythymidine (AZT; zidovudine) (Fig. 3 *e* and *f*) showed large intracellular vacuoles with a sickle-like shape. In addition, ≈5% of AZT-treated cells displayed ultrastructural features of apoptosis (Fig. 3*f*). These data confirmed the lack of toxicity of SPC3 under our experimental conditions, in particular when compared with other antiviral agents.

Inhibition by SPC3 of HIV-1 Infection in Normal Human Lymphocytes. In agreement with the data obtained with CEM cells, SPC3 inhibited the infection of PBMCs by HIV-1(LAI) only when added after the phase of incubation with the virus (data not shown). Under these conditions, the peptide was also able to inhibit infection of PBMC by various isolates including two zidovudine-resistant viruses (Table 2). Interestingly, SPC3 was particularly active against syncytium-inducing cytopathic isolates (SI phenotype). At the morphological level, the infection of PBMCs with HIV-1 induced both single-cell apoptosis and formation of multinucleated giant cells (Fig. 4*b*). When the cells were cultured in the presence of SPC3, added 1 h after the initial contact with HIV-1, these signs of cytopathicity were no longer observed (Fig. 4 *c* and *d*). Most importantly, the finding of mitotic cells in PBMC cultures chronically treated with

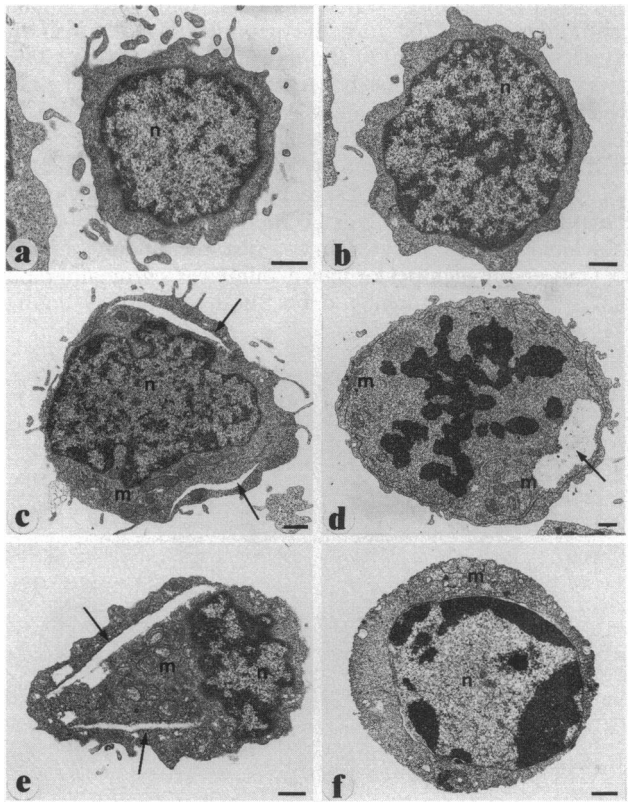

FIG. 3. Morphology of CEM cells upon treatment with SPC3: Comparison with other antiviral agents. (*a*) Ultrastructure of a typical untreated CEM cell. (*b*) Typical CEM cell cultured with 20 μM SPC3 for 4 days. (*c* and *d*) Treatment of CEM cells with 10 μM dideoxyinosine (4 days) is associated with the occurrence of sickle-like vacuoles (arrows) in ≈20% of the cells. (*e* and *f*) CEM cells treated with 5 μM AZT for 4 days. About 20% of the cells show severe signs of cytopathy including large vacuoles (*e*) (arrows) and apoptosis (*f*). n, Nucleus; m, mitochondria. [Bars = 1 μm, except in *d* (2 μm).]

Table 2. Post-binding treatment by SPC3 inhibits the infection of PBMCs by laboratory and clinical HIV-1 isolates

Virus	Phenotype	RT activity, cpm/ml		p24, pg/ml	
		− SPC3	+ SPC3	− SPC3	+ SPC3
HIV-1(LAI)	Laboratory strain, SI	95,520	1,910	7,238	<10
HIV-1(80)	Seroconversion isolate, NSI	99,360	33,700	17,170	5,035
HIV-1(133)	CSF isolate, NSI	746,110	174,190	102,000	22,880
HIV-1(J1)	AZT-resistant isolate, SI	63,830	3,580	10,370	12
HIV-1(JVR)	AZT-resistant isolate, SI	99,310	760	18,940	<10

PBMC were exposed to 100 $TCID_{50}$ of the indicate HIV-1 isolate for 1 h at 37°C. After thorough washing, the cells were cultured in the absence or presence of SPC3 (5 μM). The state of infection was assessed after 7 days of culture by both RT activity and p24 production in cell-free culture supernatants. The phenotype of each virus was determined by syncytium-forming assay in MT-2 cells. CSF, cerebrospinal fluid; NSI, non-syncytium-inducing virus; SI, syncytium-inducing virus.

SPC3 (Fig. 4*d*) confirmed the apparent lack of peptide-induced cytotoxicity.

Anti-HIV Activity of SPC3 in $CD4^-/GalCer^+$ Cells. The mechanism of action of SPC3 was then analyzed in $CD4^-/GalCer^+$ HT-29 cells. When SPC3 was added in competition with HIV-1(NDK), a marked inhibition of infection was observed (Fig. 5). In contrast to the results obtained with $CD4^+$ lymphocytes, SPC3 had no effect on the infection of HT-29 cells when added after the initial contact with the virus: the presence of SPC3 during the whole phase of viral exposure was necessary to obtain an inhibition of infection.

Interaction of SPC3 with gp120 Receptors. To understand the molecular mechanisms involved in the antiviral activity of SPC3 in $CD4^+$ and $CD4^-/GalCer^+$ cells, we studied the effect of the peptide on the interaction between HIV-1 gp120 and its receptors. Binding of gp120 to CD4 was analyzed by using an ELISA gp120-capture assay with CD4-coated plates. At concentrations up to 100 μM, the peptide did not affect the interaction between gp120 and its binding site on the CDR2 domain of CD4 (data not shown). The anti-CD4 mAb Leu3A, which recognizes this domain, was used as a positive control in the assay. As reported (25), this mAb could block gp120 binding to CD4. These data, which show that SPC3 does not act at the level of gp120–CD4 recognition, are consistent with the lack of activity of SPC3 as a competitive inhibitor of HIV-1 attachment to lymphocytes. In contrast, the binding of gp120 to GalCer on high-performance thin-layer chromatography plates was strongly inhibited by SPC3 but not by control peptides that do not inhibit HIV-1 infection of HT-29 cells (data not shown). This agrees with the infection data and suggests that SPC3 acts as a competitive inhibitor of HIV-1 attachment to the surface of $CD4^-/GalCer^+$ cells, as proposed (26).

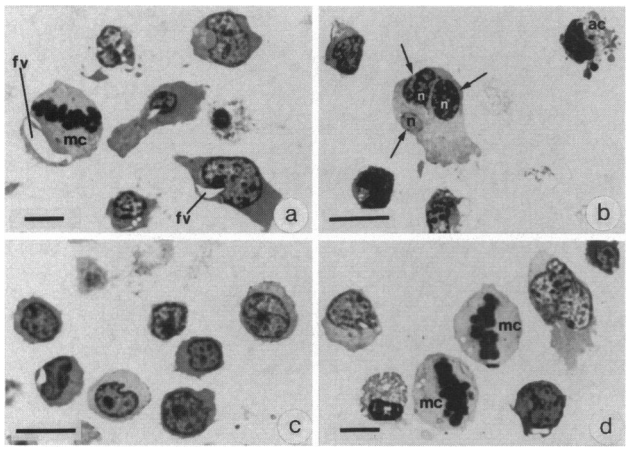

FIG. 4. SPC3 prevents the cytopathic effects associated with HIV-1 infection of PBMCs. PBMCs were exposed to HIV-1(LAI) and, subsequently, treated (or not treated) with 5 μM SPC3 for 7 days. The morphology of SPC3-treated (*c* and *d*) or untreated (*a* and *b*) infected cells was analyzed in semi-thin sections at the optical microscope level. HIV-1-infected PBMCs showed cells with follicular vacuoles (fv) (*a*) and small syncytia (arrows) and apoptotic cells (ac) (*b*). None of these features were observed in PBMCs exposed to HIV-1 and treated with SPC3 (*c* and *d*). Note the presence of mitotic cells (mc) (*d*). n, Nucleus. (Bars = 10 μm.)

DISCUSSION

SPC3 has been designed to mimick a conserved part of the HIV-1 V3 loop, the GPGRAF motif (27). This peptide has a broad anti-HIV activity in $CD4^+$ lymphocytes and macrophages and $CD4^-/GalCer^+$ epithelial cells (15, 18, 26, 28, 29). In $CD4^+$ lymphocytes, SPC3 is able to prevent HIV-1 infection when added as late as 1 h after the initial contact of the cells with the virus. This result is consistent with kinetic analyses of HIV-1 penetration into $CD4^+$ cells showing that virus entry takes place over several hours, the binding to the cell surface being the rate-limiting step (30, 31). Our data show that SPC3 does not interfere with CD4-mediated virus attachment but acts at a post-binding step necessary for HIV-1 entry into $CD4^+$ cells. Since the peptide is able to block the cell–cell fusion induced by HIV-1 (15, 18), it is likely that it also inhibits HIV-1 entry into $CD4^+$ lymphocytes by interfering with the fusion of the viral envelope with the plasma membrane of the target cell. The fact that a minimal post-binding treatment of 2 h with SPC3 is sufficient to obtain the antiviral effect is consistent with a blockade of an early step of the viral cycle. Of particular relevance to our data is the observation that both HIV–cell fusion and cell–cell fusion can be blocked by anti-V3 antibodies after binding of gp120 to CD4 (25). In this regard, the post-binding inhibition of HIV-1 entry induced by SPC3 is a property that would be expected from a molecule designed to mimick the V3 loop.

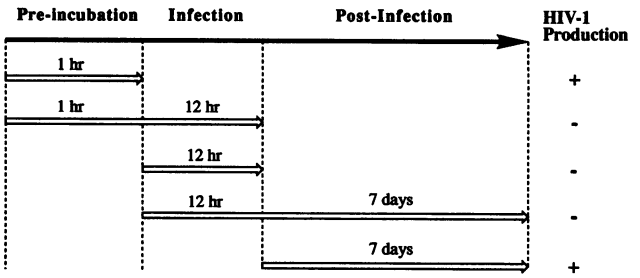

FIG. 5. Effect of SPC3 on HIV-1 infection in HT-29 cells. The arrows indicate the time and duration of treatment of HT-29 cells with 10 μM SPC3. In all cases, the cells were exposed to HIV-1(NDK) at a MOI of 0.005 $TCID_{50}$ per cell for 12 h at 37°C. The state of infection of the cells was assessed 7 days after infection by RT activity and p24 measurements in cell-free supernatants. −, Undetectable levels of both RT activity (<2000 cpm/ml) and p24 production (<10 pg/ml) in SPC3-treated infected cells, whereas virus production was detected in untreated infected cells (RT activity from 25,000 to 50,000 cpm/ml; p24 production from 200 to 400 pg/ml); +, levels of p24 production and RT activity were similar in SPC3-treated and untreated control infected HT-29 cells.

In contrast with the results obtained in CEM cells and PBMCs, SPC3 blocked the infection of $CD4^-/GalCer^+$ HT-29 cells when present in competition with the viral inoculum and had no effect when added after virus exposure. Thus, SPC3 clearly acts differently in $CD4^+$ lymphocytes and in $CD4^-/GalCer^+$ epithelial cells. These data show that the peptide is not able to prevent the infection of HT-29 cells after the attachment of the virus. Moreover, SPC3 was found to significantly decrease the binding of gp120 to the GalCer receptor (26). Therefore, it is likely that the antiviral activity of SPC3 in HT-29 cells results from an inhibition of GalCer-mediated virion binding to the surface of these $CD4^-$ cells.

Inasmuch as SPC3 mimicks a biologically active form of the V3 loop, the different facets of its antiviral activity could be related to (*i*) the involvement of the V3 loop in the interaction between gp120 and GalCer on the surface of $CD4^-/GalCer^+$ cells (10, 11) and (*ii*) the role of the V3 loop in the post-binding events that follow HIV-1 attachment to $CD4^+$ cells leading to virus fusion (25). In the latter case, it is likely that the V3 loop interacts with an accessory binding site necessary for the fusion to proceed. The nature of such a coreceptor has remained elusive. Due to the ability of the V3 loop to recognize GalCer, one should consider the possibility of a glycolipidic coreceptor for HIV-1 in $CD4^+$ lymphocytes.

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1. Sioda, T., Levy, J. A. & Cheng-Mayer, C. (1991) *Nature (London)* **349,** 167–169.
2. Hwang, S. S., Boyle, T. J., Lyerly, H. K. & Cullen, B. R. (1991) *Science* **253,** 71–74.
3. Bolognesi, D. P. (1993) *Adv. Virus Res.* **42,** 103–148.
4. Rusche, J. R., Javaherian, K., McDanal, C., Petro, J., Lynn, D. L., Grimaila, R., Langlois, A., Gallo, R. C., Arthur, L. O., Fischinger, P. J., Bolognesi, D. P., Putney, S. D. & Matthews, T. J. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3198–3202.
5. Goudsmit, J., Boucher, C. A., Meloen, R. H., Epstein, L. G., Smit, L., Van der Hoek, L. & Bakker, M. (1988) *AIDS* **2,** 157–164.
6. Tateno, M., Gonzalez-Scarano, F. & Levy, J. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 4287–4290.
7. Harouse, J. M., Kunsch, C., Hartle, H. T., Laughlin, M. A., Hoxie, J. A., Wigdhal, B. & Gonzalez-Scarano, F. (1989) *J. Virol.* **63,** 2527–2533.
8. Harouse, J. M., Bhat, S., Spitalnik, S. L., Laughlin, M., Stefano, K., Silberberg, D. H. & Gonzalez-Scarano, F. (1991) *Science* **253,** 320–323.
9. Yahi, N., Baghdiguian, S., Moreau, H. & Fantini, J. (1992) *J. Virol.* **66,** 4848–4854.
10. Cook, D. G., Fantini, J., Spitalnik, S. L. & Gonzalez-Scarano, F. (1994) *Virology* **201,** 206–214.
11. Yahi, N., Sabatier, J. M., Nickel, P., Mabrouk, K., Gonzalez-Scarano, F. & Fantini, J. (1994) *J. Biol. Chem.* **269,** 24349–24353.
12. Palker, T. J., Clarck, M. E., Langlois, A. J., Matthews, T. J., Weinhold, K. J., Randall, R. R., Bolognesi, D. P. & Haynes, B. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1932–1936.
13. De Rossi, A. Pasti, M., Mamano, F., Panozzo, M., Dettin, M., Di Bello, C. & Chieco-Bianchi, L. (1991) *Virology* **184,** 187–196.
14. Nehete, P. M., Arlinghaus, R. H. & Sastry, K. J. (1993) *J. Virol.* **67,** 6841–6846.
15. Fantini, J., Yahi, N., Mabrouk, K., Van Rietschoten, J., Rochat, H. & Sabatier, J. M. (1993) *C. R. Acad. Sci. (Paris)* **316,** 1381–1387.
16. Tam, J. P. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5409–5413.
17. Nardelli, B., Lu, Y. A., Shiu, D. R., Dempierre-Defoort, C., Profy, A. T. & Tam, J. P. (1992) *J. Immunol.* **148,** 914–920.
18. Yahi, N., Fantini, J., Mabrouk, K., Tamalet, C., De Micco, P., Van Rietschoten, J., Rochat, H. & Sabatier, J. M. (1994) *J. Virol.* **68,** 5714–5720.
19. Merrifield, R. B. (1986) *Science* **232,** 341–347.
20. Sabatier, J. M., Zerrouk, H., Darbon, H., Mabrouk, K., Benslimane, A., Rochat, H., Martin-Eauclaire, M. F. & Van Rietschoten, J. (1993) *Biochemistry* **32,** 2763–2770.
21. Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Axler-Blin, C., Brun-Vezinet, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220,** 868–870.
22. Spire, B., Sire, J., Zachar, V., Barré-Sinoussi, F., Galibert, F., Hampe, A. & Chermann, J. C. (1989) *Gene* **81,** 275–284.
23. Harada, S., Koyanagi, Y. & Yamamoto, N. (1985) *Science* **229,** 563–566.
24. Faure, E., Yahi, N., Zider, A., Cavard, C., Champion, S. & Fantini, J. (1994) *Virus Res.* **34,** 1–13.
25. Moore, J. P., Bradford, A., Jameson, A., Weiss, R. A. & Sattentau, Q. J. (1993) in *Viral Fusion Mechanisms,* ed. Bentz, J. (CRC, Boca Raton, FL), pp. 233–289.
26. Yahi, N., Sabatier, J. M., Baghdiguian, S., Gonzalez-Scarano, F. & Fantini, J. (1995) *J. Virol.* **69,** 320–325.
27. LaRosa, G. J., Davide, J. P., Weinhold, K., Waterbury, J. A., Profy, A. T., Lewis, J. A., Langlois, J. G., Dreesman, G. R., Boswell, R. N., Shadduck, P., Holley, L. H., Karplus, M., Bolognesi, D. P., Matthews, T. J., Emini, E. A. & Putney, S. D. (1990) *Science* **249,** 932–935.
28. Benjouad, A., Fenouillet, E., Gluckman, J. C. & Sabatier, J. M. (1994) *Antiviral Chem. Chemother.* **5,** 195–196.
29. Fantini, J., Yahi, N., Mabrouk, K., Van Rietschoten, J., Rochat, H. & Sabatier, J. M. (1994) *Lett. Peptide Sci.* **1,** 17–24.
30. Orloff, G. M., Orloff, S. L., Kennedy, M. S., Maddon, P. J. & McDougal, J. S. (1991) *J. Immunol.* **146,** 2578–2587.
31. Rieber, E. P., Federle, C., Reiter, C., Krauss, S., Gürtler, L., Eberle, J., Deinhardt, F. & Riethmüller, G. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10792–10796.