Inhibition of Human Immunodeficiency Virus Type 1 Infection and Syncytium Formation in Human Cells by V3 Loop Synthetic Peptides from gp120

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Because V3 loop-specific antibodies have been shown to inhibit human immunodeficiency virus type 1 (HIV-1) infection of human cells and because specific mutations in the V3 loop render the virus ineffective for infection and syncytium formation, we tested the anti-HIV effects of V3 loop peptides from different HIV-1 strains. We obtained evidence that V3 loop synthetic peptides of 8 to 15 amino acids at nanogram concentrations efficiently blocked HIV-1 IIIB infection of several human T-cell lines and of freshly prepared normal human T cells. More importantly, syncytium formation by three different primary clinical HIV isolates was inhibited by the V3 loop peptide from HIV-1 IIIB at a concentration of 1 $\mu\text{g/ml}$. Concentrations of V3 peptides up to 50 $\mu\text{g/ml}$ were not toxic to any of the human cells studied. Additionally, V3 peptides incubated in normal human serum or plasma exhibited biological and physical stability for up to 24 h. Taken together, these results suggest that the V3 loop peptides have medical utility as therapeutic reagents to either prevent HIV-1 infection in humans or reduce the spread of virus infection in HIV-infected individuals. These findings are especially significant because a number of reports in the literature indicate that the V3 loop region in gp120 plays an important role in the initial stages of HIV-1 infection of cells.

The envelope glycoprotein of human immunodeficiency virus type 1 (HIV-1) plays a central role in the life cycle and pathogenesis of the virus. It is synthesized as a precursor polypeptide, gp160, which is cleaved by a host cell protease to generate the surface envelope glycoprotein gp120 and the transmembrane glycoprotein gp41 (8, 18). Viral infection begins with the envelope glycoprotein binding to the CD4 receptor on uninfected cells. This step is followed by a critical second step in which the lipid bilayer of the virus fuses with the lipid bilayer of the target cell (16, 26). Expression of gp120 at the cell surface has also been shown to induce cell-to-cell fusion, or syncytium formation, in appropriate cell types bearing CD4 (14, 25).

Although the mechanism by which the HIV-1 envelope glycoproteins trigger membrane fusion is poorly understood, it is well established that proteolytic processing of the precursor gp160 is required for HIV-1 infection of cells (8, 27). Several reports in the literature have shown that the V3 loop region of gp120 is essential for HIV-1 infection because (i) specific mutations introduced into the V3 loop will inhibit infectivity and syncytium formation (3, 4, 9), (ii) the V3 loop region is the target for a trypsin-related protease on the host cell surface (6, 11, 20), and (iii) antibodies to the principal neutralizing domain in the V3 loop region inhibit HIV-1 infection of cells without interfering with the binding of HIV-1 gp120 to its cellular receptor, the CD4 molecule (10, 12, 15, 21, 23). It has therefore been proposed that after gp120 present on the viral surface binds to the CD4 receptor on the target cell membrane, the V3 loop region in gp120 is proteolytically cleaved by a cell surface protease, leading to a conformational change in the gp120-gp41 protein complex on the viral surface (6, 11, 20). Such a change is hypothesized to be responsible for exposing the fusogenic domain of the transmembrane protein gp41,

resulting in the fusion of the viral-particle membrane with the cell membrane.

Recently, Koito et al. (11) reported that synthetic peptides (of 24 and 36 amino acids) corresponding to the principal neutralizing domain of HIV-1 IIIB inhibit syncytium formation by interacting with a proteinase-like molecule at the cell surface. However, De Rossi et al. (2) reported that synthetic peptides (of 24 amino acids) from principal neutralizing domains of HIV-1 MN and IIIB enhanced infection of Molt-3 cells by different HIV-1 strains, presumably through a CD4-dependent mechanism. We examined the effects of synthetic peptides from the V3 loop regions of different HIV-1 strains and observed that peptides of 8 to 15 amino acids exhibit efficient inhibition of virus infection of various human T cells as well as of virus-induced syncytium formation.

We tested a 15-amino-acid synthetic peptide, designated R15K, from the V3 loop region in HIV-1 IIIB for its effect on infection of the human T lymphoblastoid cell lines MT-4 and CEM by HIV-1 IIIB. In these experiments, the cells were pretreated with various concentrations of the peptides for 15 min before being infected with the virus. The amount of virus used is equivalent to 10 infectious virus particles per cell. This calculation was based on endpoint dilution analysis of virus stock and is equal to approximately 10 times the dose required for maximum cytopathic effect on MT-4 cells. We measured the infectivity in terms of virus production by assaying for reverse transcriptase activity in the culture medium after 7 days of infection as described by Popovic et al. (22). While infection of MT-4 cells was blocked by 90% at a peptide concentration of 150 ng/ml (0.15 $\mu\text{g/ml}$) (Fig. 1A), as little as 8 ng/ml of R15K completely inhibited infection of CEM cells by HIV-1 IIIB (Fig. 1B). The lack of cytotoxicity of R15K peptide at these concentrations was demonstrated in MT-4 cells by determining total viable cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay (1, 5) (Fig. 1A) whereas the viability of CEM cells was estimated by trypan blue dye exclusion (unpublished results). Several peptides

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FIG. 1. Peptide R15K inhibits infection of human T-cell lines by HIV-1. (A) MT-4 cells (5×10^4 per well) were preincubated in triplicate wells of a 96-well microtiter plate with various concentrations of the negative control peptide or R15K peptide for 15 min at 37°C and then infected with HIV-1 at 10 infectious particles per cell. Other controls included cells alone and cells infected with HIV-1 but without R15K. The percent reduction in the amount of reverse transcriptase (RT) activity in the culture medium of cells incubated with various concentrations of R15K was determined 7 days after infection by the method of Popovic et al. (22). The toxicity of R15K for the MT-4 cells was also determined in the same experiment by incubating the uninfected cells with medium alone or with various concentrations of R15K and calculating percent viability by the MTT dye reduction assay (1). (B) CEM cells (10^5 per well) were seeded in a 48-well plate and were infected with HIV-1 at 10 infectious particles per cell in the presence of various concentrations of R15K. The RT activity of the culture medium was measured after 7 days of incubation. (C) Primary human T cells (10^5 per well) were incubated in triplicate wells of a 96-well microtiter plate with medium alone, control peptide S13T, or various concentrations of R15K and then infected with HIV-1 at 10

TABLE 1. Amino acid sequences of synthetic peptides tested for inhibition of HIV infection^a

Peptide	Source	Amino acid sequence ^b
R15K	HIV IIIB <i>env</i>	³¹⁵ RIQRGPGRAFTIGK ³²⁹
N24G	HIV IIIB <i>env</i>	³⁰⁸ NNTRKSIRIQRGPGRAFTIGKIG ³³¹
R8K	HIV IIIB <i>env</i>	³²² RAFVTIGK ³²⁹
T13Q	HIV RF <i>env</i>	³¹⁷ TKGPGRVIYATGQ ³²⁹
H13N	HIV MN <i>env</i>	³¹⁷ HIGPGRAFYTTKN ³²⁹
F14F	HIV IIIB <i>env</i>	²¹⁵ FEPIPIHYCAFPGF ²²⁸
K14L	HIV IIIB <i>gag</i>	²⁸ KYKLKHIVWASREL ⁴¹
E12K	HIV IIIB <i>pol</i>	⁴⁶⁷ ELELAENREILK ⁴⁷⁸
C15P	HIV IIIB <i>pol</i>	²⁰⁵ CTEMEKEGKISKIGP ²¹⁹
S13T	<i>c-mos</i> proto-oncogene	¹⁵⁸ STRTPEDSNLSLT ¹⁷⁰
I15M	Scrambled R15K	IFPGKRTIVAGIRGM

^a Peptides were synthesized by Merrifield's solid-phase method (19), either on a modified Vega 250 automatic peptide synthesizer or by the bag method as described by Houghten (7).

^b Amino acid sequences are according to LaRosa et al. (12).

were used as negative control reagents in these experiments (Table 1), including a peptide similar to R15K but with a scrambled amino acid sequence (I15M), one peptide (K14L) from the *gag* gene, two peptides (E12K and C15P) from the *pol* gene, one peptide (F14F) from a different region of the *env* gene, and finally one peptide (S13T) from the *c-mos* proto-oncogene. No significant level of inhibition of HIV infection was observed with any of these negative control peptides.

We also studied the ability of the R15K peptide at various concentrations to inhibit HIV-1 IIIB infection of primary human T cells. Human T cells isolated from normal donors by standard techniques (17) were stimulated for 72 h with phytohemagglutinin, washed three times, and resuspended at 10^6 /ml in Dulbecco's modified Eagle's medium containing glutamine, 10% heat-inactivated fetal calf serum, and 20 U of human interleukin 2 (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml. Aliquots (100 μ l) of the cell suspension were preincubated at 37°C for 15 min with various concentrations of R15K in triplicate wells of a 96-well microtiter plate. These cells were then infected with 10 infectious particles of HIV-1 IIIB per cell and incubated for 9 days before being assayed for reverse transcriptase activity in the culture medium. R15K inhibited reverse transcriptase production by 50% at 0.12 μ g/ml and by 90% at 1.25 μ g/ml (Fig. 1C). R15K was not toxic to these cells at concentrations up to 20 μ g/ml, but at 50 μ g/ml, 50% of the cells were viable (Fig. 1C). The scrambled R15K peptide (I15M), when tested at different concentrations (0.01 to 1 μ g/ml) in experiments with MT-4 cells and fresh human T cells, showed no inhibitory effect.

We also tested whether the R15K peptide would directly affect the infectivity of the virus. In these experiments, we preincubated the virus stock with the peptide at 1 μ g/ml for 4 h at 37°C and subsequently pelleted the virus by ultracentrifugation according to standard protocol. The resulting virus was then compared with untreated virus for infection of MT-4 and H9 cells, and we observed no change in infectivity as measured by both virus-induced cytopathic effect (in MT-4 cells) and reverse transcriptase activity in the culture medium (unpub-

infectious particles per cell. After 9 days, RT activity in culture medium was determined. The total numbers of viable cells at the end of 9 days of incubation in the presence of various concentrations of the peptide were measured by trypan blue dye exclusion.

TABLE 2. Effect of V3 loop peptides from different HIV-1 strains on infection of primary human T cells by HIV-1 IIIB^a

Cell type and treatment	Reverse transcriptase activity ^b with cells from subject:		
	1	2	3
Uninfected cells	509.52 ± 18	118.75 ± 7	279.44 ± 32
Infected cells plus:			
Medium only	5,425.30 ± 174 (0)	27,142.85 ± 2,116 (0)	36,340.2 ± 1,186 (0)
R15K	407.45 ± 5.2 (100)	5,814.51 ± 212 (79)	6,940.56 ± 214 (81)
N24K	382.25 ± 19 (100)	6,690.26 ± 390 (76)	4,399.40 ± 216 (89)
R8K	1,876.51 ± 44 (72)	7,080.50 ± 215 (74)	8,971.14 ± 602 (76)
T13Q	2,172.19 ± 171 (66)	6,424.20 ± 340 (77)	17,097.25 ± 303 (53)
H13N	1,713.72 ± 44 (75)	6,424.20 ± 340 (77)	19,094.55 ± 183 (48)
S13T	4,948.63 ± 178 (10)	24,824.35 ± 89 (9)	ND

^a Primary human T cells isolated from fresh human blood were preincubated with peptides (1 µg/ml) and then infected with HIV-1 IIIB as described in the legend to Fig. 1. On day 9, reverse transcriptase activity was measured.

^b Data are mean counts per minute ± standard error of triplicate samples. The numbers in parentheses are percent inhibition. ND, not determined.

lished results). These results strongly suggest that the infection-inhibitory effects of V3 peptide are not due to an adverse effect on the virus.

Since R15K peptide was effective against infection of normal human T cells, we tested synthetic peptides T13Q and H13N, derived from the V3 loop regions of HIV-1 RF and MN strains, respectively, for their ability to inhibit infection of primary human T cells by HIV-1 IIIB. These results were compared with the inhibitory effects of HIV-1 IIIB V3 loop peptides of various lengths (R15K, N24G, and R8K). All the peptides were added to the cells at a concentration of 1 µg/ml before infection with HIV-1 IIIB. Inhibition of virus infection was determined by assaying the reverse transcriptase activity in culture medium, and the results for cells of three different normal donors are shown in Table 2. The V3 loop peptides from HIV-1 RF and MN strains inhibited HIV-1 IIIB infection by 53 to 77% and 48 to 77%, respectively. In the same experiment, the control peptide S13T did not significantly inhibit infection. Thus, these results indicate that V3 loop

peptides from HIV-1 MN and RF strains exhibit cross-reactivity in terms of infection inhibition. Another important observation from these experiments is that while the 24- and 15-amino-acid peptides (N24G and R15K, respectively) from the V3 loop region of HIV-1 IIIB produced 76 to 100% inhibition, R8K also inhibited HIV-1 IIIB infection of primary human T cells by 72 to 76%. These results clearly indicate that the HIV infection-inhibitory property of the V3 loop peptides from HIV-1 IIIB is within this 8-amino-acid region.

In order to further confirm the HIV infection-inhibitory effect of the 8-amino-acid V3 loop peptide, R8K, we tested infection of four different human T-cell lines by using this peptide at 1 µg/ml. We observed that the R8K peptide was effective in inhibiting HIV-1 IIIB infection of H9 cells by 82%, CEM cells by 85%, MT-4 cells by 88%, and VB cells by 87% as measured by determining the reverse transcriptase activity in the culture medium after 7 days of infection. Additionally, we performed Western blot (immunoblot) analysis to detect p24 protein in these cell lines after infection in the presence or

FIG. 2. (A) Western blot analysis of inhibition of expression of HIV-1 *gag* proteins p55 and p24 by peptide R8K in different human T-cell lines. Total cell lysates of various cell lines infected with HIV-1 IIIB after pretreatment with peptide R8K at 1 µg/ml or no pretreatment were separated on a 10% polyacrylamide gel, transferred to a nitrocellulose filter, and treated with a 1/200 dilution of serum from an HIV-seropositive AIDS patient and ¹²⁵I-labeled protein A. Molecular weight markers (in thousands) are shown on the right, and various HIV-1 protein bands are indicated on the left. Samples in lanes 1 to 4 were cell lysates of H9, CEM, VB, and MT-4, respectively, infected with HIV-1 IIIB in the absence of peptide R8K, while lanes 5 to 8 are corresponding cultures infected in the presence of R8K. Percent inhibition of reverse transcriptase (RT) activity by R8K in each of the cell lines is indicated. (B) Western blot analysis of extracts from H9, CEM, VB, and MT-4 cells (lanes 9 to 12, respectively) without virus infection and peptide pretreatment. The experimental method was similar to that described for panel A.

absence of R8K at 1 $\mu\text{g/ml}$. Nine days postinfection, the cell extracts were prepared and separated on 10% polyacrylamide gels. The proteins transferred onto nitrocellulose filters were reacted with serum from an HIV-positive AIDS patient and ^{125}I -labeled protein A, and the results are presented in Fig. 2. It is clear from the data that, concurrent with the inhibition of reverse transcriptase in these cell lines, there is also inhibition of p55 and p24 formation. These results, along with the data from Table 2 showing inhibition of infection of human T cells isolated from three different healthy donors, indicate that the effective amino acid sequence is contained within the R8K peptide. Analyses of shorter peptides and of peptides with single-amino-acid changes should provide further understanding of the mechanics of the role of the V3 peptides in the inhibition of HIV-1 infection of human cells.

Since V3 loop peptides from three HIV-1 strains exhibited inhibition of HIV-1 IIIB infection of human T cells (Table 2), we tested their effects on syncytium formation. For these experiments, HeLa-CD4 cells were infected with control vaccinia virus (vSC8) or vaccinia virus recombinants that express gp160 from the IIIB (vPE16) or RF (vRF222) strain of HIV-1 (all reagents were obtained through the AIDS research and reference reagent program of the National Institute of Allergy and Infectious Diseases). HeLa-CD4 cells were plated at a density of 10^6 per well of a six-well flat-bottom plate (Costar no. 3506) and allowed to grow for 40 h at 37°C , at which time the cell monolayer was 80% confluent. Various control or V3 loop peptides at a concentration of 1 $\mu\text{g/ml}$ in 1 ml of medium were added to the monolayers and incubated for 30 min at 37°C . The peptide solutions were removed, and cell monolayers were washed three times with complete medium. Subsequently, vaccinia virus stocks appropriately diluted to obtain a multiplicity of infection at 100 in a total volume of 1 ml were added, and incubation was continued for an additional 2 h. At this time, the vaccinia virus inoculum was replaced with 3 ml of fresh medium, and cultures were incubated for 18 h. At the end of the incubation period, syncytia were observed under the microscope at a magnification of $\times 100$ and photographed. While HeLa-CD4 cells preincubated either with medium alone or with peptide before infection with control vaccinia virus showed no syncytia (Fig. 3A and D), cells not preincubated with peptide when infected with vaccinia virus recombinant vPE16 or vRF222 resulted in formation of 80 to 90 syncytia per well of a six-well plate (Fig. 3B and C, respectively). Efficient inhibition of syncytium formation was observed in cells pre-treated with peptides R15K (100% inhibition, no syncytia) and T13Q (80 to 90% inhibition, 8 to 16 syncytia) before infection with recombinant vaccinia viruses vPE16 and vRF222, respectively (Fig. 3E and F). These experiments were repeated at least three times, and each time similar results were obtained with less than 5% difference in numbers of syncytia with various treatments. Thus, V3 loop peptides from HIV-1 IIIB and RF proved effective for inhibition of syncytium formation in cells expressing envelope protein from the respective HIV-1 strain.

We also tested the effectiveness of R15K peptide against syncytium formation by different primary clinical HIV-1 isolates referred to as R006, R042, R043, and R086 (obtained as very early passage of VB cell culture medium from Miles Cloyd, University of Texas Medical Branch, Galveston). HeLa-CD4 cells infected with primary isolates R006, R042, and R043 exhibited 25 to 30 syncytia per well of a six-well plate (Fig. 4A, B, and C), while cells treated with R15K at a concentration of 1 $\mu\text{g/ml}$ showed no syncytium formation (100% inhibition) by these clinical HIV-1 isolates (Fig. 4D, E, and F). However, although syncytia were observed with isolate R086 (28 to 30

FIG. 3. Inhibition of syncytium formation by V3 loop peptides. Monolayers of HeLa-CD4 cells were incubated with medium alone (A, B, and C), medium containing R15K (D and E), or T13Q (V3 peptide from HIV-1 RF) (F) at 1 $\mu\text{g/ml}$ for 30 min at 37°C . The cell monolayers were washed before infection with control vaccinia virus (vSC8) (A and D) or recombinant vaccinia virus expressing gp160 from HIV-1 strain IIIB (vPE16), (B and E) or RF (vRF222) (C and F). After 18 h of incubation, cells were observed for syncytia (arrows) under the microscope at $\times 100$ magnification and photographed. Final magnification, $\times 90$.

per well), no inhibitory effect was observed (28 to 30 syncytia per well; unpublished results) with this V3 loop peptide (R15K) at the concentration tested. Thus, the V3 loop peptide R15K was effective not only against laboratory isolates of HIV-1 but also against three of four clinical isolates for inhibiting syncytium formation. These data strongly suggest that the inhibitory effects observed with R15K and other V3 loop peptides are not limited to blocking HIV-1 infection of cells, as R15K can inhibit the fusion of CD4-expressing normal cells with HIV-infected cells expressing the envelope protein, a phenomenon suggested to be responsible for HIV-induced pathology in AIDS patients (13, 14, 25, 27).

These results are in agreement with those of Koito et al. (11), who reported that synthetic peptides (of 24 and 36 amino acids) corresponding to the major HIV-1 IIIB neutralizing epitope of the V3 loop of gp120 (amino acids 303 to 338) inhibited syncytium formation between the HIV-1-infected CCRF-CEM and uninfected Molt-4 cells in a dose-dependent manner. However, in their studies, the 36-amino-acid peptide was effective at a concentration of 100 μM (approximately 300 $\mu\text{g/ml}$) while the 24-amino-acid peptide (amino acids 308 to 331) was required at a concentration of 300 μM (approximately 720 $\mu\text{g/ml}$) to achieve the same level of inhibition of syncytium formation. However, neither of these peptides was tested by these authors for its capacity to inhibit HIV infection of cells. On the other hand, in our studies, we observed 80 to

FIG. 4. R15K inhibits syncytium formation by primary clinical HIV-1 isolates. Monolayers of HeLa-CD4 cells were incubated with medium alone (A, B, and C) or medium containing R15K at 1 μ g/ml (D, E, and F) for 30 min at 37°C. The cell monolayers were washed before infection with primary clinical isolates of HIV-1 R006 (A and D), R042 (B and E), and R043 (C and F). After 72 h of incubation, cells were observed under the microscope for syncytia (arrows) at $\times 100$ magnification and photographed. Final magnification, $\times 78$.

100% inhibition of syncytium formation in HeLa-CD4 cells at much lower concentrations (1 μ g/ml or less) of V3 loop peptides from two different HIV-1 strains. At this time, we do not know the reason for this discrepancy between our results and those of Koito et al. (11) regarding effective concentrations of V3 peptides for inhibition of syncytium formation. However, in a subsequent publication from the same group it was shown that a 15-amino-acid peptide exhibited strong inhibition of HIV-1 IIIB-induced syncytium formation at only 1 μ M (6). This peptide was from trypstatin, a protease inhibitor which exhibits 40% sequence homology to the 15-amino-acid V3 loop peptide designated R15K in our studies. These results suggest that the length of the peptide and the additional amino acid sequence are important in determining the effective inhibitory concentration of V3 loop peptides.

However, both the results presented here and those of Koito et al. (11) are different from the results reported by De Rossi et al. (2), who observed that synthetic peptides 24 amino acids in length from V3 loop regions of HIV-1 IIIB and MN strains enhanced infection of Molt-3 cells by different HIV-1 strains through a CD4-dependent mechanism. In their experiments, the V3 loop peptide from HIV-1 MN showed significant enhancement of infection of Molt-3 cells by the homologous virus strain at concentrations ranging between 5 and 20 μ M (approximately 12 to 48 μ g/ml). In the same study, a 24-amino-

FIG. 5. Biological stability of R15K peptide in fetal calf serum. R15K was incubated at 37°C in fetal calf serum at a concentration of 100 μ g/ml for 24 h. At different time intervals, aliquots of peptide corresponding to 1 μ g/ml (final concentration) were added to MT-4 cells in a 96-well microtiter plate. After 15 min of incubation with peptide, the cells were infected with HIV-1 as described in the legend to Fig. 1. Seven days after infection, the total number of viable cells was determined by the MTT dye reduction assay (1), and RT activity in the culture medium was assayed by the method of Popovic et al. (22). The controls included cells incubated in medium alone with and without virus infection and cells infected with virus and incubated with R15K peptide that was not preincubated in fetal calf serum. The results are expressed as percent cell viability or percent reverse transcriptase (RT) activity in MT-4 cells that were infected with HIV-1 in the absence of R15K.

acid peptide from the HIV-1 IIIB strain less efficiently enhanced infection by strain IIIB while a homologous peptide from strain RF was completely ineffective. More importantly, in these studies, a 10-amino-acid peptide corresponding to the middle portion (10 amino acids) of the HIV-1 IIIB V3 loop did not show enhancement of infection. This 10-amino-acid peptide is completely homologous to the C-terminal portion of the R15K peptide in our studies (and the sequence of the R8K peptide also overlaps with this peptide). It is difficult to reconcile our findings with those of De Rossi et al. (2).

In order to gain some understanding of the stability of the inhibitory activity of V3 peptides, we incubated the R15K peptide in fetal calf serum at 37°C. At different intervals during the incubation, aliquots at a final concentration of 1 μ g/ml (approximately 0.6 μ M) were tested for inhibition of HIV-1 infection of MT-4 cells. At the end of the 7-day incubation period, both cell viability and the amount of reverse transcriptase present in the culture medium were measured (Fig. 5). It is clear that R15K retained its full-strength inhibitory activity for as long as 4 h of incubation in serum. Even after 24 h of incubation, R15K retained 50% of its inhibitory activity. We obtained similar results with the V3 loop peptide from HIV-1 MN incubated in either fetal calf serum or human serum (unpublished results). The retention of efficient inhibitory effect at these relatively low concentrations bodes well for the use of R15K and other V3 loop peptides as therapeutic reagents. Additionally, we observed that V3 loop peptides incubated in human serum or plasma retained both physical stability (as measured by high-pressure liquid chromatography analysis) and biological activity (unpublished results).

In view of the clinical importance of HIV in AIDS, numerous attempts to develop effective therapeutic reagents have been made in recent years. Most of these approaches were

based on the rationale that virus-specific enzymes such as HIV reverse transcriptase should be suitable targets. Our results provide support for potential therapeutic application of V3 loop peptides because they not only are capable of blocking HIV infection of a number of human cell lines and of primary human T cells isolated from fresh healthy human blood samples but also inhibit cell-to-cell spread of HIV. Also, the V3 loop peptides as a mixture could provide the added advantage of being an immunotherapeutic reagent because, as we recently demonstrated in case of R15K (24), they are capable of inducing HIV-1-specific cytotoxic T-lymphocyte responses that can effectively kill virus-infected cells. Thus, V3 loop peptides have the potential to be medically useful both to block viral spread and HIV-induced pathology and to increase specific cytotoxic T lymphocytes for destruction of virus-infected cells.

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