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In vitro inhibition of R5 HIV-1 infectivity by X4 V3-derived synthetic peptides

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Abstract. The aim of the present study was to investigate the inhibitory effect of synthetic peptides derived from the principle neutralizing domain of the V3 loop of the HIV-1 gp120 in the infectivity rates of HIV-1 variants with different tropism. Assessment of the viral infectivity was determined by detection of soluble HIV $p24^{gag}$ antigen in the culture supernatants of PM-1 T cells and primary macrophages after *in vitro* infection with the R5, Ba-L and X4, NL4.3 variants in the presence or absence of soluble V3-derived synthetic peptides. Our results showed a clear inhibition of Ba-L infectivity in both the PM-1 T cells and primary macrophages. The degree of inhibition was related to the number of basic amino acids in the peptide. The most effective inhibitory peptide, at a concentration of 50 ng/ml, was the one with the highest cationic potential, achieving over 60% inhibition to the PM-1 T cell line and over 90% to primary macrophages. The same peptides did not affect the NL4.3 infectivity. In addition to our previously reported observations on the electrostatic nature of the V3-CCR5 interaction, we show here that V3-like peptides from the more electropositive X4 variants may be useful as effective antagonists and potential infectivity blockers of the R5 variants.

Introduction

The HIV-1 envelop glycoprotein gp120 mediates viral entry by binding to target cell CD4 and chemokine receptors (1-5). Different virus isolates and strains display distinct biological phenotypes for infection depending on the expression patterns of the major co-receptors (CCR5 and CXCR4) on the target cell. Macrophage tropic viruses are thought to be responsible for the initial infection, predominate during the asymptomatic phase and persist throughout the infection. These isolates use CCR5 as a co-receptor and are referred to as R5 variants. Naïve and resting memory $CD4^+$ T lymphocytes can be infected directly by isolates that utilize specifically CXCR4 as co-receptor (T cell line adapted or X4 variants) which evolve from R5 strains during disease progression (6). There are also transient strains, which are referred to as R5X4.

The gp120-CD4 co-receptor interaction is also considered to be the most potent inducer of cell apoptosis, acting preferentially on uninfected memory $CD45RO^+/CD4^+$ T lymphocytes (7-9). We have reported that lipopeptides containing the principle neutralizing domain of the V3 loop of the HIV-1 gp120 when exposed to monocyte-derived macrophages interact during the process of antigen presentation with the CCR5 of the responding effector $CD4^+/CD45RO^+$ T lymphocytes (10,11). These V3 peptides interacted with a 22 a.a. synthetic peptide from the amino terminal domain of CCR5. The nature of this interaction is electrostatic and appears to play a prominent role in the pathogenesis of the virus (12). Indeed, we have postulated that the degree of V3-mediated co-activation of the responding T lymphocytes is directly associated with the cationic potential of the V3 peptide (13).

The above model predicts that peptides with higher cationic potential than the corresponding V3 domain of the infecting R5 strain should interfere with the binding process and consequently with viral infectivity. In the present study we addressed this question using the R5 strain Ba-L and the X4 strain NL4.3.

Materials and methods

HIV-1 strains. Virus-containing supernatant fractions from cultures of M tropic Ba-L and T cell line tropic NL4.3 HIV-1 strains were used to infect target cell cultures.

Cell cultures. Infectivity assays were performed using: i) the PM-1 T cell line, a derivative of HUT-78 neoplastic T cell line expressing CD4, CXCR4 and transfected to express CCR5;

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Table I. Synthetic peptides used as HIV-1 infection inhibitors.

gp120/V3 peptides from HIV-1 strains	Sequence															Size
V3 peptides from HIV-1 strains																
LAI	R	K	S	I	R	I	Q	R	G	P	G	R	A	F	Y	15mer
MN	•	•	R	•	H	•	-	-	•	•	•	•	•	•	•	13mer
SF2	•	•	•	•	N	•	-	-	•	•	•	•	•	•	•	13mer
SF128	•	•	•	•	Y	•	-	-	•	•	•	•	•	•	•	13mer
SF162	•	•	•	•	T	•	-	-	•	•	•	•	•	•	•	13mer
De novo peptides																
LAI+1	V	G	•	•	L	•	•	•	•	•	•	A	•	•	•	15mer
LAI+9	•	•	•	•	•	R	•	•	R	•	K	•	•	K	•	15mer
LAI scrambled	A	V	R	F	S	K	I	G	R	•	R	Q	G	R	I	15mer

•, conserved amino acid residue; -, amino acid absence.

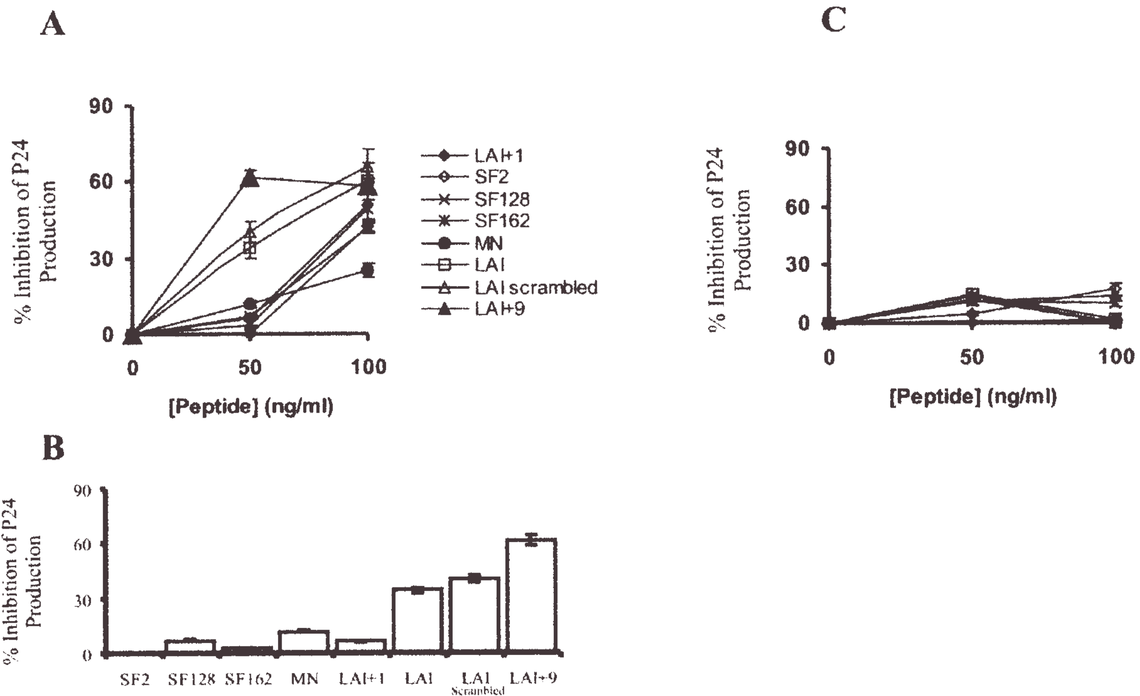

Figure 1. Inhibition of PM-1 T cell line infection by HIV-1 using V3 peptides, as assessed with secreted HIV-1 p24 protein: (A), inhibition using the R5 HIV-1 strain Ba-L and peptides at 0, 50, and 100 ng/ml; (B), comparison of inhibition efficiency at 50 ng/ml V3 peptide on Ba-L; (C), inhibition using the X4 HIV-1 strain NL4.3 and peptides at 0, 50, and 100 ng/ml HIV-1 strains. Each value represents a mean of tests in triplicate.

and ii) with primary blood macrophages isolated from peripheral blood mononuclear cells (PBMCs) of HIV-1 and CMV sero-negative donors, after centrifugation on a Ficoll-Pacque gradient (14). The macrophage fraction was enriched to approximately 98% purity by CD14 positive selection using specific magnetic separation according to the manufacturer's instructions (MACs protocol, Miltenyi Biotec, Germany).

Inhibitor peptides. Eight HIV-1 gp120/V3 peptide variations from the semi-conserved region (a.a. 304-318) were synthesized using F-moc/tBu chemistry (15). Five of them derived from the subtype B (SF2, SF128, SF162, MN, and LAI) strains, and three variations derived from LAI peptide sequence containing *de novo* amino acid substitutions (Table I).

The V3 peptides we used were from HIV-1 strains selected for comparison reasons to have the minimal differences in their amino acid sequences, containing essentially the GPGRAFY motif at the carboxy terminal. The corresponding V3 domains from the infecting strains Ba-L and NL4.3 only differed from the SF2 and LAI peptides at the carboxy end valine that was substituted in the corresponding strains by tyrosine (V318Y).

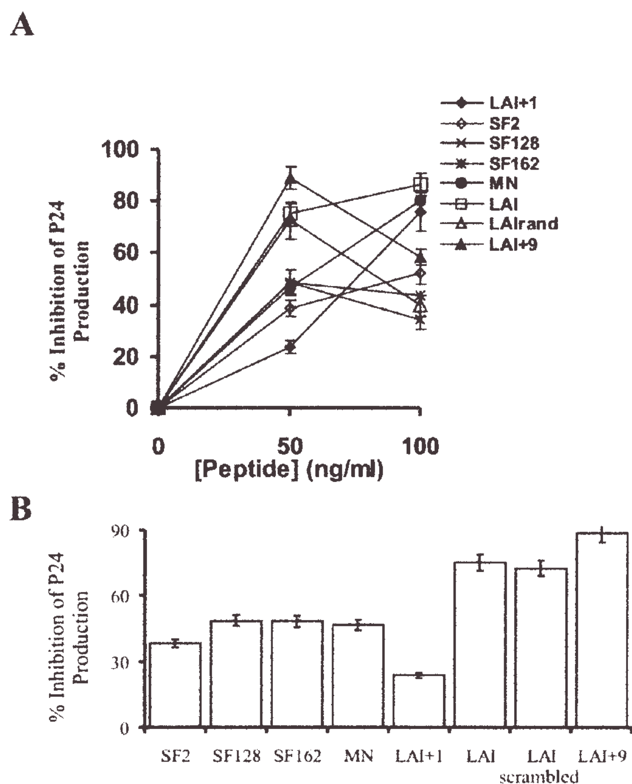

Figure 2. Inhibition of human primary macrophage infection by Ba-L (R5) HIV-1 using V3 peptides, as assessed with secreted HIV-1 p24 protein: (A), inhibition using the peptides at 0, 50, and 100 ng/ml; (B), comparison of inhibition efficiency at 50 ng/ml V3 peptide on HIV-1. Each value represents a mean of tests in triplicate.

Infectivity assays and p24 assessment. Purified primary macrophages or PM-1 T cells were cultured in RPMI-1640 supplemented with 5% human serum (HS) or 2% foetal calf serum (FCS) respectively, in the absence or presence of V3 peptides (50 or 100 ng/ml) added 12 h prior to HIV-1 infection. Filtered stock supernatants obtained from primary HIV-1 infected cultures were tested for the presence of p24 antigen and appropriate volumes of positive culture supernatants in a moi of 0.1 were used to infect 5×10^4 primary macrophages/well or 2×10^4 PM-1 T cells/well, and incubated overnight at 37˚C. The cells were then washed twice with 50 mM PBS 1x (pH 7.4) and resuspended in RPMI-1640 supplemented with 5% HS or 2% FCS respectively. The cells were cultured for a further week, sampling for p24 at the end of this period. Assessment of the viral growth was determined by estimation of soluble p24 antigen in the culture supernatants using an in-house p24 assay (16,17).

Results and Discussion

The presence of V3 and *de novo* designed peptides (Table I) appeared to inhibit the infection of CCR5-transfected PM-1 T cells by Ba-L (Fig. 1). Characteristically, infected cultures in the absence of peptides produced 118.6 ng/ml p24, whereas in the presence of the most effective peptide, LAI+9, at a concentration of 50 ng/ml, produced 45.9 ng/ml p24. The inhibition effect (Fig. 1A) was far too strong at the higher peptide concentration (100 ng/ml) to assess reliably the inhibition efficiency. At the lower concentration (50 ng/ml), the interference of viral infection was distinct and clearly associated with the number of basic amino acids in the inhibitory peptides (Fig. 1A and B). In contrast, the same peptides did not effectively inhibit the infection of PM-1 T cells by NL4.3 (Fig. 1C). These data indicate either direct interaction of the peptides with CCR5, or a greater sensitivity of the Ba-L strain to local electrostatic changes at the cell surface that alters significantly its infectivity.

We further investigated the inhibition effect of the peptides with human primary macrophages (Fig. 2). We observed similar patterns with Ba-L (Fig. 2A), achieving over 90% inhibition with the peptide LAI+9 at 50 ng/ml (Fig. 2B). NL4.3 was not tested, as it does not infect macrophages *in vitro*. It is worth noting that in both cell culture and primary macrophage experiments, removing the peptides just prior to adding the viral particles restored p24 production to no-peptide control culture levels (data not shown).

These results support the proposed prediction that peptides with cationic charge higher than that of the infective strain can effectively compete and interfere with the process of viral binding. It was interesting to note that our polycationic X4 V3 peptides were more effective inhibitors of the infective R5 strain than of the X4 strain, suggesting a possibility of R5 viruses being more sensitive to electrostatic charges. This notion may well be supported by reports that polyanionic compounds such as heparin, dextran sulphate and glycosaminoglycan sulphates enhance HIV-1 infection (18-20). In addition, the precise role of V3 function in relation to co-receptor usage, particularly during the transition from CCR5 to CXCR4, and the impact in infectivity remain under debate (21,22). There is no evidence, however, that V3-like peptides from X4 variants cannot be considered as potential inhibitors of R5 strains. Entry of the virus into the host cell is a complex process, not fully understood. Emerging data suggest that other components such as Nef may also have an impact on virion infectivity that is independent of co-receptor tropism (23).

Our previous (10,12,13), and present reports suggest that V3 and V3-like peptides can interfere with both the function of antigen presentation and the infection process of R5 HIV-1 strains via surface CCR5. There is no apparent structure-related restriction in this phenomenon. The cationic V3 domain and the external anionic N-terminal of CCR5 appear to be a major interaction interface between the viral gp120 and the co-receptor CCR5 during the viral binding process (24,25). This is supported by reports indicating that only CCR5-Nt peptides containing sulfotyrosines were capable of inhibiting the fusion and entry processes of R5 isolates to the host cell (26). Our results suggest that peptides with higher cationic potential have also higher affinity for CCR5 and thus compete effectively against the viral V3 loop of R5 variants.

It is conceivable that V3-like peptides exert allosteric changes to the tertiary conformation of CCR5 (27), thus being potentially useful as CCR5 antagonists and possible blockers of HIV infection. A more extensive study is, however, needed with a larger number of different R5 and X4 strains to confirm these findings, and more importantly, to assess the potential

genetic escape, if any, from the V3-like peptides (21). Finally, the model of cationic peptides is not unique to HIV-1 pathogenicity, as a similar mechanism has been described that potentially links innate with acquired immunity (28,29).

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