Apelin peptides block the entry of human immunodeficiency virus (HIV)

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Abstract The orphan G protein-coupled receptor APJ has been shown to be a coreceptor for human and simian immunodeficiency virus (HIV and SIV) strains. We have determined that some HIV and SIV strains use APJ as a coreceptor to infect the brain-derived NP-2/CD4 cells. Because apelin is an endogenous ligand for the APJ receptor, we examined the inhibitory effects of apelin peptides on HIV infection, and found that the apelin peptides inhibit the entry of some HIV-1 and HIV-2 into the NP-2/CD4 cells expressing APJ. The inhibitory efficiency has been found to be in the order of apelin-36 > apelin-17 > apelin-13 > apelin-12.

Key words: Human immunodeficiency virus; Simian immunodeficiency virus; Apelin; APJ; Coreceptor

1. Introduction

Recent studies have shown that human immunodeficiency virus (HIV) entry into target cells requires binding of the viral envelope protein to CD4, followed by interaction with one of several coreceptors. The macrophage-tropic HIV-1 uses CCR5 as a coreceptor, while T cell line-tropic (T-tropic) HIV-1 uses CXCR4 [1–5]. Dual-tropic HIV-1 that can infect T cell lines and macrophages uses both CCR5 and CXCR4, and occasionally other chemokine receptors such as CCR2b and CCR3 [3,4]. The endogenous ligands for CCR5, RANTES, MIP-1α, and MIP-1β, block HIV-1 entry [6,7], whereas stromal cell-derived factor-1 (SDF-1) inhibits the entry of viruses that utilize CXCR4 as a coreceptor [8,9].

HIV-2 and simian immunodeficiency virus (SIV) strains also use coreceptors for their entry. Most SIV strains use CCR5 as a coreceptor [10–12], while many HIV-2 strains use CCR5, CXCR4, or both [13–15]. Several HIV and SIV strains can use other chemokine receptors such as CCR8 [16,17], GPR15, STRL33/Bonzo [18,19], and US28 [20] as coreceptors. It has recently been reported that the orphan G protein-coupled receptor APJ [21] is a coreceptor for the T-tropic and dual-tropic HIV-1 and SIV strains [22–24]. Several viruses utilize APJ as a viral coreceptor almost as efficiently as CCR5 and CXCR4 in cell-to-cell infection [23]. APJ is widely expressed in many regions of the central nervous system including hippocampus, striatum, thalamus, cortex, cerebellum, and spinal cord [25] as well as in NT2N neurons [23]. The APJ transcripts are also detected in the spleen, thymus, prostate, testis, ovary, small intestine, and colonic mucosa. Furthermore, APJ transcripts are detected in CD4-positive T cells such as C8166 [23].

The novel peptide apelin was discovered as an endogenous ligand for the APJ receptor [26]. The 36-amino acid peptide, apelin-36, was isolated from bovine stomach extracts by monitoring the increase in the extracellular acidification rate induced by the receptor–ligand interaction in the APJ-expressing cells. Subsequently, apelin-36 has been shown to be derived from the C-terminal portion of the 77-amino acid preproprotein. In further studies, it was found that synthetic apelin-13 and apelin-17, the C-terminal fragments of apelin-36, exhibit much higher acidification-rate-promoting activities than apelin-36 [26]. These results suggest that the C-terminal portion of apelin-36 may be responsible for its receptor binding and biological activity.

In this study, we have shown that APJ acts as a coreceptor for cell-free HIV and SIV using brain-derived glioma NP-2 cells expressing CD4 [27]. Because apelin is an endogenous ligand for the APJ receptor, we examined the effects of apelin peptides on the entry of HIV in association with CD4, and found, for the first time, that these peptides block cellular entry of HIV.

2. Materials and methods

2.1. Virus strains

The HIV-1 (IIIB, GUN1, GUN1v, GUN4, and GUN7), HIV-2 (CBL-20, CBL-21, CBL-23, GH-1, and ROD/B), and SIV (agmTYO-1, mac251, and mndGB-1) strains were propagated in Molt-4 T cells. The HIV-1 (GUN2, GUN3, GUN12, GUN12v) and HIV-2 SBL6669 strains were produced in C8166 T cells. HIV-1 Ba-L strain was propagated in the peripheral blood lymphocytes prepared after Ficoll-Hypaque centrifugation of the blood from healthy donors.

2.2. Chemical synthesis of apelin peptides

Apelin-36, apelin-17, apelin-13, apelin-12, and apelin-11 were synthesized by a solid-phase synthetic technique using an automatic peptide synthesizer (Model 431, Applied Biosystems) and purified by HPLC after deprotection. Structures of the synthetic peptide preparations were confirmed by mass spectrometric measurements using a matrix-assisted laser desorption ionization mass spectrometer (Kompact MALD III, Kratas) and by amino acid sequence analysis using a protein sequencer (Model 477A, Applied Biosystems).

2.3. Preparation of the NP-2 cells expressing human APJ

The 1143-bp cDNA encoding APJ was cloned from a Molt-4 cDNA library by specific PCR primers containing *Bam*HI (5′) and

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*Not*I (3′) cloning sites. The PCR product was cloned into a pCR 2.1 TA vector (Invitrogen) and the sequence of the product was confirmed by DNA sequence analysis. The cDNA obtained from the plasmid was subcloned into a retroviral vector pMX-puro that contains a puromycin selection marker. The plasmid DNA was transfected into ϕNX-A packaging cells to make pseudotyped virus stocks to infect NP2/CD4 and NP2 cells [27]. APJ-transduced NP-2 and NP-2/CD4 cells were subsequently selected in puromycin-containing medium. The surviving cells were then examined for their susceptibilities to HIV and SIV infection. The expression of APJ mRNA in the puromycin-resistant cells was confirmed by a reverse transcription polymerase chain reaction (RT-PCR) method using a specific PCR primer pair. NP-2/CD4 cells expressing human CCR5 and CXCR4 were established as described elsewhere [27].

2.4. Assay of cell-free HIV infection

Reverse transcriptase (RT) activities of the virus stocks of HIV and SIV strains were determined as described previously [28]. NP-2, NP-2/CD4, NP-2/APJ, and NP-2/CD4/APJ cells were seeded at a density of $3–5\times10^4$ cells/ml. On the following day, the cells were inoculated with the virus stock corresponding to an RT activity of 1×10^5 cpm at 37°C for 2 h. Cells were washed and cultured at 37°C for up to 10 days. Culture supernatants were harvested on day 0 (2 h after infection), day 5, and day 10, and subjected to the RT assays as described [28,29]. Viral antigens expressed in infected cells were detected using an indirect immunofluorescence assay (IFA) [27].

2.5. Inhibition of HIV infection by apelin peptides

NP-2/CD4/APJ cells were seeded as described above and then pretreated with varying concentrations of synthetic apelin peptide (apelin-36, apelin-17, apelin-13, or apelin-12) for 1 h at 37°C. The cells were then infected with the HIV or SIV stock (corresponding to an RT activity of 1×10^4 cpm) for 2 h and cultured in the medium containing the peptide. On day 4 or day 7 after infection, the percentage of viral antigen-positive cells was determined by IFA. Inhibition of the infection was calculated from numbers of viral antigen-positive cells (%) in the infected cultures with and without peptide treatment.

3. Results

3.1. APJ as a coreceptor for the HIV and SIV strains

Since APJ has been reported to be a coreceptor for HIV-1 and SIV [22–24], we examined infection of NP-2/CD4/APJ cells with cell-free HIV and SIV. The abilities of these viruses to infect the APJ-expressing NP-2/CD4 cells varied markedly when their infectivities were followed during 10 days after infection by IFA (Fig. 1). HIV-2 strains, CBL-23 and ROD/B, most efficiently infected the cells, while GH-1 and SBL6669 infected with less efficiency. HIV-1 (GUN1, GUN2, GUN4, GUN7, GUN12) also infected the cells with less efficiency. Among the SIV strains examined, only SIV mndGB-1 infected the cells. None of the HIV and SIV strains tested in this study infected the NP-2, NP-2/APJ, or NP-2/CD4 cells.

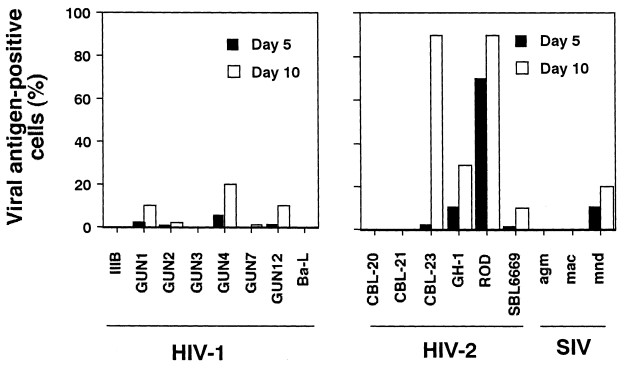

Fig. 1. Infection of the APJ-expressing NP-2/CD4 cells with HIV-1, HIV-2, and SIV. The APJ-expressing NP-2/CD4 cells were seeded at $3–4\times10^4$ cells per well and inoculated with each virus stock (1×10^5 cpm RT activity). After incubation for 5–10 days, the cells were smeared and fixed, and infection (percentages of virus antigen-positive cells) was determined by IFA.

We also assessed the syncytium formation activities of the HIV and SIV strains to examine infection capabilities to the cells. We found that HIV-2 (CBL-23 and ROD/B) strains induced extensive syncytium formation in NP-2/CD4/APJ cells, while the other HIV and SIV strains examined formed either few syncytia or no syncytium. These results were well correlated with those for IFA.

3.2. HIV and SIV infection measured by RT activity

The virus production in culture fluids was detected by an RT assay. Virus production rapidly reached a plateau after infection of NP-2/CD4/APJ cells with the HIV-2 (ROD/B and CBL-23) strains, but the production levels were lower in the HIV-2 (GH-1 and SBL6669) and SIV mndGB-1 strains than in the HIV-2 (ROD/B and CBL-23) strains.

3.3. Blocking of HIV infection by apelin peptides

Apelin peptides are endogenous ligands for the coreceptor APJ. We have studied whether apelin peptides are capable of blocking HIV infection. As shown in Fig. 2, it was found that apelin peptides efficiently inhibited infection of HIV-1 (GUN4 and GUN12v) and HIV-2 ROD/B strains in NP-2/CD4/APJ cells. The order of inhibitory efficiency of the infection was

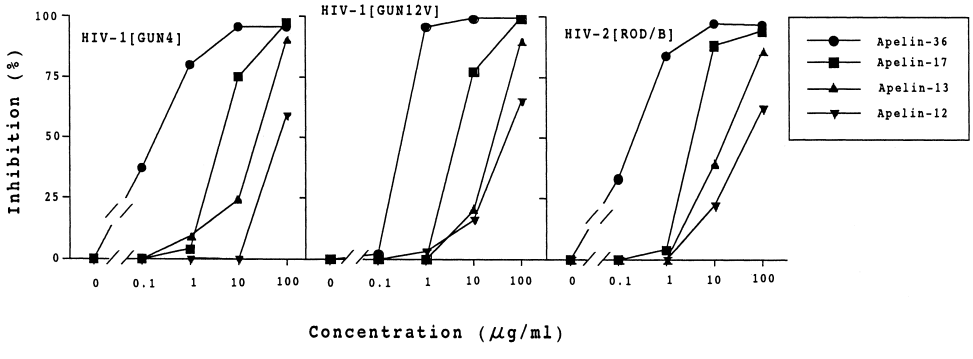

Fig. 2. Inhibitory effects of apelin peptides on the infection of NP-2/CD4/APJ cells with HIV-1 (GUN4 and GUN12v) and HIV-2 ROD/B. NP-2/CD4/APJ cells were preincubated with the peptide at the indicated concentrations and then infected with HIV. After incubation for 4 days (HIV-1 GUN4 and HIV-2 ROD/B) or 7 days (HIV-1 GUN12v), numbers of virus antigen-positive cells in the infected culture were determined by IFA.

Table 1
Effects of apelin peptides on inhibition of HIV infection and acidification rate promotion

Peptide	Amino acid sequence	IC_{50} (μg/ml)	EC_{50} (nM)
Apelin-36	LVQPRGPRSGPGPWQGGRRKFRRQRPRLSHKGPMPF	0.3	20.0
Apelin-17	KFRRQRPRLSHKGPMPF	4.8	2.5
Apelin-13	QRPRLSHKGPMPF	26.0	0.4
Apelin-12	RPRLSHKGPMPF	63.0	ND
Apelin-11	PRLSHKGPMPF	–	–

IC_{50} represents the peptide concentration that induces the half-maximal inhibition of HIV infection in NP-2/CD4/APJ cells. The mean values for infection by HIV-1 (GUN4 and GUN12v) and HIV-2 ROD/B are shown. EC_{50} represents the median effective peptide concentration in acidification-rate-promoting activity. EC_{50} of the APJ-expressing CHO cells [26] was determined using a Cytosenser. ND, not done. Apelin-11 failed to block HIV infection and also to promote acidification rate.

apelin-36 > apelin-17 > apelin-13 > apelin-12. Apelin-11 exhibited no blocking effect. Apelin-36 induced 50% inhibition of HIV infection at a concentration of 0.3 μg/ml, while apelin-12 did so at 63 μg/ml (Table 1). This means that apelin-36 is 200-fold more potent than apelin-12 in inhibiting HIV infection. To determine whether the inhibitory effect of apelin on HIV infection was specific to the APJ receptor, the effect of apelin peptides on HIV-2 ROD/B infection was examined for the NP-2/CD4 cells expressing CCR5 or CXCR4. No significant inhibition was observed with the presence of either CCR5 or CXCR4, suggesting that the blocking of HIV entry by apelin peptides may be mediated specifically through the APJ receptors. Furthermore, MIP-α, MIP-1β, RANTES, and SDF-1 did not block the infection by HIV-2 ROD/B in NP-2/CD4/APJ cells.

4. Discussion

HIV requires CD4 and a coreceptor to enter the target cells. The G protein-coupled receptor APJ has been reported to be a coreceptor for several HIV-1 and SIV strains. In this study, we found that apelin peptides were able to inhibit infection by cell-free HIV-1 (GUN4 and GUN12v), and HIV-2 ROD/B in the brain-derived NP-2/CD4 cells expressing APJ. The inhibitory effects of the apelin peptides on HIV entry appeared to be related to the molecular size of the peptides. Apelin-36 showed the highest infection-blocking efficiency followed by apelin-17, apelin-13 and apelin-12, in that order. Apelin-11, which does not bind to the APJ receptor, failed to block HIV entry, indicating that specific binding to this receptor may be required for an apelin-related peptide to block entry. The efficiency of apelin peptides at blocking HIV entry was in sharp contrast to that of the acidification-rate-promoting activity in the cells in which apelin-13 or apelin-17 exhibited much higher activity than apelin-36 (Table 1). These results suggest that the structural requirement for apelin peptides is different between blocking of HIV infection and exhibiting biological activity. Lee et al. [30] have recently identified the domains of the CCR5 molecule responsible for the interaction with the viral envelope protein gp120 using monoclonal antibodies against CCR5. Their results show that the N-terminal domain of CCR5 is important for binding to gp120, while the extracellular loops of CCR5 are important for inducing conformational changes in gp120 that lead to membrane fusion and virus infection [30]. Our results indicate that apelin-36 more effectively blocks HIV infection than apelin-12 or apelin-13, suggesting that not only the C-terminal portion of apelin-36 which is responsible for the APJ receptor binding, but also the N-terminal portion contributes significantly to the blocking of HIV entry.

The use of alternative coreceptors such as APJ may help to explain HIV tropism and certain kinds of pathogenesis in vivo. Since apelin is the endogenous ligand for the APJ receptor, it will be important to study whether high levels of endogenous apelin are associated with a delayed progression of HIV-related diseases. AIDS patients frequently develop serious neurological disorders such as AIDS dementia complex. Since APJ is expressed widely in many regions of the brain [25] and is also expressed in NT2N neurons [23], the results of the present study suggest that brain cells having both CD4 and APJ may be potential target cells for certain HIV infections in the central nervous system.

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