Stimulation of Enveloped Virus Infection by Beta-Amyloid Fibrils

Woj M. Wojtowicz†, Michael Farzan‡§, John L. Joyal†, Kara Carter†, Gregory J. Babcock‡§, David I. Israel†, Joseph Sodroski†§, & Tajib Mirzabekov†**

†Praecis Pharmaceuticals, Inc., 830 Winter Street, Waltham, Massachusetts 02451-4100, USA and ‡Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, the §Department of Pathology, Division of AIDS, Harvard Medical School, and the †Department of Immunology and Infectious Disease, Harvard School of Public Health, Boston, Massachusetts 02115, USA

** To whom correspondence should be addressed: Praecis Pharmaceuticals, Inc., 830 Winter Street, Waltham, MA 02451-4100. Tel.: 781-795-4213; Fax: 781-795-4494; E-mail: tajib.mirzabekov@praecis.com.
Alzheimer’s disease (AD) is characterized by deposition of beta-amyloid peptide (Aβ) into plaques in the brain, leading to neuronal toxicity and dementia. Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system (CNS) can also cause a dementia, and amyloid deposition in the CNS is significantly higher in HIV-1-infected individuals compared with uninfected controls. Here we report that Aβ fibrils stimulated, by 5-20 fold, infection of target cells expressing CD4 and an appropriate coreceptor by multiple HIV-1 isolates, but did not permit infection of cells lacking these receptors. Aβ enhanced infection at the stage of virus attachment or entry into the cell. Aβ fibrils also stimulated infection by amphotrophic Moloney leukemia virus (A-MuLV), herpes simplex virus (HSV), and viruses pseudotyped with the envelope glycoprotein of vesicular stomatitis virus (VSV). Other synthetic fibril-forming peptides similarly enhanced viral infection and may be useful in gene delivery applications utilizing retroviral vectors. These data suggest that Aβ deposition may increase the vulnerability of the CNS to enveloped viral infection and that amyloidogenic peptides could be useful in enhancing gene transfer by enveloped viral vectors.
The efficiency of gene delivery using retroviral vectors is often a limiting factor in attempts to express exogenous genes in both cultured mammalian cells and in vivo. Facilitators such as polybrene (hexadimethrine bromide) (1) and DEAE-dextran (2) have been utilized to increase the efficiency of viral infection. Vectors pseudotyped with the envelope glycoproteins of various viruses are advantageous for targeting exogenous genes to specific cell types that express the cognate receptor molecules. Envelope glycoproteins of vesicular stomatitis virus (VSV) and amphotropic murine leukemia virus (A-MuLV) utilize ubiquitously-expressed receptors and are useful for transduction of varied cell types (3,4). Envelope glycoproteins that require cell-type specific receptors, such as the gp120-gp41 of human immunodeficiency virus-1 (HIV-1), can provide a useful tool for targeting exogenous genes to specific cell types. HIV-1 envelope glycoproteins facilitate the fusion of viral and cellular membranes through sequential binding of CD4 and a chemokine receptor, principally CCR5 or CXCR4 (5-7).

The accumulation of $A\beta_{1-40}$ and $A\beta_{1-42}$ proteolytic fragments of Amyloid-β precursor protein (APP), is a molecular marker characteristic of Alzheimer’s disease (AD) (8-10). HIV-1 infection of microglia in the CNS leads to HIV-associated dementia (HAD) in approximately 20-30% of late-stage AIDS patients (11) and HIV-1 replication in the brain has been observed to co-localize with sites of APP accumulation (12,13). The occurrence of APP-rich lesions coincides with the presence of HAD (12).

HIV-1 infection of the CNS and subsequent infection in the brain occurs by mechanisms that remain poorly understood. It is believed that infection of macrophages, microglia, and possibly astrocytes leads to indirect neuronal injury and death, providing the basis for the development of
HAD, a syndrome of cognitive and motor dysfunction diagnostically similar to AD-related dementia (11,14,15). A positive relationship between cerebrospinal fluid viral load and the extent to which patients with HAD or minor cognitive/motor disorder experience cognitive dysfunction has been described (16,17). Aβ-rich neuritic plaques are also observed to occur with greater prevalence in HIV-1-infected individuals compared with uninfected controls, although an etiological relationship between HAD and plaques has not been established (18). Additionally, HIV-1-infected individuals bearing the ApoE4 allele, a genetic risk factor for AD that correlates with elevated Aβ levels (19), are twice as likely to be demented or have peripheral neuropathy than individuals lacking this allele (20). ApoE4 is an AD susceptibility factor, particularly for individuals harboring herpes simplex virus (HSV) in the brain (21,22). HIV-1 infection of the CNS, systemic immune suppression, and increased permeability of the blood brain barrier (11) promote opportunistic HSV (23) and cytomegalovirus infection (24).

Whether the proteolytic fragments of APP, a common molecular marker of dementing disease states including AD, are implicated in the mechanism of HIV-1 brain infection remains unclear. We wished to address whether a relationship exists between the presence of APP proteolytic fragments and HIV-1 infection. Here we report that the amyloidogenic APP fragments Aβ1-40 and Aβ1-42, as well as other synthetic amyloidogenic peptides, significantly enhanced infection by HIV-1 and viruses with other envelope glycoproteins. The effect was stronger than the enhancement of infection observed using polybrene. These findings are suggestive of a model which may explain how neuritic damage caused by HIV-1 infection in the brain and subsequent Aβ deposition induced by this damage may facilitate further HIV-1 infection. Additionally, they
suggest a use for synthetic amyloidogenic peptides in both laboratory and clinical viral delivery systems.
EXPERIMENTAL PROCEDURES

Peptides and Fibrils. Lyophilized beta-amyloid (Aβ) 1-40 and 1-42 fragments (California Peptide Research, Inc. and New England Peptide, Inc.), Aβ_{40-1} reverse fragment (Sigma-Aldrich), and small molecular weight peptides, PPI-2480 and PPI-2566 (Praecis Pharmaceuticals Inc.), were dissolved in DMSO to 5 mM and subsequently diluted to 200 µM in PBS (10 mM HEPES, pH 7.4 for Aβ_{1-42}). Peptides were used fresh or incubated under conditions that support fibril formation as follows. Aβ_{1-40}, PPI-2480, and PPI-2566 peptide solutions were incubated for 8 days at 37° C, sonicated, aliquotted, and stored at –20° C. Fibril formation for the Aβ_{1-42} peptide solution was allowed to proceed for 24 hrs at room temperature with stirring. The efficiency of fibril formation was verified by reaction with Congo Red or electron microscopy. Purity of all peptides was >98%.

Cell lines and culture. All cell lines were grown at 37° C and 5% CO₂ in Dulbecco’s modified Eagle medium (GibcoBRL) containing 10% fetal bovine serum (Sigma-Aldrich) and 100 µg/ml penicillin-streptomycin (Mediatech, Inc.) (complete DMEM) supplemented with antibiotics as noted. Cf2Th canine thymocytes, 293T human embryonal kidney (HEK), and NIH-3T3 mouse embryonal fibroblast cells were obtained from the American Type Culture Collection (ATCC CRL 1430, 1573 and 1658, respectively). Stable cell lines included Cf2Th expressing human CD4 and CCR5 (Cf2Th-CD4/CCR5) (25) grown in medium supplemented with 0.5 mg/ml Geneticin (GibcoBRL) and 0.15 mg/ml Hygromycin B (Roche Diagnostics Corp.), Cf2Th-CD4 (26) with 0.15 mg/ml Hygromycin B, Cf2Th-CCR5 (30) with 0.5 mg/ml Geneticin, and GHOST(3)-CD4/CXCR4 human osteosarcoma cells expressing human CD4 and CXCR4 (27)
with 0.5 mg/ml Geneticin, 50 µg/ml Hygromycin B and 1 µg/ml Puromycin (Sigma-Aldrich). SupT1-CCR5 cells (28) were cultured in complete RPMI medium (GibcoBRL) supplemented with 0.2 µg/ml Puromycin.

Recombinant Reporter Viruses. Recombinant HIV-1 reporter viruses were constructed by cotransfection of 293T-HEK cells with vectors expressing the pCMVΔP1ΔenvpA HIV-1 Gag-Pol packaging construct (29), the envelope glycoproteins of AMLV, VSV, and HIV-1 isolates (ADA, YU2, JR-FL and HXBc2), and a reporter gene at a DNA weight ratio of 1:1:3 using Effectene reagents (Qiagen). Cotransfection produced replication-defective (single-round) virions capable of expressing HIV-1 tat and the firefly luciferase gene under control of the HIV-1 long terminal repeat (LTR), or the green fluorescent protein (GFP) gene under control of the cytomegalovirus immediate-early promoter (CMV). Viruses pseudotyped with VSV-G, A-MuLV, and HIV-1 envelope glycoproteins were produced by cotransfecting the pHCMV-G (30), SV-A-MLV-Env (31), or pSVIIIenv (32-35) plasmids, respectively. Production of the VSV-G and A-MuLV recombinant viruses also required cotransfection of pCMV-Rev, a plasmid expressing the HIV-1 Rev protein (36). Thirty hrs following transfection, the virus-containing cell supernatants were harvested, filtered (0.45 µm), aliquotted and kept frozen until use. The reverse transcriptase (RT) activities of all viruses were quantified and normalized by cpm as described previously (37). Replication-deficient A-MuLV (Retropack, Clonetech) and HSV (HD-2) (38) vectors containing β-galactosidase reporter genes were produced using NIH-3T3 cells, according to the manufacturer’s protocol; infection efficiencies were estimated by reporter gene activity in the target cells. Luciferase and β-galactosidase activity was quantitated as described in Promega protocols using an EG&G Berthold Microplate Luminometer LB 96V.
**Infection by Single-Round Viruses Expressing Luciferase.** Target cells for viral entry were seeded in 96-well luminometer-compatible tissue culture plates (Dynex) at a density of $6 \times 10^3$ cells/well and incubated for 24 hrs. The medium was removed from the target cells and replaced with fresh complete DMEM containing RT-normalized units of recombinant virus. The amounts of virus varied depending upon the envelope glycoproteins used for pseudotyping: VSV-G, 1K cpm; A-MuLV, 30K cpm; ADA, YU2, JR-FL, 89.6, ADA-ΔV1/V2, and HXBc2 HIV-1 envelope glycoproteins, 10K cpm. Varying amounts of $\alpha$β$_{1-40}$, $\alpha$β$_{1-42}$ (1.25-20 µM), PPI-2566 or PPI-2480 (1-100 µM) were added with the recombinant viruses, to a final infection volume of 50 µl. The anti-CCR5 antibody 2D7 (39) (BD PharMingen) or TAK-779, a small molecular weight nonpeptide compound that specifically binds CCR5 (40) (Takeda Chemical Industries, Ltd.), was also included in some assays. Target cells were incubated with the infection medium for 48 hrs. Following this incubation, the medium was aspirated from each well and the cells were lysed by addition of 30 µl passive lysis buffer (Promega Corp.), agitation, and 2 freeze-thaw cycles. The luciferase activity of each well was measured for 10 sec following addition of 100 µl luciferase buffer (15 mM MgSO$_4$, 15 mM KPO$_4$, pH 7.8, 1 mM ATP, 1 mM DTT) and 50 µl 1 mM D-luciferin potassium salt (BD PharMingen) using an EG&G Berthold Microplate Luminometer LB 96V.

**Infection by Single-Round Viruses Expressing GFP.** SupT1-CCR5 target cells were seeded in 24-well tissue culture plates (Falcon) at a density of $5 \times 10^4$ cells/well with medium containing RT-normalized units of GFP-expressing recombinant virus (VSV-G, 3K cpm; ADA, 150K cpm; YU2, 150K cpm) and varying amounts of $\alpha$β$_{1-40}$ or $\alpha$β$_{1-42}$ (62.5 nM-1 µM) in a final volume of
0.4 ml. The infection medium-cell mixture was incubated for 48 hrs, 1 ml of fresh complete RPMI was added to each well, and the cells were incubated for an additional 24 hrs. The cells were then harvested, washed with PBS, fixed in 10% formalin, and analyzed by fluorescence-activated cell sorting using a Becton Dickinson FACScan with CellQuest software.

**Infection by Single-Round Viruses Expressing β-galactosidase.** Cf2Th cells were infected with an A-MuLV vector expressing β-galactosidase without additives, in the presence of 8 µg/ml of polybrene, or in the presence of 10 µM pre-aggregated Aβ40-1 reverse fragment or Aβ1-40. The precipitable fraction of Aβ1-40 was recovered by pelleting pre-aggregated Aβ1-40 at 15,000 x g for 5 min at 4°C, after which the supernatant was removed and retained. The precipitated peptide fibrils were resuspended in PBS, washed two more times and resuspended in the starting volume. The β-galactosidase expression in the target cells 24 hrs after infection was estimated using a chemiluminescent assay (Galacto-Star, Tropix, Inc.). Cf2Th cells were also infected with a single-round HSV virus vector (HD-2) (38) containing the β-galactosidase reporter gene in the presence of 5 or 10 µM pre-aggregated Aβ1-40. Cells were stained according to the Promega protocol and counted under the microscope 24 hrs following infection.

**FACS analysis of fibril interactions with liposomes and cells.** Unilamellar small liposomes (liposomes) similar in size to HIV-1 were prepared from a 2/1 (M/M) mixture of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine supplemented with 1% fluorescent rhodamine-lissamine B-phosphatidylethanolamine (Avanti Polar Lipids), as previously reported (41). Rhodamine-labeled liposomes were incubated with Cf2Th cells in the presence and absence of peptide fibrils
in the medium used for the viral entry assay (DMEM + 10% fetal bovine serum) supplemented with 0.02% NaN₃ for 1 hr at 37°C. Similarly, fluorescent Aβ₁₋₄₀ fibrils (FITC-Aβ) were incubated with Cf2Th cells either lacking or expressing CD4 and/or CCR5, in the absence and presence of recombinant HIV-1 gp120 envelope glycoprotein from the JR-FL isolate. Following incubation, the cells were washed with PBS containing 2% bovine serum albumin and the association of rhodamine-labeled liposomes or FITC-Aβ with cells was analyzed using FACScan, as described above. Cf2Th-CD4/CCR5 cells were also incubated without additives or with unlabeled pre-aggregated Aβ₁₋₄₀, as above, and the CD4 and CCR5 cell surface expression was detected using the anti-CD4 antibody RPA-T4-PE (BD PharMingen) and the anti-CCR5 antibody 2D7-PE (BD PharMingen) at a final concentration of 10 nM.
RESULTS

\(A\beta_{1-40}\) and \(A\beta_{1-42}\) Enhance Signal of Viral Entry. To investigate whether the presence of \(A\beta\) affects HIV-1 infection of target cells, recombinant replication-defective HIV-1 vectors expressing firefly luciferase or GFP were used. These single-round viruses were pseudotyped with the envelope glycoproteins of various HIV-1 isolates, or with those of VSV or A-MuLV. The receptors for VSV and A-MuLV are ubiquitously expressed. Entry of viruses pseudotyped with the HIV-1 envelope glycoproteins is dependent on the presence of CD4 or a chemokine receptor, CCR5 or CXCR4; the viruses pseudotyped with the VSV or A-MuLV envelope glycoproteins do not require CD4 or chemokine receptor expression on the target cells. Pre-aggregated \(A\beta_{1-40}\) and \(A\beta_{1-42}\) fibrils dramatically increased infection of Cf2Th-CD4/CCR5 cells by HIV-1 pseudotyped with the envelope glycoproteins of three CCR5-using primary HIV-1 isolates (ADA, YU2, JR-FL) in a dose-dependent manner (Fig. 1). \(A\beta_{1-40}\) similarly increased infection of GHOST(3)-CD4/CXCR4 cells by HIV-1 pseudotyped with the envelope glycoproteins of the CXCR4-using isolate, HXBc2 (Fig. 1). Similar results were obtained by infecting a human T-lymphocyte cell line stably expressing CCR5 (SupT1-CCR5) with GFP-expressing viruses pseudotyped with ADA and YU2 envelope glycoproteins (data not shown). \(A\beta_{1-40}\) was more potent than \(A\beta_{1-42}\) and increased the entry of viruses by 2-10 times in a concentration range of 1-5 \(\mu\)M, and by 5-30 times at a concentration of 20 \(\mu\)M. Infection of cells by viruses pseudotyped with the A-MuLV or VSV envelope glycoproteins was also enhanced. The relatively lower enhancement observed with VSV-G-pseudotyped virus may be due to the substantially greater efficiency with which this virus infects cells in the absence of \(A\beta\). These data show that \(A\beta\) can substantially increase the efficiency of infection of cells by HIV-1.
pseudotyped with the envelope glycoproteins of a wide range of HIV-1 isolates, as well as with those of other enveloped viruses.

**Aβ₁₋₄₀ Enhances an Early Step in Virus Infection.** To elucidate whether the enhancement of virus infection by Aβ was mediated by an increased efficiency of early or late events in the virus life cycle, incubation of the recombinant viruses with the Cf₂Th-CD4/CCR5 target cells was carried out for only 4 hrs, followed by washing. The target cells were incubated with 20 µM Aβ₁₋₄₀ concurrently with virus (+/-), immediately following removal of virus (-/+), or throughout both time periods (+/+)) (Fig. 2). After the wash, the cells were incubated for an additional 48 hrs, at which time luciferase activity was measured. Enhancement of infection was observed only when Aβ₁₋₄₀ was present during the initial 4-hr incubation of virus and cells. These data suggest that Aβ exerts its effect at an early stage of viral infection. Because the first 4 hrs of HIV-1 infection involves virus attachment and entry into the host cell, Aβ likely enhances these processes.

**Enhancement of Viral Infection by Aβ is Receptor-Mediated.** Aβ has been shown to exert a destabilizing effect on cellular membranes (42,43). Therefore, Aβ might facilitate fusion of the target cell and viral membrane in a manner that would circumvent the dependence of the virus on its receptors. To investigate this possibility, we examined infection of Cf₂Th, Cf₂Th-CD4, Cf₂Th-CCR5, and Cf₂Th-CD4/CCR5 cells by CCR5-dependent HIV-1 isolates. No infection by CCR5-dependent HIV-1 isolates was observed in the presence or absence of Aβ with cells lacking CD4 and/or CCR5 (Fig.3a), whereas infection by viruses pseudotyped by VSV and A-MuLV envelope glycoproteins, which do not require these cellular receptors, was enhanced by
Aβ on all cells examined. Aβ-enhanced CCR5-dependent viral entry remained sensitive to inhibition by CCR5 ligands, including the 2D7 antibody (39) (Fig. 3b) and the small-molecule antagonist TAK-779 (40) (data not shown). These data demonstrate that HIV-1 infection in the presence of Aβ remains dependent on the expression of CD4 and a chemokine coreceptor.

Other Fibril-Forming Peptides Enhance Viral Infection. Aβ aggregates into fibrils (8,42,44-46). We investigated whether other fibril-forming peptides unrelated to Aβ could enhance virus infection. Fig. 4 shows that two such peptides, PPI-2480 (AGAKWSWELTWVGG) and PPI-2566 IRQAMCNISRADWND), which form fibrils similar to Aβ1-40 and Aβ1-42 (Fig. 5b-f), also enhanced the infection efficiency of recombinant HIV-1 virus pseudotyped with the envelope glycoproteins of the ADA and YU2 HIV-1 isolates by 5-20 fold. The stimulation by these fibrils also required the expression of viral entry coreceptors (data not shown). These compounds enhanced infection of HIV-1 virus pseudotyped with the VSV-G protein by approximately two-fold. A number of control peptides of varying sequences and lengths that did not form fibrils had no effect on HIV-1 infection. An example is the peptide PPI-1966 shown in Figs. 4 and 5f. These data demonstrate that the ability of a peptide to enhance viral infection correlates with its propensity to form fibrils in solution. Interestingly, the peptides that most potently enhance infection (Aβ1-40 and PPI-2480) formed shorter fibrils (Figs. 5b and 5d), while peptides forming longer fibrils (Aβ1-42 and PPI-2566, Figs. 5c and 5e) were less efficient.

Fibril-Forming Peptides Promote Lipid Vesicle Association with Cells. To investigate further the mechanism by which these fibril-forming peptides stimulate viral infection, we modeled the enveloped virus interaction with cells using liposomes. Rhodamine-labeled liposomes,
approximately the size of HIV-1 (47), were incubated with cells under the conditions of viral infection in the presence and absence of \( \text{A}\beta_{1-40}, \text{A}\beta_{1-42}, \text{PPI-2566}, \text{PPI-2480} \) and PPI-1966. The liposome-cell mixtures were then analyzed by FACScan (Fig. 5a). Each of the fibril-forming peptides that enhanced infection also promoted irreversible association of liposomes with cells. The peptides did not cause the formation of syncytia, nor did they promote liposome-to-cell fusion, as judged by the failure of the rhodamine dye in the liposomes to distribute into the cell membrane (data not shown). Consistent with their relative ability to enhance infection, \( \text{A}\beta_{1-40} \) promoted the adherence of liposomes better than \( \text{A}\beta_{1-42} \). PPI-1966, which Fig. 5f shows cannot form fibrils, had no effect on the association of liposomes with cells (data not shown). Utilizing fluorescent \( \text{A}\beta_{1-40} \) fibrils (FITC-A\( \beta \)), we found that FITC-A\( \beta \) associated with cell surfaces independent of CD4 or CCR5 expression (data not shown). The presence of recombinant HIV-1 envelope glycoprotein (JR-FL gp120) in the medium did not promote the association of FITC-A\( \beta \) with cell membranes (data not shown). Additionally, the presence of \( \text{A}\beta_{1-40} \) did not induce changes in cell surface expression of CD4 or CCR5 (data not shown). These data support a model in which \( \text{A}\beta \) and other fibril-forming peptides enhance viral infection by mediating a physical association of viral envelopes with the cell lipid bilayer.

**Magnitude of Infection Enhancement by the Precipitable Fraction of A\( \beta \) Exceeds the Effect of Polybrene.** As shown in Figs. 1-3, infection by HIV-1 pseudotyped with the envelope glycoprotein of A-MuLV was enhanced by \( \text{A}\beta \). Infection by complete A-MuLV was also strikingly enhanced, from 30-50 fold, in the presence of pre-aggregated \( \text{A}\beta_{1-40} \) (Fig. 6a). This effect was 2-3-fold greater than that observed for polybrene, a cationic polymer commonly used to increase the efficiency of retroviral gene delivery systems (48). In this experiment, \( \text{A}\beta_{1-40} \)
fibrils were precipitated by multiple centrifugation and washing steps, and compared with the supernatant of the first centrifugation. Fig. 6a demonstrates that the precipitable Aβ₁₋₄₀ fraction, but not any residual soluble peptide, enhanced A-MuLV infection comparably to Aβ₁₋₄₀ that had not been centrifuged. Conversely, the Aβ₄₀−₁ reverse fragment did not enhance the infection efficiency of A-MuLV. These data underscore the substantial enhancement of retroviral infectivity by Aβ₁₋₄₀, and demonstrate that the precipitable, and presumably fibril-forming, fraction of Aβ mediates its ability to enhance infection.

Aβ Weakly Stimulates Infection by an Enveloped Virus Other than a Retrovirus. Because HSV has been suggested to play a role in AD and is a major opportunistic infection observed in late-stage HIV-1 infection, we investigated the ability of Aβ to enhance HSV infection. A dose-dependent enhancement of the infection mediated by an HSV vector was observed (Fig. 6b). However, relative to the enhancement observed with retroviruses, Aβ₁₋₄₀ was substantially less efficient in enhancing HSV infection. This less pronounced ability of Aβ to enhance HSV infection may be a consequence of differences in accessibility or composition of the HSV lipid membrane.
DISCUSSION

Here we describe an enhancement of enveloped virus infection by amyloidogenic APP proteolytic fragments $\text{A} \beta_{1-40}$ and $\text{A} \beta_{1-42}$. The requirement that the $\text{A} \beta$ fragments be present during the contact of the virus with the target cell suggests that a very early phase of infection is stimulated by the peptides. Enhancement of infection was observed for viruses containing several different envelope glycoproteins that utilize unrelated receptors, suggesting that enhancement does not require specific protein-protein interactions. Consistent with this, these peptides substantially enhanced the association of liposomes with cells. A common element among the viruses assayed in this study is the presence of a lipid envelope bilayer. It is therefore likely that the mechanism by which these peptides enhance entry includes their propensity to promote an interaction between the viral and cellular lipid membranes. The extent to which the reported membrane-destabilizing properties of $\text{A} \beta$ participate in the observed enhancement of viral fusion remains unclear. The requirement for appropriate receptors on the target cell is not bypassed by $\text{A} \beta$, suggesting that receptor-triggered changes in the envelope glycoproteins are still crucial for achieving the fusion of the viral and target cell membranes.

The entry enhancement observed herein is mediated by the precipitable amyloidogenic fraction of $\text{A} \beta$. Interestingly, other synthetic amyloidogenic peptides unrelated to $\text{A} \beta$ similarly enhanced viral infection, whereas synthetic non-amyloidogenic peptides had no entry-enhancing effect. These results suggest that fibril formation may be important for
the viral enhancement effect. Additional studies will be required to determine the other properties of amyloidogenic peptides that contribute to enhancement of virus infection.

Our observations could have relevance to neuropathogenesis. Neuritic plaques, a primary component of which is Aβ, are more detectable in HIV-1-infected individuals than uninfected individuals (18). Additionally, HIV-1-infected individuals are prone to an HIV-associated dementia that is correlated with high viral loads in the cerebrospinal fluid (16,17). Immune cells, in particular microglia and macrophages, which are important target cells of HIV-1 in the brain, are commonly recruited to neuritic plaques (11). Our observations suggest that regions of high Aβ, such as those in the vicinity of plaques, would be a highly favorable environment for virus transmission. It has been observed that sites of HIV-1 replication in the brain co-localize with sites of APP accumulation (12,13), a possible consequence of HIV-1-induced neuronal injury. If high local APP levels also result in the production of Aβ, neuronal injury may both recruit immune cells and promote their infection. The observation of more frequent and severe HAD in individuals bearing the ApoE4 allele (20) is also consistent with a role for Aβ in HAD. Taken together with the data herein, these observations suggest that testing the effect of inhibitors of Aβ production in primate models of HAD (49) is warranted.

The infection of viruses pseudotyped with the envelope glycoproteins of VSV, A-MuLV, HIV-1, and HSV was enhanced by Aβ. It has been reported that HSV is detectable in a greater percentage of AD patients than in age-matched controls, and that the combination of HSV and the ApoE4 allele disposes to AD more than either factor alone (21,22).
These observations are consistent with a contribution of HSV to the pathogenesis of AD. Although the enhancement of HSV by Aβ was significantly less pronounced than that observed for the two retroviruses studied, the possibility that an enveloped virus contributes to AD pathology merits further study.

The magnitude of virus entry enhancement by these amyloidogenic peptides raises the possibility that the effect reported herein may be useful in applications such as gene therapy using viral vectors. Particularly in desirable target cells, viral titers and infection rates are frequently limiting. Aβ1-40 is less neurotoxic than Aβ1-42 (20,50), but under the conditions assayed it is more potent in promoting infection. It is therefore possible that the ability of a peptide to promote infection is independent of its pathogenic properties. Experiments aimed at identifying synthetic peptides that promote infection with the same or greater efficiency as Aβ, but with lower cytotoxicity, are underway.
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REFERENCES

FIGURE LEGENDS

Fig. 1. **Aβ stimulates infection by recombinant HIV-1 viruses.** The efficiency of infection of Cf2Th-CD4/CCR5 or GHOST(3)-CD4/CXCR4 cells by recombinant luciferase-expressing HIV-1 viruses pseudotyped with CCR5-using HIV-1 envelope glycoproteins (ADA, YU2, JR-FL) or the CXCR4-using HIV-1 envelope glycoprotein (HXBc2), respectively, was determined by measuring luciferase activity in the cells. Viruses with VSV-G and A-MuLV envelope proteins, which utilize ubiquitously-expressed receptors, were included for comparison. Increasing concentrations of aggregated Aβ₁₋₄₀ (left) and Aβ₁₋₄₂ (right) peptides were incubated with the virus and target cells. Luciferase activities were normalized relative to those observed for each recombinant virus in the absence of peptide. Results are representative of the median values obtained from independent assays performed in duplicate or triplicate.

Fig. 2. **Aβ acts at an early stage of viral infection.** Cf2Th-CD4/CCR5 cells were incubated with recombinant HIV-1 pseudotyped with HIV-1 (ADA, YU2, JR-FL) or A-MuLV envelope glycoproteins for 4 hrs at 37°C, after which the cells were washed to remove virus. Aβ₁₋₄₀ was absent (-/-), present only during virus incubation (+/-), present only after virus was washed away (-/+), or present throughout the assay (+/+). The luciferase activity in the target cells 48 hrs after incubation with the virus is indicated.

Fig. 3. **HIV-1 infection in the presence of Aβ is dependent on coreceptors.** (a) Cf2Th cells expressing only CCR5 (□), only CD4 (△), both receptors (◆) or neither receptor
were used as target cells for infection. Infection by recombinant HIV-1 viruses pseudotyped with the envelope glycoproteins of CCR5-using HIV-1 isolates (ADA and YU2), as well as the envelope glycoproteins of A-MuLV and VSV, was assessed in duplicate or triplicate by measuring the luciferase activity in the target cells. Average values are shown. (b) Infection of Cf2Th-CD4/CCR5 cells by recombinant HIV-1 pseudotyped with the envelope glycoproteins of the CCR5-using HIV-1 isolates ADA and YU2 was carried out in the presence of 10 μM Aβ with increasing concentrations of the 2D7 anti-CCR5 monoclonal antibody.

Fig. 4. Synthetic fibril-forming peptides enhance infection of recombinant HIV-1. Cf2Th-CD4/CCR5 cells were infected with recombinant HIV-1 pseudotyped with ADA (▲), YU2 (○) and VSV (□) envelope glycoproteins in the presence of varied concentrations of amyloidogenic peptides PPI-2480 (AGAKWSWWELTWVGG) or PPI-2566 (IRQAMCNISRADWND). Infection was also performed in the presence of more than 20 different non-amyloidogenic peptides of 8-17 amino acids long. None of them, including the peptide shown in the figure, PPI-1966 (APMGSDPPTA), affected viral entry. All peptides were amidated at the C-terminus and incubated under fibril-forming conditions. Results of independent assays are reported as previously described in the legend to Figure 1.

Fig. 5. Peptides that enhance viral infection form fibrils and promote lipid vesicle association with cells. (a) Cf2Th cells were incubated with fluorescent liposomes (final concentration of 1 mg of lipid/ml) in the presence of 10 μM pre-aggregated
amyloidogenic peptides Aβ1-42 (gray), PPI-2566 (diagonal lines), Aβ1-40 (black), PPI-2480 (dots) or no peptide (white) and analyzed using FACScan. Non-amyloidogenic peptides, including PPI-1966, did not promote lipid vesicle association with cells (data not shown). (b-f) Negatively stained electron micrographs of amyloidogenic peptides, Aβ1-40, Aβ1-42, PPI-2480, and PPI-2566 are shown (b-e, respectively). The non-amyloidogenic peptide PPI-1966 used for a control is also depicted (f). The scale bar represents 100 nM (f).

Fig. 6. Aβ fibrils stimulate infection by viruses other than HIV-1. (a) Cells were infected with a recombinant A-MuLV vector expressing β-galactosidase in the absence of additive or in the presence of 8 µg/ml of polybrene or 10 µM of pre-aggregated Aβ40-1 reverse fragment or Aβ1-40. The precipitable fraction of Aβ1-40 was compared with any soluble fraction of Aβ1-40 remaining in the supernatant following centrifugation. β-galactosidase expression 24 hrs after incubation of viruses and cells was used to evaluate the efficiency of viral infection. (b) Cf2Th cells were infected with a recombinant HSV vector (HD-2) containing the β-galactosidase reporter gene in the presence of 5 or 10 µM of pre-aggregated Aβ1-40. The results shown in (a) and (b) are the mean values obtained from duplicate experiments.
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