Alzheimer’s disease is characterized by deposition of β-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 can also cause a dementia, and amyloid deposition in the central nervous system 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 amphotropic Moloney leukemia virus, herpes simplex virus, and viruses pseudotyped with the envelope glycoprotein of vesicular stomatitis virus. 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 central nervous system 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 type 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 Aβ40–42 and Aβ1–42 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 central nervous system leads to HIV-associated dementia (HAD) in ~20–30% of late-stage AIDS patients (11), and HIV-1 replication in the brain has been observed to colocalize 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 central nervous system and subsequent infection in the brain occur 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 diagnosed similarly 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 as 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 central nervous system, 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 Aβ40–42 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 that may ex-
plaque 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 use for synthetic amyloidogenic peptides in both laboratory and clinical viral delivery systems.

**EXPERIMENTAL PROCEDURES**

**Peptides and Fibris**—Lipophilized Aβ$_{1–40}$ and Aβ$_{1–42}$ fragments (California Peptide Research, Inc. and New England Peptide, Inc.), Aβ$_{1–40}$ reverse fragment (Sigma), and low molecular weight peptides PPI-2480 and PPI-2566 (Praceis Pharmaceuticals, Inc.) were dissolved in Me$_2$SO to 1 mg/ml and subsequently diluted to 200 μM in phosphate-buffered saline (10 mM HEPES, pH 7.4, for Aβ$_{1–40}$; 15 mM HEPES, pH 8.0, 1 mM ATP, and 1 mM dithiothreitol and 50 μM of 1 mM t-luciferin potassium salt (BD PharMingen) using an EG&G Berthold Microplate Luminometer LB 98V.

**Infection by Single-round Viruses Expressing GFP**—SupT1-CCR5 target cells were seeded in 24-well tissue culture plates (Falcon) at a density of $5 \times 10^5$ cells/well with medium containing reverse transcriptase-normalized units of GFP-expressing recombinant virus (VSV-G, 3,000 cpm; ADA, 150,000 cpm; YU2, 150,000 cpm) and varying amounts of Aβ$_{1–40}$ or Aβ$_{1–42}$ (62.5 nM to 1 μM) in a final volume of 0.4 ml. The infection medium-cell suspension was incubated for 48 h, 1 ml of fresh complete RPMI 1640 was added to each well, and the cells were incubated for an additional 24 h. The cells were then harvested, washed with phosphate-buffered saline, 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**—C272 cells were infected with an A-MuLV vector expressing β-galactosidase without additives, in the presence of 8 μg/ml Polybrene or in the presence of 10 μM pseudokazotide Aβ$_{1–40}$, reverse fragment or Aβ$_{1–42}$. The precipitable fraction of Aβ$_{1–40}$ was obtained by pelleting preaggregated Aβ$_{1–40}$ at 15,000 × g for 5 min at 4°C, after which the supernatant was removed and retained. The precipitated peptide fibrils were resuspended in phosphate-buffered saline, washed two more times, and resuspended in the starting volume. The β-galactosidase expression in the target cells 24 h after infection was estimated using a chemiluminescence assay (Galacto-Star, Tropix, Inc.). C272 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 preaggregated Aβ$_{1–40}$. Cells were stained according to the Promega protocol and counted under the microscope 24 h after infection.

**Fluorescence-activated Cell-sorting Analysis of Fibril Interactions with Liposomes and Peptide Aggregates**—Synthetic amyloidogenic peptides 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 reported previously (41). Rhodamine-labeled liposomes were incubated with C272 cells in the presence and absence of peptide fibrils in the medium used for the viral entry assay (Dubelco's modified Eagle's medium + 10% fetal bovine serum) supplemented with 0.02% NaN$_3$ for 1 h at 37°C. Similarly, fluorescent Aβ$_{1–40}$ fibrils (FITC-Aβ) were incubated with C272 cells either lacking or expressing CD4 and/or CCR5, in the absence in the presence of recombinant HIV-1 gp120 envelope glycoprotein from the JR-FL isolate. After incubation, the cells were washed with phosphate-buffered saline containing 2% bovine serum albumin, and the association of rhodamine-labeled liposomes or FITC-Aβ with cells was analyzed using FACSscan, as described above. C272-CDC4/C5R5 cells were also incubated without additives or with unlabeled preaggregated Aβ$_{1–40}$ as described above, and the CD4 and CCR5 cell surface expression was detected using the anti-CD4 antibody RPA-T4-F6 (BD Pharmingen) and the anti-CCR5 antibody 2D7-F6 (BD Pharmingen) at a final concentration of 10 μM.

**RESULTS**

Aβ$_{1–40}$ and Aβ$_{1–42}$ Enhance Signal of Viral Entry—To investigate whether the presence of Aβ 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. Preaggreated Aβ$_{1–40}$ and Aβ$_{1–42}$ fibrils dramatically increased infection of C272-CDC4/C5R5 cells by HIV-1 pseudotyped with the...
envelope glycoproteins of three CCR5-using primary HIV-1 isolates (ADA, YU2, and JR-FL) in a dose-dependent manner (Fig. 1). $\Delta\beta_{1-40}$ 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). $\Delta\beta_{1-40}$ was more potent than $\Delta\beta_{42-42}$ 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 HIV-1 by pseudotyped with the envelope glycoproteins of a wide range of HIV-1 isolates, as well as with those of other enveloped viruses.

$\Delta\beta_{1-40}$ Enhances an Early Step in Virus Infection—To elucidate whether the enhancement of virus infection by A$\beta$ was mediated by an increased efficiency of early or late events in the virus life cycle, incubation of the recombinant viruses with the C2Th-CD4/CCR5 target cells was carried out for only 4 h, followed by washing. The target cells were incubated with 20 $\mu$M $\Delta\beta_{1-40}$ concurrently with virus (+/-), immediately after virus removal (−/+), or throughout both time periods (+/+). (Fig. 2.) After the wash, the cells were incubated for an additional 48 h, at which time luciferase activity was measured. Enhancement of infection was observed only when $\Delta\beta_{1-40}$ was present during the initial 4-h incubation of virus and cells. These data suggest that A$\beta$ exerts its effect at an early stage of viral infection. Because the first 4 h of HIV-1 infection involves virus attachment and entry into the host cell, A$\beta$ likely enhances these processes.

Enhancement of Viral Infection by A$\beta$ Is Receptor-mediated—A$\beta$ has been shown to exert a destabilizing effect on cellular membranes (42, 43). Therefore, A$\beta$ 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 C2Th, C2Th-CD4, C2Th-CCR5, and C2Th-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 A$\beta$ 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 A$\beta$ on all cells examined. A$\beta$-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 A$\beta$ remains dependent on the expression of CD4 and a chemokine coreceptor.

Other Fibril-forming Peptides Enhance Viral Infection—A$\beta$ aggregates into fibrils (8, 42, 44–46). We investigated whether other fibril-forming peptides unrelated to A$\beta$ could enhance virus infection. Fig. 4 shows that two such peptides, PPI-2480 (AGAKWSWELTWVG) and PPI-2566 (IRQAMCNISRAD), which form fibrils similar to A$\beta_{1-40}$ and A$\beta_{42}$ (Fig. 5, b–f), also enhanced the infection efficiency of recombinant HIV-1 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 pseudotyped with the VSV-G protein by ∼2-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 ($\Delta\beta_{1-40}$ and PPI-2480) formed shorter fibrils (Fig. 5, b–f).
FIG. 3. HIV-1 infection in the presence of Aβ is dependent on co-receptors. a, C82Th 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 C82Th-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 μg Aβ with increasing concentrations of the 2D7 anti-CCR5 monoclonal antibody.

and d), whereas peptides forming longer fibrils (Aβ1-42 and PPI-2566, Fig. 5, c and e) were less efficient.

Fibril-forming Peptides Promote Lipid Vesicle Association with Cells—To further investigate 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 Aβ1-40, Aβ1-42, PPI-2480, PPI-2566, 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 irreversibly associated 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, Aβ1-40 promoted the adherence of liposomes better than Aβ1-42, PPI-1966, which Fig. 5f shows cannot form fibrils, had no effect on the association of liposomes with cells (data not shown). Utilizing fluorescent Aβ1-40 fibrils (FITC-Aβ), we found that FITC-Aβ 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-Aβ with cell membranes (data not shown). Additionally, the presence of Aβ1-40 did not induce changes in cell surface expression of CD4 or CCR5 (data not shown). These data support a model in which Aβ 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 Aβ 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 Aβ. Infection by complete A-MuLV was also strikingly enhanced, from 30–50-fold, in the presence of preaggregated Ab1-40 (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, Ab1-40 fibrils were precipitated by multiple centrifugation and washing steps and compared with the supernatant of the first centrifugation. Fig. 6a demonstrates that the precipitable Ab1-40 fraction, but not any residual soluble peptide, enhanced A-MuLV infection comparably to Ab1-40 that had not been centrifuged. Conversely, the Ab40-1 reverse fragment did not enhance the infection efficiency of A-MuLV. These data underscore the substantial enhancement of retroviral infectivity by Ab1-40 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 Aβ1–40 and Aβ1–42. The requirement that the Aβ 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 Aβ participate in the observed enhancement of viral fusion remains unclear. The requirement for appropriate receptors on the target cell is not bypassed by Aβ, 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 Aβ. Interestingly, other synthetic amyloidogenic peptides unrelated to Aβ similarly enhanced viral infection, whereas synthetic nonamyloidogenic 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 more detectable in HIV-1-infected individuals than uninfected individuals.
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 ββ, 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 colocalize 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 ββ 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 ββ in HAD. Taken together with the data herein, these observations suggest that testing the effect of inhibitors of ββ 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 Oza for amyloidogenic peptides, and Malcolm Gefter for critical comments. We thank Dr. David Knipe for the kind gift of HD-2, Maria ter for supplying Cf2Th, 293T human embryonal kidney, and NIH-3T3 cells. Oza for amyloidogenic peptides, and Malcolm Gefter for critical comments. We thank Dr. David Knipe for the kind gift of HD-2, Maria ter for supplying Cf2Th, 293T human embryonal kidney, and NIH-3T3 cells.