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Semen-Derived Amyloid Fibrils Drastically Enhance HIV Infection

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SUMMARY

Sexual intercourse is the major route of HIV transmission. To identify endogenous factors that affect the efficiency of sexual viral transmission, we screened a complex peptide/protein library derived from human semen. We show that naturally occurring fragments of the abundant semen marker prostatic acidic phosphatase (PAP) form amyloid fibrils. These fibrils, termed Semen-derived Enhancer of Virus Infection (SEVI), capture HIV virions and promote their attachment to target cells, thereby enhancing the infectious virus titer by several orders of magnitude. Physiological concentrations of SEVI amplified HIV infection of T cells, macrophages, ex vivo human tonsillar tissues, and transgenic rats in vivo, as well as *trans*-HIV infection of T cells by dendritic or epithelial cells. Amyloidogenic PAP fragments are abundant in seminal fluid and boost semen-mediated enhancement of HIV infection. Thus, they may play an important role in sexual transmission of HIV and could represent new targets for its prevention.

INTRODUCTION

HIV-1, the causative agent of AIDS, has infected about 60 million people and caused over 20 million deaths. More than 80% of these HIV-1 infections are acquired through sexual intercourse. Despite its dramatic spread in the human population, the efficiency of HIV-1 transmission via the sexual route is surprisingly poor. For instance, the risk of male-to-female intravaginal HIV-1 transmission is estimated at about 1 event per 200–2000 coital acts (Gray et al., 2001). This rate is about 10-fold increased during acute infection when the viral load is particularly high (Pilcher et al., 2004). Moreover, the presence of other sexually transmitted diseases and sexual practices associated with bleeding and lesions of the mucosal barrier can increase this risk to up to 3% per sexual contact (Galvin and Cohen, 2004). Nevertheless, the poor transmissibility of HIV-1 is clearly a major factor restricting the AIDS pandemic.

Globally, most infections result from genital exposure to semen (SE) of HIV-positive men (Royce et al., 1997). Women who acquired HIV-1 through vaginal intercourse constitute almost 60% of new infections in Africa (reviewed in Haase, 2005). The infectivity of HIV-1 in male genital fluid together with the susceptibility of the host, the type of sexual practice, and the viral load are major determinants of sexual transmission (Chakraborty et al., 2001; Gupta et al., 1997; Pilcher et al., 2004). The factors modulating HIV infectiousness in SE are poorly understood (reviewed in Miller and Shattock, 2003).

To identify natural agents that might play a role in sexual transmission of HIV/AIDS, we screened a complex peptide/protein library derived from human seminal fluid (SE-F) for novel inhibitors and enhancers of HIV infection. We found that fragments of prostatic acidic phosphatase (PAP) drastically enhance HIV infection. Functional and structural analyses showed that these peptides form amyloid fibrils that capture HIV particles and strongly enhance the number of productively infected cells by promoting virion-cell attachment and fusion. In agreement with a relevant role in vivo we found that semen and

seminal fluid also drastically enhance HIV infection and provide evidence that fibril-forming PAP fragments contribute to this effect. Our data support that amyloidogenic peptides are abundant in semen and promote sexual transmission of HIV/AIDS.

RESULTS

Purification of an Enhancer of HIV-1 Infection from Semen

Our recent isolation of a novel HIV-1 entry inhibitor (Münch et al., 2007) shows that screening of peptide libraries from human body fluids is a useful approach to discover as-yet-unknown molecules modulating HIV-1 infection. To identify factors that play a role in sexual transmission of HIV-1, we analyzed a complex peptide/protein library derived from pooled human SE. This library encompassed 294 fractions and ought to represent all peptides and small proteins (MW < 50 kDa) present in seminal fluid (SE-F). We found that fraction 29 of pH pool 7 significantly enhanced HIV-1 infection (Figure 1A). This fraction contained only small amounts of peptide/protein (Figure 1A, left inset). Mass spectrometry (MS) after one additional round of purification identified several peptides in the active fractions, ranging from 4028 to 4551 Da (Figure 1A, right inset). Peptide sequencing identified them as fragments of PAP (Table S1). This protein is produced by the prostatic gland, secreted in large amounts (1–2 mg/ml) into SE (Rönnberg et al., 1981), and used as a SE marker (Graves et al., 1985). All peptides mapped to the same region of PAP but differed in length from 34 to 40 residues. The predominant form of 4551 Da (Figure 1A, right inset) corresponds to amino acids 248 to 286 of PAP (EMBL accession number AAB60640).

Next, we verified that chemically synthesized PAP peptides also enhance HIV-1 infection, whereas control peptides, including a sequence scrambled variant of the predominant PAP fragment (PAPscr), had no effect (Figure 1B and Table S1). The PAP peptides promoted HIV-1 infection in the absence of fetal bovine serum (Figure S1) indicating that no serum cofactor is required. If not mentioned otherwise, all subsequent experiments were performed with the synthetic peptide corresponding to the major form detected in the SE-F (PAP248-286). Examination of CEMx174 5.25 M7 (CEMx M7) cells containing the GFP reporter gene under the control of the HIV-1 promoter (Hsu et al., 2003) by fluorescence microscopy (Figure 1C) and flow cytometry (Figure 1D) confirmed that PAP248-286 drastically increases the number of HIV-1-infected cells. In contrast, it did not affect the transcriptional activity of the HIV-1 LTR promoter or Env-mediated cell-cell fusion (Figure S2). Fresh PAP248-286 solutions were inactive in promoting HIV-1 infection. After overnight incubation, however, they enhanced HIV-1 infection more efficiently and were less cytotoxic than the polycation polybrene (Figure 1E), commonly used to boost HIV-1 infection or retroviral gene transfer. In comparison, full-length PAP neither promoted HIV-1 infection nor inhibited the enhancing activity of PAP248-286 (Figure 1F).

PAP Fragments form Amyloid Fibrils that Promote HIV Infection

We noted that PAP248-286 and other PAP fragments increased HIV-1 infection only when the solutions became turbid either spontaneously during storage or after agitation and found that the precipitate contains the active form (Figure 2A). It has been previously shown that amyloid fibrils associated with Alzheimer's disease enhance HIV-1 infection (Wojtowicz et al., 2002). Thus, we examined whether PAP fragments also form amyloid fibrils. We found that agitation of fresh PAP248-286 solutions induced a strong increase in Thioflavin T binding (Figure 2B), in green birefringence upon staining with Congo red (data not shown), and in β sheet content (Figure S3). Electron microscopy confirmed effective fibril formation (Figure 2C), and X-ray powder diffraction demonstrated reflections at 4.7 and 10.6 Å, which correspond to the regular interstrand spacing and intersheet distances, respectively (Figure 2D). All these properties are typical for amyloid fibrils (reviewed in Nilsson, 2004). Length variations at the N terminus of the PAP fragments did not impair fibril formation or enhancement of HIV-1 infectivity, whereas deletion of the four C-terminal (LIMY) residues reduced both effects (Figures 1B, S4, and S5). However, the PAP247-282 fragment lacking the LIMY region did not exert efficient transdominant-negative effects on fibril formation by other PAP fragments (Figure S4). Notably, PAP fragments were substantially more potent in enhancing HIV-1 infection than other amyloidogenic peptides (Figure S5). Since the amyloid fibrils strongly enhanced the infectivity of HIV-1 we refer to them herein after as Semen-derived Enhancer of Virus Infection (SEVI).

To assess whether SEVI interacts directly with HIV-1 particles, we preincubated virus stocks with the fibrils for 5 min in a small volume and subsequently added the virus/SEVI mixture to the cell culture, thereby diluting it 50-fold. Diluting the HIV-1/SEVI mixture did not reduce the magnitude of infectivity enhancement (Figure S6A), implying that SEVI efficiently bound to the virions. However, preincubation of the target cells with SEVI also enhanced HIV-1 infection, even after extensive washing (Figure S6B). To clarify whether SEVI promotes a physical interaction of virions with target cells, we incubated TZM-bl cells with HIV-1 particles in the presence or absence of SEVI. Viral binding was assessed by the amount of cell-associated p24 after extensive washing. SEVI significantly enhanced binding of both wild-type HIV-1 particles and virions lacking Env, although the absolute levels of cell-associated p24 were about 30-fold lower in the absence of Env (Figure 2E). To directly visualize the effects of SEVI, we monitored infection of TZM-bl cells microscopically using fluorescently labeled HIV-1 virions. We found that SEVI drastically enhanced virion binding to the cells and the coverslips (Figure 2F). Parallel bright-field phase images demonstrated that SEVI fibrils are loaded with