Among the modes of transmission available to the cytomegalovirus (CMV) is sexual transmission, primarily via semen. Both male-to-female (M-F) and male-to-male (M-M) sexual transmission significantly contribute toward the spread of CMV infections in the global population. Semen plays an important role in carrying the viral particle that invades the vaginal or rectal mucosa, thereby initiating viral replication. Both seminal plasma (SP) and semen can enhance HIV-1 infection in cell culture, and two amyloid fibrils, semen-derived enhancer of viral infection (SEVI) and amyloids derived from the semenogelins (SEM amyloids), have been identified as seminal factors sufficient to enhance HIV-1 infection (J. Munch et al., Cell 131:1059–1071, 2007; N. R. Roan et al., Cell Host Microbe 10:541–550, 2011; F. Arnold et al., J. Virol. 86:1244–1249, 2012). Whether SP, SEVI, or SEM amyloids can enhance other viral infections has not been extensively examined. In this study, we found that SP, SEVI, and SEM amyloids strongly enhance both human CMV (HCMV) and murine CMV infection in cell culture. SEVI and SEM amyloids increased infection rates by greater than 10-fold, as determined by both flow cytometry and fluorescence microscopy. Viral replication was increased by 50- to 100-fold. Moreover, viral growth curve assays showed that SP, SEVI, and SEM amyloids sped up the kinetics of CMV replication such that the virus reached its replicative peak more quickly. Finally, we discovered that SEM amyloids and SEVI counteracted the effect of anti-gH in protecting against CMV infection. Collectively, the data suggest that semen enhances CMV infection through interactions between semen amyloid fibrils and viral particles, and these interactions may prevent HCMV from being neutralized by anti-gH antibody.

**T**opical microbicides that prevent sexual transmission of viruses could significantly reduce sexually transmitted diseases. People who are infected with human cytomegalovirus (HCMV) can shed the virus in their body fluids, including semen (1, 2). HCMV replicates in the genital tract, is sexually transmitted, and is highly prevalent worldwide (3). Viral load in semen is directly related to the transmission of HCMV from male-to-male (M-M) and from male-to-female (M-F) (1). In the United States, ca. 30 to 50% of women have never been infected with HCMV. About 1 to 4% of previously uninfected women are infected with HCMV during pregnancy. Upon infection, about one-third of pregnant women will pass HCMV to their fetuses or infants (4, 5). HCMV can cause birth defects, making it a significant public health problem (6). In addition, HCMV infection causes life-threatening diseases in immunocompromised hosts, such as individuals with HIV/AIDS, and is associated with HIV disease progression in both treated and untreated individuals (7, 8).

No effective drugs against CMV-mediated diseases in infants are available, and no vaccine is effective in preventing CMV infection. For these reasons, fresh approaches for developing microbicides effective against CMV could have important benefits for the health of both adults and infants. Identifying risk factors for the transmission of CMV during sexual intercourse and understanding how semen is involved in the transmission of CMV are important elements in the development of innovative strategies against CMV infection, especially in terms of designing nontoxic, effective topical microbicides against the virus. Although it is apparent that semen is an important carrier of HCMV, the effects of semen on CMV transmission remain unknown.

Semen contains proteolytic cleavage products of prostatic acid phosphatase (PAP) and semenogelatin (SEM) that form amyloid fibrils in semen. The PAP-derived amyloids were named semen-derived enhancer of viral infection (SEVI) and were the first semen amyloids shown to enhance HIV infection (9). A subsequently identified second set of peptides that form HIV-enhancing amyloid fibrils are derived from SEM and referred to as SEM amyloids (10). Whether other sexually transmitted viral infections can be enhanced by seminal plasma (SP) or semen amyloids has remained largely unexplored.

In the present study, we discovered that SP, SEVI, and SEM amyloids can enhance both HCMV and murine CMV (MCMV) infection of permissive cells. We also observed that the fibrils can interact directly with viral particles and protect viruses from being neutralized by antibodies against glycoprotein H (gH).

**MATERIALS AND METHODS**

**Tissue culture and viruses.** NIH 3T3 (from the American Type Culture Collection [ATCC]), U-251 MG, and MRC-5 (ATCC no. CCL171) cells, permissive to infection by MCMV and HCMV, respectively, were maintained in Dulbecco Modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin.

MCMVE5gfp was generated from the BACmid Sm3fr (11) by tagging IE2 using bacterial artificial chromosome (BAC) techniques. MCMVgfpSVH was made by tagging GFP to the N terminus of IE1 and IE2 using bacterial artificial chromosome (BAC) techniques. Briefly, we inserted galk between the first and second amino acid codons of the MIE gene. Then, the galk was replaced with the open reading frame.
of GFP so that GFP and the MIE genes were fused in frame. The BAC DNA was sequenced and confirmed to be correct and transfected into MRC-5 cells to produce the HCMVgfpSVH virus.

**Reagents.** SEVI was synthesized by the genomic and the proteomics core laboratories at the University of Pittsburgh. SEM amyloids (amino acid residues 49 to 107) (10) were synthesized by Celtek Peptides. Synthetic peptides were dissolved in phosphate-buffered saline (PBS) or serum-free MEM and agitated overnight at 1,400 rpm at 37°C using an Eppendorf Thermomixer, as described previously (9). Aβ1-42 amyloids (catalog no. A9810) were purchased from Sigma-Aldrich (St. Louis, MO). Semen samples were pooled from 10 different individuals. To generate seminal plasma, the pooled semen samples were centrifuged at 700 × g for 5 min, and the supernatant was divided into aliquots and stored at −80°C and until use. The process of obtaining and using of semen samples and the informed consent forms were fully reviewed and approved by the committee on human research at Ponce School of Medicine and Health Sciences before the initiation of the study (IRB number 081106-YY).

**Antibodies.** The antibodies used for Western blotting (WB) and immunofluorescence (IF) are listed below. A monoclonal antibody against tubulin (T-9026) was purchased from Sigma-Aldrich (1:1,000 for WB); polyclonal antibodies against GAP (sc-8334) and monoclonal antibodies against HCMV pp65 (3A12), gB (2F12), and gH (monoclonal antibody 0861) were purchased from Santa Cruz Biotecnology, Inc. (Santa Cruz, CA; 1:1,000 for WB to probe GFP); monoclonal antibodies against MCMV IE1 and E1 were provided by Stipan Jonjic (Croatia; 1:50 for IF and 1:200 for WB) (14). The monoclonal antibodies against HCMV pp65, MCMV gB, m25, and gH were generous gifts from J. D. Shanley (University of Connecticut; 1:250 for WB) (15, 16). The monoclonal antibody against HCMV IE1/2 (MAB810) was purchased from Sigma-Aldrich. The rabbit antibody against HCMV UL112/113 was a gift from J. H. Ahn (Korea University, Seoul, Korea).

**Purification of MCMV and HCMV.** MCMV and HCMV were amplified in NIH 3T3 and MRC-5 cells, respectively. The viral supernatant was centrifuged at 8,000 × g for 20 min to remove cell debris. The clarified medium was transferred into SW27/28 Ultraclear centrifuge tubes that prevented binding of the most prevalent virus. SEVI and SEM amyloids have been demonstrated to enhance HIV infection (9, 10). To determine whether these amyloids can also enhance CMV infection, we used HCMVgfpSVH and MCMVEG5gfp (12, 19), both of which express GFP-fused immediate-early (IE) proteins (GFP-IE3 and IE4) and used to detect additional proteins.

**PFU assay.** Virus titers were determined by the plaque assay, essentially as described previously (12), but with some slight modifications. Supernatants containing serially diluted viral particles were added to confluent NIH 3T3 (MCMV) or MRC-5 (HCMV) monolayers in six-well plates. After adsorption for 2 h, the medium was removed, and the cells were washed twice with serum-free DMEM and overlaid with phenol-free DMEM containing 5% FCS, 0.5% low-melting-point agarose (Gibco), and 1% penicillin-streptomycin. The numbers of plaques that were stained red were counted and are reported as PFU/ml. Mean PFU were determined after averaging the numbers of PFU from different dilutions. A Student t test was used to statistically analyze differences between the groups; a P value lower than 0.005 was used as the threshold for a significant difference.

**RESULTS**

**SEVI and SEM amyloids increase CMV infection rates.** Herpesviruses are commonly detected in semen samples, with CMV being the most prevalent virus. SEVI and SEM amyloids have been demonstrated to enhance HIV infection (9, 10). To determine whether these amyloids can also enhance CMV infection, we used HCMVgfpSVH and MCMVE5gfp (12, 19), both of which express MIE proteins fused to GFP, thereby allowing infection to be assessed by flow cytometry (Fig. 1) and fluorescence microscopy (Fig. 2). Infections were carried out in permissive cells (MRC-5 for HCMVgfpSVH and NIH 3T3 for MCMV5gfp).

For MCMV5gfp infection of NIH 3T3, we treated the virus in the presence of titrations of SEVI or SEM amyloids at 37°C for 1 h and then added the pretreated virus to NIH 3T3 cells (multiplicity of infection [MOI] = 0.1). At 16 h after infection, the cells were washed, treated with trypsin, fixed, and quantitated by flow cytometry. Mock-infected cells with or without amyloid treatment served as autofluorescence controls. As can be seen in Fig. 1A, SEVI and SEM amyloids both strongly enhance CMV infection in a dose-dependent manner. The percentages of MCMV5gfp-infected NIH 3T3 cells are indicated in the lower right-hand corners. In the absence of amyloid treatment, the MCMV infection
Flow cytometry to detect viral infection rate. (A) MCMV infection in NIH 3T3 cells. NIH 3T3 cells were cultured in a six-well plate. When the cells were 80% confluent, they were mock infected or infected with MCMVE5gfp (12) at an MOI of 0.1. Virus was preincubated with 0, 2, 5, or 10 μg of SEM amyloids or SEVI/ml for 1 h at 37°C before infection. The cells were fixed at 16 h postinfection and measured by flow cytometry to detect the percentage of GFP-positive (infected) cells. The percentage of infected cells is shown in the lower right-hand corner of each plot. (B) HCMV infection in MRC-5 cells. MRC-5 cells were mock infected or infected with HCMVgfpSVH at an MOI of 0.1 for 16 h. Prior to infection, the virus was incubated with 0, 2, 5, or 10 μg of SEM amyloids or SEVI/ml at 37°C for 1 h. Infection rates were detected by flow cytometry, as described in panel A, and the percentage of infected cells is shown in the lower right-hand corner of each plot. Mock-infected cells in the absence or presence of 10 μg of SEM/ml or 10 μg of SEVI/ml were used to control for autofluorescence. These experiments were performed in duplicate, with one set of the results reported in the figure. The results are representative of one of three independent experiments.
rate was 2.42%. As the amount of SEM amyloids was increased from 2 to 10 μg/ml, infection rates increased from 7.8 to 29.2%. Similarly, increasing SEVI levels from 2 to 10 μg/ml increased infection rates from 6.23 to 23.5%.

For HCMV infection of MRC-5 cells, cell-free virus was treated at 37°C for 1 h with different titrations of SEVI or SEM amyloids (Fig. 1B). Pretreated HCMV was then added to MRC-5 cells in a six-well plate. Sixteen hours later, cells were treated with trypsin and fixed with 1% paraformaldehyde, and the total cells and GFP-positive cells were quantified. As can be seen in Fig. 1B, the basal infection rate in the absence of SEVI or SEM amyloid was 1.68% and increased to 18.2% after treatment with 10 μg of SEM amyloids/ml and to 18.5% after treatment with 10 μg of SEVI/ml.

SEVI and SEM amyloids enhance HCMV infection of U-251 MG epithelial cell-like cells. HCMV can infect epithelial cells in vivo (8). In order to determine whether SEVI or SEM amyloid can enhance CMV infection of epithelial cell-like cells, we next examined HCMV infection of U-251 MG cells in the absence or presence of semen amyloids. U-251 MG cells seeded on coverslips were infected with HCMVgfpSVH at an MOI of 0.5 for 16 h and then fixed with 1% paraformaldehyde. After a PBS wash, the cells were stained with DAPI to identify nuclei. The coverslips were transferred onto slides and then visualized using confocal microscopy. CMV-infected cells were identified by GFP expression, whereas DAPI was used to quantify the total number of cells.

Consistent with the flow cytometry data, HCMV infection was

FIG 2 Visualization of HCMV infection in U-251 MG cells. (A to C) The epithelial cell-like cell line U-251 MG was infected with HCMVgfpSVH at an MOI of 0.5 for 16 h in the absence of amyloids (A1 to A3) or after pretreatment with either 10 μg of SEM amyloids/ml (B1 to B3) or 10 μg of SEVI/ml (C1 to C3). The cells were fixed with 1% paraformaldehyde and stained with DAPI. The slides were observed under a fluorescence microscope (×10 amplification lens), and pictures were taken to show infected cells (GFP, A1 to C1) and total cells (DAPI, A2 to C2). The merged pictures are shown in A3 to C3. The percentages of GFP-positive (infected) cells are shown in the upper right-hand corners of A1 to C1. Scale bars, 20 μm. (D) Imaging experiments were performed three independent times. We counted a total of 1,000 cells for each experiment and averaged the percentages of GFP-positive cells. The averages and standard errors are shown in this panel. Statistically significant differences are indicated at the top (P < 0.005).

rate was 2.42%. As the amount of SEM amyloids was increased from 2 to 10 μg/ml, infection rates increased from 7.8 to 29.2%. Similarly, increasing SEVI levels from 2 to 10 μg/ml increased infection rates from 6.23 to 23.5%.

For HCMV infection of MRC-5 cells, cell-free virus was treated at 37°C for 1 h with different titrations of SEVI or SEM amyloids (Fig. 1B). Pretreated HCMV was then added to MRC-5 cells in a six-well plate. Sixteen hours later, cells were treated with trypsin and fixed with 1% paraformaldehyde, and the total cells and GFP-positive cells were quantified. As can be seen in Fig. 1B, the basal infection rate in the absence of SEVI or SEM amyloid was 1.68% and increased to 18.2% after treatment with 10 μg of SEM amyloids/ml and to 18.5% after treatment with 10 μg of SEVI/ml.

SEVI and SEM amyloids enhance HCMV infection of U-251 MG epithelial cell-like cells. HCMV can infect epithelial cells in vivo (8). In order to determine whether SEVI or SEM amyloid can enhance CMV infection of epithelial cell-like cells, we next examined HCMV infection of U-251 MG cells in the absence or presence of semen amyloids. U-251 MG cells seeded on coverslips were infected with HCMVgfpSVH at an MOI of 0.5 for 16 h and then fixed with 1% paraformaldehyde. After a PBS wash, the cells were stained with DAPI to identify nuclei. The coverslips were transferred onto slides and then visualized using confocal microscopy. CMV-infected cells were identified by GFP expression, whereas DAPI was used to quantify the total number of cells.

Consistent with the flow cytometry data, HCMV infection was
drastically enhanced by SEVI and SEM amyloids (Fig. 2). Whereas basal infection levels were low in the absence of semen amyloids (Fig. 2A), infection was substantially increased in the presence of either SEM amyloids (Fig. 2B) or SEVI amyloids (Fig. 2C). The percentages of GFP-positive cells are shown in the upper right-hand corners of plots in Fig. 2A to C1. A total of 1,000 cells were counted in each experiment to obtain the percentage of GFP-positive cells, and the numbers were averaged from three independent experiments. As shown in Fig. 2D, the differences between the virus-only group and the SEVI- or SEM amyloid-treated groups were significant (P < 0.005).

**Viral protein production is enhanced by SP, SEVI, and SEM amyloids.** To determine whether the enhancement of CMV infection mediated by semen amyloid fibrils results in increased viral protein production, we infected NIH 3T3 or MRC-5 cells with MCMV or HCMV, respectively, and detected viral protein production by WB. Four groups of cells are shown in Fig. 3: (i) virus infection alone (that is, virus not exposed to SP or amyloids), (ii) virus treated with SP at a dilution of 1:1,000, (iii) virus treated with SEM amyloids (5 μg/ml), and (iv) virus treated with SEVI (5 μg/ml). At 24 h after infection, whole-cell lysates were fractionated by SDS-PAGE and analyzed by WB with anti-CMV antibodies.

**FIG 3** Western blot assay to detect viral protein production. (Left) NIH 3T3 cells were mock infected or infected with either MCMV/E5gfp alone (MOI = 0.1) or MCMV/E5gfp treated with SP, SEM amyloids, or SEVI for 24 h. Whole-cell lysates were prepared for Western blot with antibodies against GFP (IE3-GFP), IE1, M112-113, and tubulin (as loading control). (Right) MRC-5 cells were mock infected or infected either with HCMV alone or HCMV treated with SP, SEM amyloids, or SEVI; all infections were carried out for 24 h at an MOI of 0.1. Whole-cell lysates were prepared for Western blot with antibodies against IE1, IE2, UL112-113, and tubulin. By comparing the density of the IE3 bands of MCMV or the IE1 bands of HCMV with that of the virus alone (with all intensities normalized to the tubulin control), the fold increase of IE3 levels for MCMV (left) and that of IE1 for HCMV (right) were calculated (Quantity One 4.5.0 software; Bio-Rad Laboratories, Richmond, CA). Normalized IE3 (MCMV, left) or IE1 (HCMV, right) levels are shown below the corresponding Western blots.
can be used as biomarkers for viral entry. Amyloid-treated cells in the absence of virus were used as controls for the autofluorescence. As shown in Fig. 4A, both SEVI and SEM amyloids increased the percentage of cells that are positive with tegument proteins upon infection, suggesting that these amyloids increased CMV entry into target cells.

To provide further support for the notion that the amyloids increased CMV infection at the stage of viral entry, we tested whether the amyloids could increase viral gene expression if added to the cell culture postentry. We infected MRC-5 or NIH 3T3 cells with HCMV or MCMV at an MOI of 0.1 for 2 h, and then washed the cells three times with MEM to remove surface-associated virions. A final concentration of 10 μg of SEVI or SEM amyloids/ml was then added to the cells. Whole-cell lysates were collected at 24 h postinfection and assessed for viral protein expression (IE1 and IE2 for HCMV; IE1 and IE3 for MCMV). As shown in Fig. 4B, neither SEVI nor SEM amyloids enhanced viral gene expression when they were added after infection had been established. These experimental results further confirm that SEVI and SEM amyloids enhance CMV infection by increasing viral entry.

Viral replication is accelerated and enhanced by SP, SEVI, and SEM amyloids. We then examined whether viral replication can be enhanced by SP and semen components. Five groups of cultures were set up and infected with virus that was preincubated at 37°C for 1 h with the following: (i) mock treatment (CMV alone, no SEM or SEVI), (ii) SP (1:1,000), (iii) SEM amyloids (5 μg/ml), (iv) SEVI (5 μg/ml), and (v) 5 μg of SEM/ml plus 5 μg of SEVI/ml. Infection of both NIH 3T3 and MRC-5 cells were carried out at an MOI of 0.1. Media were collected every day for 6 days (for MCMV) or 9 days (for HCMV) to monitor CMV replication by PFU counts (Fig. 5). Collected viral samples were freeze-thawed for three cycles and then centrifuged at 8,000 × g for 20 min to remove the cellular debris. Supernatants were assayed for viral production in NIH 3T3 (for MCMV) and MRC-5 (for HCMV) cells. As shown in Fig. 5, both MCMV (lower) and HCMV (upper) infections were strongly enhanced by a 1:1,000 dilution of SP (20-fold for MCMV and 10-fold for HCMV), 5 μg of SEM amyloids/ml (100-fold for MCMV and 50-fold for HCMV), and 5 μg of SEVI/ml (50-fold for both viruses). The less-pronounced effect on viral replication by SP might be caused by the lower amount of SEVI and SEM amyloids in the diluted SP. According to some estimations, the concentration of SEVI in semen is ~35 μg/ml (9) and that of SEM amyloids 90 to 3,000 μg/ml (10), suggesting that the diluted SP used in this experiment contained only 0.035 μg of SEVI/ml and 0.09 to 3 μg of SEM amyloid/ml. High concentrations of SP were not used in this experiment since such concentrations have been shown to be toxic to cell culture when used for prolonged periods (20). We also tested whether SEM amyloid and SEVI could have a synergistic effect on enhancing CMV infection. Combining SEVI and SEM amyloids led to an additive, and not a synergistic, effect on enhancing HIV infection.

SEVI and SEM amyloids interact with CMV viral particles. One mechanism by which semen amyloids enhance viral infection is promoting the binding of virions (17, 18) to cellular targets. SEVI and SEM amyloids directly interact with HIV particles to form fibril-virus complexes (9, 10, 17). We next sought to determine whether SEVI and SEM amyloids could also directly interact with CMV particles. To answer the question, we performed a viral binding assay. CMV viral particles (5 × 10⁷ particles/ml) were incubated (i) in the absence of added factors, (ii) with 50 μg of Aβ(1–42) amyloids/ml, (iii) with 50 μg of SEVI/ml, or (iv) with 50 μg of SEM amyloid fibrils/ml. All treatments were carried out at 37°C for 1 h. The mixtures were then centrifuged at 700 × g for 5 min, and the resulting pellets were washed twice with serum-free MEM. Pellets were then lysed in gel loading buffer and assessed by Western blotting. As demonstrated in Fig. 6, viral particles incubated with only buffer or with Aβ amyloids failed to be pelleted; in contrast, SEM and SEVI fibrils efficiently pelleted viral particles after low-speed centrifugation. Only viral surface proteins (gB and gH) and tegument protein (m25 of MCMV and pp71 of HCMV) were pulled down, whereas the nonstructural protein IE1, which is in infected cells but not present in the viral particle, was not pelleted. These results suggest that CMV particles directly bind both SEVI and SEM amyloids.

SEVI and SEM amyloids protect viruses from being neutralized by anti-gH antibodies. Several antibodies against CMV glycoproteins have been tested for their ability to neutralize CMV. The major targets of the neutralizing antibody response to HCMV are the glycoproteins gB, gM/gN, and gH/gL/gO, all of which mediate entry into host cells (21). In our laboratory, we have two monoclonal antibodies against gB and gH. We found that only the anti-gH antibodies significantly neutralized CMV; in contrast, the
anti-gB monoclonal antibody had little neutralizing effect on either MCMV or HCMV infection (data not shown).

The physical interaction between SEVI and SEM amyloids with CMV particles prompted us to speculate that the amyloids might overcome the effects of antibody-mediated neutralization. To test this conjecture, we examined whether the amyloids protected against neutralization by anti-gH (22). We set up the following five treatment groups (Fig. 7): (i) virus only (HCMV or MCMV), (ii) virus incubated with anti-gH antibody for 30 min, (iii) virus incubated with anti-gH antibody for 30 min and then with SEVI or SEM amyloids for another 30 min, (iv) virus incubated with SEVI or SEM amyloids for 30 min and then with anti-gH antibody for another 30 min, and (v) virus incubated with only SEVI or SEM amyloids for 30 min. Infection rates were determined by flow cytometry. The results showed that adding either SEVI or SEM amyloids before the addition of anti-gH significantly reduced the neutralizing effects of the antibody on HCMV and MCMV. Therefore, we postulate that SEM amyloids and SEVI not only enhance CMV infection but might also protect the virus from antibody-mediated neutralization.

DISCUSSION
Clinical significance of sexually transmitted CMV. Although the majority of herpes simplex virus and CMV infections are clinically mild or asymptomatic, CMV infection in two populations can result in serious disease and mortality. First, primary infection during the fetal or perinatal period can be neurologically devastating, even fatal, for the unborn child (23,24). A CMV-negative woman who becomes infected with CMV during pregnancy has a high probability of transmitting CMV to her fetus (24). CMV causes birth defects (6) and therefore poses a significant public health problem. The precise reasons for the increased severity of the disease early in life are not clear and may involve aspects of immune defense. The severity of CMV-caused diseases is directly influenced by viral load (6). Significantly, one method of CMV acquisition by pregnant women is via sexual intercourse (6). The second population at high risk for CMV infection consists of immunocompromised individuals, including AIDS and organ transplantation patients (6). Many cases of HIV infection are associated with CMV infection, mainly at the stage when the CD4+ T cell...
count is <50 cell/mm³ (25). Over three-fourths of HIV-infected men who have sex with men have at least one herpesvirus detected in their semen, with CMV being the most prevalent of these (3, 13, 26). CMV infection in AIDS patients can cause myelitis, retinitis, encephalitis, and hepatitis. CMV myelitis is often associated with CMV infection in the peripheral nervous system, whereas patients with CMV encephalitis always have high titers of CMV in the cerebrospinal fluid (25). Although nonsexual routes of CMV transmission are common, sexual transmission also contributes toward the global spread of CMV, with semen being the primary vehicle for both male-to-female (M-F) and male-to-male (M-M) transmission (1, 6). Understanding the molecular details underlying semen-mediated CMV infections will therefore aid in the development of anti-CMV strategies for minimizing or eliminating M-M and M-F sexual transmission of CMV, which can decrease overall CMV transmission rates.

**Semen is an important vector for CMV transmission.** In order to be successfully transmitted and thereby infect genital cells, viruses have to survive in both vaginal and seminal fluids. Recent studies demonstrated that semen enhances the HIV infection of multiple cell types in vitro (9, 10, 17, 27). Two different semen-derived amyloid fibrils were identified as factors sufficient to enhance HIV infection (9, 10). In the present study, we found that SP and semen amyloid fibrils (SEVI and SEM amyloids) enhanced both HCMV and MCMV infection of permissive cells (Fig. 1 and 2). Semen amyloid-mediated enhancement of CMV infection was dose dependent. SP and purified amyloids not only caused higher viral gene expression but also led to increased viral production (Fig. 3 and 5). The enhancement of CMV infection by SP and the amyloids is likely caused by the direct binding and clustering of the virions, since the fibrils can bind CMV (Fig. 6) in a manner similar to the way in which the fibrils bind HIV (17). Overall, semen amyloids increased viral replication 50- to 100-fold, which could have significant implications for semen-mediated transmission of CMV.

**SEVI and SEM amyloids interact with CMV and overcome the neutralizing effects of antibodies.** Both neutralizing and nonneutralizing CMV-specific antibodies have been identified (28, 29). The major targets of the neutralizing antibody response to HCMV are glycoproteins gB, gM/gN, and gh/G/GO, all of which mediate entry into host cells (21, 30). In our assays, monoclonal antibodies against HCMV (gB and gH) and MCMV (gB and gH) were tested for neutralization activity. In the absence of complement, there was no neutralizing effect mediated by anti-gB. In contrast, anti-gH antibodies neutralized viral infection (Fig. 6) in the absence of complement, a finding consistent with previously reported results (31). Interestingly, in the present study we found that the semen amyloids can overcome the neutralizing effects of anti-gH antibodies. When CMV was first incubated with SEVI or SEM amyloids and then with anti-gH antibody, the neutralizing effects were significantly reduced (Fig. 6). These results may provide an explanation for why the antiviral components in vaginal fluid are not effective at blocking CMV infection.

**Conclusion and perspectives.** Our results suggest that (i) SEVI and SEM amyloids, as well as SP, enhance CMV infection, (ii) SEVI and SEM amyloids represent novel micrvice targets for CMV infection, and (iii) SEVI and SEM amyloids can prevent neutralization of CMV by antibodies. It is possible that the combination of inhibitors targeting the activities of SEVI and SEM amyloids and anti-gH antibodies may have a synergistic effect in protecting against CMV transmission. It would therefore be worthwhile to test a novel, innovative approach to CMV microbiocide development by testing agents that target SEVI, SEM, or both. Our studies point to a potential strategy of inhibiting the semen amyloid-mediated infection of CMV by preventing the formation of amyloids in semen. This approach of targeting host-derived factors is in contrast to traditional microbicidal strategies that target the virus itself. It has been shown that the genital tract of a man infected with HIV-1 is an anatomic compartment that supports CMV replication (26, 32). That being the case, it would be intriguing to investigate the extent to which semen from AIDS patients enhances CMV transmission.

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