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Interference Between Human Herpesvirus 7 and HIV-1 in Mononuclear Phagocytes

Richard W. Crowley,1 Paola Secchiero, Davide Zella, Andrea Cara, Robert C. Gallo,2 and Paolo Lusso3

Human herpesvirus 7 (HHV-7) uses CD4 as a cellular membrane receptor and thereby interferes with infection of CD4+ T cells by HIV-1. We studied the interactions between HHV-7 and a macrophage-tropic HIV-1 isolate (HIV-1Mab) in terminally differentiated human peripheral blood monocyte-derived macrophages, another critical target for infection by HIV-1 in vivo. Exposure of macrophages to HHV-7 alone yielded no signs of virus replication or cytopathic effects. Nevertheless, when macrophages were pre-exposed to either live or UV-inactivated HHV-7 and subsequently infected with HIV-1Mab, a significant dose-dependent inhibition of HIV-1 replication was documented. At day 7 postinfection, the average level of HIV-1 p24 Ag in cultures from five different donors was reduced by 91.7 ± 8.3% by pretreatment with live HHV-7 and by 91.8 ± 8.2% by pretreatment with UV-inactivated HHV-7. Moreover, the synthesis of HIV-1 proviral DNA in macrophages pretreated with HHV-7 was completely inhibited during the early stages after infection, suggesting that HHV-7 blocks HIV-1 at the level of interaction with the CD4 receptor. Consistent with this concept, both macrophage and CD4+ T cell cultures with pre-established HIV-1 infection were not susceptible to inhibitory effects of HHV-7. The proliferative response of PBMC to mitogens was only marginally inhibited by exposure to HHV-7 before mitogen stimulation, indicating that the inhibition of HIV-1 infection was not due to a negative effect on cell proliferation. These data demonstrate that HHV-7 is a powerful inhibitor of HIV-1 infection in cells of the mononuclear phagocytic lineage, despite its inability to replicate actively in such cells. The Journal of Immunology, 1996, 156: 2004–2008.

Materials and Methods

Purification of peripheral blood macrophages

Terminally differentiated monocyte-derived Mφ were obtained from the peripheral blood of healthy adult blood donors and enriched by adherence on plastic. Briefly, unfractionated mononuclear cells, purified by density gradient centrifugation on Ficoll-Paque (Pharmacia, Piscataway, NJ), were initially seeded in T-75 flasks (Costar, Cambridge, MA) at a concentration of 10^7/ml in the presence of recombinant human GM-CSF (Boehringer Mannheim, Indianapolis, IN) at 50 IU/ml. Every 3 days, one-half of the medium was removed from the flasks, together with one-half of the non-adherent cell population, and replaced with fresh medium containing 10% fetal bovine serum (Life Technologies, Inc., Gaithersburg, MD) and GM-CSF. At day 10 to 14 of culture, depending on the confluence of the adherent macrophages, nonadherent cells were completely removed, and the adherent monolayer was extensively washed with prewarmed PBS and recultured with complete medium containing GM-CSF.

Abbreviations used in this paper: HHV, human herpesvirus; GM-CSF, granulocyte-macrophage colony-stimulating factor; MOI, multiplicity of infection; SQ-PCR, semiquantitative PCR; Mφ, macrophages; IFA, immunofluorescence assay; Q-PCR, quantitative PCR.
Purification of CD4+ T cells

Peripheral blood CD4+ T cells were negatively selected by using magnetic beads coated with goat anti-mouse IgG antiseraum (Dynal, Oslo, Norway). Approximately 10^6 cells were incubated with a mixture of mAb directed against the surface Ags CD8, CD4, CD45R, and the yB TCR heterodimer (YB-81) (T-Cell Diagnostics, Cambridge, MA) for 30 min, washed once with PBS, and then placed in a 15-ml tube at 4°C with the beads at a cell:bead ratio of 1:40. After 30 min of continuous rotation, the beads were removed with a magnetic device (Dynal), and the residual cells were washed twice and cultured in complete medium containing 1 μg/ml of purified PHA (Burroughs-Wellcome, Cambridge, MA). One day later, 20 μl of partially purified human IL-2 (Boehringer Mannheim) were added.

Viruses and infection

The HHV-7 isolate AL (4, 6) was grown in PHA-stimulated CD4+ T lymphocytes. At the peak of the cytopathic effect (7 to 10 days after infection), the culture supernatants were collected, filtered, and stored at −70°C. The MOI (the number of infectious viral particles divided by the number of cells) was determined by exposing triplicate cultures of CD4+ T cells to serial 1:10 dilutions of the viral stock. An indirect immunofluorescence assay (IFA) was performed after 7 days as previously described (4), using mAb 9ASD12 (23), which was originally developed against HHV-6 but cross-reacts with HHV-7 (5). UV inactivation of the virus was performed as described (4).

Isolates. After HHV-7 (AL) was grown in M+ cultured in T-75 flasks, and the HHV-7 clones (24) were grown in PHA-stimulated CD4+ T lymphocytes. The virus yield of these cultures was tested by measuring the level of extracellular p24 Ag at day 10 postinfection. Supernatants containing >10 ng/ml of p24 were stored as viral stocks. The release of HIV-1 p24 Gag protein was measured by commercial ELISA (Dupont NEN, Boston, MA).

Cell proliferation assays

PBMC (5 × 10^7/ml) were treated with either complete medium or HHV-7 for 1 h and then washed before mitogen stimulation (PHA 1 μg/ml, OKT3 mAb 25 ng/ml). Cultures were incubated at 37°C with 5% CO2 for 48 or 96 h before being pulsed with 1 μCi of [3H]Tdr (Dupont NEN) for 18 h. Cells were then harvested onto glass fiber filters and radioactivity was measured by a liquid scintillation counter.

Experiments of co-infection with HHV-7 and HIV-1

For experiments of pre-exposure to HHV-7, 10 ml of the HHV-7 stock, at an approximate MOI of 5, were incubated with the macrophages in T-75 flasks at 4°C. For experiments of MOI-dependent inhibition of HIV-1 infection by HHV-7, serial twofold dilutions of the HHV-7 stock, starting at an approximate MOI of 5, were incubated with the macrophages in T-75 flasks at 4°C. After 1 h, stock cultures of the HIV-1 stock (1 ng/ml of p24) were added for 1 h at 37°C. The monolayers were then washed carefully four times with prewashed PBS and recultured in medium containing GM-CSF. Cell-free supernatants were collected at days 7, 10, and 13 for HIV-1 p24 assay. HHV-7 expression was evaluated in parallel by indirect IFA.

For experiments of postexposure to HHV-7, the M+ monolayers were initially infected with HIV-Iad, (1 ng/ml of p24 per 10^6 cells) and cultured in medium containing GM-CSF. After 10 days, the cultures were washed extensively and 10 ml of the HHV-7 stock were added without further washing. Control cultures were washed and mock-treated with 10 ml of uninfected medium. For the postexposure experiments in enriched CD4+ T lymphocytes, the cells were exposed to HIV-1 (1 ng of p24 per 10^6 cells) for 1 h at 37°C. washed, and immediately exposed to HHV-7 at a MOI of either 5 or 0.5 for 1 h at 37°C. Complete medium containing 20 μl of IL-2 was then added without additional washing. Supernatants were collected at days 2, 4, and 6 for p24 assay.

Quantitative PCR (Q-PCR) for HHV-7 and semiquantitative PCR (SQ-PCR) for HIV-1

The number of HHV-7 or HIV-1 viral genome equivalents in M+ cultures were measured by a specific Q-PCR assay and a SQ-PCR assay for each virus, respectively. For quantitation of HHV-7, total cellular DNA was extracted by crude digestion with protease K buffer (0.1 mg/ml protease K, 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.5% Tween 20, 0.5% Nonidet P-40) as previously described (25). For the quantitation of the viral genomes, a fixed amount (100 genome equivalents) of a plasmid carrying homologous sequences flanked by the HHV-7-specific PCR primer binding sequences was used as an internal standard in each reaction tube (25). An external standard curve was generated by co-amplifying serial dilutions of a plasmid (positive control) carrying the wild-type sequence of HHV-7 (25) together with a fixed amount of the respective internal nonhomologous template. This technique allows for normalization of the results, independently from the variability of the efficiency of amplification in each tube, and to verify immediately the possible presence of Taq polymerase inhibitors. The conditions of reaction for HHV-7 DNA quantification have been described (25). For quantification of HIV-1, DNA was extracted and PCR amplification was performed as described (26, 27). Briefly, HIV-1 was amplified from total DNA by using a pair of primers flanking the region spanning the 5’ long terminal repeat and the gag region of HIV-1. The number of copies of viral HIV-1 genome was calculated based on the standard curve generated with serial dilutions of a control plasmid containing the full-length genome of HIV-1. The conditions of reaction for HIV-1 DNA quantification were as follows: denaturation at 92°C for 1 min, annealing and elongation at 65°C for 2 min (for 30 cycles). The amplification products were transferred onto nitrocellulose filters, hybridized with labeled specific probes and densitometrically quantified. The absolute quantities of genome equivalents contained in each test sample were interpolated from the standard curve after quantification with a PhosphorImager (Molecular Dynamics).

Results

Lack of productive infection of human monocary-derived macrophages by HHV-7

To evaluate the ability of HHV-7 to replicate in cells of the mononuclear phagocytic system, adherent cultures of monocary-derived M+ were exposed to HHV-7 at the MOI of 5 for 1 h at 37°C, extensively washed with PBS, and recultured with GM-CSF. HHV-7 infection was assessed by indirect IFA and Q-PCR. No detectable viral Ag expression was seen at any time after infection (data not shown). Similarly, by Q-PCR, less than 10^2 HIV-7 genome equivalents per 20 cells was measured at day 6 postinfection, with no increase at day 12 (data not shown). These results demonstrate that HHV-7 fails to replicate in primary monocary-derived M+, most likely as a consequence of a block at the level of virus entry.

Interference with HIV-1 infection of primary macrophages by HHV-7

Adherent monocary-derived M+ were exposed to HHV-7 at the MOI of 5 for 1 h before infection with HIV-1 and the extracellular release of the HIV-1 p24 Ag was monitored at days 7, 10, and 13 postinfection. As illustrated in Figure 1, the release of p24 was significantly inhibited in cultures pre-exposed to either live HHV-7 or UV-inactivated HHV-7, compared with mock-treated cultures. The average percent inhibition in M+ from five different donors pre-exposed to live HHV-7 was 91.7 ± 8.3, 91.5 ± 1.4, and 85.9 ± 4.7% at days 7, 10, and 13 postinfection, respectively. For UV-inactivated HHV-7, the average percent inhibition was 91.8 ± 8.2, 80.4 ± 3.63, and 81.8 ± 2.2% at days 7, 10, and 13, respectively.

MOI-dependent inhibition of HIV-1p24 infection by FHV-7 in primary human macrophages

Terminal differentiated adherent monocary-derived M+ were exposed to different amounts of HHV-7 for 1 h before infection with HIV-1, and extracellular p24 release was monitored at days 7, 10, and 13 postinfection. As seen in Figure 2, p24 release was inhibited by pre-exposure of M+ cultures to HHV-7 at high MOI. At an HHV-7 MOI of 5, 2.5, and 1.25, inhibition was 93.5, 83.7, and 55.0%, respectively, at day 7; 98.0, 94.7, and 86.8%, respectively, at day 10; and 99.0, 98.6, and 91.8%, respectively, on day 13 postinfection. Inhibition of HIV-1 was progressively less efficient with the decrease of the MOI of HHV-7. At an HHV-7 MOI of 0.6, 0.3, and 0.15, p24 inhibition was 49.2, 63.4, and 24.2%, respectively, at day 7; 77.4, 28.8, and 0%, respectively, at day 10; and 79.3, 46.1, and 0%, respectively, at day 13.
Inhibition of proviral synthesis by HHV-7

To investigate at which level infection by HIV-1 was blocked, the number of HIV-1 proviral genome equivalents was measured by SQ-PCR in Mø infected with HIV-1, with or without pretreatment with HHV-7. Adherent Mø were exposed to HHV-7 for 1 h at 4°C, then infected with HIV-1 for 1 h at 37°C. The cells were subsequently washed four times with PBS and recultured with GM-CSF. After 1, 5, 11, 24, or 96 h, the cells were scraped from the flasks, washed in PBS, pelleted, dried, and stored at -80°C until PCR analysis. In HIV-1-infected Mø with no HHV-7 pretreatment, the HIV-1 proviral DNA, as measured by SQ-PCR, increased progressively in the early hours after exposure to the virus. At 1, 5, 11, 24, and 96 h after infection, the number of proviral DNA copies per 10⁵ cells was 1, 30, 400, 1000, and above 10,000, respectively (Fig. 3). In contrast, in Mø pretreated with HHV-7 before infection with HIV-1, the HIV-1 DNA was undetectable or close to background levels for the entire time period.

Lack of inhibition of the proliferative response of human PBMC by pre-exposure to HHV-7

To elucidate whether the HIV-1-inhibitory activity of HHV-7 was related to a negative effect on cellular proliferation, we studied the proliferative responses of human PBMC exposed to HHV-7 for 1 h before mitogen stimulation in vitro. The amount of HHV-7 used in these experiments (MOI = 5) was the same as the highest MOI used in HIV-1 inhibition studies. As illustrated in Table I, PBMC from two different donors, pre-exposed to HHV-7, maintained their ability to proliferate following stimulation with either OKT3 or PHA, both in the presence and in the absence of exogenous IL-2. Low level stimulation was observed in unstimulated PBMC from one donor treated with HHV-7, compared with mock-treated cells. This effect may be due to the presence of residual exogenous IL-2 in the viral inoculum. The highest level of inhibition (37.8%) was observed in PBMC from one donor (donor 2) stimulated with OKT3 and IL-2. In the other donor, however, the proliferation of PBMC stimulated with OKT3 and IL-2 was inhibited by only 11.0%. These levels of inhibition of cellular proliferation are low when compared with the near-total inhibition of HIV-1 infection observed after pretreatment of CD4⁺ T cells or Mø with HHV-7 at the same MOI.

Failure of HHV-7 to interfere with pre-established HIV-1 infection of primary macrophages and CD4⁺ T cells

To elucidate whether HHV-7 exerts its inhibitory effect on HIV-1 before or after the entry of HIV-1 into the cells, p24 release was measured in Mø with pre-established HIV-1 infection and subsequently exposed to HHV-7. In cultures from three different donors, the extracellular release of HIV-1 p24 after HHV-7 treatment was not markedly different compared with control, untreated cultures (Fig. 4).

Additionally, we studied the effects of HHV-7 treatment after the entry of HIV-1 into purified peripheral blood CD4⁺ T cells pretreated in vitro with PHA. Purified CD4⁺ T cells from three different donors were exposed first to HIV-1 and 1 h later to HHV-7 at the MOI of 5 or 0.5. Treatment with HHV-7 at either MOI caused no significant reduction of the p24 release at days 2 and 4 (Fig. 5). At the MOI of 0.5 there was even a slight enhancement of HIV-1 Ag production (24% higher than the control) at day 4 postinfection. The highest degree of inhibition (44%) was observed at day 6 in cells infected with HHV-7 at the highest MOI, most likely reflecting the dramatic cytopathic effect induced by active HHV-7 replication in CD4⁺ T cell cultures.

Discussion

Infection with HHV-7, the second discovered T lymphotropic human herpesvirus, has a very high prevalence in the human population and has not been associated thus far to any disease in adults. We recently found that HHV-7 is the only other virus known, besides the human and simian immunodeficiency retroviruses, to use the CD4 glycoprotein as a major cellular membrane receptor (4). Indeed, a dramatic reciprocal interference was documented between the two viruses in CD4⁺ T cells (4). These observations raised the question as to whether an analogous interference may occur in mononuclear phagocytes, the second major target cells for HIV-1 infection. Along with CD4⁺ T lymphocytes, the cells of the...
FIGURE 3. SQ-PCR for HIV-1 DNA in macrophages infected with HIV-1BaL with or without pretreatment by HHV-7. **Upper panel,** Bars represent HIV-1 DNA intensity quantitated by Phospholmager analysis from the amplified product shown in the bottom part of the figure. **Bottom panel,** DNA was extracted from 10⁶ cells at the hours indicated and amplified for the presence of HIV-1 by using primers spanning the gag region of the HIV-1 genome. Quantitation of HIV-1 DNA during PCR amplification was obtained by comparison with a standard curve of serial dilutions of pHXB2 (Rip7) plasmid DNA shown in the right part of the figure.

Table 1. *Effect of pre-exposure to HHV-7 on the incorporation of [³H]Tdr by stimulated human PBMC*

<table>
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<th>Stimuli Used</th>
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<th>PHA + IL-2</th>
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<td>66,024</td>
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In this report, we demonstrate that infection of human Mφ by HIV-1 is dramatically inhibited by pre-exposure to HHV-7. Both live and UV-inactivated HHV-7 induce this effect, suggesting that active HHV-7 infection is not required for interference with HIV-1. Moreover, after pretreatment of Mφ with HHV-7 and subsequent exposure to HIV-1, SQ-PCR documented the lack of HIV-1 proviral DNA synthesis in the early hours after HIV-1 infection. This finding demonstrates that the inhibition of HIV-1 infection by HHV-7 occurs at a very early stage, which is consistent with blocking at the level of virus attachment to the cells. Furthermore, titration experiments show that the inhibition of HIV-1 infection by pre-exposure to HHV-7 is MOI dependent. Theoretically, the possibility that additional mechanisms, such as negative signaling mediated through binding of HHV-7 to CD4 (32) or to other membrane components, may contribute to the observed HIV-1 suppression by HHV-7, cannot be completely ruled out. However, this hypothesis is unlikely in light of the results obtained by treating with HHV-7 cells with pre-established HIV-1 infection. For example, in Mφ previously infected with HIV-1, the extracellular release of HIV-1 Ag was not significantly inhibited by subsequent exposure to HHV-7. Similar results were obtained in CD4+ T cells pre-exposed to HIV-1 and immediately thereafter treated with HHV-7. We demonstrated that mitogen-induced proliferation of human PBMC was only marginally inhibited by pre-exposure to HHV-7, indicating that the HIV-1 inhibition was not...
due to a major suppressive effect of HHV-7 on cellular proliferation. Altogether, these findings suggest that HHV-7 blocks the early interaction of HIV-1 with susceptible cells, yet cannot block the expression of HIV-1 after it enters the cells.

Our data indicate that productive infection of Mφ by HHV-7 does not occur, at least in vitro. The inability of HHV-7 to infect Mφ, despite their expression of CD4, suggests that other receptors may be required for viral entry, as previously seen in experiments demonstrating the lack of HHV-7 productive infection in epithelial cells (HeLa) expressing human CD4 (4). Nevertheless, experiments with UV-inactivated HHV-7 demonstrated that productive infection by HHV-7 is unnecessary for inhibiting HIV-1 infection. The results of this study suggest that strategies to exploit the ability of HHV-7 to block HIV-1 infection of both CD4+ T cells and Mφ could be devised. We are currently trying to identify and clone the protein of HHV-7 that binds to CD4. This protein or parts of it can be used to directly block the entry of HIV-1 into CD4+ cells, or be incorporated into a delivery system to transfer specific antiviral genes or compounds selectively to CD4+ T cells and mononuclear phagocytes.

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References