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# Human Herpesvirus 6 Inhibits Human Immunodeficiency Virus Type 1 Replication in Cell Culture

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**The SF strain of human herpesvirus 6 ($HHV\text{-}6_{SF}$) isolated from the saliva of a human immunodeficiency virus (HIV)-infected individual was shown to inhibit HIV type 1 (HIV-1) replication in both peripheral blood mononuclear cells and purified $CD4^+$ lymphocytes. This suppression of HIV-1 replication led to decreased cytopathic effects of HIV-1 and prolonged survival of $CD4^+$ cells in culture. Even low levels of HHV-6 added to peripheral blood mononuclear cells showed an inhibitory effect on HIV-1 replication. These results differ from those previously reported showing enhanced HIV-1 production following infection with another strain of HHV-6.**

We previously described the high prevalence of human herpesvirus 6 (HHV-6) in the saliva of healthy individuals (5). The prototype virus strain characterized, $HHV\text{-}6_{SF}$, has biologic and molecular features somewhat different from those of other reported HHV-6 strains, but like all other isolates, it preferentially grows in $CD4^+$ lymphocytes (5a). Both human immunodeficiency virus type 1 (HIV-1) and HHV-6 can be found in the same individual, but these viruses apparently use a different receptor on $CD4^+$ cells for infection (5a, 9). Other investigators have previously reported that coinfection of peripheral blood mononuclear cells (PMC) or established T-cell lines with both viruses leads to an enhanced replication of HIV-1 (8). This observation supports the finding that HHV-6 infection stimulates chloramphenicol acetyltransferase enzyme production when linked to the HIV-1 long terminal repeat (2, 8). We also studied the interaction of these two viruses in PMC and purified $CD4^+$ lymphocytes. Our results differ from those previously published, since we found that dual infection of these cultures leads to a marked decrease in HIV replication as well as an inhibitory effect of HIV-1 on HHV-6 expression.

For our studies, the PMC (6) and $CD4^+$ lymphocytes (11) were obtained from the blood of adult HIV-1-seronegative individuals whose PMC have not shown the presence of replicating HHV-6. The cells ($3 \times 10^6$) were cultured as described previously (6) in 2 ml of RPMI 1640 medium containing 5% heated (56°C, 30 min) fetal calf serum, 2 mM glutamine, 1% antibiotics (100 U of penicillin per ml, 100 μg of streptomycin per ml), and 5% interleukin-2 (Electronucleonics, Silver Springs, Md.). In the initial experiments, 0.1 ml of $HHV\text{-}6_{SF}$ (with a titer of $10^{6.4}$ infectious particles per ml) and 0.1 ml of $HIV\text{-}1_{SF33}$ (reverse transcriptase [RT] activity, $10^6$ cpm/ml) were added at the same time to the cultures. Control cultures received the same quantity of either virus alone. After 24 h, the cultures were centrifuged and the cells were suspended in fresh medium without the viruses. Every 3 days, the clarified supernatant was evaluated for HIV-1 by RT assays (1) and the cells were suspended in fresh medium. A portion of the cells was examined for HHV-6 by indirect immunofluorescence assays (IFA) as described previously (4, 5). In some studies, cells were also evaluated by IFA for HIV antigens with a monoclonal antibody to the viral core (4). Other experiments were conducted with the $HIV\text{-}1_{SF2}$ strain by using similar procedures. This virus differs from $HIV\text{-}1_{SF33}$ in that it is less cytopathic and does not grown as rapidly in cell culture (6).

The results indicated that $HHV\text{-}6_{SF}$ infection inhibits HIV-1 replication. In the first experiments, after 1 week $HIV\text{-}1_{SF33}$ had an RT activity of 19,800 cpm/ml in the HHV-6 coinfected cells compared with over $10^6$ cpm/ml in the culture containing the HIV-1 strain alone. During this observation period, HHV-6-staining cells increased from 5 to 100%, with no substantial cell death in the cultures, as measured by trypan blue dye exclusion and viable cell counts. Moreover, by 11 days, HIV-1 was no longer recoverable from the supernatant of the dually infected cultures, as measured by inoculation of the fluid onto fresh PMC cultures. Three other experiments conducted in the same manner gave comparable results.

Similar observations were made as well when $3 \times 10^6$ $CD4^+$ lymphocytes purified by panning (11) were dually infected with the herpesvirus and the $HIV\text{-}1_{SF33}$ or $HIV\text{-}1_{SF2}$ strains (Table 1). Inhibition of the more rapidly growing and more cytopathic $HIV\text{-}1_{SF33}$ strain was noted somewhat sooner than that of the $HIV\text{-}1_{SF2}$ strain. Substantial reduction in replication of both HIV-1 strains in culture occurred within 4 to 7 days after infection. Moreover, HIV-1 infection also decreased the replication of HHV-6. In the dually infected cultures, the herpesvirus antigen-positive cells increased at a slower rate, reaching 100% on day 11, compared with day 7 in the $HHV\text{-}6_{SF}$-infected cultures alone (Table 1). By day 11, the cell viabilities of both infected cultures had decreased to 60 to 79% of the uninfected controls, with the exception of $HIV\text{-}1_{SF2}$-infected cultures, which remained at 93%. These results reflect the known cytopathic properties of HHV-6 and $HIV\text{-}1_{SF33}$.

Flow cytometry studies (7) with the Leu3a and Leu2a monoclonal antibodies to CD4 and CD8 cell markers (Becton Dickinson and Co., Mountain View, Calif.) were conducted on $CD4^+$ cell populations infected by each virus alone and those dually infected to note any effect on cell subset survival. The relative number of $CD4^-/CD8^-$ cells increased

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TABLE 1. Effect of HHV-6 on HIV-1 replication in $CD4^+$ lymphocytes

| Virus | Measure of virus replication at[a]: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 4 | | Day 7 | | Day 11 | | | | | |
| | HIV RT | HHV-6 Ag | HIV RT | HHV-6 Ag | HIV RT | HHV-6 Ag | Viability | $CD4^+$ | $CD8^+$ | $CD4^-/CD8^-$ |
| $HHV\text{-}6_{SF}$ | — | — | — | 100 | — | 100 | 73 | 94 | 5 | — |
| $HIV\text{-}1_{SF2}$ | 0.8 | — | 104.9 | — | 206 | — | 93 | 68 | 4 | 27 |
| $HIV\text{-}1_{SF2}$ + $HHV\text{-}6_{SF}$ | 1.6 | 1 | 3.5 | 25 | 1.9 | 100 | 79 | 97 | 2 | 1 |
| $HIV\text{-}1_{SF33}$ | 32.6 | — | 183.3 | — | 148 | — | 60 | 57 | 14 | 29 |
| $HIV\text{-}1_{SF33}$ + $HHV\text{-}6_{SF}$ | 14.5 | — | 30.5 | 50 | 20.2 | 100 | 64 | 87 | 3 | 10 |
| None | — | — | — | — | — | — | 95 | 98 | 2 | — |

[a] Values indicate the following: RT, RT activity ($10^4$ cpm/ml); Ag, HHV-6 antigen expression (percentage of cells), as detected by IFA (see text); viability, percentage of cells, as measured by trypan blue dye exclusion. T-cell subsets ($CD4^+$, $CD8^+$, and $CD4^-/CD8^-$) were assessed by flow cytometry analysis (7) and expressed as the percentage of total cells examined. A representative experiment of three independent studies is presented. —, None detected.

in the HIV-1-infected cultures, most likely reflecting $CD4^+$ cell death and/or down modulation of the CD4 antigen in HIV-1-infected cells (3). The absence or low levels of these $CD4^-/CD8^-$ cells in the dually infected cultures and the continued presence of high numbers of $CD4^+$ cells supports the lack of substantial HIV-1 replication and cytopathology in these cultures.

We next examined whether low input levels of HHV-6 would have a similar effect on HIV replication. High titers ($10^6$ cpm/ml) of $HIV\text{-}1_{SF2}$ and $HIV\text{-}1_{SF33}$ (0.1 ml) were inoculated onto PMC. At the same time, 0.1 ml of $HHV\text{-}6_{SF}$ undiluted or diluted 10- and 100-fold (from a stock of $10^{6.4}$ infectious particles per ml) was added to separate cultures (Table 2). The initial low level antigen detected at 5 days in the undiluted HHV-6-infected control cultures most likely reflects defective interfering particles (5a). The results again demonstrated that HHV-6 infection had a dramatic inhibiting effect on HIV-1 replication. Even when very limited HHV-6 infection was noted (day 5), with 1% of the cells staining for HHV-6 antigen, the reduction in RT activity was evident (Table 2). The same observation was made after 1 week of culture. At both time periods, viable cell counts were similar for both infected and uninfected cells (>95% viable). Moreover, as observed with the $CD4^+$ cells (Table 1), $HIV\text{-}1_{SF33}$ had some inhibitory effect on HHV-6 replication. In three independent studies, this inhibitory effect of the two virus types on each other was noted to varying degrees. The reduction in RT activity was not always linear (Table 2), but the reduction in HIV-1 replication was evident throughout. Moreover, the effect of HIV-1 on HHV-6 expression was not consistently noted with the $HIV\text{-}1_{SF2}$ strain.

TABLE 2. Effect of varying quantities of $HHV\text{-}6_{SF}$ on HIV-1 replication

| Virus | Virus replication at[a]: | | | |
|---|---|---|---|---|
| | Day 5 | | Day 7 | |
| | Ag[b] | RT[c] | Ag | RT |
| $HHV\text{-}6_{SF}$ | | | | |
| $10^0$ | 8 | | 80 | |
| $10^{-1}$ | 20 | | 30 | |
| $HIV\text{-}1_{SF2}$ | — | 88 | — | 436.9 |
| +$HHV\text{-}6_{SF}$ ($10^0$) | 8 | 34 | 75 | 74.5 |
| +$HHV\text{-}6_{SF}$ ($10^{-1}$) | 1 | 12.8 | 30 | 83.1 |
| +$HHV\text{-}6_{SF}$ ($10^{-2}$) | 1 | 13.4 | 10 | 234.2 |
| $HIV\text{-}1_{SF33}$ | — | 311.4 | — | 474.6 |
| +$HHV\text{-}6_{SF}$ ($10^0$) | 5 | 99.0 | 80 | 56.2 |
| +$HHV\text{-}6_{SF}$ ($10^{-1}$) | 1 | 166.4 | <1 | 276.3 |
| +$HHV\text{-}6_{SF}$ ($10^{-2}$) | 1 | 161.1 | 1 | 126.4 |

[a] Procedures are described in text. —, Not detected. A representative experiment of three independent studies is presented.

[b] Values indicate the percentage of PMC staining for HHV-6 antigen by IFA (4).

[c] Values indicate HIV-1 RT activity ($10^3$ cpm/ml) in the culture supernatant (1).

This inhibition of HIV-1 replication by $HHV\text{-}6_{SF}$, as well as the effect of HIV-1 on $HHV\text{-}6_{SF}$ expression, was further examined in timed experiments in which one virus was added to PMC 24 h earlier than the other. In these studies, a dramatic reduction in HIV-1 replication was found in the PMC cultures previously inoculated with $HHV\text{-}6_{SF}$. Similarily, $HHV\text{-}6_{SF}$ expression, as demonstrated by antigen production, was substantially inhibited by prior infection of the PMC with $HIV\text{-}1_{SF33}$ (Fig. 1). These observations were made when no substantial decrease in cell viability was noted by trypan blue dye exclusion.

These results in cell culture contrast with those previously reported for HHV-6 (8). Our data indicate that $HHV\text{-}6_{SF}$ infection inhibits HIV-1 replication in PMC and purified $CD4^+$ lymphocytes before any evidence of viral cytotoxicity. The reason for the discrepancy between our results and those of others in which enhanced HIV-1 replication was noted is not clear. It may be related to the HIV-1 or HHV-6 strains used. $HHV\text{-}6_{SF}$ does differ biologically from the other HHV-6 strains described elsewhere (5a). Our results suggest, however, that in contrast to observations made with previous biologic and molecular studies (2, 8), infection with this herpesvirus in vivo can lead to an inhibition of HIV-1 replication. Moreover, our findings indicate a difference in the interaction of this herpesvirus with HIV-1 compared with those of other herpesviruses, such as Epstein-Barr virus, in which enhancement of HIV-1 replication appears to take place (10).

The mechanism for this viral inhibition is not known. Because HHV-6 antigen can be found in more than 80% of the cells at the time that some HIV-1 replication can be detected, we assume the inhibition occurs intracellularly. In addition, in some experiments before HIV-1 replication was completely suppressed, cells could be detected that were dually infected, although, as expected, the expression of HIV-1 antigens was reduced. Furthermore, these two viruses use different cellular receptors (5a, 9). Finally, the

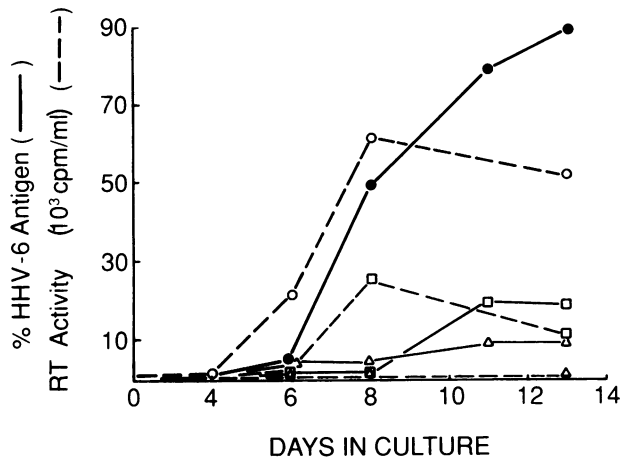


FIG. 1. Human PMC ($6 \times 10^6$ cells in 2 ml of medium) were inoculated with $HHV\text{-}6_{SF}$ ($10^5$ infectious particles) or $HIV\text{-}1_{SF33}$ ($10^5$ cpm). After 24 h, the cells were washed and split into duplicate cultures. One $HHV\text{-}6_{SF}$ culture was superinfected with $HIV\text{-}1_{SF33}$; one $HIV\text{-}1_{SF33}$ culture was superinfected with HHV-6. Every 2 to 3 days, the four cultures were centrifuged and a portion of the cells was examined for HHV-6 antigens by IFA (5); the culture fluid was assayed for particle-associated RT activity (1). The data from the single- and dually infected cultures are given. The superinfecting virus, when inoculated onto fresh PMC, gave curves similar to those shown for the cultures receiving the viruses at time zero. Symbols: ●, $HHV\text{-}6_{SF}$ alone; ○, $HIV\text{-}1_{SF33}$ alone; △, $HHV\text{-}6_{SF}$ followed by $HIV\text{-}1_{SF33}$ 24 h later; □, $HIV\text{-}1_{SF33}$ followed by $HHV\text{-}6_{SF}$ 24 h later. A representative experiment from two independent studies is presented.

timed experiments indicate that infection with one virus can inhibit replication of the other virus. It is conceivable, however, that dual infection is not needed and an inhibitory cytokine or some cell surface phenomenon blocks the virus spread and replication. In any case, the results demonstrate that the presence of both viruses is inhibitory in culture and this observation should be considered when culturing fluids or cells for HIV-1. The sensitivity for HIV detection could be decreased if HHV-6 is present in the sample.

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