Transactivation of the Human Immunodeficiency Virus Promoter by Human Herpesvirus 6 (HHV-6) Strains GS and 2-29 in Primary Human T Lymphocytes and Identification of Transactivating HHV-6(GS) Gene Fragments

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Transactivation of the Human Immunodeficiency Virus Promoter by Human Herpesvirus 6 (HHV-6) Strains GS and Z-29 in Primary Human T Lymphocytes and Identification of Transactivating HHV-6(GS) Gene Fragments

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Human herpesvirus 6 (HHV-6) can activate the human immunodeficiency virus (HIV) promoter and accelerate cytopathic effects in HIV-infected human T cells. This study examines the regions of the HIV promoter required for HHV-6 transactivation in a heterogeneous population of primary human T lymphocytes with or without antigenic stimulation. Two different strains of HHV-6, GS and Z29, transactivated the HIV promoter. The GS strain transactivated the promoter in both stimulated and resting T cells, while the Z29 strain increased HIV promoter activity only in stimulated T cells. Three DNA clones containing HHV-6(GS) genomic fragments transactivated the HIV promoter in cotransfected T cells. A 21.4-kb DNA clone, pZVB70, showed the highest transactivating ability, while two other DNA fragments, pZVB10 (6.2 kb) and pZVH14 (8.7 kb), showed lower activity. One of these clones, pZVH14, activated the HIV promoter construct containing a mutation in the NfκB site. However, this mutated NfκB promoter was not transactivated during HIV-6(GS) infection or after cotransfection with pZVB70 or pZVB10. These data indicate that the NfκB sites of the HIV promoter are essential for its transactivation during HHV-6(GS) infection. By increasing HIV promoter activity in primary T lymphocytes, HHV-6 may consequently increase HIV replication, leading to an increase in the cytopathic effect on infected human T cells.
different cloned HHV-6(GS) DNA fragments that transactivate the HIV promoter after cotransfection into human T cells.

MATERIALS AND METHODS

DNA and plasmids. The HIV chloramphenical acetyltransferase (CAT) plasmid has been described in detail previously (19). Briefly, the construct contains both the 3' untranslated region and the repeat sequences of the HIV LTR aligned with the CAT gene. The -147 CAT plasmid was constructed by deleting a fragment of the HIV LTR at the Avai site as described elsewhere (13). The -57 CAT plasmid was kindly provided by Joseph Sodroski (Harvard Medical School, Boston, Mass.) (33). The HIV LTR plasmid containing the mutated NFkB site was kindly provided by Gary Nabel (27). BamHI plasmid libraries of the HHV-6 genome were generated as described previously (17).

HHV-6 viral strains and preparation. The human T-cell lines HSB-2 (ATCC CCL120.1 CCRF-HSB-2) and Molt-3 (ATCC CRL 1552) were grown in RPMI 1640 medium supplemented with fetal bovine serum and antibiotics. Two strains of HHV-6 were used in this study. HHV-6(GS) was a gift from R. C. Gallo, National Cancer Institute, Bethesda, Md.; HHV-6(Z29) was a gift from P. Pellet, Centers for Disease Control, Atlanta, Ga. HHV-6(GS) was grown in HSB-2 cells, and HHV-6(Z29) was grown in Molt-3 cells. Infected cells were collected 6 to 8 days postinfection when more than 80% of the cells were positive for viral antigens in an indirect immunofluorescence assay (IFA) by using HHV-6 specific monoclonal antibodies (3). These infected cells were frozen and thawed twice, and the 50% tissue culture infectivity dose was determined by procedures described previously (3, 41) on HSB-2 cells for HHV-6(GS) and Molt-3 cells for HHV-6(Z29).

Human T lymphocytes. Fresh mononuclear cells were isolated from the peripheral blood of purified protein derivative (PPD)-immune, normal human donors by using Hypaque-Ficoll (13). These cells (10^6 per well) were then stimulated for 6 days in RPMI with tuberculin PPD (5 U/ml) and then treated with dimethyl sulfoxide (10%) for exactly 1 min. After washing, the cells were placed in fresh RPMI 1640 medium containing 10% human serum. A portion of the transfected T cells was then infected with HHV-6(GS) or HHV-6(Z29) at a multiplicity of infection of 0.01 by incubating the T-cell pellet for 1 h at 37°C with cell lysates of HHV-6(GS) or HHV-6(Z29). In certain experiments, HHV-6(GS) DNA clones were cotransfected with the HIV promoter constructs into primary T lymphoblasts or the T-cell line HSB-2 for 3 h at 37°C. The cells were then treated with dimethyl sulfoxide (10%) for exactly 1 min. After washing, the cells were placed in fresh RPMI 1640 medium containing 10% human serum. A portion of the transfected T cells was then infected with HHV-6(GS) or HHV-6(Z29) at a multiplicity of infection of 0.01 by incubating the T-cell pellet for 1 h at 37°C with cell lysates of HHV-6(GS) or HHV-6(Z29). In certain experiments, HHV-6(GS) DNA clones were cotransfected with the HIV promoter constructs into primary T lymphoblasts or the T-cell line HSB-2. The transfected and/or infected T cells (2 × 10^6 per well) were then added to either irradiated, adherent, autologous mononuclear cells (10^6 per well) alone or cells that had been incubated overnight with tuberculin PPD (5 µl/ml) in 96-well flat-bottom plates as described earlier (13). These cell populations are referred to as resting transfected T cells or stimulated transfected T cells, respectively. Five days later, the transfected T cells were collected, washed twice with phosphate-buffered saline, counted, and used in CAT assays. Since a background level of CAT activity is observed in fresh T-cell blasts as a result of antigenic stimulation, cells were collected 5 days after transfection in order to lower this background level of CAT activity.

CAT assays. Transfected T cells, collected after 5 days, were resuspended in 100 μl of sonication buffer (0.25 M Tris HCl, pH 7.4). The cell number was adjusted to 2 × 10^5/100 μl so that the same number of cells was used in each CAT assay. Cellular extracts were prepared by a single freeze (−70°C)-thaw (37°C) cycle and 1 min of sonication, followed by centrifugation to remove cell debris. CAT assays using [14C]chloramphenicol and thin-layer chromatography have been described in detail previously (12). CAT assays using [3H]acetylpropionate and thin-layer chromatography have been described by Neuman et al. (30). Briefly, 50 μl of the cell lysates, [3H]acetyl-CoA, and chloramphenicol was mixed in 100 mM Tris HCl, pH 8.0. An organic scintillation cocktail (Econofluor; Hewlett-Packard) was layered over this mixture, and the samples were incubated at 37°C. The samples were counted in a beta scintillation counter at hourly intervals after initiation of the assay. Units of CAT activity in each cell lysate were calculated according to a standard curve established with a CAT sample (Pharmacia) of known enzymatic activity.

RESULTS

HHV-6(GS) transactivates the HIV LTR promoter in primary human T lymphoblasts. The primary T lymphoblasts used in this study have been described previously (13) and consist of approximately 85% CD3+, 59% CD4+, and 32% CD8+ cells. These lymphoblasts were transfected with the HIV CAT construct, containing the HIV promoter, by using DEAE-dextran. After transfection, a portion of the T cells was infected with HHV-6(GS) and a portion with HHV-6(Z29), and the samples were incubated at 37°C. The cells were collected 5 days after transfection with HHV-6(GS), 30 to 40% of the cells reacted with HHV-6-specific monoclonal antibodies (3) by IFA. This result indicated that the transfected cells were productively infected by HHV-6(GS). None of the uninfected lymphocytes reacted with HHV-6-specific monoclonal antibodies, indicating the absence of endogenous HHV-6 virus. After 5 days in culture, lysates were prepared from the transfected cells and CAT activity was measured (Fig. 1). Lysates from transfected T cells without antigen (PPD) stimulation and without HHV-6 infection converted 79% of the chloramphenicol substrate to its acetylated forms, as shown by thin-layer chromatography (Fig. 1A). This lysate contained a total of 0.12 U of CAT activity (Fig. 1B and Table 1). Transfection of T-cell blasts activated with antigen showed higher CAT activity than did unstimulated T cells. Lysates from these cells converted 79% of the chloramphenicol to its acetylated forms and contained a total of 0.46 U of CAT activity (Fig. 1C). This represents a fourfold increase in CAT activity after antigenic stimulation (Table 1). These experiments confirm our previous studies, which showed that antigen activation of primary T cells can activate the HIV promoter (13).

Infection of these transfected T cells with HHV-6(GS) resulted in transactivation of the HIV promoter in both resting and stimulated T cells (Fig. 1A and C). When lysates from unstimulated, GS-infected T-cell lymphoblasts were tested for CAT activity, there was a conversion of 75% of the
substrate, corresponding to 0.64 U of CAT activity, while lysates from activated, infected T cells showed a 99% conversion of the substrate and 2.61 U of CAT activity (Fig. 1A and Table 1). These values represent 5- and 22-fold increases in CAT activity without and with antigenic stimulation, respectively. Since CAT assays with $[^{3}H]$acetyl-CoA are more quantitative and convenient (30), subsequent cell lysates were all measured by this rapid method.

These data show that infection of primary T lymphoblasts with HHV-6(GS) activates the HIV promoter in both resting and stimulated primary T cells. Although the activation in resting cells is not as pronounced (5- to 7-fold) as in antigen-activated T cells (22- to 25-fold), the data clearly demonstrate that HHV-6(GS) can infect unstimulated primary T cells and will activate the HIV promoter, which otherwise remains quiescent. Although activation of resting, primary T cells by HHV-6 may have also contributed to the low level of transactivation of the HIV promoter, this alone cannot account for all of the transactivation activity observed.

**Transactivation of the HIV promoter in antigen-activated primary human T lymphocytes by HHV-6(Z29).** Soon after the GS strain of HHV-6 was identified, a new variant strain of HHV-6 was identified which was designated HHV-6(Z29) (5). HHV-6(Z29) is related to the GS strain both antigenically and genetically (17, 25, 41). Both strains infect human T lymphocytes but seem to have a preference for distinct subsets. These subsets are still not clearly defined. The Z29 strain can effectively infect the Molt-3 T-cell line but not HSB-2, while the GS strain infects HSB-2 but not Molt-3. Both HHV-6 strains can infect primary human phytohemagglutinin-stimulated blasts, but HHV-6(Z29) failed to replicate in unstimulated peripheral blood lymphocytes (41). In this study, primary T lymphoblasts specific for the antigen tuberculin PPD were transfected with the HIV CAT construct and then infected with HHV-6(Z29). After 6 days, the cells were collected and tested for infection with HHV-6(Z29) by IFA using cross-reactive HHV-6(GS) monoclonal antibodies (41). T lymphocytes stimulated with PPD during the HHV-6(Z29) infection had enlarged cells and were pos-

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**FIG. 1.** CAT enzymatic activity from primary T-cell lysates. (A) Thin-layer chromatography of $[^{14}C]$chloramphenicol reacted with cell lysates from stimulated T cells (PPD+) and resting T cells (PPD-) either infected with HHV-6 (+) or uninfected (−). (B) Standard curve of CAT enzymatic activity obtained by using a rapid method that utilizes $[^{3}H]$acetyl-CoA. This standard curve is used to calculate the amount of CAT activity in cell lysates. As little as 0.01 U of enzymatic activity can be detected per lysate. (C) CAT activity measured in T-cell lysates by the rapid method.

**TABLE 1. Evidence that the enhancer region of the HIV LTR is required for HHV-6(GS) transactivation**

<table>
<thead>
<tr>
<th>Promoter</th>
<th>CAT activity (total U in cell lysate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected cells</td>
</tr>
<tr>
<td></td>
<td>−</td>
</tr>
<tr>
<td>HIV CAT</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>-147 CAT</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>-57 CAT</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Mutant NFkB-CAT</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

* Various promoters were transfected into primary T cells by using DEAE-dextran. The transfected cells were either cultured directly with autologous, irradiated mononuclear cells or infected with HHV-6(GS) and then added to feeder cells. Antigen was then added to appropriate cultures. Cells were incubated for 5 days, collected, and lysed, and CAT activity was measured. Transfected cells were infected with HHV-6(GS) at multiplicity of infection of 0.01. Some cell cultures were given the antigen PPD (+), while others were given medium (−).

* Cells from at least two different donors were transfected with each promoter. Cells from each donor were transfected twice.

* Values in parentheses represent the increase in CAT activity based on the enzymatic activity found in noninfected, resting T cells.
and 0.011 for donor 4.

Total units of CAT activity in unstimulated T cells are 0.09 for donor 2 and 0.011 for donor 4. As noted above, in the absence of antigen stimulation showed no viral antigen-positive cells.

After 6 days of culture, transfected T lymphocytes were collected, washed, and lysed, and the CAT activity was measured from the cell lysates. The results showed that the HIV CAT promoter was activated only in HHV-6(Z29)-infected human T lymphocytes that had been stimulated with antigen. This was explained by the finding that productive infections of T lymphocytes with HHV-6(Z29), as determined by IFA with HHV-6-specific antibodies, occurred only in cells stimulated by antigen. Unstimulated cells did not support viral infection, nor did they demonstrate HIV CAT promoter activation (0.09 U of CAT activity in donor 2; 0.011 U of CAT activity in donor 4). As noted above, in primary T cells not infected with HHV-6(Z29), the HIV promoter was activated by antigenic stimulation (19-fold in donor 2, with 1.75 U of CAT activity; 140-fold in donor 4, with 1.54 U of CAT activity) (Fig. 2). When the transfected T cells were infected with HHV-6(Z29) in the absence of antigen, no increase in CAT activity was observed (0.97-fold in donor 2, with 0.087 U of CAT activity; 0.7-fold in donor 4, with 0.008 U of CAT activity). However, when the transfected T cells were infected with HHV-6(Z29) and activated with PPD, a 46- to 404-fold increase in total CAT activity and 3-fold increase in activity over antigenic stimulation alone were noted (4.18 U of total CAT activity in donor 2; 4.44 U of total CAT activity in donor 4). These data indicate that HHV-6(Z29) transactivated the HIV LTR in antigen-stimulated, primary, human T lymphocytes and that transactivation by HHV-6(Z29) requires productive infection of primary T lymphocytes.

**Transactivation of the HIV promoter in human T lymphocytes by HHV-6(GS)** depends on the presence of a functional NfκB enhancer site. Earlier studies on the activation of the HIV promoter in primary human T lymphocytes showed that activation of the HIV promoter was influenced by the NfκB enhancer region as well as an upstream region that exerts negative regulation (13). Several different constructs of the HIV promoter, described in Fig. 3, were transfected into PPD-specific human T lymphoblasts. The HIV promoter designated -57 CAT contains one of the Sp1 sites as well as the TATA site. This promoter has been shown to be active in other cell types (COS-7; data not shown), but little or no promoter activity has ever been observed in primary T lymphocytes (13) (Table 1). The promoter designated -147 CAT retained the NfκB binding region as well as the three Sp1 sites and the TATA box (Fig. 3). This promoter does not have the negative regulatory region, and therefore the CAT activity expressed after transfection into primary T lymphoblasts is somewhat higher than with the intact promoter (Table 1). T lymphoblasts transfected with -147 CAT and then infected with HHV-6(GS) consistently showed evidence of transactivation (9- to 17-fold increase in CAT activity). This finding indicates that the negative regulatory region is not primarily involved in the increased promoter activity due to HHV-6(GS) infection. These results also show that the DNA sequences responsible for HHV-6(GS) transactivation must be within the -147 to -57 region. To further locate the specific promoter region responsible for HHV-6 transactivation, the HIV LTR plasmid containing a mutated NfκB CAT promoter was transfected into primary T lymphocytes. This promoter contained two sequences...
TABLE 2. Evidence that HHV-6(GS) plasmids can transactivate the HIV promoter.

<table>
<thead>
<tr>
<th>T-cell donor</th>
<th>Antigenic stimulation</th>
<th>Plasmid</th>
<th>Antigenic stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>HIV-CAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-CAT + HHV-6(GS) plasmid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>1.8(45)</td>
<td>pZVB70</td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>0.26(2)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.15</td>
<td>1.95(13)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>1.24(12)</td>
<td>pZVH14</td>
</tr>
<tr>
<td>6</td>
<td>0.24</td>
<td>1.95 (8)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.19</td>
<td>1.46 (8)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.02</td>
<td>1.35 (68)</td>
<td>pZVB10</td>
</tr>
<tr>
<td>9</td>
<td>0.01</td>
<td>1.54 (154)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.02</td>
<td>1.7 (85)</td>
<td>pZVB9</td>
</tr>
<tr>
<td>11</td>
<td>0.38</td>
<td>1.91 (5)</td>
<td>pZVB15</td>
</tr>
<tr>
<td>12</td>
<td>0.01</td>
<td>1.54 (154)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.2</td>
<td>1.24 (12)</td>
<td>pZVB43</td>
</tr>
<tr>
<td>14</td>
<td>0.15</td>
<td>1.95 (13)</td>
<td>pZVH14</td>
</tr>
<tr>
<td>15</td>
<td>0.19</td>
<td>1.46 (8)</td>
<td></td>
</tr>
</tbody>
</table>

* Plasmids containing HHV-6(GS) DNA were cotransfected with HIV CAT into primary T cells. CAT activity was measured after 5 days from cell lysis. Antigen activation of transfected cells was as described for Table 1.

* See Table 1, footnote c.

within the NFkB sites that had been changed from GGG to CTC (Fig. 3), changes that in the NFkB site eliminate the activation of the HIV promoter by the NFkB protein (27). This mutation in the NFkB sites abolishes the HIV promoter activity induced in primary T lymphoblasts both by antigen and by HHV-6(GS) infection. Although no CAT activity could be detected in the lysates from these cells, IFA indicated that the population of T cells transfected with the mutant NFkB promoter and infected with HHV-6(GS) contained enlarged cells highly positive for HIV-6(GS) protein (data not shown). This result showed that the transfected T cells were productively infected with HHV-6(GS) and suggests that HHV-6(GS) requires the NFkB region for transactivation of the HIV promoter.

At least three different DNA segments cloned from the HHV-6(GS) genome can transactivate the HIV promoter when cotransfected into primary T lymphocytes. The HHV-6(GS) genome was digested with the restriction enzyme BamHI or HindIII, and the resulting fragments were ligated into the Bluescript vector (17). Six different HHV-6(GS) DNA fragments representing about 40% of the total viral genome and varying in length from 3.3 to 21.4 kb (17) were used in these studies. Each HHV-6(GS) fragment was cotransfected with HIV CAT into the primary T-cell populations, and CAT activity was measured in cell lysates 5 days later. The largest fragment, pZVB70 (21.4 kb), showed a dramatic transactivating effect on the HIV promoter (Table 2). In the absence of antigen, this fragment increased HIV promoter activity 26 to 101 times over the activity expressed in unstimulated T cells. When the cells were stimulated with antigen, there was a 77- to 271-fold increase in total CAT activity, which was 6- to 42-fold over the antigenic stimulation alone. It was the major DNA fragment from HHV-6(GS) that showed this level of activity, and so it was designated a high transactivator of the HIV promoter.

Two other HHV-6(GS) fragments, designated pZVH14 and pZVB10, marginally transactivated the HIV promoter (Table 2) when expressed in the absence of antigen stimulation. The DNA clone pZVH14 contained a 8.7-kb fragment from HHV-6(GS), while clone pZVB10 contained a 6.2-kb fragment. Since these HHV-6(GS) DNA clones showed only low-level activation of HIV CAT in the absence of antigen and promoter activity in antigen-stimulated cells increased only slightly, we have designated these clones low transactivators. The highest transactivation was observed when cotransfected T lymphocytes were stimulated with antigen, suggesting an additive effect of antigenic stimulation and the HHV-6(GS) gene product(s) on HIV promoter activity. Table 2 also shows data for three fragments cloned from HHV-6(GS) that failed to transactivate the HIV promoter. These DNA clones were designated pZVB9 (11.8-kb fragment), pZVB43 (8.3-kb fragment), and pZVB15 (3.3-kb fragment). From these results, it can be concluded that HHV-6 contains at least three different gene segments that can transactivate the HIV promoter.

To confirm the data observed in primary T lymphocytes, these fragments were also cotransfected with HIV CAT into the human T-cell line HSB-2 (Fig. 4A). As previously demonstrated (14), infection of the transfected HSB-2 cells with HHV-6(GS) resulted in an increase in CAT activity (threefold), indicating that the HIV promoter was also transactivated in a T-cell line. Cotransfection of HHV-6(GS) DNA clone pZVB70 and HIV CAT resulted in a dramatic increase in CAT activity (58-fold; Fig. 4A). Plasmid pZVB70 transactivated HIV CAT to a much greater extent than did HHV-6(GS). It was also observed that plasmid pZVH14, when cotransfected with HIV CAT into the HSB-2 T-cell line, activated promoter activity to a higher level than did HHV-6(GS).
for primary T cells, pZVB10 failed to activate the HIV
promoter in HSB-2 cells.

To determine whether mutations in the NFkB site of the
HIV promoter affected the transactivation by cloned HHV-
6(GS) DNA fragments, the three transactivating fragments
were cotransfected into primary T cells with the promoter
containing the NFkB mutation. The resulting promoter
activities are shown in Table 3. Mutation in the NFkB site
completely abolished the ability of the pZBV70 and pZVB10
clones to transactivate. However, with the pZVH14 clone, a
slight but consistent transactivation was observed (four-
to-sevenfold) that was less than the transactivation seen with
the intact HIV promoter. The transactivation of the mutated
NFkB promoter by pZVH14 was not dependent on antigenic
stimulation of the T cells, since it was not enhanced by the
presence of antigen. Similar results were observed when
these cloned DNA fragments were cotransfected with the
mutated NFkB promoter into HSB-2 cells (Fig. 4B). The two
DNA clones, pZVB70 and pZVB10, failed to transactivate
the mutated NFkB promoter, but pZVH14 showed slight
transactivation (8- to 10-fold). This finding confirmed the
results in primary T lymphocytes. Together, these data
suggest that the transactivating gene(s) included within the
pZVH14 clone may not totally depend on an intact NFkB site
but may also use other target DNA region(s) in the promoter.

**DISCUSSION**

Our studies demonstrate that two strains of HHV-6, GS
and Z29, can infect and activate the HIV promoter in
primary, human T lymphocytes. However, these two strains
of HHV-6 showed distinct differences in ability to transac-
tivate the HIV promoter, related to the ability of the two
strains to productively infect resting T lymphocytes. HHV-
6(GS) infected both stimulated and unstimulated human T
cells, while HHV-6(Z29) infected only stimulated T cells.
This observation confirms the results of Wyatt et al. (41),
who showed that HHV-6(Z29) failed to replicate in nonacti-
vated peripheral blood lymphocytes. HHV-6(Z29) was un-
able to infect resting T cells, and no transactivation of the
HIV promoter was observed. However, HHV-6(GS) trans-
activated the HIV promoter even in resting T cells. These
data suggest that active replication of HHV-6(Z29) and
subsequent production of its gene products are required for
transactivation of the HIV promoter. It is also possible that
HHV-6(Z29) requires essential cellular elements that are
provided by antigenic stimulation of T cells for its replica-
tion, and thus the activation of the HIV promoter by Z29
may require cellular cooperation. Ensoli et al. (8) have
previously shown that HHV-6(GS) infection of HIV-infected
T cells increases the steady-state level of HIV mRNA that
parallels CAT enzymatic activity. It is quite possible that
similar mechanisms are responsible for the increase in CAT
enzymatic activity observed here. Studies are currently
under way to examine the mechanism of action by the cloned
gene fragments.

Certain difficulties are inherent in these types of experi-
ments. We have observed differences in HIV promoter
activation between different donors and even from the same
donor when cells were donated at different times (6 months
later). Without HHV-6 infection or antigenic stimulation, the
background CAT activity ranged between 0.01 and 0.38 total
U of enzyme activity. Because the increase in promoter
activity is based on the background activity between donors
or experiments, this parameter cannot be used to compare
promoter activity but must be used to measure transactiva-
tion within a single experiment. This was not expected, since
test experiments require many steps of long incubation
periods. Because of the large number of peripheral blood
mononuclear cells needed for each experiment (>100 × 10⁶),
donors were only bled every 6 weeks. However, each
transfection was done at least twice on two different healthy
adult human donors. Therefore, when transactivation was
observed consistently in all experiments, we concluded that
there was activation of the HIV promoter in these situations.

A number of experiments were performed to identify the
region of the HIV promoter required for transactivation by
HHV-6(GS). In earlier studies, several deletions or muta-
tions in the HIV promoter were used to determine which
promoter regions were essential for control of the promoter
activity during primary in vitro T-cell stimulation (13). These
altered promoters were also used in this study, and the
results showed that the NFkB region was essential for
transactivation during HHV-6(GS) infection. Thus, an HIV
promoter containing mutated sequences within the NFkB
region was no longer activated by HHV-6(GS) infection. It
is not known whether HHV-6(GS) directly activates the NFkB
cellular protein or, alternatively, a HHV-6(GS) gene prod-
uct(s) binds directly to the NFkB region. However, these
results indicate that this particular promoter region seems
to be essential for HHV-6(GS) transactivation during an
active infection. Similar results were observed when the
human T-cell line HSB-2 was transfected with the mutated
NFkB promoter and then infected with HHV-6(GS). HHV-
6(GS) is not unique in using the NFkB site, since it has been
noted that other herpesviruses, such as herpes simplex virus
type 1, cytomegalovirus, and Epstein-Barr virus, also act on
the NFkB region of the HIV promoter (22).

The genome of HHV-6(GS) has been estimated to be
about 170 kb in length (17, 18), which should code for more
than 70 proteins. A number of DNA cloned fragments from
the GS genome were examined in this study to determine
whether they code for products that transactivate the HIV
promoter. Several HHV-6(GS) DNA clones, comprising
about 40% of the viral genome, were individually cotrans-
fected with HIV CAT into primary human T lymphocytes.
Three different non-cross-hybridizing DNA clones transac-
tivated the HIV promoter, and at present it is not known
whether other HHV-6 DNA fragments are also able to
transactivate the HIV promoter. The DNA clone showing
the highest level of transactivation (pZVB70) contained 21.4

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**TABLE 3. Transactivation of the mutant NFkB promoter with HHV-6(GS) DNA clones**

<table>
<thead>
<tr>
<th>T-cell donor</th>
<th>Antigen stimulation</th>
<th>Mutant NFkB</th>
<th>Mutant NFkB + HHV-6(GS) plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasmid</td>
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<tr>
<td>3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>pZVH14 0.07 (7)</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>pZVH70 0.04 (4)</td>
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<td>3</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>pZVH70 0.01</td>
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<tr>
<td>2</td>
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<td>&lt;0.01</td>
<td>pZVB10 0.01</td>
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<tr>
<td>4</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>pZVH14 0.01</td>
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- HHV-6(GS) DNA clones were cotransfected with the mutant NFkB promoter (see Fig. 3) into primary T cells. Cells were incubated for 5 days as
described for Table 1. Total CAT activity was measured in cell lysates.
- See Table 1, footnote c.
kb of HHV-6(GS) DNA. It is possible that more than one gene product expressed from this plasmid is responsible for the high level of transactivating ability of this plasmid. Since these fragments were not inserted into a eukaryotic expression vector, their expression requires endogenous promoter activity. It can thus be inferred from these data that at least some of the HHV-6 promoters must be active in both stimulated and unstimulated primary T cells. Two other plasmids containing HHV-6(GS) fragments also transactivated the HIV promoter in primary T cells. These plasmids were pZVH14, which contained an 8.7-kb viral fragment, and pZVB10, which contained a 6.2-kb fragment. To determine the nature of the open reading frames present in these fragments, the DNA was sequenced (data not shown). From this information, several genes have been identified and are currently being subcloned and tested for transactivating ability on the HIV promoter in human T cells.

Although the pZVH14 clone was less active than pZVB70, it transactivated HIV CAT in the T-cell line HSB-2 and in primary T cells. Of interest was the observation that this fragment, pZVH14, was unique in transactivating the mutant NFκB promoter even in the absence of antigenic stimulation. No such activity was seen after GS infection of T cells or transfection of primary T cells with the two transactivating DNA fragments from GS, pZVB70 and pZVB10. These data also suggest that the gene products expressed from pZVH14 are able to act on regions other than the NFκB site, independent of antigenic stimulation. This also implies that gene expression from this DNA fragment is possible even without overt cellular stimulation. Since transactivation of the mutant NFκB was not observed during an actual infection with HHV-6(GS), the gene(s) contained within the pZVH14 DNA fragment must be under some type of regulatory control. A recent study has shown that the pZVH14 genomic fragment also has oncogenic properties (32). At present it is not known whether the gene product(s) the induce transformation in NIH 3T3 cells is the same product(s) responsible for transactivation of the HIV promoter.

The immunodeficiency disease AIDS is a chronic disease caused by the human retrovirus HIV. The time from HIV infection to the clinical manifestation of AIDS can range from 2 months to more than a decade (2, 15, 16). An important determinant of the length of this latency period is the rate of HIV replication. Factors that accelerate HIV replication increase the progression of the disease. After HIV infection of human T cells in vitro, the virus undergoes a form of latency until activated by mitogens or specific antigens (42). In vitro stimulation of T cells increases HIV promoter activity (13, 40), which can terminate HIV latency. Thus, activation of the HIV promoter can be a factor in the length of the latency period. On the basis of their ability to activate the HIV promoter, other DNA viruses, such as herpes simplex virus type 1, cytomegalovirus, Epstein-Barr virus (22), papovaviruses (11), adenoviruses (28), and hepatitis B virus (36), have been implicated as possible cofactors in AIDS. Recent studies have shown that the herpesvirus cytomegalovirus enhances the replication of HIV in infected cells (37), and cells productively infected with both CMV and HIV have been found in the brains of AIDS patients (29). These studies indicate that it is possible for DNA viruses, especially herpesviruses, to activate HIV even in vivo.

HHV-6 infects primarily CD4+ human T cells, the same cells infected with HIV. Several investigators have shown that peripheral blood mononuclear cells can be productively coinfected with HHV-6 and HIV (6, 24, 26). In fact, many of the initial HHV-6 strains were isolated from individuals suffering from AIDS (25, 35, 39). Therefore, it seems likely that coinfection of human T cells with HHV-6 and HIV could accelerate the immunodeficiency disease caused by HIV. Ensioli et al. (8) showed that T-cell line HSB-2 could be productively coinfected with HHV-6 and HIV, resulting in accelerated cell death and increased HIV transcription. In the present study, the HIV promoter was activated in HHV-6-infected, primary human T lymphoblasts. In contrast, recent reports have shown that coinfection of peripheral blood mononuclear cells with HHV-6 and HIV suppressed HIV replication (6, 24). The differences between these studies may be due to the nature of the cells that were infected, dosage of virus, and time of infection. In the present study, 85 to 89% of the transfected cells were human T lymphoblasts, whereas the other studies (6, 24) used stimulated peripheral blood mononuclear cells which contained a variety of cell types. It has been demonstrated that HHV-6 infects numerous cell types present in peripheral blood mononuclear cells, including monocytes and B cells (25, 26, 39). Since the mechanism of HIV inhibition by HHV-6 in these studies is not known, it still remains possible that HHV-6 infection of cell types other than T cells contributes to suppression of HIV replication through cytokine production.

This study has demonstrated that HHV-6 gene products can activate the HIV promoter in primary human T lymphoblasts. Therefore, an active HHV-6 infection of human T cells can activate an otherwise quiescent HIV promoter. This promoter activation can lead to an increased HIV replication and consequently enhances T-cell death. In addition, the replication of HHV-6 itself is cytotoxic for T cells, which would start a cycle of increased T-cell death. By this proposed mechanism, an active HHV-6 infection would accelerate the progression of AIDS.

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