Reciprocal Regulatory Interaction between Human Herpesvirus 8 and Human Immunodeficiency Virus Type 1*

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Human herpesvirus 8 (HHV8) is the primary viral etiologic agent in Kaposi’s sarcoma (KS). However, individuals dually infected with both HHV8 and human immunodeficiency virus type 1 (HIV-1) show an enhanced prevalence of KS when compared with those singularly infected with HHV8. Host immune suppression conferred by HIV infection cannot wholly explain this increased presentation of KS. To better understand how HHV8 and HIV-1 might interact directly in the pathogenesis of KS, we queried for potential regulatory interactions between the two viruses. Here, we report that HHV8 and HIV-1 reciprocally up-regulate the gene expression of each other. We found that the KIE2 immediate-early gene product of HHV8 interacted synergistically with Tat in activating expression from the HIV-1 long terminal repeat. On the other hand, HIV-1 encoded Tat and Vpr proteins increased intracellular HHV8-specific expression. These results provide molecular insights correlating coinfection with HHV8 and HIV-1 with an unusually high incidence of KS.

Human herpesvirus 8 (HHV8), a Kaposi’s sarcoma-associated herpesvirus, is the first human member of γ2-herpesviruses (1–3). HHV8 has been identified in Kaposi’s sarcoma (KS) (4–8), primary effusion lymphoma (also termed body cavity-based lymphoma or BCBL) (9, 10), and multicentric Castleman’s disease (11). Epidemiological surveys report that the seroprevalence for HHV8 ranges from 0 to 20% in the general population of the Western world (12). Recently, HHV8 seropositivity was observed to be increased in populations exposed to sexually transmitted diseases, such as syphilis or human immunodeficiency virus (HIV), suggesting that HHV8 is also a sexually transmitted pathogen (13). As the etiologic agent for KS, HHV8 generally results in latent infection in its natural host cells (14). Based on several extensive studies on the association of HIV and the development of KS, it is now understood that HHV8 is required but not necessarily sufficient for the development of KS (15). In fact, the incidence rate of KS among HIV-1-infected individuals is up to 100,000-fold higher than that among the general population, and 300-fold higher than that for persons with other types of acquired immunosuppression. These findings suggest that immunosuppression likely plays a role as a cofactor with HHV8 in the development of KS; however, they also suggest that HIV-1 infection, independent of its immunosuppressive effects, contributes to HHV8-induced KS (16). Accordingly, it has been proposed by several investigators (17–19) that the role of HIV-1 in KS potentially involves two additional events: HIV-1-induced cytokines production and production of HIV-1 Tat protein. Indeed, cytokines produced by HIV-1-infected cells can induce lytic cycle replication of HHV8, and Tat can activate the vascular epithelial growth factor receptor KDR in endothelial cells (20).

Currently, it cannot be excluded that there may be many yet characterized interactions between HHV8 and HIV-1 that explain the dramatically higher incidence of KS in coinfected individuals. Thus, additional HIV proteins and/or cellular factors induced by HIV-1 could contribute to KS pathogenesis with possibly complex reciprocal effects between HHV8 and HIV-1. Consistent with these thoughts, many herpesviruses such as Epstein-Barr virus (21, 22), cytomegalovirus (23–25), human herpesvirus 6 (26–28), and human herpesvirus 7 (29, 30) have been described to influence HIV-1 replication and expression. To decipher mechanistic interplays between HHV8 and HIV-1, several relevant findings need to be considered. Typically, HHV8 is found in B lymphocytes, keratinocytes, epithelial cells, KS tumor cells, and endothelial cells (31). By contrast, the predominant host cells for HIV-1 are CD4+ T lymphocytes, dendritic cells, and mononuclear phagocytes (32, 33). Interestingly, Moir and colleagues (34) have shown recently that the up-regulation of CD4 and CXCR4 on B lymphocytes mediated by CD40 stimulation leads to increased susceptibility of these B lymphocytes to T-tropic HIV-1 infection. Conversely, dendritic cells and macrophages have been found to be susceptible cells for HHV8 infection (35, 36). Additionally, HHV8-infected B lymphocytes are further infectable by HIV-1 through a cell-cell-mediated pathway (37). Therefore, it stands to reason that in vitro HHV8 and HIV-1 genomes could coexist in some cells in dually infected individuals.

With the goal of elucidating better intracellular interactions between HHV8 and HIV-1, we queried for potential reciprocal effects on gene expression between HHV8 and HIV-1. We found that when the HHV8-positive primary effusion lymphoma cell line BCBL-1 (38) was fused to HIV-1-latent ACH2 cells (39, 40),...
Inc.) according to the manufacturer’s instructions. RNA (50 μg) from cells were finally resuspended into complete medium (46). Polyethylene glycol suspension was centrifuged at 100 g for 2 min, thereafter, 10 ml of RPMI 1640 containing 5% (v/v) dimethyl sulfoxide. The cell/polyethylene glycol mixture was resuspended into 1 ml of 100% (w/v) polyethylene glycol 4000 and 2 μg/ml PMA was added to stimulate HHV8 expression (2). Cells and Culture Conditions—BCBL-1 and ACH2 were obtained from the National Institutes of Health AIDS Research Reagent and Reference Program. Jurkat cells were purchased from the American Type Culture Collection. BCBL-1, ACH2, and Jurkat cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in the presence of 5% CO2. HeLa cells were maintained in Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum. For induction of HIV expression in ACH2 cells, 50 ng/ml TPA and 2 μg/ml PHA were added to complete medium (44). BCBL-1 cells were treated with 20 ng/ml TPA for 3 days to stimulate HHV8 expression (2).

Cloning of HHV8 KIE cDNAs—BCBL-1 cells, which had been chemically induced into lytic cycles, were harvested after exposure to 3 mM sodium butyrate for 4 h. Poly(A)+ RNA was isolated with a QuickPrep Micro mRNA purification kit (Amersham Pharmacia Biotech, Piscataway, NJ) and was reverse-transcribed using a Fast-Run RT-PCR kit (Promega, Madison, WI) to yield flag-tagged pKIE2, pKIE3, pKIE1–50, pKIE1–8.2, and pKIE1–8a. Recognition sites for restriction enzymes are underlined; boldface nucleotides denote the start (5’-end oligonucleotides) and stop codons (3’-end oligonucleotides). PCR primers used for KIE cDNA amplification were 35 cycles at 94 °C, 1 min; 53 °C, 1 min; and 72 °C, 1 min and that for KIE3, KIE1–50, and KIE1–8.2 were 35 cycles at 94 °C, 1 min; 50 °C, 1 min; and 72 °C, 1.5 min. The PCR-generated fragments of the HHV8 KIE cDNAs were subcloned into pCMV Tag2B (Promega, Madison, WI) to yield tag-flagged pKIE2, pKIE3, pKIE1–50, pKIE1–8.2, and pKIE1–8a.

RESULTS

Reciprocal Activation of Transcription between HHV8 and HIV-1—To better understand functional interactions between HHV8 and HIV-1, a series of cell fusion experiments was done. We took advantage of the fact that ACH2 and BCBL cells are latently infected with HIV-1 and HHV8, respectively. Both genomes can be activated from latency by treatment of cells with phorbol ester, TPA. By fusing together ACH2 and BCBL cells, heterokaryons can be created, which simultaneously contain both viral genomes. In this manner, potential interactions...
between the two viruses in the same cell could be assayed. When ACH2 cells were fused with TPA-induced BCBL-1 cells, RNase protection assay showed that HIV-1 TAR-containing RNAs were markedly increased when compared with control samples (Fig. 1A). Because all HIV-1 transcripts contain TAR sequence, this finding suggests that some element associated with the HHV8 genome could activate transcription from the HIV-1 LTR. Indeed, the BCBL-1-mediated transcriptional enhancement was considerably stronger than that achieved by

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**Fig. 1. RNase protection assay (RPA) of the expression of HHV8 and HIV-1 in cell-cell fusions of ACH2 and BCBL-1 cells.** A, 50 µg of total RNA was isolated from HIV-latently-infected ACH2 cells either without or with chemical (TPA/PHA) induction (lanes 1, 2) or HHV8-harboring BCBL-1 cells either without or with TPA induction prior to cell fusion (lanes 3, 4). RNAs were then incubated with 3 × 10⁶ cpm of antisense HIV TAR (upper panel) and human β-actin probes (lower panel) in RPA. B, RPA of total RNA (50 µg) from BCBL-1 cells either without or with TPA induction (lanes 1, 2) and ACH2 cells either without or with chemical induction prior to fusion with BCBL-1 (lanes 3, 4). RNA expression was quantitated using antisense HHV8 VIL-6. Protection of human β-actin mRNA was used as a normalizing control. Size markers are indicated at the left of the panel. The ratio of integrated density values (IDV) of target signal against β-action is shown under the RPA results.

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chemically inducing ACH2 cells with TPA and PHA. Thus the observed effect is likely a functional consequence of an HHV8 gene product and not a trivial result of TPA carryover from the induced BCBL-1 cells. Conversely, in a reciprocal experiment, when HHV8 gene expression was analyzed, the level of vIL-6 RNA was increased significantly when BCBL-1 cells were fused with TPA-induced ACH2 cells (Fig. 1B).

**KIE2 Gene of HHV8 Activates HIV-1 Transcription Synergistically with Tat**—We next attempted to define the HHV8 responsive element within the HIV-1 LTR. Six scanning mutants that abrogated separately the NF-κB sites, the Sp1 sites, or the TATA element were utilized (Fig. 2A). Although the basal activities of each of the mutant promoters were different when each was transfected into TPA-stimulated BCBL-1 cells, similar levels of HHV8-associated induction were observed. These results indicate that HHV8-responsiveness involves complex, rather than simple sequence motif(s). Control transfection of the same mutant promoters in HeLa cells (Fig. 2B) with or without TPA failed to show comparable activation consistent with activation being specific for BCBL-1-associated HHV8 gene product(s).

To further clarify how HHV8 activates HIV-1, we sought to identify responsible viral gene product(s). Although current understanding of HHV8 gene functions remains incomplete, we reasoned, based on analogy to other herpesviruses, that immediate-early genes of HHV8 are plausible candidate activators of HIV-1. Hence, we cloned five immediate-early open-reading frames (ORFs) from HHV8 and tested for their effects on the HIV-1 LTR. Our results showed that out of these five ORFs, only KIE2 activated the HIV-1 LTR (Fig. 3A). To check whether activation by KIE2 of HIV-1 LTR correlates with that observed in BCBL-1 cells (Fig. 2) we tested the series of linker-scanning LTR mutants used above. Similar to the activation profiles in Fig. 2, KIE2 activated each linker-scanning mutant comparably (Fig. 3B).

Because the magnitude of activation by KIE2 alone was less than that observed in the ACH2-BCBL-1 fusion experiments (Fig. 1) we considered whether there could be a synergy be-

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**Fig. 3.** HHV8 KIE2 activates HIV-1 LTR. A, Jurkat cells were transfected with 0.2 μg of HHV8 KIE expression vectors and 0.1 μg of pLTR-luc. B, Jurkat cells were transfected with 0.1 μg of pKIE2 and 0.1 μg of wild type HIV-1 LTR or LTR mutants. Cells were harvested 72 h post-transfection and normalized for β-galactosidase. The activity of pLTR-luc alone was arbitrarily set as 100.
Jurkat cells were transfected with Numbers luciferase assay. Activation reached a level comparable to that observed in the synergistic activation was observed for the HIV-1 LTR (Fig. 4).

Indeed, when KIE2 and Tat were coexpressed, a significantly synergistic activation was observed for the HIV-1 LTR promoter. The results in each figure are from one experiment performed in triplicates ± S.D.

FIG. 4. KIE2 acts synergistically with Tat on the HIV-1 LTR.

The concept of reciprocal interaction between HHV8 and HIV-1 potentially offers an explanation for the rapid development of KS in HHV8-infected individuals whom are subsequently infected with HIV-1. Thus, in this dually infected subset, the ratio of HIV-1 RNA expression and KS progression quickly, with a 60% increase for each year of HIV-1 infection (52). If, as suggested here, HIV-1 infection might transcriptionally activate HHV8 genome from latency, and, if such activation over the basal HIV-1 LTR promoter. The results in each figure are from one experiment performed in triplicates ± S.D.

FIG. 5. Expression of major capsid protein is stimulated by HIV-1 protein Vpr and Tat in BCBL-1 cells. At 72 h post-transfection, RNAs from BCBL-1 cells transfected with Vpr or Tat expression vectors were isolated for RT-PCR. The PCR products of major capsid protein (270 bp) and 18 S rRNA (314 bp) were analyzed on a 2.5% agarose gel.

Efforts to identify more direct interactions between HHV8 and HIV-1 have, to date, centered largely on HIV-1-induced cytokines and the secretion of Tat protein from HHV8-infected cells (53, 54). Intracellular interactions between the two viruses have been generally discounted, because a prevailing view has been that the two viruses infect different cell types. However, several points argue that such an assumption might not necessarily be warranted. First, it remains unclear as to what are the range of HHV8 susceptible cells in vivo. Second, recent ex vivo infection studies clearly support that both HHV8 and HIV-1 can efficiently infect cells of the monocyte/macrophage lineage (35, 36). Finally, Spearman and colleagues (37) have intriguingly demonstrated that HHV8-genome containing Lell B lymphocytes, in fact, can be infected by HIV-1 via a cell-cell pathway and that such infected B lymphocytes could support productive HIV-1 replication.

Here we have tried to define which virally encoded component of HIV-1 and HHV8 might reciprocally influence each other’s expression inside cells. Our cell fusion results (Fig. 1) indicate that when both genomes exist in the same cellular environment there is a bilateral transcriptional effect between the two viruses. Among the many ORFs that are encoded by HHV8, we found that the KIE2 protein was sufficient for singularly inducing transcription from the HIV-1 LTR (Fig. 3). On the other hand, persons infected with both HHV8 and HIV-1 have up to a 100,000-fold increase, compared with infection with HHV8 alone, in risk for developing KS (16). Based on this statistic, a reasonable deduction is that HHV8 is necessary but insufficient for producing KS and that HIV-1 is an important cofactor, which promotes HHV8 induced KS.

How might HIV-1 contribute to the development of KS? The results from a large multicenter study on HHV8 and HIV-1 infected men established an unmistakable link between HIV-1-associated immunosuppression and HHV8-engendered KS (52). However, in the same study it is interesting that when immunosuppression was excluded, an independent association was revealed between HIV-1 RNA expression and KS progression (52). The latter observation provided the impetus for us to systematically explore potentially more direct interactions between HHV8 and HIV-1.
tion, then one could imagine how such a feedback-cycle might accelerate disease manifestation. Indeed consistent with this scenario, an interruption of this cycle through treating HIV-1 with reverse transcriptase (RT) or protease inhibitors has been shown to efficaciously reduce the regression of KS (45). Future investigation toward understanding how to further intervene against the molecular interplay between HHV8 and HIV-1 should usefully advance the treatment of KS.

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