Activation of Gene Expression by Human Herpes Virus 6

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Introduction

Human herpes virus type 6 (HHV-6) was first detected by Salahuddin et al. [6] and has been isolated from patients with lymphoproliferative and immunosuppressive conditions. HHV-6 is the causative agent of exanthem subitum and the majority of the adult population has been infected with this virus.

HHV-6 is able to grow in lymphoblastoid cell lines. Previous studies have shown that coinfection with human immunodeficiency virus (HIV) leads to accelerated cell death [5] and that infection with HHV-6 increases expression from the HIV long terminal repeats (LTR).

We set out to study the effect of HHV-6 infection on transcription directed by the human T-cell leukaemia virus type 1 (HTLV-1) LTR as there is the potential for these two viruses to interact in vivo as both have similar cell tropisms.

Activation of HTLV-1 LTR CAT by HHV-6

In order to determine if infection with HHV-6 could increase expression of an HTLV-1 LTR CAT construct, transfection experiments were carried out. HIV LTR CAT and HTLV-1 LTR CAT were introduced into J. Jhan cells (a T-lymphocyte cell line) using the diethylaminoethyl (DEAE) dextran transfection method. Following transfection, media were added to the cells from uninfected or HHV-6-infected cultures. The cells were incubated for 2 days at 37 °C then harvested and the CAT activity assayed. An exceptionally marked increase in CAT activity was observed from both the HTLV-1 LTR and HIV LTR CAT constructs (Fig. 1).

Transcription mediated by the HTLV-1 LTR is upregulated by tax, a 40-kDa protein encoded by HTLV-1. To examine the effect of coactivation with tax and HHV-6, cells were transfected with HTLV-1 LTR CAT and either mock-infected, infected with HHV-6 or cotransfected with a plasmid carrying the HTLV-1 *tax* gene. The results (Fig. 2) show that when both methods of activation are used together a very strong response is observed which is greater than the sum of the individual activations. This result shows that tax and HHV-6 act synergistically and implies they have different mechanisms of activation.

Activation of Gene Expression During HHV-6 Infection Does Not Require Specific Promoter Regulatory Sequences

In order to study the sequences required for HHV-6 activation we constructed a deletion mutant of HTLV-1 LTR CAT by removing sequences upstream of 55 bp from the initiation site. The resultant plasmid pH2 CAT contains the TATA box but lacks upstream regulators. When pH2 CAT was introduced into cells its expression was increased by 41.8-fold by

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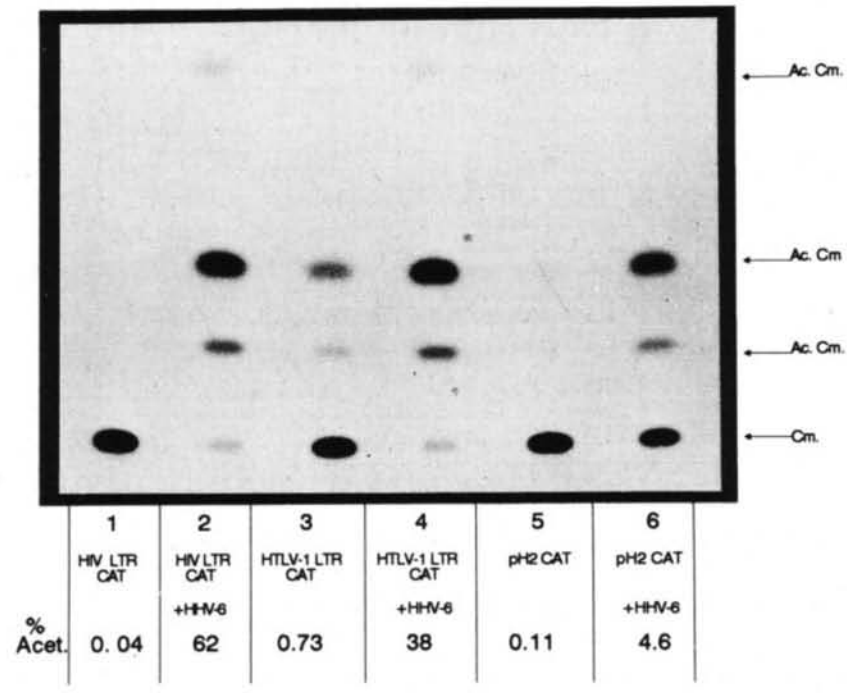

Fig. 1. Activation of promoter CAT constructs in transfected J. Jhan cells. Plasmids used and HHV-6 infections are indicated. The percentage acetylation of chloramphenicol/μg protein per hour of assay is shown (*Ac.Cm:* acetylated chloramphenicol; *Cm:* chloramphenicol)

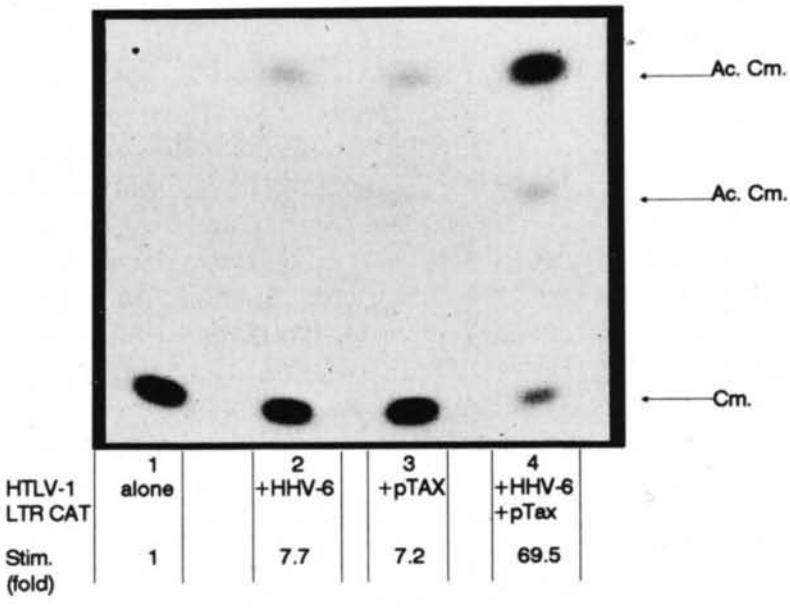

Fig. 2. CAT assays showing induction of HTLV-1 LTR CAT with HHV-6 and tax. Average-fold increase in CAT activity (for three experiments) compared to cells transfected with HTLV-1 LTR CAT alone is shown

HHV-6 infection, while in the same set of experiments HTLV-1 LTR CAT was stimulated by 52.0-fold (Fig. 1), although basal levels of pH2 CAT expression were lower. This result showed that upstream regulators are not required for HHV-6 activation.

A more detailed investigation of herpesvirus promoter elements required for activation by HHV-6 was carried out by utilizing a series of fine deletion mutants of the HSV-1 glycoprotein D gene [2] (Fig. 3). The constructs were transfected into J. Jhan cells and infected with HHV-

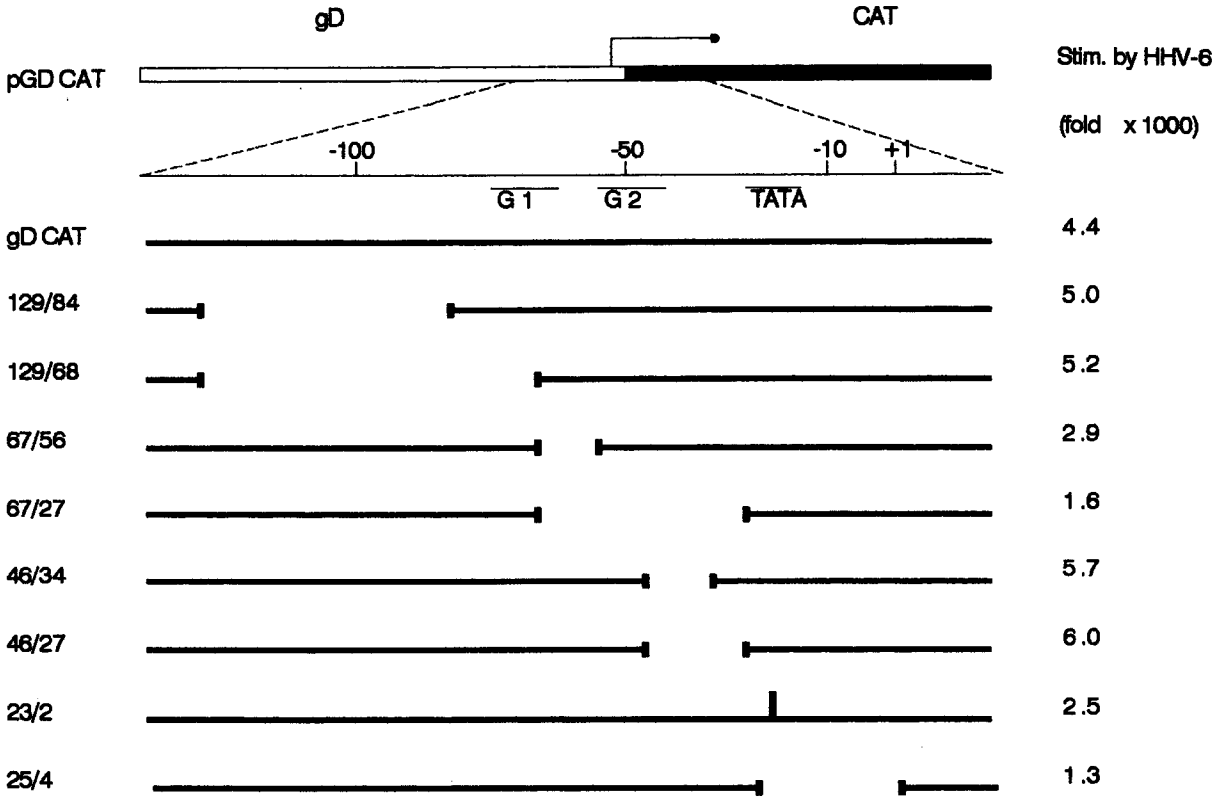

Fig. 3. Deletion mutants of HSV-1 pgD CAT. Promoter sequences of the gD gene are shown, indicating positions of the TATA box and GA-rich sequences (*G1* and *G2*). Stimulation (*stim*) in CAT activity due to HHV-6 infection is shown

6, or mock-infected. The level of activation of all pgDCAT constructs is very high (Fig. 3) and although some variation is observed this difference is small compared to the total level of induction. These results show that no essential promoter sequences are required for activation.

HHV-6 Infection Does Not Increase Levels of HTLV-1 mRNA

The C8166 cell line contains integrated defective HTLV-1 proviruses, one of which is able to synthesize the doubly spliced form of mRNA which encodes tax. Later species of RNA and infectious virus are not produced. To study the effect of HHV-6 on HTLV-1 RNA levels cytoplasmic RNA was isolated from cells which had been infected for 2 days with HHV-6 or from uninfected cells and was analysed by S1 nuclease mapping. A probe which spanned the mRNA start site was hybridized to the RNA and electrophoresed on a denaturing gel following S1 digestion. Both samples of C8166 RNA (Fig. 4, lanes 2 and 3) gave rise to a hybrid band of 58 bp which corresponds to correctly initiated HTLV-1 mRNA. The intensity of this band was not greater in the HHV-6 infected sample showing that infection did not affect levels of accumulated HTLV-1 mRNA.

HHV-6 Activation is Dependent On Gene Construct

We examined the effect of HHV-6 infection on the expression of different constructs under the regulation of the HSV-1 thymidine kinase (TK) promoter to determine if the nature of the reporter gene played any role in HHV-6 activation. The TK CAT constructs, like all other CAT constructs tested were strong-

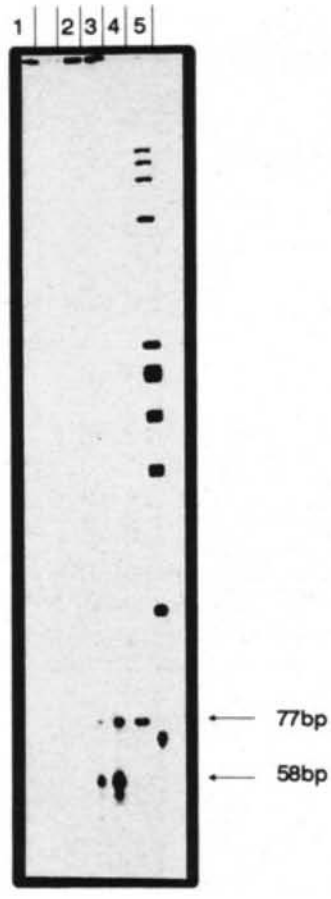

Fig. 4. S1 analysis of cytoplasmic RNA samples following electrophoresis on an 8% denaturing polyacrylamide gel. Samples used for S1 analysis were: J. Jhan RNA (lane *1*), HHV-6-infected C8166 RNA (lane *2*), mock-infected C8166 RNA (lane *3*). Markers were 77 bp probe (lane *4*) and *Hae*II-digested phi X DNA (lane *5*)

ly induced by infection (by 160-fold) (Table 1). However, the growth hormone (GH) gene controlled by the TK promoter, pTKGH, was only induced by 1.3-fold, showing this construct to have a very low, if any, response to infection. A similar level of activation was observed when both plasmids were cotransfected with a plasmid encoding the pseudorabies virus transactivator (pPRV IE), showing pTKGH can be efficiently expressed. These results reveal that induction of gene expression by HHV-6 is dependent on the recorder gene used. Expression of the CAT gene is strongly increased while very little change in GH levels is observed.

Conclusions

Our results show that activation by HHV-6 depends on the recorder gene used and that the role of the promoter or enhancer sequences are, at most, of minor importance. Induction of gene expression by HHV-6 is very likely to occur post transcription since any transcriptional effect would be expected to be independent of the reporter gene used.

The data we have obtained differ from that of Ensoli et al. [1] who studied activation of the HIV LTR by HHV-6. These workers found induction to occur at the level of transcription and that particular sequences within the HIV LTR were responsible. They also noted that an

Table 1. Activation of different reporter gene constructs by HHV-6

Plasmid[a]	CAT activity[b]	GH concentration (ng/ml)	Activation[c]
pTKCAT	0.12	–	–
pTKCAT + HHV-6	19.30	–	160.8
pTKCAT + pPRV IE	2.47	–	20.6
pTKGH	–	–	–
pTKGH + HHV-6	–	8.7	1.3
pTKGH + pPRV IE	–	106.6	14.6

[a] Plasmids were transfected into J. Jhan cells.
[b] Percentage acetylation per microgram of protein per hour of assay.
[c] Fold increase in activity due to HHV-6 infection.

HTLV-1 LTR CAT construct was unresponsive to HHV-6. The differences between our results and those of Ensoli et al. [1] are likely to stem from differences in the activity or expression of the transactivating polypeptides between the two strains of virus.

Our results, however, bear a strong similarity to those obtained by Kenney et al. [3, 4] who studied the BMLF1 gene, which encodes an immediate early transactivator of the Epstein Barr virus (EBV). These workers find activation of all CAT constructs by BMLF1, including those with no recognizable eukaryotic promoter, and they observe no increase in CAT mRNA levels. Most significantly they find that only CAT constructs and not GH constructs are induced by BMLF1. The similarity between these results and those obtained for HHV-6 suggests that the HHV-6 transactivator may be a homologue of BMLF1.

References

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