Studies of viral co-factors for human immunodeficiency virus in vitro and in vivo

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Introduction

The incubation period from initial human immunodeficiency virus (HIV) infection to the development of AIDS and then death varies markedly between individuals (Darby et al., 1996). Increased plasma levels of HIV are associated with more rapid progression to AIDS and death, while reduction of HIV load with anti-retroviral drugs has a corresponding clinical benefit in delaying progression to AIDS and death (Mellors et al., 1996). Factors which modulate the viral load of HIV could therefore impact either positively or negatively on disease progression. Co-infection of cells (or people) with other viruses, particularly herpesviruses, has the potential to effect such modulations (Griffiths, 1992). In this review, I will summarize the mechanisms for interaction which have been studied in vitro and in vivo.

Mechanisms of possible herpesvirus/HIV interactions

Table 1 summarizes the general types of interactions which could stimulate the replication of HIV (Webster et al., 1989; Griffiths, 1992). It classifies them, firstly, according to whether the co-factor virus infects the same cell as HIV or a neighbouring cell and, secondly, according to their overall effect on activating HIV proviral DNA or altering the tropism of HIV.

In Table 2, the mechanisms described above have been tabulated against the list of human herpesviruses which are now known. It will be apparent from this table that many virus/mechanism combinations remain to be studied. Nevertheless, of those that have been investigated, many demonstrate that herpesviruses can stimulate replication of HIV, although there are important exceptions.

Transactivation

Several viruses use transactivation to control their own replication. Studies have shown that herpes simplex virus (HSV), hepatitis B virus (HBV), human T-cell leukaemia virus (HTLV), human herpesvirus 6 (HHV-6), Epstein–Barr virus (EBV), cytomegalovirus (CMV) and adenovirus can all activate the HIV LTR (Davis et al., 1987; Gendelman et al., 1986; Kenney et al., 1988; Lusso et al., 1989; Rice & Mathews, 1988; Siddiqui et al., 1989; Siekevitz et al., 1987). This could represent a ‘final common pathway’ whereby convergent evolution has selected a variety of viruses able to modulate the cellular environment to facilitate their own intracellular replication. For example, by making available activated transcription factors, one virus could then produce an intracellular milieu favourable for the replication of another virus such as HIV. The co-factor virus could encode a transactivator protein directly, or a protein which increases the availability of cellular transcription factors such as NFκB (Yurochko et al., 1995). This suggests that the design of inhibitors of factors such as NFκB could have marked effects on the activation of HIV, irrespective of which virus was responsible for making NFκB available, i.e. such a drug could inhibit the effects of multiple co-factor viruses. Note, however, that NFκB is strongly required by HIV for its own activation (Alcami et al., 1995; Grilli et al., 1993), so that such inhibitors could also inhibit HIV, even if no co-factor viruses were involved.

In contrast, there is strong evidence that, under certain circumstances, herpesviruses can inhibit HIV replication. In a series of elegant studies, D. Spector and colleagues have shown that active CMV replication can compete with that of HIV and decrease the amount of retrovirus produced (Koval et al., 1991, 1995; Moreno et al., 1997). The experiments show that, in vitro, permitting CMV replication to proceed fully to productive infection can decrease HIV infection, whereas abortive CMV infection can significantly increase HIV replication. When the retrovirus genome is integrated (mimicking the natural situation), the transactivating ability of CMV is markedly reduced compared to its effect on cells transiently transfected with a plasmid containing CMV. In the same manner, HHV-6 has been shown to decrease HIV replication (Carrigan et al., 1990; Levy et al., 1990). The implication of these findings is that abortive (or slow) herpesvirus infections in a co-infected cell may facilitate HIV replication, whereas full productive replication may paradoxically decrease HIV replication.

Recent experiments have shown that the US11 gene product of HSV can act as a chaperon, escorting unspliced
Table 1. Interactions between HIV and potential co-factor viruses which could lead to increased HIV replication

Modified from Webster et al. (1989).

<table>
<thead>
<tr>
<th>Effect of interaction</th>
<th>HIV and co-factor virus infect:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Same cell</td>
</tr>
<tr>
<td>Activate proviral HIV DNA</td>
<td>Transactivation</td>
</tr>
<tr>
<td>Alter tropism of HIV RNA</td>
<td>Pseudotype formation</td>
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<td></td>
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</tbody>
</table>

Table 2. In vitro evidence for co-factor herpesvirus stimulating HIV replication

✓, Data support stimulation; ×, data reject stimulation (or show inhibition); blank, no data.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Transactivation</th>
<th>Cytokine release</th>
<th>Alternative HIV receptor</th>
<th>CD4 or co-receptor up-regulation</th>
<th>Antigen presentation</th>
<th>Pseudotype formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV</td>
<td>✓</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>VZV</td>
<td>✓</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>CMV</td>
<td>✓</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV-6</td>
<td>✓</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV-7</td>
<td>✓</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV-8</td>
<td>×</td>
<td>×</td>
<td></td>
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</tr>
</tbody>
</table>

mRNAs from the nucleus to the cytoplasm, where they can associate with polyribosomes for translation. This activity is strongly reminiscent of that of rev for unspliced mRNAs of HIV, and experiments have revealed that US11 could perform this function for HIV (Diaz et al., 1996).

Up-regulation of CD4 and/or co-receptor expression

Since CD4 is the major component of the cellular receptor for HIV, viruses which modulate CD4 expression could affect HIV replication. Both HHV-6 and HHV-7 are tropic for CD4+ lymphocytes, although only HHV-7 uses CD4 as its receptor (Lusso et al., 1994; Yasukawa et al., 1995). Comparison of the effects of these two herpesviruses on HIV infection is instructive. Lusso et al. (1991) showed that HHV-6 could upregulate CD4 expression on CD8+ cells which could then be infected with HIV. Thus, HHV-6 has the potential to increase HIV replication. In contrast, since HHV-7 shares the CD4 receptor molecule with HIV, it has been shown to compete with HIV for binding to cells and so decreases HIV replication (Lusso et al., 1994).

Likewise, the recent discovery (Deng et al., 1996; Dragic et al., 1996) that cellular chemokine receptors act as the second signal required for HIV internalization after CD4 binding suggests the possibility that homologues encoded by herpesviruses could provide the same function. Recently, experiments have shown that gene US28 of human CMV, when incorporated into cells expressing CD4 but resistant to HIV entry, transferred the ability for HIV to be internalized and initiate infection (Pleskoff et al., 1997).

Induction of alternative HIV receptors

It is also possible that herpesviruses could either encode proteins or activate cellular proteins capable of acting as alternative receptors for HIV. Thus, McKeating et al. (1990) showed that HIV coated in non-neutralizing antibody could bind to fibroblasts expressing the Fc receptor of CMV. This effect was not blocked by monoclonal antibodies against CD4 or by an excess of soluble CD4 itself but was blocked by normal IgG.

Pseudotype formation

Pseudotypes such as a virion containing the RNA of HIV enveloped with the surface glycoproteins derived from a co-
factor virus would be able to infect cells using the receptor for the co-factor virus instead of the CD4 molecule. Once the HIV RNA had gained access to the cell it would replicate as HIV thereafter.

Pseudotype formation with HIV-1 has been described for HSV, HTLV, HIV-2 and vesicular stomatitis virus (Landau et al., 1991; Le Guern & Levy, 1992; Lusso et al., 1990; Zhu et al., 1990). Pseudotypes between EBV and HIV-1 were not detected when lymphoblastoid cell lines were infected with both viruses (Van Kuyk & Mosier, 1995). Lerner-Tung et al. (1991) used electron microscopy and immunogold staining to identify guinea-pig CMV pseudotypes with an endogenous retrovirus.

There is one report (Margalith et al., 1995) which is consistent with the production of pseudotypes between human CMV and HIV in patients infected with both viruses. Samples of urine were inoculated onto fibroblasts and examined for the presence of HIV proviral DNA. Positive signals were seen only when the urine also contained evidence of CMV infection. The most likely explanation for this is that cells in the renal tract co-infected with HIV and CMV produced not only CMV virions but pseudotypes of HIV coated in CMV surface glycoproteins which then bound to cellular receptors on the fibroblasts and allowed the HIV RNA to be reverse-transcribed to proviral DNA. This explanation has been accepted for Table 2.

**Cytokine release**

The paracrine effects of cytokines stimulated by co-factor viruses could activate latent HIV proviral DNA through signal transduction. Clouse et al. (1989) stimulated monocytes from healthy laboratory staff with preparations of killed herpesviruses. The conditioned medium produced by CMV and EBV, but not HSV, varicella-zoster virus (VZV) or HHV-6, activated HIV replication.

In contrast, expression of the vMIP-I gene from HHV-8 in cell cultures decreased their production of HIV-1, but not HIV-2, presumably because the expressed chemokine blocked the cellular chemokine co-receptor for HIV-1 (Moore et al., 1996). Recently, vMIP-II gene, PCR-amplified from a Kaposi’s sarcoma biopsy, was used to express recombinant chemokine; this displaced authentic MIP1α and decreased cellular production of p24 antigen (Kledal et al., 1997).

**Antigen presentation**

T-memory cells containing HIV provirus require immunological stimulation to activate productive HIV infection. Some of these cells will be committed to the recognition of cognate antigens from viruses other than HIV. Release of antigens from a cell infected with a co-factor virus could thus activate HIV replication in bystander cells in proportion to the frequency of T-memory cells specific for the co-factor virus. Experiments have shown that peripheral blood mononuclear cells stimulated with CMV antigens released tumour necrosis factor α only if the cells were taken from individuals seropositive for CMV (Peterson et al., 1992).

Evidence has been presented that CMV-infected monocytes can expand the T-cell receptor Vβ repertoire, possibly through a CMV-encoded superantigen. CD4 cells selected for the altered Vβ12 receptor replicated HIV more efficiently than other T-cells (Dobrescu et al., 1995).

**Implications for the whole individual**

Given all of these results, it is impossible to be precise about the net effect of herpesvirus/HIV interactions because both positive and negative interrelationships are plausible, the effects may be different in different cell types, and they may occur differentially as immune function wanes and permits increased herpesvirus replication in individuals. Fig. 1 therefore summarizes the types of general interactions which can be envisaged. The upper part (Basic scheme) is non-controversial and outlines current concepts about the pathogenesis of AIDS, while the lower part (Variations) embellishes this general scheme to include the following features.

**HIV as an opportunist**

If the immune system functions to control herpesvirus replication, then why should decreased immune function not lead to increased HIV replication as well as increased herpesvirus replication? This could provide the potential for HIV to cause opportunistic disease in its own right; for example, AIDS dementia complex caused by HIV infection of the brain would be a candidate opportunistic HIV disease.

The therapeutic implication of this proposal is that initiation of anti-retrovirus therapy in late-stage HIV disease may produce clinical benefits through inhibition of HIV opportunistic disease, rather than by interference with the rate at which HIV damages the immune system. Such clinical trials, whether performed years ago with zidovudine (Fischl et al., 1987) or more recently with protease inhibitors, should document whether they are inhibiting the ability of HIV to induce progressive immune damage or its ability to cause opportunistic disease.

**HIV as a co-factor virus**

If herpesviruses can drive HIV replication, why should HIV not drive herpesviruses? For example, HIV infection stimulates CMV (Skolnik et al., 1988) and HIV tat can stimulate the replication of HHV-6 (Siekzowski et al., 1995). It is striking that most of the viruses postulated to be co-factors also act as opportunists in their own right. The therapeutic implications of this are that anti-herpes drugs might inhibit progression of HIV disease (reviewed in Stein & Graham, 1996), while anti-retroviral drugs might decrease herpesvirus disease. For
example, the recent availability of protease inhibitors with potent activity against HIV might be expected to delay the time until opportunistic herpesvirus disease occurs and current clinical anecdotes support this possibility. Two possible explanations are apparent: either the protease inhibitors have improved immune function, leading to better control of opportunistic herpesviruses, or they have decreased the opportunity for HIV to drive the replication of opportunistic viruses to the levels required to cause disease in their own right.

**Negative co-factors**

As described earlier, CMV can, under some circumstances, decrease HIV replication (Koval et al., 1991). However, it would be foolhardy in the extreme to consider administering CMV to HIV-positive patients given its proven ability to produce serious opportunistic disease (Gallant et al., 1992). Likewise, although there is no definitive proven opportunistic disease caused by HHV-6, the anecdotal evidence from case reports urges caution when considering the use of this virus to inhibit HIV replication (Qavi et al., 1995). Similarly, HHV-8-encoded cytokines may down-regulate HIV by occupying cellular chemokine receptors (Moore et al., 1996; Kledal et al., 1997) but the strong association of HHV-8 with Kaposi’s sarcoma precludes its consideration for therapeutic purposes. Thus, for these three viruses, therapeutic possibilities are limited to identification of pharmaceutical compounds which could mimic in vivo their negative co-factor effects in vitro.

In contrast, HHV-7 has the characteristics required of a ‘negative co-factor’ virus, since there is little evidence to date that it causes opportunistic disease in immunocompromised transplant or AIDS patients and direct evidence that it can inhibit HIV replication (Lusso et al., 1994) in vitro. Consideration could therefore be given to the possibility of administering HHV-7 to HIV-positive patients already HHV-7 seropositive, with the intention of competing with HIV in vivo. Clearly, considerable safety and regulatory hurdles would have to be overcome before this could be contemplated in practice but HHV-7 may have a potential role as a vector for gene therapy targeted at CD4+ cells. Indeed, under the cover of potent anti-retroviral chemotherapy, HHV-7 vectors might find a role in targeting CD4+ cells with the aim of activating HIV proviral DNA from long-lived reservoirs of infection. Meanwhile, the mutually antagonistic interaction between HIV and HHV-7 suggests that caution should be exercised in giving anti-herpes compounds with activity against HHV-7 to patients with HIV infection.

By competing with HHV-7 for the CD4 receptor, HIV could decrease HHV-7 infection and so decrease any opportunistic disease associated with this virus. If HHV-7 is shown to cause disease in transplant patients, but not in AIDS patients, then the possibility should be considered that HIV is acting in this way as a ‘negative co-factor’.

**Results of in vivo experiments to determine if such interactions are plausible**

Several investigators have attempted to find in vivo correlates of the herpesvirus/HIV interactions described in vitro. The results in Table 3 show that most parameters have not been studied for most herpesviruses; nevertheless, some useful data have been obtained.

**Herpesviruses increase HIV plasma load**

Only acute HSV infection has been studied to date in a small number of patients. The results show that HIV-positive
Table 3. *In vivo* evidence for co-factors

<table>
<thead>
<tr>
<th>Virus</th>
<th>↑ HIV RNA</th>
<th>↑ Organ</th>
<th>↑ Cell</th>
<th>↑ AIDS</th>
<th>↑ death</th>
<th>↑ death</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>✓/✗</td>
<td>✓/✗</td>
<td>✓/✗</td>
<td>✓</td>
<td>✓/✗</td>
<td>✓/✗</td>
</tr>
<tr>
<td>HHV-6</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>HHV-7</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
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<td></td>
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<tr>
<td>HHV-8</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
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</tbody>
</table>

* Ab, Antibody; V, co-factor virus.

Patients with acute genital herpes have a transient increase in HIV plasma load which returns towards baseline once the genital herpes episode has resolved (Mole *et al.*, 1997).

**Herpesvirus serostatus affects outcome**

Two studies have investigated whether CMV seropositivity at the time of HIV seroconversion affects the time to development of AIDS in haemophiliacs. The first cohort described a relative risk for CMV of 3.2, which was reduced to 2.5 after controlling for age using a Cox proportional hazards model (Webster *et al.*, 1989). The second study, using the same laboratory techniques, reported no significant association with CMV, either before or after controlling for age (Rabkin *et al.*, 1993). There are differences in patient selection between the studies but, at this time, it is not possible to determine whether a true effect is identified by CMV serostatus. The first study has now progressed sufficiently to allow an analysis for death, and CMV seropositivity is also associated with more rapid progression to death (Sabin *et al.*, 1995).

**Active herpesvirus infection affects outcome**

Some individuals with serological evidence of previous infection may not reactivate their latent herpesviruses. Given the *in vitro* mechanisms for interactions with HIV described in Table 1, it would be expected that selection of individuals with active herpesvirus infections would identify a group with stronger association with progression of HIV disease than the presence of latent herpesviruses detected serologically. However, there is a confounding interpretation; those with active herpesvirus infection may progress rapidly because they have profoundly damaged immune function, which permits herpesvirus reactivation.

One study has addressed this problem by measuring the CD4 counts of patients with active semen CMV infection, and controlling for the CD4 variable (Detels *et al.*, 1994). The results showed that the population of 164 men who donated at least four semen samples could be divided into three groups: those who were persistently positive on CMV culture; those who were intermittently positive; and those who were persistently CMV culture-negative. As expected, the CD4 counts were significantly lower among those with persistent CMV infection. Nevertheless, once this factor was controlled for using a Cox proportional hazards model, persistent CMV infection was still significantly associated with more rapid progression to AIDS.

A recent study has shown that, among patients presenting with first episode CMV retinitis, the time to death was significantly shorter among those with CMV viral loads above the median (measured by quantitative–competitive PCR) than in those with loads below the median (Bowen *et al.*, 1996).

**Prevalence of herpesviruses in autopsy tissue**

All of the interactions described earlier require that co-factor viruses should be either in the same cells as HIV or in closely related bystander cells. One approach to investigating the possible significance of co-factor effects is thus to determine whether any given herpesvirus co-infects tissues with HIV. The only pragmatic and ethical approach to obtaining multiple tissues from individual patients is to collect them at autopsy. Accordingly, several studies have used different methods to detect herpesviruses in autopsy samples. While the presence of a given herpesvirus does not guarantee that it was acting as a co-factor, the reciprocal relationship is informative. Thus, VZV was not detected by culture in any of 464 tissues from 29 autopsies, so it would be difficult to sustain the argument that VZV infection was so prevalent that this virus was a candidate co-factor (Pillay *et al.*, 1993). The same tissues revealed HSV in 11% and CMV in 66% of patients. Subsequent studies using the more sensitive PCR revealed CMV in 100% of patients, with HIV/CMV co-infection of organs documented in 21–69% of specific tissues (Webster *et al.*, 1995). Autopsy tissues
have also revealed the presence of HHV-6 (Corbellino et al., 1993; Knox & Carrigan, 1994) at significantly higher viral loads than those found in HIV-negative controls (Clark et al., 1996).

As described earlier, some of the possible HIV/herpesvirus interactions require co-infection of individual cells. To date, CMV has been examined and co-infection of cells from retina, brain and lungs has been identified (Belec et al., 1990; Finkle et al., 1991; Nelson et al., 1988; Skolnik et al., 1989).

Conclusions

In this review, I have summarized the work of many groups of investigators using molecular biological, cell biological and clinicopathological methodologies. Most of this work indicates that herpesviruses can interact with HIV in vitro, that such interactions are plausible in vivo, and that inhibition of co-factor herpesviruses may provide clinical benefit. With the discovery of HHV-8, the subject of co-factors looks ready for a renaissance.

References


Webster, A., McLaughlin, J. E., Johnson, M. A., Emery, V. C. & Griffiths,


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