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Herpesviruses and AIDS

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Herpesviruses and retroviruses are distinct taxonomically, yet have the potential for multiple bidirectional interactions. *In vitro*, these interactions have largely been studied in terms of herpesvirus up-regulation of HIV genome expression and/or transmissibility. This can be demonstrated *in vitro* through experiments showing transactivation, CD4 up-regulation, *Fc* receptor induction, pseudotype formation, cytokine production, and antigen presentation. In addition, once HIV has induced immunosuppression several herpesviruses can reactivate, and so cause disease in their own right.

The in-vivo correlates of these phenomena are difficult to study in humans but evidence is reviewed to support the concept that herpesviruses may be activating HIV, so that potential clinical benefit could be obtained through the use of antiviral drugs active against herpesviruses. Whether the clinical benefits seen in particular trials could be ascribed to inhibition of herpesvirus disease, or to herpesvirus infection driving HIV pathogenesis, remains a controversial area.

Herpesviruses and AIDS

Humans can be infected by any one, or a combination, of eight herpesviruses (see Table I) which share sufficient morphological and biochemical characteristics to justify their inclusion within the family *Herpesviridae*. Each is able to establish latency within the host, yet displays unique features which differentiate it from other members of the same family.

AIDS continues to afflict increasing proportions of populations worldwide and is clearly caused by members of the *Retroviridae* human immunodeficiency virus type 1 or type 2 (HIV-1 or HIV-2). These viruses cause progressive loss of cell-mediated immune function by mechanisms which remain obscure even one decade after the first isolation of the causative viruses.

When viewed from the perspective of viral taxonomy, there is no obvious connection between members of the *Retroviridae* and *Herpesviridae*. Yet, as will be discussed below, there are many ways in which these viruses could potentially interact when viewed from the perspective of individual cells in the human body. Thus, once HIV has damaged the cellular immune system, herpesviruses could reactivate to cause opportunistic disease in their own right. In contrast, herpesviruses could potentially interact with HIV at the cellular or molecular level to accelerate the rate at which HIV

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causes AIDS and/or death; this is termed a co-factor relationship. Although the aim of this paper is to categorise diseases into opportunist or co-factor polar positions on a continuum or herpesviruses/HIV interactions, it will be clear that this is not possible for all herpesviruses at the present time owing to incomplete knowledge about pathogenesis.

It will also be obvious that a co-factor relationship could act in the reverse direction; that is, HIV could drive herpesvirus replication to accelerate the rate at which opportunistic disease occurs. Patients with AIDS do experience a higher rate of some herpesvirus infections (e.g. CMV retinitis) than do patients immunocompromised following organ allografting. Whether these can be considered examples of reverse co-factor activity or whether they will ultimately be explained by other pathogenetic mechanisms remains to be defined. Furthermore, the possibility that the total burden of disease experienced by a patient with AIDS results from multiple cycles of bi-directional interactions between HIV and herpesviruses should not be ignored.

Opportunists

Herpesviruses typically reactivate and cause disease when patients with normal immunity are unwell, so it is not surprising that these viruses cause the same types of disease once HIV has debilitated the host (Nelson, Ghazal & Wiley, 1990). These diseases may be more extensive and more severe than those caused in patients without HIV infection, or they may cause less disease. Finally, some herpesvirus diseases are unique to AIDS, having been described only recently in this patient group. Each virus will now be considered in turn.

Herpes simplex virus (HSV). In patients with HIV infection, HSV type 1 or type 2 may cause typical herpetic vesicles on oral or genital mucosa respectively. A survey of ulcers in patients with AIDS (Bagdades *et al.*, 1992) revealed that HSV was most frequently isolated once the CD4 count had declined below 50×10^6 L. However, instead of healing after 5–7 days, such recurrences, especially of the ano-genital area, may progress to extensive ulceration involving a greater surface area of skin and deeper tissues. An initial diagnosis of "bed sores" may be made, especially in a debilitated patient, so that effective antiviral chemotherapy may even not be considered.

HSV may also cause infection of visceral organs including oesophagitis, pneumonitis and hepatitis which are rare in patients with normal immunity (Nelson *et al.*, 1990). Clearly, appropriate specimens must be obtained from patients presenting with symptoms referable to these sites to allow the virus to be detected and antiviral treatment to be given.

One way of assessing the potential pathogenicity of HSV for patients with AIDS is to seek evidence of HSV infection in autopsy tissues but those series relying upon histopathological criteria have not reported HSV as commonly occurring in patients dying of AIDS (Morgello *et al.*, 1987; Klatt & Shibata, 1988). However, by collecting several tissue samples prospectively from autopsies, we cultured HSV in one or more specimens from 11% of 47 patients (Pillay *et al.*, 1993). Although these patients died with HSV infection, review of their medical records failed to identify any particular clinico-pathological associations to suggest that they had died because of HSV infection. Clearly, larger prospective series must be undertaken to address this question adequately.

Table I. Classification of human members of the *Herpesviridae* and their genomic sizes

Subfamily	Name	Approximate size of genome (kbp)
α-Herpesvirinae	HSV1	152
	HSV2	154
	VZV	125
β-Herpesvirinae	CMV	229
	HHV6	160–170
	HHV7	140–150
γ-Herpesvirinae	EBV	172
	HHV8	?

Varicella zoster virus (VZV). Most patients infected with HIV have previously experienced chickenpox, so little evidence is available about whether HIV infection alters the course of chickenpox. However, the increasing number of children infected with HIV and who are varicella naive may alter this state of affairs.

Shingles due to reactivation of VZV is a major presenting sign of underlying HIV infection, especially in developing countries. The average CD4 count at which shingles develops in patients at risk has not been studied systematically. However, one report (Glesby *et al.*, 1993) of a cohort of 1044 patients with CD4 counts less than 250×10^6 L suggests that the prognosis is no different for patients with HIV infection than for a similar patient matched for CD4 count when subjected to proportional hazards analysis. Thus, shingles is an indicator of underlying HIV infection, but is not a determinant of future disease progression. Shingles may become chronic and lead to hyperkeratotic, verrucous lesions (Hoppenjans *et al.*, 1990) and may also lead to zoster encephalitis (Rosenblaum, 1989).

VZV infection of the eye can present as acute retinal necrosis or necrotising retinitis (Margolis *et al.*, 1991) and, since most patients have a preceding history of ophthalmic zoster, controlled trials should address whether antiviral prophylaxis would benefit such patients.

Table II. Mechanisms by which herpesviruses could interact with HIV to accelerate progression of AIDS and/or death

Mechanism	Herpesvirus for which each mechanism has been demonstrated *in vitro*	Reference
Transactivation	HSV	Gendelman *et al.* (1986)
	HHV6	Lusso *et al.* (1989)
	EBV	Kenney *et al.* (1988)
	CMV	Davis *et al.* (1987)
CD4 upregulation	HHV6	Lusso *et al.* (1991)
Fc receptor	CMV	McKeating, Griffiths & Weiss (1990)
Pseudotype formation	HSV	Zhu, Chen & Huang (1990)
Cytokine production	CMV, EBV	Clouse *et al.* (1989)
Antigen presentation	CMV	Peterson *et al.* (1992)

Epstein-Barr virus (EBV). The receptor for EBV is complement receptor-2 (CD21), so the virus is preferentially taken up into cells of B-cell lineage, although it also infects epithelial cells. EBV is adapted to increased proliferation of B-cells by the presence of genes homologous to interleukin-10 which can drive B-cell proliferation. Likewise, it encodes a homologue to *bcl*-2 which can deactivate the cellular defence mechanism of apoptosis (Milner, Johnson & Gregory, 1992). Throughout, EBV persists in a circular episomal form which is replicated and transmitted to each daughter cell. The full complement of latency-associated genes is not expressed however, so that HLA Class I molecules may be unable to present specific peptides which would otherwise lead to destruction of the cell by cytotoxic T-lymphocytes (Gregory, 1992).

It is well recognised in patients with iatrogenic immunodeficiency, that EBV is causally-associated with B-cell lymphomas (Randhawa *et al.*, 1992). Initially, the failure to control B-cell proliferation leads to polyclonal B-cell activation driven by EBV. At this stage, the patient may present clinically with 'lymphoma', manifest by a swollen lymph node. Removal of immunosuppressive treatment, with or without acyclovir, may allow natural cell-mediated immune responses to eliminate the incipient tumour. If this fails or, if immunosuppressive treatment is not reduced, clones of B-cells with increased proliferative capacity due to gene rearrangement may be selected for. Once the 'lymphoma' has evolved to the stage of selecting monoclonal tumours with somatic gene rearrangements, reduction of immunosuppressive treatment and prescription of acyclovir are unlikely to be helpful and the patient may die from disseminated true lymphoma.

In addition to its well recognised role in B-cell lymphomas, EBV DNA has been found in some T-cell lymphomas (Harabuchi *et al.*, 1990) and Hodgkin's tissue samples (MacMahon *et al.*, 1991). It will be important to differentiate an aetiological role for EBV from an association whereby EBV preferentially replicates in those cells induced by a different pathological process.

EBV infection of the lateral border of the tongue appears to result from full expression of EBV lytic cycle genes and leads to oral hairy leukoplakia (Ryon *et al.*, 1993). This is a relatively trivial condition which has been reported to respond to acyclovir therapy (Herbst *et al.*, 1989).

Cytomegalovirus (CMV). CMV is the major herpesvirus causing opportunistic disease in patients with AIDS (Gallant *et al.*, 1992) and CMV retinitis is second only to *Pneumocystis carinii* pneumonia as an opportunistic infectious agent.

CMV can also cause ulceration anywhere in the gastrointestinal tract although ulceration of the oesophagus and colon are most common (Gallant *et al.*, 1992). CMV has also been reported to cause encephalitis and polyradiculopathy/cauda equina syndrome. Polymerase chain reaction in cauda equina syndrome is the only way of diagnosing these infections (Cinque *et al.*, 1992; Wolf & Spector, 1992). It is interesting to note that CMV pneumonitis is unusual in AIDS (Bozzette *et al.*, 1992), even though the virus can be found frequently in their lungs at autopsy (Pillay *et al.*, 1993). This is consistent with the hypothesis that CMV pneumonitis in bone marrow allograft recipients is an immunopathological condition (Grundy, Shanley & Griffiths, 1987) where CMV triggers abnormal cell-mediated immune responses which damage the lung; presumably, AIDS patients cannot mount the abnormal response required to cause this disease. Note, however, that CMV pneumonitis has been reported in two cases with high CD4 counts in whom HIV seroconversion illness was suspected (Squire *et al.*, 1992), suggesting that the ability to mount the immunopathological response is still intact soon after initial HIV infection.

CMV infection has also been associated anecdotally with other clinical conditions, among which CMV adrenalitis is of some interest (Grinspoon & Bilezikian, 1992). The symptoms experienced by many AIDS patients are both constitutional and non-specific, so the possibility that they might be suffering from Addison's disease is intriguing and supported by some clinical testing of endocrine function.

At autopsy, CMV has been identified histopathologically in 25% of cases. Using cell culture, we have detected CMV in 66% of 47 patients undergoing autopsy (Pillay *et al.*, 1993). Sufficient cases were available to permit a clinico-pathological assessment of the potential significance of this finding. Statistical associations between CMV detection in central nervous system tissue and undiagnosed encephalopathy as well as detection of the virus in the gastrointestinal tract and undiagnosed diarrhoea were found. Further studies are needed to examine these possible disease associations and to define the risks associated with CMV. If the results are confirmed, then controlled trials of anti-CMV therapy for these two clinical conditions would be justified.

Human herpesvirus 6/7 (HHV). Only anecdotal associations between HHV-6 and disease in HIV-infected individuals have been reported. It is to be expected that the same will soon become true for HHV-7! Clearly, prospective studies are required to determine their true significance.

Human herpesvirus 8 (HHV-8). The technique of representational difference analysis has recently identified DNA sequences from a new herpesvirus in tissue from those with AIDS-KS and Kaposi's sarcoma (KS) (Chang *et al.*, 1994). While more work is required to determine if the virus causes KS, it aptly illustrates the difficulty of classifying a particular herpesvirus disease as an opportunist or co-factor. If HHV-8 causes KS once old age or HIV have induced immunodeficiency, then it would be an opportunist. However, if HHV-8 requires upregulation by HIV to cause KS, then it would be a co-factor, as described below.

Co-factors

In-vitro studies. Multiple mechanisms have been described by which a herpesvirus could interact with HIV to drive its replication (or *vice versa*). These have been discussed in detail elsewhere (Griffiths 1992) and are summarised in Table II. Essentially, they can be divided into mechanisms which up-regulate latent HIV genomes (transactivation, cytokine release, antigen presentation), or which alter the tropic range of HIV (pseudotype formation, Fc receptor formation, CD4 up-regulation). Each of these mechanisms of interaction is complex, as will be illustrated by discussing the studies of transactivation. Several herpesvirus gene products can transactivate HIV in co-transfection studies. However, we do not know if the physical form or sub-cellular location of the plasmids used in such experiments mimic authentically those of the HIV proviral DNA and herpesvirus DNA *in vivo*. For example, Koval *et al.* (1991) showed that CMV could transactivate HIV in co-transfection studies, but that CMV infection decreased production of HIV. It is, of course, possible that HIV is transactivated in some cells *in vivo* and inhibited in others. Inhibition is more likely when the herpesvirus is fully productive, especially when it replicates more rapidly than HIV to destroy the cell before productive HIV infection has become established. Stimulation is more likely when herpesvirus replication is not fully permissive (including the possibility of defective genomes), or when the herpesviruses replicate slower than HIV. In summary, for those interactions involving co-infection, an effective co-factor virus would be one that replicates as slowly, or more slowly than, HIV.

In-vivo studies. The natural history of HIV infection has two major clinical end-points: namely, development of AIDS and death. These two end-points can be used in clinical cohort studies to determine if the presence of a particular herpesvirus is associated with accelerated progression of HIV disease. When designing these studies, it is important to be able to distinguish between an opportunist and a co-factor relationship between HIV and the herpesviruses. For example, patients might be recruited at time of HIV seroconversion and divided into those serologically positive for EBV. Follow-up for several years might show that more of the seropositive individuals develop AIDS than do the seronegative patients. If so, it would be important to determine the AIDS-defining events in all patients. This is important because if an excess of B-cell lymphomas accounted for all of the excess cases of AIDS, this would reflect opportunistic disease caused by EBV. The same conclusion would apply if the patients were followed to death and excess cases among those seropositive for EBV were attributed to B-cell lymphomas. Thus, co-factor relationship with a herpesvirus can only be clearly invoked as an explanation for accelerated HIV disease progression once opportunism has been excluded (Griffiths, 1992).

Given this background, we set out to determine if there was any evidence for CMV acting as co-factor in a population of haemophiliacs infected with HIV. Our *a priori* hypothesis was that those serologically positive for CMV at the time of HIV seroconversion would develop AIDS more rapidly than those who were seronegative for CMV and that serological status for HSV would have no effect since, as described earlier, this virus replicates so rapidly that it would be likely to destroy the target cell rather than stimulate HIV replication. The results supported this hypothesis because the relative risk for developing AIDS among the CMV seropositives was 3.2 compared with 0.8 for the HSV seropositives. Furthermore, the CMV association was statistically independent of age because the relative risk of 2.5 after controlling the age by logistic regression was still statistically significant (Webster *et al.*, 1989). Continued follow-up of the patients has also shown a significant association between CMV and death (Sabin *et al.*, 1995). Rabkin *et al.* (1993) did not find an association between initial CMV serostatus and progression of AIDS. The reasons for the differences between these two studies are not clear.

Haemophilia cohorts are important because, unlike other risk groups, they contain sufficient numbers of CMV seronegative individuals to provide a comparison with seropositive patients. However, it is important to note that a CMV co-factor effect would be of relevance to most communities infected with HIV, since almost all individuals are co-infected with CMV.

CMV has also been found in children with congenital HIV infection (Frenkel *et al.*, 1990). Data from the small number of cases to date suggest that progression of HIV disease may be higher in those co-infected with CMV. If this observation is correct, there are two possible explanations: CMV may drive HIV replication in the mother leading to transmission of a greater inoculum of HIV; or CMV may drive HIV infection in the child leading to accelerated pathogenicity. Prospective studies of pregnant women infected with HIV will be required to differentiate between these two possibilities.

If a herpesvirus is to interact with HIV, both viruses must be found in close contact within target organs of the body. It is therefore of interest that, in a series of 47 autopsies of patients dying of AIDS we were able to culture CMV from at least one organ in 66% of cases (Pillay *et al.*, 1993). Subsequent PCR studies showed CMV and HIV co-infection of 52% of organs (Webster *et al.*, 1995). Further studies are required

to determine how frequently individual cells are coinfected with these two viruses (Wiley & Nelson, 1988).

Similar studies have shown that HHV-6 can be found frequently in patients dying from AIDS (Corbellino *et al.*, 1993; Knox & Carrigan, 1994). Thus, at least two herpesviruses β- have the potential to interact readily with HIV.

Therapeutic studies. In addition to helping our understanding of the pathogenesis of AIDS, the concept of herpesvirus co-factors suggests the possibility of conducting controlled trials of antiviral chemotherapy against the co-factor. Results of such trials must be interpreted with caution, however, since several putative co-factor herpesviruses are opportunists in their own right. Thus, one could only be certain that a drug was providing clinical benefit by suppressing a co-factor if opportunism caused by that virus was unaffected (Griffiths, 1992).

This is exactly the outcome in two placebo-controlled double-blind trials of high dose oral acyclovir conducted by Cooper *et al.* (1993) and Youle *et al.* (1994), since the drug had no effect on opportunistic CMV disease yet did produce a significant survival benefit. Further support is provided by an observational study by Stein *et al.* (1994), although they found a survival benefit among patients taking low dose acyclovir. These results suggest that a virus susceptible to acyclovir (presumably a herpesvirus) is involved in the pathogenesis of AIDS. The question of dose is being examined further in an AIDS Clinical Trials Group Sponsored Controlled Study (Protocol 204), comparing low dose acyclovir, high dose acyclovir and valaciclovir, and the results may provide some insight into which herpesvirus(es) is/are mediating this effect (Wood, 1996).

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