HIV-1 and HHV-6 Antigens and Transcripts in Retinas of Patients With AIDS in the Absence of Human Cytomegalovirus

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Purpose. The purpose of this study was to define the agents involved in the development of acquired immune deficiency syndrome (AIDS)-associated retinitis. To achieve this goal, the authors determined the frequency and proximity of the simultaneous presence of human immunodeficiency virus (HIV)-1, human herpesvirus (HHV)-6, and human cytomegalovirus (HCMV) in retinas of patients with AIDS with and without AIDS-associated retinitis.

Methods. Retinal sections from 50 globes from patients with AIDS were analyzed for the presence of viral antigens and transcripts. Group 1 contained 13 globes from patients with HCMV infection. Group 2 contained 20 globes from patients with retinal lesions of uncertain etiology in which HCMV antigen and transcripts were not detected. Group 3 contained 17 globes from patients with no retinal lesions.

Results. Retinal sections from all 13 globes (group 1) were positive for HCMV antigens and HIV-1 antigens and transcripts. Six of the 13 retinas were also positive for HHV-6 antigens and transcripts. Sections from 13 of the 20 globes (group 2) were positive for HIV-1 antigens and transcripts, and 5 of these 13 were also positive for HHV-6 antigens and transcripts. Multiple areas in sections from two of the HIV-1-positive retinas showed coinfection with HHV-6. All 17 globes (group 3) were positive for HIV-1 antigens and transcripts. Ten of these 17 retinas were also positive for HHV-6 antigens. Human cytomegalovirus antigens were not detectable in retinas from groups 2 and 3. No viral antigens or transcripts were detectable in retinal sections from 10 HIV-1 negative donors.

Conclusion. The coexistence of HIV-1 and HHV-6 activity in more than 50% of retinas without HCMV infection suggests that HIV-1 and HHV-6 alone or in combination may predispose retinal tissue to other opportunistic agents such as HCMV during the development of AIDS-associated retinitis. Invest Ophthalmol Vis Sci. 1995; 36:2040-2047.

Before death, more than 50% of patients infected with human immunodeficiency virus (HIV-1) experience significant visual loss caused by AIDS-associated retinitis.1 It also has been reported that human cytomegalovirus (HCMV) infection of the retina is a late manifestation of AIDS and a poor prognostic sign of this deadly disease.2 The etiology and mechanism(s) involved in the development of AIDS-associated retinitis are unknown. It is of critical importance to understand the pathobiology of this disease process to develop a rational approach to therapeutic intervention. Several investigators have demonstrated the presence of HIV-1 and human herpesviruses in retinal lesions from patients with AIDS.3–8 Recently, we have reported the simultaneous presence of HIV-1 and human herpesvirus type 6 (HHV-6) DNA sequences, transcriptional activity, and antigens in retinal lesions from patients with AIDS with HCMV retinitis.9–11 Similarly, Reux et al12 demonstrated the presence of HHV-6 antigens in retinas of two patients with AIDS. Interestingly, HHV-6 is selectively tropic for CD4+ T-lymphocytes in vivo, and it has been shown...
that coinfection of cells with HIV-1 and HHV-6 leads to an increased expression of HIV-1 antigens in vitro.13–16

Dual infection with HIV-1 and herpesviruses, specifically HHV-6, may have implications with regard to the pathogenic mechanisms involved in the development of AIDS-associated retinitis. The purpose of this study was to determine whether HIV-1 and HHV-6 are capable of infection or coinfection of retinal tissue without HCMV, which may lead to other opportunistic infection, such as HCMV, in the late stages of AIDS-associated retinitis. In the current study, retinal sections from 50 globes from 29 patients (with and without HCMV infection of retinas) were analyzed for the presence of viral antigens and transcripts. Though preliminary, the results of this study indicate that HIV-1 and HHV-6 are capable of infecting retinal cells with no HCMV infection.

Table 1. Viral Antigens and Transcripts in Retinal Sections From Patients With AIDS

<table>
<thead>
<tr>
<th>Globes Analyzed</th>
<th>CMV</th>
<th>HIV-1</th>
<th>HHV-6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antigens</td>
<td>Transcripts</td>
<td>Antigens</td>
</tr>
<tr>
<td>Group 1: 13 globes with CMV lesions</td>
<td>13</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>Group 2: 20 globes with lesions of unknown etiology</td>
<td>0,0</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Group 3: 17 globes with no lesions</td>
<td>0,0</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

ON = optic nerve. No viral antigens or transcripts were detected in retinal sections from 10 HIV-1 negative donors. Optic nerves from all 50 globes were tested for viral antigens and transcripts.
FIGURE 2. Retinal sections from AIDS globes with HCMV retinitis (group 1). Viral antigens were detected by immunoperoxidase staining using monoclonal antibodies and viral transcripts were detected by in situ hybridization using RNA probes. Human immunodeficiency virus (HIV)-1 and human herpesvirus (HHV)-6 positive cells are indicated by arrows. (A) HHV-6 antigens. (B) HHV-6 transcripts, dark field. (C) HHV-6 transcripts; confocal image of b.

FIGURE 3. Retinal sections from AIDS globes containing microlesions with no human cytomegalovirus infection (group 2). Viral antigens were detected by immunoperoxidase staining using monoclonal antibodies, and viral transcripts were detected by in situ hybridization using RNA probes. Human immunodeficiency virus HIV-1-positive and human herpesvirus (HHV)-6-positive cells are indicated by arrows. (A) HIV-1 antigens. (B) HIV-1 transcripts. (C) HHV-6 antigens.

MATERIALS AND METHODS
Globes
Fifty globes from patients with AIDS and 10 globes from donors who were HIV-1 negative were provided
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FIGURE 4. Retinal sections from AIDS globes containing microlesions with no human cytomegalovirus infection (group 2). Viral antigens were detected by immunoperoxidase staining using monoclonal antibodies, and viral transcripts were detected by in situ hybridization using RNA probes. Human immunodeficiency virus (HIV)-1-positive and human herpesvirus (HHV)-6-positive cells are indicated by arrows. (A) HHV-6 antigens. (B) HIV-1 transcripts in vicinity of same area as shown in (a). (C) HHV-6 antigens. (D) HIV-1 antigens in vicinity of same area as shown in (C).

after death by the Lions Eye Bank of Texas and the Eye Bank of University of Texas. Globes were received in RPMI 1640 + 5% fetal bovine serum (Gibco BRL, Gaithersburg, MD) within 24 to 72 hours of the patient's death. All donor material used in this study was collected at autopsy using the guidelines of the institutional review board. The globes were dissected and examined for lesions under a dissecting microscope. Fifty globes from 29 patients with AIDS were divided into three groups: Group 1 contained 13 globes from patients with HCMV infection (HCMV antigens were detectable in retinas); Group 2 contained 20 globes from patients with retinal lesions of unknown etiology (HCMV antigens or transcripts were not detectable in retinas); group 3 contained 17 globes from patients with no visible retinal lesions (HCMV antigens or transcripts were not detectable in retinas).

Retinal blocks (2X2 mm) containing lesions and suspected areas were fixed in 10% buffered formalin, processed, embedded in paraffin, and sectioned. Four-micron sections were placed on RNase-free polylysine coated slides and used for immunohistochemical staining and in situ hybridization.

Cells and Viruses

CCRF-HSB2 T-lymphocytes and human embryonic lung cells purchased from ATCC (Rockville, MD) were propagated and maintained as recommended by the supplier. The peripheral blood mononuclear cells (PBMC) were prepared by the Ficol–Hypaque gradient method as described previously. HHV-6 (GS) and HIV-1 (Bal, IIIB) were provided by Dr. Robert Gallo of the National Institutes of Health. Human cytomegalovirus (Ad169) was purchased from ATCC. For positive and negative controls, infected and uninfected cell pellets (peripheral blood mononuclear cells for HIV-1, CCRF-HSB2 for HHV-6, and human embryonic lung cells for HCMV) were fixed in 10% neutral buffered formalin and processed as described previously.

Immunoperoxidase Staining

Deparaffinized retinal sections with appropriate positive and negative controls were incubated with 0.3% hydrogen peroxide and rinsed twice with phosphate-buffered saline (PBS). Slides were then treated with pepsin (3 mg/ml in 0.2 N HCl) for 10 minutes at room temperature and washed twice in PBS containing 0.2% Triton X-100. The sections were incubated with 10% goat serum at 37°C for 4 hours. Monoclonal antibodies to p17 and p24 for HIV-1, antibodies to early and late proteins of HHV-6, and antibodies to late protein of HCMV (Chemicon, Temecula, CA) were diluted and overlaid on tissue sections and incubated overnight at 37°C in a moist chamber. The sections were rinsed with PBS containing 0.2% Triton X-100 and incubated for 1.5 hours at 37°C with a biotin-labeled secondary antibody (goat antimouse immunoglobulin G). The slides were then incubated for 1.5 hours at 37°C with the avidin-biotin-horseradish-peroxidase complex, and sites of peroxidase activity were visualized after incubation in 3-amino-9-ethylcarbozle with 0.01% hydrogen peroxide. The slides were counterstained for 10 seconds in Mayer's hematoxylin, then six drops of crystal mount (Fisher Scientific, Springfield, NJ) were applied to each section and baked at 60°C for 30
FIGURE 5. Optic nerve sections from AIDS globes with no visible lesions and no human cytomegalovirus infection (group 3). Viral antigens were detected by immunoperoxidase staining using monoclonal antibodies, and viral transcripts were detected by in situ hybridization using RNA probes. Human immunodeficiency virus (HIV)-1-positive and human herpesvirus (HHV)-6-positive cells are indicated by arrows. (A) HIV-1 antigens. (B) HIV-1 transcripts. (C) HHV-6 antigens.

minutes. The sections were mounted in Permount (Fisher Scientific) and examined.

In Situ Hybridization
Four-micron sections were placed on RNase-free polylsine-coated slides and subjected to in situ hybridization using $^{35}$S-labeled RNA probes. The pBH10R3 plasmid containing a 9-kb fragment of HIV-1 DNA was digested with EcoRI, and the pZVH14 plasmid containing a 8.7-kb fragment of HHV-6 was digested with EcoRV. The fragments were used to prepare $^{35}$S-labeled RNA probes with Riboprobe kits as recommended by the supplier (Promega, Madison, WI). Slides were briefly rinsed in 2 X saline sodium citrate (SSC), acetylated for 10 minutes, and treated with glycinate aldehyde groups for 30 minutes. The slides were then washed in 2 X SSC, dehydrated in ethanol, and air dried. The hybridization mixture containing the $^{35}$S-labeled RNA probe was added to each section, coverslipped, and incubated for 3 hours in a humidified incubator at 52°C. The slides were rinsed in 2 X SSC and incubated in prewarmed 50% formamide in 2 X SSC (vol/vol) for 20 minutes at 52°C. They were then rinsed in 2 X SSC four times and treated with RNase to digest single-stranded RNA. They were then rinsed, dehydrated in ethanol, and air dried.

For autoradiography, the slides were dipped in NTB-2 Kodak (Rochester, NY) emulsion, dried, and exposed for 2 to 3 days. For developing, slides were placed in chilled Kodak D-19 developer, water, and chilled fixative. After the slides were stained with Giemsa and mounted, they were screened for the presence of HIV-1 and HHV-6 transcripts using a BioRad (Richmond, CA) MRC-500 confocal laser scanning microscope.

RESULTS

Group 1
The globes for this group were from patients with AIDS with HCMV retinitis. Retinal blocks containing large and clinically advanced lesions were analyzed for the presence or absence of HCMV antigens and HIV-1, HHV-6 antigens, and transcripts. Retinal sections from all 13 globes were positive for HCMV antigens and HIV-1 antigens and transcripts (Table 1, Figs. 1a to 1c). Only one optic nerve was positive for HCMV antigens (Table 1, Fig. 1d). Six of the 13 retinas (46%) were also positive for HHV-6 antigens and transcripts (Table 1, Fig. 2).

Group 2
Retinas from this group had microlesions of uncertain etiology. Lesions in the retinal sections were visible only under the microscope. Retinal sections from 13 of 20 globes (65%) were positive for HIV-1 antigens and transcripts (Table 1, Figs. 3a, 3b). Only five of these 13 retinas (38.5%) were also positive for HHV-6 antigens and transcripts (Table 1, Fig. 3c). Multiple areas in two of those five retinas showed coinfection with HIV-1 and HHV-6 (Fig. 4). Optic nerves from
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**Group 3**

Retinas from this group were devoid of lesions even when examined under a high power objective. Optic nerves from five globes (30%) were positive for HIV-1 antigens and transcripts (Table 1, Figs. 5a, 5b). One of the five HIV-1 positive optic nerves was also positive for HHV-6 antigens and transcripts (Table 1, Fig. 5c). Retinal sections from all 17 globes showed HIV-1 antigens and transcripts (Table 1, Figs. 6a, 6b), and HHV-6 antigen-positive cells were detectable in only 10 of the 17 (59%) HIV-1 positive retinas (Table 1, Fig. 6c). No HCMV antigens or transcripts were detectable in retinas of the 17 globes analyzed.

The retinal cell type predominantly infected with HIV-1 and HHV-6 was Müller cell (data not shown). In addition, no viral antigens or transcripts were detectable in retinal sections from 10 donors who were HIV-1 negative.

**DISCUSSION**

Though preliminary, the results of this study demonstrate that HIV-1 and HHV-6 are capable of infecting certain retinal cells with no HCMV infection. The presence of HIV-1 and HHV-6 activity in more than 50% of retinal tissues with no HCMV infection may have etiologic implications with regard to the pathogenic mechanism involved in the development of AIDS-associated retinitis in persons with HIV-1 infection.

Although several herpesviruses have been observed routinely in AIDS-associated retinitis, the actual etiology of this disease process has not been determined. It is unknown whether HIV-1 alone or in conjunction with herpesviruses, such as HHV-6, is associated with the pathologic changes observed during the development of AIDS-associated retinitis. It has been suggested that HIV-1 may directly or indirectly damage retinal tissue and interact with opportunistic pathogens leading to the variety of ocular abnormalities observed with AIDS. Interestingly, HIV-1 has been isolated from and demonstrated within lesional and nonlesional retinal tissue. These data suggest that HIV-1 alone may not cause clinically advanced retinal lesions.

AIDS is a complex disorder; several cofactors may be involved in its pathogenesis. Some of these cofactors may involve the herpes group viruses. Several human herpesviruses (HSV-1, HCMV, and Epstein–Barr virus) have been shown to transactivate HIV-1 long terminal repeat (LTR)-directed gene expression in vitro. However, none of these pathogens has the ability to invade and replicate efficiently in CD4+ T-lymphocytes. Therefore, their mode of activation of HIV-1 is unclear. On the other hand, HHV-6 and...
HHV-7 are selectively tropic and replicate well in CD4+ T-lymphocytes. It has been shown that the expression of HIV-1 and the destruction of CD4+ T-lymphocytes was increased in cultures coinfected with HHV-6 and HIV-1 compared to cultures with HIV-1 infection alone. Even though Carrigan et al reported the suppression of HIV-1 replication in the presence of HHV-6, the same study showed that the rate of cell destruction was increased in cultures coinfected with both viruses. This observation was not surprising because HHV-6 itself is cytopathic to CD4+ lymphocytes that might accelerate cell death and because enhancement of HIV-1 expression might be HHV-6 dose dependent. It has been shown also that HHV-6 infection induces nuclear factors that specifically bind to the enhancer region of HIV-1 LTR. DNA sequences able to cause activation of HIV-1 LTR in vitro have been identified in the immediate early locus of HHV-6. Recently, preliminary data from another study in our laboratory indicated that when HIV-1 and HHV-6 interact, they may activate each other.

Similar bidirectional activation between HIV-1 and HCMV, and between HIV-1 and HHV-6, have been reported. Another study indicated that compared to HCMV infection, the rate of productive HHV-6 infection was significantly increased in tissues from nine patients with AIDS. Because of the unique biologic characteristics of HHV-6—specifically its immunotropic nature, similar cell tropism, and positive interaction with HIV-1—a catalytic role for HHV-6 during the progression of HIV-1 infection to full-blown AIDS has been proposed.

The most significant findings of this study are the presence of HIV-1 and HHV-6 activity in retinas without HCMV infection and the dual infection of multiple sites of retinas from two patients with AIDS with HIV-1 and HHV-6. However, it cannot be determined with certainty whether individual cells from these areas were coinfected. In addition, the retinal cells with frequent HIV-1 and HHV-6 activity were ganglion cells. Similar observations have been reported by other investigators. Overall, these observations suggest that HIV-1 and HHV-6, alone or in combination, may predispose retinal tissue to other opportunistic agents, such as HCMV, in the late stages of AIDS-associated retinitis. It is also very likely that HIV-1, along with other pathogens such as HHV-6, may be responsible for the viral retinitis seen in certain patients with AIDS with no HCMV infection (unpublished observations, 1994). A suitable animal model or in vitro studies involving infection of retinal cells with HIV-1 alone or in combination with HHV-6–HCMV may clarify the role of these pathogens in the development of AIDS-associated retinitis.

Key Words
AIDS-associated retinitis, HCMV, HCMV retinitis, HHV-6, HIV-1, human retina

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References