Active Replication of Human Immunodeficiency Virus Type 1 by Peripheral Blood Mononuclear Cells Following Coincubation With Herpes Viruses

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Patients with acquired immunodeficiency syndrome (AIDS) commonly suffer from opportunistic infections associated with members of the herpes virus family. To investigate whether certain of these other viruses might have an effect on the ability of the human immunodeficiency virus type 1 (HIV-1) to replicate, we coincubated peripheral blood mononuclear cells (PBMC) from nine HIV-1-seropositive donors with live preparations of various herpes viruses. In seven of nine cases, exposure of PBMC to preparations of either HSV-1, HSV-2, or CMV stimulated the cells to become active producers of HIV-1, as determined by reverse transcriptase activity and by the presence of infectious progeny virus. This increased production of HIV-1 particles appeared to be a consequence of mitogenic proliferation and of herpes virus-encoded transacting factors. These results supplement earlier findings on the molecular activation of the HIV-1 genome by both HSV and CMV genetic elements and point to a possible role for these viruses in the pathogenesis and ultimate clinical outcome of HIV-1 infections.

KEY WORDS: cytomegalovirus, lymphocyte stimulation, transacting factors

INTRODUCTION

HIV-1 is the etiological agent of AIDS [Barre-Sinoussi et al., 1983; Gallo et al., 1984; Levy et al., 1984]. The virus can selectively infect and kill T helper-inducer lymphocytes that bear the CD4 (T4) epitope at their surface [Dalgleish et al., 1984; Gallo et al., 1984; Levy et al., 1984]. These cells, but not the T lymphocyte subset that bears the CD8 (T8) epitope (suppressor-cytotoxic cells), are preferentially destroyed following infection by HIV-1, leading to a noticeable decrease in the T4/T8 ratio [Weiss et al., 1985].

In HIV-1–infected individuals, viral replication may occur over long periods in the absence of clinical symptoms and despite the presence of an anti-HIV-1 immune response [Jaffe et al., 1985]. It has been demonstrated that mitogen-stimulated T4 cells are much more susceptible to HIV-1 infection and replication than are unstimulated lymphocytes. Furthermore, the replication of HIV-1 in cultured cells was itself found to be proportional to the extent of T-cell activation [Folks et al., 1986]. Lymphocytes from healthy donors who had been immunized with tetanus toxoid displayed enhanced susceptibility to HIV-1 infection, when the cells were first activated in vitro by this same antigen [Margolick et al., 1987]. It has thus been hypothesized that stimulation of the immune system, by bacterial and/or viral pathogens, may play a role as a cofactor in the pathogenesis of AIDS [Montagnier et al., 1984] by enhancing the efficiency of HIV-1 infection [Zagury et al., 1986]. This might help to explain why only a proportion of individuals infected by HIV-1 have developed full-blown AIDS, and it could also account for the broad spectrum of clinical outcomes that are seen in infected individuals.

Members of the herpes family of viruses have been suggested as possible cofactors in the development of AIDS because of their documented immunosuppressive effects, ubiquitous presence, and, in some cases, lymphotropism. Moreover, herpes virus infections are very common in homosexual men, who constitute a high-risk group for development of AIDS [Siegal et al., 1981; Quinnan et al., 1984]. HSV is commonly isolated from AIDS patients, who often suffer from more severe HSV-induced lesions than those of otherwise healthy individuals [Siegal et al., 1981]. In addition, AIDS patients
generally have a high frequency of CMV-associated disease [Welch et al., 1984], elevated levels of anti-CMV antibodies [Halbert et al., 1986], and are active shedders of this virus [Polk et al., 1987].

We have therefore studied the possible role of these viruses with regard to stimulation of HIV-1 replication in the PBMC of HIV-1 seropositive donors. The results indicate that HSV-1, HSV-2, and CMV are each able to trigger overt replication of HIV-1.

MATERIALS AND METHODS

Subjects

The study population consisted of eight AIDS patients being treated at the Dental Clinic of the Jewish General Hospital (Montreal, Canada) and of one asymptomatic, seropositive child followed at the Department of Pediatrics, Hôpital Ste-Justine (Montreal, Canada). All of these individuals, except for the child, fulfilled the criteria for AIDS of the Centers for Disease Control [1987]. None of these patients was on therapy at the time of study. All patients had immunological evaluations that included Ab titers for each of CMV and HSV as determined by complement fixation. Levels of Ab against hepatitis B surface antigen (HBsAg) were determined by ELISA assay (Abbott Laboratories, North Chicago, IL).

Viruses

Clinical isolates of HSV-1, HSV-2, and CMV were obtained from the Virology Laboratory of the Montreal Children's Hospital. Two different isolates of CMV (DL-4 and LB-1) were utilized; both had been derived from neonatal cases of congenital cytomegalic disease.

HSV-1 and HSV-2 were expanded to high titer in Vero cells. At times of extensive cytopathology, culture fluids from infected monolayers were clarified by low-speed centrifugation and filtered through Millipore filters (0.45 μM). Titration of HSV-1 and HSV-2 was carried out by plaque assay on monolayers of Vero cells [Rapp, 1963].

CMV was also passaged on Vero cells. After 5–7 days, by which time the observed cytopathology involved at least 90% of the monolayer, culture fluids were clarified and filtered. Virus was titrated by plaque assay [Wentworth and French, 1970] and stored at -70°C until used.

Coincubation of Cells With Various Stimuli

Peripheral blood mononuclear cells (PBMC) from HIV-1–infected individuals were isolated by Ficoll-Paque (Pharmacia, Piscataway, Nj) density gradient centrifugation [Boyum, 1968]. The cells were resuspended to a concentration of 1 × 10⁶ cells/ml in culture medium (RPMI-1640, supplemented with L-glutamine [2 mM], penicillin [250 U/ml], streptomycin [250 μg/ml], and fetal calf serum [10% v/v]) and seeded into each well (1.5 ml) of a 24 well tissue culture plate (Flow Laboratories, McLean, VA). The cells were placed into contact with a variety of potential viral stimuli including HSV-1 (multiplicity of infection [moi], 0.00012), HSV-2 (moi, 0.00012), and CMV (moi, 0.00002). In some experiments, we examined what effect either anticytokine antibodies or viral antigens might have on HIV-1 replication by coincubation of cells with anti-Leu 4 (final concentration, 1:1,000) and HBsAg (Heptavax-B vaccine [0.2 μg]). Culture controls were PBMC from HIV-1–infected individuals coincubated with conditioned medium from Vero cells. The cells were incubated at 37°C under 5% CO₂ for 48 hr, following which they were centrifuged and resuspended in antigen-free culture medium, supplemented with 5% recombinant interleukin-2 (r-IL-2, Boehringer-Mannheim, West Germany) and polybrene (2 μg/ml). Thereafter, this supplemented medium was used to reseed the cells twice weekly. Beginning on the seventh day following such coincubation, culture fluids were harvested at regular intervals, pelleted by ultracentrifugation, and assayed for reverse transcriptase activity by a previously published procedure [Hoffman et al., 1985].

Lymphocyte Proliferation

PBMC from HIV-1–infected patients were cultured in flat-bottomed microtiter plates, using three replicate samples per assay (1.5 × 10⁶ lymphocytes/0.15 ml/well). Either these cells were cultured in the absence of exogenous stimulant, or stimulation was attempted by including any of several live viral preparations and/or anticytokine monoclonal antibodies. Blastogenic responses were measured by adding 1 μCi/well of tritiated thymidine (specific activity, 30 Ci/mmol; New England Nuclear, Boston, MA), 4 hr prior to culture termination. The results are expressed as net cpm per well.

Infectivity of HIV-1 Recovered Following Cell Stimulation

The infectivity of HIV-1, isolated after coincubation of PBMC with herpes viruses, was assessed using umbilical cord blood mononuclear cells from healthy donors as targets. Such cells were isolated and stimulated with PHA-P₁ (0.1%) for 48 hr and were then treated with polybrene (2 μg/ml) for 20 min at 37°C, resuspended at a concentration of 10⁶ cells/ml in growth medium, and dispensed into a 24 well tissue culture plate at a volume of 1.5 ml. All HIV-1 reverse transcriptase-positive samples isolated following coincubation of PBMC with herpes viruses were inoculated onto the previously stimulated cord blood mononuclear cells. Supernatant fluids were harvested and assessed for reverse transcriptase activity.

RESULTS

Table I is a summary of the clinical and immunological features of the nine patients studied. All patients but two (Nos. 4 and 9) had a positive antibody titer against CMV. Only two patients (5 and 9) did not have detectable antibodies against HSV. All patients were seronegative for hepatitis B virus (data not shown).
The most common opportunistic organisms diagnosed in the studied population were Candida albicans (six cases) and herpes simplex virus (three cases).

The results in Table II show that certain of the viral preparations employed were able to generate a lymphocyte blastogenic response when coincubated with cells from HIV-1-seropositive donors was carried out. In contrast, controls consisting of PBMC from HIV-1-infected patients, not so coincubated with HSV and/or CMV preparations, showed little, if any, blastogenic response. It is noteworthy that mononuclear cells from six of nine patients tested showed some degree of blastogenic response to each of two CMV strains employed as antigen. Interestingly, the only instances in which the various CMV antigenic preparations were found to be completely devoid of stimulatory activity were in the case of two patients (4 and 9) who were seronegative for anti-CMV antibodies. The results in Table II also indicate that the various HSV antigenic preparations employed were unable to serve as inducers of lymphocyte blastogenesis. This is despite the fact that seven of the nine patients studied were seropositive for HSV-1 and/or HSV-2. This finding is consistent with previous observations from our laboratory that showed that specific HSV-mediated lymphocyte proliferation, in the case of AIDS patients, could only be elicited after addition to cultures of exogenous interleukin-2 [Wainberg et al., 1987]. HB,Ag was similarly unable to elicit any blastogenic response, as demonstrated in each of three cases in which this antigen was used (data not shown). In contrast, the use of antibodies directed to the CD3 antigen (anti-Leu 4) gave rise, in most cases, to a very strong blastogenic response.

The major aim of this study was to demonstrate whether coincubation of PBMC from HIV-1-infected individuals with viruses such as CMV and HSV might be able to trigger production of HIV-1 on the part of cells that had been infected by this agent but that were not actively engaged in HIV-1 replication. The results in Table III show that active production of HIV-1 was elicited in seven of nine cases in which patient cells were coincubated with live CMV, as demonstrated by the presence of reverse transcriptase activity in culture fluids. Those patients for whom only one strain of CMV is listed were able to respond to that strain only but not to the other strain of CMV utilized or to any of the other viral preparations tested. The various CMV preparations used were able to effect active replication of HIV-1 in the case of seven patients. One of the CMV strains utilized, LB-1, appeared to induce HIV-1 recovery with relative efficiency and permitted the isolation of infectious HIV-1 in each of seven cases studied. In contrast, the other CMV strain employed, DL-4, was able to elicit HIV-1 recovery in two cases only. Positive recovery of HIV-1 was achieved only once, out of the nine cases studied, when HSV-1 and HSV-2 were used as stimulating agents. Recovery of HIV-1 could not be accomplished using HB,Ag as stimulus (results not shown). HIV-1 was recovered in only one instance in which anti-Leu 4 was used as a mitogen. The time to HIV-1 culture positivity in these studies ranged between 11 and 32 days (Table III).

The potential infectiousness of HIV-1 recovered following coincubation of PBMC with these various herpes viruses was also evaluated. The results of Table III show that such isolated HIV-1 was able to infect PHA-stimulated cord blood mononuclear cells from healthy donors in each instance.

**DISCUSSION**

It has been hypothesized that a variety of factors might enhance replication of HIV-1 by virus-infected cells [Quinnan et al., 1984; Siegal et al., 1981]. We postulated that members of the herpes family of viruses might fulfill such an enhancing role in the presence of PBMC, latently infected by HIV-1. To test this hypothesis, PBMC from HIV-1-infected individuals were coincubated with low-passage clinical isolates of herpes viruses including CMV, HSV-1, and HSV-2. We now report that such coincubation, primarily with CMV can cause active replication of HIV-1 in latently infected cells and/or in cells that produce low levels of HIV-1. Such active replication was never observed when conditioned medium from Vero cells, used to grow herpes viruses, was employed as a stimulant.

The technique commonly used to isolate HIV from asymptomatic, infected individuals and from patients with AIDS and/or ARC involves an initial stimulation step by PHA, a lectin with polyclonal activation properties for T lymphocytes [Montagnier, 1986]. The fact that we were able to recover HIV-1 only once when using anti-Leu 4 as a mitogen, despite high blastogenic responses in six cases, is intriguing. This monoclonal antibody acts, like PHA, by causing PBMC to enter mitosis, an event thought to be requisite to retroviral replication. Early methods of HIV-1 isolation did not use, as in the present study, coculture of PHA-stimulated normal PBMC with the patient's PHA-stimulated cells. Instead, only PHA-stimulated PBMC from AIDS and ARC patients were employed and yielded recovery rates less than 50% (Gallo et al.,
TABLE II. Blastogenic Responses to Viral Preparations and to Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Patients (cpm × 10^{-3})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CMV (DL-4)</td>
<td>59.10</td>
</tr>
<tr>
<td>CMV (LB-1)</td>
<td>20.23</td>
</tr>
<tr>
<td>HSV-1</td>
<td>3.85</td>
</tr>
<tr>
<td>HSV-2</td>
<td>19.74</td>
</tr>
<tr>
<td>Anti-Leu 4</td>
<td>79.76</td>
</tr>
<tr>
<td>Unstimulated control</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*Those entities employed as stimuli of lymphocyte mitogenesis included live preparations of CMV, HSV, and anti-Leu 4 monoclonal antibodies.

N.T.: not tested.

TABLE III. Recovery of HIV-1 From Cultures of Patient Mononuclear Cells Following Coincubation With Live Herpes Viruses

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stimulant</th>
<th>No. of days required for culture positivity</th>
<th>Reverse transcriptase activity at time of initial HIV-1 recovery (cpm/ml × 10^{-6})</th>
<th>Reverse transcriptase activity when primary isolates of HIV-1 were inoculated onto cord blood lymphocytes (cpm/ml × 10^{-6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CMV (LB-1)</td>
<td>25</td>
<td>1.20</td>
<td>3.79</td>
</tr>
<tr>
<td>3</td>
<td>CMV (DL-4)</td>
<td>11</td>
<td>0.10</td>
<td>2.57</td>
</tr>
<tr>
<td>4</td>
<td>CMV (LB-1)</td>
<td>11</td>
<td>0.09</td>
<td>2.66</td>
</tr>
<tr>
<td>5</td>
<td>HSV-1</td>
<td>25</td>
<td>0.02</td>
<td>4.99</td>
</tr>
<tr>
<td>6</td>
<td>HSV-2</td>
<td>32</td>
<td>0.07</td>
<td>4.50</td>
</tr>
<tr>
<td>7</td>
<td>CMV (LB-1)</td>
<td>18</td>
<td>0.05</td>
<td>1.66</td>
</tr>
<tr>
<td>8</td>
<td>CMV (DL-4)</td>
<td>25</td>
<td>0.69</td>
<td>3.87</td>
</tr>
<tr>
<td>9</td>
<td>CMV (LB-1)</td>
<td>25</td>
<td>0.22</td>
<td>2.75</td>
</tr>
<tr>
<td>10</td>
<td>CMV (LB-1)</td>
<td>18</td>
<td>0.03</td>
<td>3.43</td>
</tr>
<tr>
<td>11</td>
<td>Anti-Leu 4</td>
<td>28</td>
<td>0.02</td>
<td>2.14</td>
</tr>
<tr>
<td>12</td>
<td>CMV (LB-1)</td>
<td>12</td>
<td>0.03</td>
<td>2.52</td>
</tr>
</tbody>
</table>

HIV-1 could not be recovered from the lymphocytes of patients 2 and 6 in spite of coincubation with each of the CMV and HSV preparations described.

A sample was considered positive when reverse transcriptase activity was >0.01 cpm/ml × 10^{-6}. Unstimulated PBMC from HIV-1-infected individuals consistently had reverse transcriptase levels <0.0001 cpm/ml × 10^{-6}.

Reverse transcriptase determinations were carried out 10 days after inoculation of PHA-stimulated cord blood lymphocytes by primary isolates of HIV-1.

In our study, the failure of anti-Leu 4 to elicit HIV-1 replication could reflect the normally low levels of infected PBMC in infected individuals [Harper et al., 1986] and the specificity of the antibodies for the CD3 epitope.

In contrast, efficient HIV-1 recovery was achieved in 78% of cases when various herpes viruses were employed. This process may involve both immunological recognition by HIV-1-infected cells of herpes antigens as nonself and molecular activation by herpes virus-transacting factors. While no blastogenic responsiveness to CMV itself (strain LB-1) was detected, as expected, with each of two CMV-seronegative individuals (subjects 4 and 9), coincubation of their lymphocytes with CMV led to production of HIV-1, suggesting that molecular activation had probably occurred. However, antigenic stimulation as a mechanism responsible for HIV-1 recovery cannot be ruled out, since most CMV-seropositive individuals showed high blastogenic responses in the presence of this virus.

The discrepancy observed in recovery of HIV-1, following coincubation of cells with either anti-Leu 4 or herpes viruses, may be explained by a dual effect of these viruses. Following antigenic stimulation, T cells that recognize herpes antigens as nonself might be stimulated to divide; such activation, in concert with the effects of herpes transacting factors, could lead to active HIV-1 replication.

It is relevant that HSV-1 can cause transcription of latent HIV-1 [Mosca et al., 1987a,b] by transactivating the long terminal repeat (LTR) region of the latter virus [Gendelman, 1986]. The HSV-1 genes responsible for such HIV-1 activation are those that encode immediate early gene products [Nabel et al., 1988]. Similarly, the immediate early gene region of CMV can also stimulate HIV-1 gene expression [Davis et al., 1987].

Serological and immunological observations further reinforce the potential role that herpes viruses might play in HIV-1-infected individuals. One report demonstrated a close association between HSV-2 and HIV-1 infection [Holmberg et al., 1988]. Another study demonstrated enhanced replication of HIV-1 following in-
fection by CMV of two chronically HIV-1-infected cell lines [Skolnik et al., 1988].

The developments that lead in vivo to productive HIV-1 replication from latent infection are still obscure. However, since reactivation and/or reactivation by herpes viruses do take place, such events may explain why some HIV-1-infected individuals develop symptoms and opportunistic diseases within a relatively short period. Indeed, the immunosuppressive state induced by HIV-1 may facilitate reactivation and/or reactivation by latent herpes viruses that may, as shown here, have an enhancing effect on HIV-1 replication. The fact that immunologically healthy individuals are commonly infected by a single strain of CMV [Spector, 1982], whereas patients with AIDS are infected by multiple CMV strains, is of interest in this regard [Drew et al., 1984].

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