Susceptibility of EBV-carrying B cell lines to infection by HIV-1: variability of production of progeny virus and expression of viral antigens

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Summary

We have examined 3 different EBV-carrying B cell lines, in terms of ability to be super-infected by the human immunodeficiency virus (HIV-1), and have followed these lines, after infection by HIV-1, over a period of 3 months. We found significant variation among different HIV-1 strains in terms of the multiplicity of infection required to initiate infection in these EBV-positive cell lines. Persistent infection by HIV-1 in the absence of detectable cytopathic effects could be demonstrated, as evaluated by a variety of techniques, including reverse transcriptase assay and immunofluorescence. The results indicate also that all of these cell lines produced progeny HIV-1 intermittently, with large amounts of virus production on some days but not others. In contrast, they were all able to continuously express p24.

Keywords: HIV-1; EBV; B cells; antigen; progeny virus.

Introduction

Infections caused by herpes viruses are common in most high risk groups for acquired immunodeficiency syndrome (AIDS), in North America, including homosexual men [9]. Epstein-Barr virus (EBV) is a member of the herpes virus family, which has been associated with AIDS because of the finding that a Burkitt's lymphoma-like disease, termed undifferentiated lymphoma is present in many AIDS patients. In addition, the presence of typical chromosomal translocations and EBV-specific DNA have been found in these undifferentiated lymphomas [6]. Infections caused by EBV, a ubiquitous lymphotropic virus, are generally self-limited. Most healthy adults continue to possess latently infected B cells following primary EBV infections. Patients with AIDS and with AIDS-associated diseases commonly suffer from defects with regard to T cell regulation of EBV-infected B cells [3,34]. It has further been demonstrated that patients with AIDS and AIDS-associated diseases commonly have high numbers of B cells that can produce anti-HIV-1 antibodies in vitro as well as antibodies against other
pathogens [34]. Commonly, such hypergammaglobulinemia is also associated with EBV infection [17]. EBV has also been isolated from the nasopharyngeal secretions of over 70% of homosexual men with AIDS-related disease [31]. Hitherto, EBV isolation at this frequency has only been seen in severely immunocompromised EBV-infected individuals and in cases of acute infectious mononucleosis [7]. Furthermore, high titers of anti-Epstein-Barr nuclear antigen (EBNA) and an absence of IgM antiviral capsid antigen (VCA) are observed in cases of EBV reactivation rather than primary infection in patients with HIV-1-induced disease [31].

Thus, it is speculated that these two viruses might contribute jointly to a worsening of immunological and clinical status in infected individuals through a reactivation of latent EBV infection by HIV-1 [27]. This thought is strengthened by the notion that reactivation of EBV is common at times of immunosuppression. Furthermore, it has been demonstrated that HIV-1 can infect EBV-transformed B cell lines [18,22,28,32]. Finally, an increase in HIV-1 production was observed in cases in which such B cells had been previously transformed by EBV [22].

We have initiated this study to further determine the susceptibility of EBV-carrying B cells to HIV-1 infection. Our data indicate that HIV-1 production by such cells can continue over long periods. However, such production is commonly intermittent with high levels of progeny virus being released into culture fluids on some days but not others. Moreover, these cells continue to express high levels of HIV-1-directed proteins in their cytoplasm, even at times when little or no progeny virus can be detected. We have further found that differences exist among EBV-carrying B cell lines with regard to the multiplicity of infection of HIV-1 required to initiate replication and production of progeny HIV-1 virions.

Materials and methods

Cell lines

Density gradient centrifugation (Ficoll-Paque, Pharmacia Inc. Piscataway, NJ) was used to separate mononuclear cells from either peripheral or umbilical cord blood [4]. A suspension of 10^6 cells/ml (0.8 ml) was incubated together with 0.2 ml of infectious EBV at 37°C in RPMI 1640 medium supplemented with L-glutamine (2 mM), streptomycin (250 µg/ml), penicillin (250 units/ml), and 10% (v/v) fetal bovine serum. Fresh medium was added twice weekly [16]. Establishment of lymphoblastoid cell lines was ascertained by the ability to sustain frequent subdivisions, presence of immunoglobulin surface markers, and formation of clumps [21].

Once established, such lymphoblastoid B cell lines were pretreated with polybrene (2 µg/ml) for 20 min and incubated with HIV-1 (multiplicity of infection = 0.00075—0.0025) at 37°C for 3 h with continuous mixing. Unbound virus was removed by centrifuging the cells which were then resuspended in fresh medium. Medium changes were carried out every 3—4 days.

In some cases, the infectivity of viruses isolated from HIV-1-infected EBV-transformed B cells was determined using each of PHA-stimulated peripheral and umbilical cord blood lymphocytes as targets [18]. The H-9 line of T lymphocytes (kindly supplied by Dr. R.C. Gallo, National Cancer Institute, Bethesda, MD) was also used as a target for progeny HIV-1.

Viruses

Cell-free culture fluids of the B95-8 marmoset cell line, cultured for 7 days without change of medium, were used as a source of infectious EBV [21]. Culture fluids of HIV-1 (III_b strain)-infected H-9 lines were used as a source of infectious HIV-1 [10].

Two clinical isolates of HIV-1, obtained from infected individuals, were also used
(viral isolates 334 and 336). Titers of HIV-1 were determined by plaque assay on a MT-4 cell line that had been pre-infected by the human T lymphotropic virus type I (HTLV-I) [13].

**Assays for cell-surface markers and viruses**

The presence of Epstein-Barr nuclear antigen (EBNA) was detected by the anti-complement immunofluorescence test [26] and viral capsid antigen (VCA) was determined by indirect immunofluorescence using sera from healthy adults. Further characterization of the cell lines was achieved by a live cell indirect immunofluorescence assay using mouse monoclonal antibodies OKT3 (Ortho Corp. Rariton, NJ) and B1 (Coulter Co. Hialeah, FL). These cell lines were also tested for the expression of CD4 antigen by indirect immunofluorescence using anti-Leu 3a (Beckton-Dickinson, Mountain View, CA). HIV-1-infected cells were monitored for viral p24 by indirect immunofluorescence [10] and for release of progeny virus by reverse transcriptase assays performed on culture fluids [14].

**Radioimmunoprecipitation**

Cells were incubated for 1 h at 37°C in culture medium devoid of L-cysteine. They were then incubated for 16 h at 37°C in minimum essential medium containing [35S]cysteine (1000 Ci/mmol, DuPont Scientific Products, Boston, MA; 125 μCi/ml). Labeled cells were lysed with radioimmunoprecipitation assay (RIPA) buffer. The supernatant of a 10,000 × g centrifugation of the cell lysate was then used in immunoprecipitation experiments. Toward this end, aliquots of lysates (200 μl) were incubated with 5 μl of serum from a HIV-1-seropositive individual for 1 h at 37°C and overnight at 4°C. Immune complexes were adsorbed on protein A-Sepharose beads. After 30 min of incubation at 4°C, these complexes were washed twice by centrifugation in RIPA buffer, denatured by heating for 3 min at 100°C and electrophoresed on 7.5—12% polyacrylamide-SDS slab gels as previously described [2].

**Results**

We were interested in evaluating the

<table>
<thead>
<tr>
<th>Table 1. Lowest MOI needed to initiate productive HIV-1 infection of EBV-transformed B cell lines or peripheral mononuclear cells.</th>
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<tbody>
<tr>
<td><strong>Cell type</strong></td>
</tr>
<tr>
<td>EBV-transformed</td>
</tr>
<tr>
<td>B cell lines</td>
</tr>
<tr>
<td></td>
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<tr>
<td></td>
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<tr>
<td>Peripheral mononuclear cells</td>
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*Peripheral mononuclear cells were from 5 different HIV-1 seronegative individuals.
types of interactions occurring between HIV-1 and EBV-carrying B cells. Accordingly, EBV-positive B cell lines were infected with either the III\textsubscript{b} strain of HIV-1 or with either of two clinical isolates of HIV-1, variants 334 and 336.

We first employed OKT3 monoclonal antibodies (Ortho Co. Rariton, NJ) in an indirect immunofluorescence assay to determine that no T cells were present in our cultures. We found using B\textsubscript{1} monoclonal antibodies (Coulter Corp., Hialeah, FL) that over 99% of the cells being followed were B cells. Moreover, we performed studies on EBV antigen expression and found that all EBV-carrying B cell lines remained EBNA-positive and VCA-negative following HIV-1 infection (data not shown). These EBV-positive B cell lines, whether infected or not by HIV-1, were devoid of CD4 surface markers as determined by indirect immunofluorescence. In contrast, CD4 antigen was detected in 54.9% of peripheral blood mononuclear cells isolated from healthy donors (data not shown).

The data of Table 1 show that approximately 10 times as many infectious HIV-1 particles were required to initiate productive HIV-1 infection of EBV-transformed B cell lines, in comparison with mononuclear peripheral blood cells of healthy donors. Average multiplicities of infection (MOI) for these two cell types were 0.0018 and 0.0002, respectively. Moreover, we observed that about the same amount of the III\textsubscript{b} strain of HIV-1 was required to initiate infection, regardless which of 3 different EBV-transformed cell lines, established from different donors, were employed as targets (lines 1, 2, 3). In contrast, variation was observed between the two clinical isolates and III\textsubscript{b} in terms of ability to initiate infection of a common EBV-transformed B cell line (line 1).

The potential infectiousness of virus released from one HIV-1-infected EBV-

<table>
<thead>
<tr>
<th>Target cells</th>
<th>Days after infection</th>
<th>% Fluorescent cells\textsuperscript{a}</th>
<th>Reverse transcriptase activity in culture fluids (cpm/ml \times 10\textsuperscript{-3})</th>
</tr>
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<tr>
<td>H-9</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>20</td>
<td>n.d.\textsuperscript{c}</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>19</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>21</td>
<td>96.5</td>
</tr>
<tr>
<td>Peripheral blood lymphocytes</td>
<td>7</td>
<td>12</td>
<td>61.3</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10</td>
<td>72.0</td>
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<tr>
<td></td>
<td>17</td>
<td>9</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0</td>
<td>0.8</td>
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<tr>
<td>Cord blood lymphocytes</td>
<td>7</td>
<td>12</td>
<td>303.4</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5</td>
<td>94.3</td>
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<td>15.5</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0</td>
<td>0.7</td>
</tr>
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\textsuperscript{a} All virus in this study was harvested from culture fluids of cell line 1.

\textsuperscript{b} Cells were stained for indirect immunofluorescence using monoclonal antibody against viral p24 antigen.

\textsuperscript{c} n.d., not done.
Fig. 1. Radioimmunoprecipitation of 35S-labeled viral proteins. Extracts used were from: lane a, HIV-1 (IIIb strain)-infected H-9 cells; lane b, uninfected H-9 cells; lane c, HTLV-IIIb-infected EBV-transformed B cell line; lane d, EBV-transformed B cell line, not infected by HIV-1. Numbers on the left are molecular size markers in kDa.

transformed B cell line (line 1) was next investigated. The data of Table 2 show that the virus present in such fluids could indeed efficiently infect susceptible H-9 cells and PHA-stimulated peripheral and umbilical cord blood lymphocytes.

Fig. 2. Reverse transcriptase activity (cpm/ml × 10^2) in culture fluids after HIV-1 infection of EBV-transformed B cell lines. Three different EBV-transformed cell lines, established from different donors (line 1, []; line 2, •; line 3, □) were infected with the IIIb strain of HIV-1. In addition line 1 was infected by clinical isolates 334 (O) and 336 (■).

Once HIV-1 infection was clearly established in the EBV-carrying cell lines, we used radioimmunoprecipitation to investigate the synthesis of HIV-1 antigens (cell line 1). As demonstrated in Fig. 1, this HIV-1-infected EBV-transformed B cell line expressed the characteristic viral antigens of HIV-1, i.e. p55, p41, p24.

The data of Fig. 2 show that enormous variation was present, from one sampling to another, of production of progeny HIV-1 by EBV-transformed cell lines, as determined by reverse transcriptase assay. Virus could be detected in the case of each line only intermittently over a 90-day period. During this time, there were several peaks of reverse transcriptase activity, followed, in most cases, by periods of minimal enzyme levels.

Expression of viral antigen, as determined by indirect immunofluorescence, correlated more or less with the level of production of progeny infectious HIV-1 detectable in culture fluids (Fig. 3). In many cases, the percentage of positive immunofluorescent
cells remained stable, in spite of low reverse transcriptase levels.

**Discussion**

Two different types of interaction may occur between HIV-1 and susceptible target cells. In the case of PHA-stimulated T lymphocytes, productive infection can involve the great majority of infected cells. Significant cytopathic changes quickly result, including giant cell formation and, ultimately, cell death occurs within 1—2 weeks [2,14,24]. A somewhat different scenario is that of persistent infection in the absence of significant cytopathic effect. This has been noted in populations of T cells which survive primary infection [15] in macrophages [12] and in monocytes [24]. The latter type of cell may play a role in asymptomatic HIV-1 infections, which, in some cases, may last for many years [8].

This paper confirms previous reports [5,22,33] that a non-cytolytic persistent infection frequently results following infection by HIV-1 of EBV-transformed B lymphocytes and that such cells produce HIV-1 over a long period. We have found that, following HIV-1 infection, EBV-carrying B cell lines remained EBNA-positive and VCA-negative. We have further determined that primary cultures established from human peripheral mononuclear cells were approximately 10 times more susceptible to infection by different HIV-1 strains than were EBV-transformed B cell lines. This lesser susceptibility on the part of the B cells to HIV-1 may be the result of undetectable surface expression of the CD4 antigen. In addition, this undetectable CD4 expression might be responsible for the relative absence of virus-induced cytopathic effects, since CD4 has been associated with both HIV-1 cell fusion and cytolysis [19,20,30].

About the same amount of the IIIb strain of HIV-1 was needed to initiate infection of each of 3 different EBV-transformed B cell lines established from different donors. In contrast, variability was observed in terms of the relative amounts of each of 3 different HIV-1 strains needed to infect a common EBV-transformed B cell line. This demonstrates the biological heterogeneity of HIV-1 strains in regard to virulence for EBV-transformed B cells. The average time needed to detect productive HIV-1 infection varied between the EBV-transformed B cells and primary cultures of mononuclear cells. In general, the latter became positive for the presence of HIV-1, p24 antigen and reverse transcriptase after 4 and 6 days, respectively. In the case of EBV-transformed B cells, the corresponding times to positivity were 17 and 23 days.

We have also shown that production of progeny HIV-1 by EBV-transformed B cell lines is intermittent. In fact, p24 antigen expression was found to be relatively constant over the duration of the study in spite of significant fluctuations in reverse transcriptase activity. This was also the case for certain lymphoblastoid T cell lines, such as H-9, with large quantities of virus being produced on some days but not others, against a constant background of p24 anti-
gen expression. In one particular case, approximately 15% of cells expressed p24 antigen, while no reverse transcriptase activity whatsoever could be detected. This could reflect a latent infection present in these cells. Latency might itself be inducible; in some instances, high levels of reverse transcriptase activity were observed following periods of zero release of progeny infectious HIV-1, as determined by reverse transcriptase. Viruses recovered at times of reverse transcriptase detection were infectious for H-9 cells and for PHA-stimulated peripheral and umbilical cord blood lymphocytes.

The fact that non-cytolytic infection of EBV-transformed B lymphocytes can occur may have clinical relevance and help to explain the progression of HIV-1-associated disease in initially asymptomatic individuals [11]. Non-cytopathically-infected B cells might persist for long periods of time. In vivo, these cells may serve as a reservoir of progeny HIV-1, at times after HIV-1 infection has compromised the numbers and/or function of the helper/inducer subset of T lymphocytes. In this regard, it is relevant that higher than average numbers of circulating EBV-carrying B cells were detected in patients with AIDS and AIDS-related disorders [3,34] and that a B-lymphocyte cell line, established from an AIDS patient, was shown to contain integrated HIV genome [25].

We are currently investigating the role of CD4 antigen as the viral receptor in B lymphocytes, by examining these cell lines for the presence of CD4 mRNA. Furthermore, we hope to determine whether specific antibodies against CD4 epitopes can block infection by HIV.

Acknowledgements

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References


