Infection of Human Thymic Lymphocytes by HIV-1

*Michel Tremblay, ††Kei Numazaki, †Hy Goldman, and ††Mark A. Wainberg

*Lady Davis Institute and Department of Medicine, Sir Mortimer B. Davis—Jewish General Hospital; McGill AIDS Centre, McGill University; and †Department of Microbiology, Montreal Children's Hospital, Montreal, Canada

Summary: We have succeeded in infecting human thymic lymphocytes with both the HIV-IIIa laboratory strain of HIV-1 as well as with a clinical isolate of this virus. Thymic lymphocytes were at least as susceptible to infection by HIV-1 as were cord blood lymphocytes, but appeared to display somewhat greater resistance to the cytopathic effects of the virus. As measured variously by each of indirect immunofluorescence for detection of viral p17, antigen capture assay for the presence of viral p24 in culture fluids, and levels of viral reverse transcriptase activity in culture fluids, infection of thymic lymphocytes could be detected as early as 2 days after infection by HIV-1, and persisted through at least 14 days of tissue culture maintenance. These findings suggest that thymic lymphocytes may be susceptible to infection by HIV-1 in vivo, and may also be relevant to our understanding of HIV-1-induced pathogenesis, particularly in pediatric populations. Key Words: Thymus—Lymphocytes—HIV-1.

The human immunodeficiency virus type 1 (HIV-1) has a selective ability to infect and replicate in cells that express CD4 antigen at their surface (1,2). In the case of T lymphocytes of the CD4 phenotype, such infection is often followed by death (3). Indeed, one of the major characteristics of HIV-1-associated disease is a profound diminution in numbers of circulating CD4 lymphocytes (4), although it is still unclear to what extent this drop is due to direct cytopathic effects of viral infection.

The thymus play a key role in the development of mature lymphocytes involved in cell-mediated immune responses (5). Maturation of thymic lymphocytes or thymocytes gives rise to the pool of peripheral T cells bearing either CD4 or CD8 markers (6). The fact that HIV-1 can replicate in and ultimately kill CD4+ cells prompted us to ask whether the thymocyte progenitors of such cells might themselves be susceptible to infection by HIV-1.

This question was deemed to be important not only because of the need to determine the susceptibility to HIV-1 of the thymic lymphocyte population, but because it has been reported that the thymus may undergo premature atrophy in HIV-1-infected newborns and infants (7). The thymus, and particularly thymic epithelium, is known to play an essential role in T cell maturation and differentiation (8). This role usually continues into the postnatal period and may persist until thymic involution is complete.

Previous work from our laboratory has shown that thymic epithelial tissue can itself be infected by a variety of different viruses including HIV-1 (9–11). This paper extends these observations to show that thymic lymphocytes can be productively infected by HIV-1.

MATERIALS AND METHODS

Thymic and Cord Blood Lymphocytes

Thymic lymphocytes (thymocytes) were obtained from the washings of thymic tissue obtained from
each of three patients (1, 3, and 5 years old) at the time of cardiovascular surgery at the Montreal Children's Hospital. The thymus tissue was minced as previously described (9), washed in RPMI 1640 medium, and thymic lymphocytes were harvested by Ficoll–Hypaque density centrifugation (12). Thymocytes were resuspended to a concentration of \(10^6\) cells/ml in RPMI-1640 medium supplemented with 10% fetal bovine serum, and stimulated with phytohemagglutinin–PI (PHA–P1) (0.1%) (Difco Laboratories, Detroit, MI, U.S.A.) for 48 h (5 ml per culture). Cord blood was obtained on a routine basis from the delivery room of the Jewish General Hospital. Mononuclear cells were separated from whole cord blood by Ficoll–Hypaque density centrifugation and were prestimulated with PHA–P1 as described above.

In the case of both thymic and cord blood cells, cultures were then washed and refed with fresh growth medium, also containing interleukin-2 (IL-2) (2% vol/vol) (Boehringer-Mannheim, Montreal, Canada).

**HIV-1 Infection of Thymic and Cord Blood Lymphocytes**

We attempted to infect both thymic and cord blood lymphocytes with each of the IIB laboratory strain of HIV-1 as well as a clinical strain of this virus (no. 334), previously isolated in our laboratory. HIV-IIB virus was obtained from 3 h culture supernatants of the H-9 T leukemia cell line, kindly supplied by Dr. R. C. Gallo, (National Institutes of Health, Bethesda, MD, U.S.A.) (13). Culture fluids were clarified by centrifugation at 2,500 \(\times\) g for 20 min at 4°C in both the case of HIV-IIB-producing H-9 cells and in the case of cultures of a CEM cell line, actively producing clinical isolate 334. These fluids were frozen in 1 ml samples at \(-70^\circ\)C, and represented common sources of HIV-1 for purposes of infection of thymic lymphocytes. On the basis of infectious center assays performed on MT-4 cells (14), the viral suspensions used each contained approximately \(5 \times 10^3\) infectious units of HIV/ml.

For purposes of infection, \(5 \times 10^6\) lymphocytes (prestimulated over 48 h with PHA) were pretreated with polybrene (2 \(\mu\)g/ml) for 20 min in 5 ml of RPMI medium, as previously described (15). The cells were washed by centrifugation at 500 \(\times\) g for 15 min at room temperature and resuspended in 0.5 ml of an undiluted suspension of infectious tissue culture fluid, and incubated at 37°C for 3 h with gentle shaking. The cells were then centrifuged again to remove unbound virus and resuspended in fresh supplemented medium (\(10^6\) cells/ml), also containing interleukin-2 (IL-2) as described above to promote cell proliferation.

The percentages of infected cells in these various cultures were ascertained using a fixed cell, indirect immunofluorescence assay. Toward this end, the cells were cytacentrifuged onto glass slides and fixed in a 1:1 acetone–methanol solution for 30 min at room temperature. For purposes of detection of viral antigen, we employed mouse monoclonal antibodies against the HIV-1 protein p17 (kindly supplied by Dr. R. C. Gallo, NIH) and fluorescein-labeled goat anti-mouse immunoglobulin conjugate, as described (15). In addition, production of progeny HIV was evaluated by testing for the presence of viral p24 in culture fluids by antigen-capture ELISA, according to the manufacturer's instructions (DuPont Laboratories, Wilmington, DE, U.S.A.) and by measurements of viral reverse transcriptase activity (15).

In general, fresh medium was added to cultures every 2–3 days, which were generally maintained at a density between 2–4 \(\times\) 10^5 cells/ml. However, in cases in which ELISA and/or reverse transcriptase measurements were to be carried out, fresh medium was utilized 24 h prior to the collection of relevant samples. Cell counts were performed using a hemocytometer, and percentages of viable cells were determined on the basis of trypan blue exclusion.

**RESULTS**

Table 1 presents the results of a study that indicates that thymic lymphocytes were highly susceptible to infection by both strains of HIV-1, as judged by the presence of reverse transcriptase activity in culture fluids. It can be seen that the levels of such enzymatic activity in cultures of infected thymocytes after 14 days were above those observed after 7 days. In contrast, infection of cord blood cells by strain 334 of HIV-1 led to cell death by 7–10 days after infection and very low counts of recoverable reverse transcriptase activity after this time. Similar results were obtained in each of two other experiments.

These data were confirmed in additional experiments in which indirect immunofluorescence examination of infected cells was carried out using monoclonal antibodies against the viral protein p17. The results of Table 2 show that a multiplicity of infec-
<table>
<thead>
<tr>
<th>Cells</th>
<th>Virus</th>
<th>m.o.i.</th>
<th>Reverse transcriptase activity (cpm/ml × 10⁻⁶)</th>
<th>% viable cells of uninfected control culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymic lymphocytes</td>
<td>HIV-III&lt;sub&gt;B&lt;/sub&gt;</td>
<td>0.001</td>
<td>37.3 ± 4.2</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>4.9 ± 0.6</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>HIV-1, strain 334</td>
<td>0.001</td>
<td>683.3 ± 121.0</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>30.0 ± 2.1</td>
<td>87</td>
</tr>
<tr>
<td>Cord blood lymphocytes</td>
<td>HIV-III&lt;sub&gt;B&lt;/sub&gt;</td>
<td>0.001</td>
<td>33.8 ± 4.9</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>4.9 ± 0.3</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>HIV-1, strain 334</td>
<td>0.001</td>
<td>3,241.8 ± 274.2</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>1,519.0 ± 132.5</td>
<td>68</td>
</tr>
</tbody>
</table>

<sup>a</sup> m.o.i., multiplicity of infection.

<sup>b</sup> Mean of two replicate samples ± SD.

<sup>c</sup> These percentages were calculated on the basis of dilutions carried out on cell cultures in comparison with uninfected controls.

**TABLE 1. Infection of human thymic lymphocytes by HIV-1**

**DISCUSSION**

The data in this report indicate that thymic lymphocytes are highly susceptible to infection by HIV-1. Both a clinical and laboratory strain of HIV-1 were utilized to demonstrate infectibility of this cell population in this study. This is the initial demonstration that lymphocytes found in the thymus may be infected by this virus.

The portal of entry of HIV-1 into these cells is probably the CD4 molecule (1,2). Flow cytometric analysis has revealed reactivity with anti-CD4 mouse monoclonal antibodies in 40–50% of thymic lymphocytes (results not shown). The use of double labeling to confirm that the infected cell population is CD4(+) would probably not be successful, given the fact that CD4 surface expression is normally reduced or eliminated following infection by HIV-1.

The fact that thymic lymphocytes are susceptible to infection by HIV-1 may have clinical relevance. HIV-1 infection of thymocytes in vivo could have a dramatic effect on subsequent levels of T cells that circulate through the body and may, in part, be responsible for the low levels of circulating T cells that are found in cases of pediatric AIDS. The relative resistance of cultured thymocytes to HIV-induced cytolysis (Table 1), in comparison with cord blood lymphocytes, is intriguing and merits further investigation.

We have further observed that the clinical strain of HIV-1 used, i.e., no. 334, appeared to replicate more efficiently and with greater cytolytic effect in the cultures than the HIV-III<sub>B</sub> laboratory isolate. Clinical strain 334 was isolated in our laboratory from the peripheral blood lymphocytes of a patient.
TABLE 3. Presence of p24 antigen in culture fluids of HIV-IIIb-inoculated thymic lymphocytes at various times after infection

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Cells</th>
<th>1 day</th>
<th>2 days</th>
<th>6 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thymic lymphocytes</td>
<td>0</td>
<td>4.4 ± 0.5</td>
<td>10.3 ± 1.4</td>
<td>14.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Cord blood lymphocytes</td>
<td>0</td>
<td>3.8 ± 0.3</td>
<td>9.6 ± 1.8</td>
<td>12.3 ± 1.6</td>
</tr>
<tr>
<td>2</td>
<td>Thymic lymphocytes</td>
<td>0</td>
<td>3.8 ± 0.5</td>
<td>14.8 ± 1.6</td>
<td>22.9 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Cord blood lymphocytes</td>
<td>0</td>
<td>4.6 ± 0.3</td>
<td>12.2 ± 0.8</td>
<td>27.3 ± 4.2</td>
</tr>
</tbody>
</table>

Data presented represent mean concentration of p24 in two replicate samples ± SD in each case.

Optical density measurements were obtained by testing 10 μl of culture fluid for the presence of p24 antigen, by means of ELISA assay. These values were subsequently converted to ng/ml on the basis of a standard curve.

with AIDS, who died within 6 months of having been diagnosed in spite of treatment with zidovudine (AZT). We do not know whether this strain is more virulent than most other clinical isolates, although efforts to characterize it further are in progress. It does not appear to be more cytopathic for other cell types, such as MT-4, than the HIV-IIIb laboratory strain.

One of the consequences of aging, in general, is the diminished T cell repertoire that correlates with a reduction in thymic function (16,17). Lymphocytes represent up to 50% of thymic tissue during adolescence, but less than 10% in elderly people (16). Because thymocytes are far more numerous in younger individuals, and are susceptible to infection by HIV-1, this may help to explain why the estimated mean incubation period for transfusion-associated AIDS in children is much shorter than that in adults. It has been reported that mean incubation periods for these two populations are 1.97 and 8.23 years, respectively (18). HIV-1 infection of thymic lymphocytes may occur during circulation of infected T cells and/or other infected cells throughout the body. It is not unreasonable to believe that such infection, in the case of pediatric cases of AIDS, may result from perinatal transmission of HIV-1, followed by rapid appearance of HIV-1 in the thymus. Earlier data have pointed to thymic atrophy as being an important feature of cases of pediatric AIDS (19). Our data, indicating infectibility in vitro of thymus-derived lymphocytes, provide further evidence for a role of the thymus in events leading to AIDS, particularly in pediatric populations.

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