Class I–unrestricted noncytotoxic anti–HTLV-I activity of CD8⁺ T cells

Masako Moriuchi and Hiroyuki Moriuchi

Although it is widely believed that viral clearance is mediated principally by the destruction of infected cells by cytotoxic T cells, noncytolytic antiviral activity of CD8⁺ T cells may play a role in preventing the progression to disease in infections with immunodeficiency viruses and hepatitis B virus. We demonstrate here that (1) replication of human T-lymphotropic virus type I (HTLV-I) is more readily detected from CD8⁺ T-cell–depleted (CD8⁻) peripheral blood mononuclear cells (PBMCs) of healthy HTLV-I carriers than from unfractionated PBMCs, (2) cocultures of CD8⁺ PBMCs with autologous or allogeneic CD8⁺ T cells suppressed HTLV-I replication, and (3) CD8⁺ T-cell antiviral activity of HTLV-I activity is not abrogated in trans-well cultures in which CD8⁺ cells are separated from CD8⁻ PBMCs by a permeable membrane filter. These results suggest that class I-unrestricted noncytotoxic anti–HTLV-I activity is mediated, at least in part by a soluble factor(s), and may play a role in the pathogenesis of HTLV-I infection. (Blood. 2000;96:1994-1995)

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Introduction

Human T-lymphotropic virus type I (HTLV-I) causes 2 distinct diseases, a T-cell malignancy designated adult T-cell leukemia/lymphoma (ATLL) and a chronic inflammatory nervous system disease designated HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP). However, the latency between viral transmission and disease progression is very long, typically several years to decades; furthermore, the majority (95% or more) of infected individuals are healthy carriers. What determines the outcome of HTLV-I infection has not been fully understood; however, CD8⁺ T-cell response to HTLV-I infection probably plays a role in disease progression. Although it has been widely considered that CD8⁺ T cells mediate antiviral activity principally by cytolytic mechanisms, noncytolytic antiviral response of CD8⁺ T cells has been demonstrated in controlling infection with human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), or hepatitis B virus. In this study, we investigated whether MHC class I-unrestricted noncytotoxic mechanism is also used by CD8⁺ T cells to control HTLV-I infection.

Study design

Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from HTLV-I seropositive or seronegative healthy individuals (Nagasaki Red Cross Blood Center, Nagasaki, Japan). CD8⁺ T cells were positively selected, as described previously. CD8⁺ T-cell-depleted PBMCs (CD8⁻ PBMCs) contained less than 1.5% CD8⁺ cells, as determined by flow cytometric analysis (data not shown). In some experiments CD8⁻ PBMCs and autologous or allogeneic CD8⁺ T cells were cultured together or separated by a semipermeable membrane filter with 0.4-µmol/L pores (Costar, Acton, MA).

Human T-lymphotropic virus type I infection

Replication of HTLV-I was determined by HTLV-I p19 Ag levels in cell-free supernatants from HTLV-I carriers' PBMCs, using a commercially available enzyme-linked immunosorbent assays (ELISA; Cellular Product, Inc, Buffalo, NY).

Results and discussion

Dramatic increase in HTLV-I p19 expression by CD8⁺ depletion

It has been shown that HIV-1 is more readily isolated from PBMCs of infected individuals when CD8⁺ T cells are depleted. CD8⁺ T-cell–mediated antiviral activity has also been demonstrated in controlling other lentiviruses such as SIV and FIV. Furthermore, CD8⁺ depletion in vivo dramatically increased plasma viral loads in SIV-infected macaques. These studies suggest that CD8⁺ T cells play a critical role in the pathogenesis of retroviral infections.

To explore the possibility that CD8⁺ T cells play a role in preventing replication of HTLV-I, another human retrovirus, CD8⁺ T cells were depleted from PBMCs of healthy HTLV-I carriers. Depletion of CD8⁺ T cells from PBMCs resulted in a significant increase in HTLV-I p19 Ag expression, and reconstitution of autologous CD8⁺ T cells suppressed viral expression in a dose-dependent manner (Table 1).

Class I-unrestricted noncytotoxic response of CD8⁺ T cells

Next, we investigated whether CD8⁺ T-cell–mediated anti–HTLV-I activity resulted principally from HTLV-I-specific class I-restricted cytotoxic T-lymphocyte response (CTL). To do so, we first performed coculture experiments in which CD8⁺ PBMCs or HTLV-I carriers were reconstituted with autologous or allogeneic CD8⁺ T cells, and p19 Ag levels in cell-free supernatants were compared. As shown in Table 1, autologous CD8⁺ T cells

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markedly, and allogeneic CD8⁺ T cells modestly suppressed HTLV-I replication. Allogeneic CD8⁺ T cells derived from HTLV-I seronegative healthy individuals had little activity against HTLV-I (data not shown); however, it was not possible in this study to determine whether Ag-specific stimulation is essential for CD8⁺ T-cell anti-HTLV-I activity. These results suggest that, although CTL response plays a critical role in controlling HTLV-I replication, there exists another antiviral activity that does not require MHC class I compatibility.

Because it is possible that anti–HTLV-I activity of allogeneic CD8⁺ T cells was attributed to CTL response mediated by partially matched class I molecules, CD8⁺ T cells were separated from CD8⁺ T cells by a semipermeable membrane filter (trans-well) auto CD8⁺ T cells were separated from one million of autologous CD8⁺ T cells (coculture), or separated from one million of autologous CD8⁺ T cells by a semipermeable membrane filter (trans-well).

Table 1. HTLV-I replication in unfractionated PBMCs, CD8⁺ PBMCs, and CD8⁺ PBMCs reconstituted with autologous or allogeneic CD8⁺ T cells

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Unfract PBMCs</th>
<th>CD8⁺ PBMCs</th>
<th>CD8⁺ PBMCs plus*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p19 Ag level in culture supernatant (pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>auto CD8⁺ cells</td>
<td>(9:1)</td>
<td>(4:1)</td>
</tr>
<tr>
<td>1</td>
<td>47</td>
<td>212</td>
<td>115 (46%)†</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>465</td>
<td>190 (59%)</td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>1120</td>
<td>512 (54%)</td>
</tr>
<tr>
<td>4</td>
<td>&lt;25</td>
<td>240</td>
<td>152 (37%)</td>
</tr>
<tr>
<td>5</td>
<td>207</td>
<td>526</td>
<td>529 (—)</td>
</tr>
<tr>
<td>6</td>
<td>91</td>
<td>493</td>
<td>480 (3%)</td>
</tr>
</tbody>
</table>

HTLV-I = human T-cell lymphotropic virus type I; PBMCs = peripheral blood mononuclear cells; unfract = unfractionated. PBMCs were obtained from 3 pairs of HTLV-I carriers (1–6). Three million unfractionated PBMCs or CD8⁺ PBMCs were propagated in RPMI 1640 medium supplemented with 10% fetal bovine serum. Cell-free culture supernatants were collected on day 7 of culture, and p19 levels were determined by ELISA.

*Where indicated, CD8⁺ PBMCs were reconstituted with autologous or allogeneic CD8⁺ T cells at the indicated ratio.
†Allogeneic cultures were performed between carriers 1 and 2, carriers 3 and 4, and carriers 5 and 6.
‡Percentage suppression is expressed as: 100-(p19 Ag level of CD8⁺ PBMC reconstituted with CD8⁺ T cells/p19 Ag level of CD8⁺ PBMC alone).

Table 2. Anti-HTLV-I response of CD8⁺ T cells that does not require direct cell-to-cell contact

<table>
<thead>
<tr>
<th>Carrier</th>
<th>CD8⁺ PBMCs</th>
<th>auto CD8⁺ cells</th>
<th>auto CD8⁺ cells (coculture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p19 Ag level in culture supernatant (pg/mL)</td>
<td>(9:1)</td>
<td>(4:1)</td>
</tr>
<tr>
<td>1</td>
<td>1280</td>
<td>100 (92%)</td>
<td>71 (95%)</td>
</tr>
<tr>
<td>2</td>
<td>4080</td>
<td>2200 (46%)</td>
<td>360 (91%)</td>
</tr>
<tr>
<td>3</td>
<td>4930</td>
<td>2020 (59%)</td>
<td>1110 (79%)</td>
</tr>
</tbody>
</table>

See Table 1 for abbreviations.

References

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