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 unfractonated PBMCs, (2) cocultures of CD8− PBMCs with autologous or allogeneic CD8+ T cells suppressed HTLV-I replication, and (3) CD8+ T-cell anti-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).1 However, the latency between viral transmission and disease progression is very often long, typically several years to decades; furthermore, the majority (95% or more) of infected individuals are healthy carriers.1 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),2 simian immunodeficiency virus (SIV),3 feline immunodeficiency virus (FIV),2 or hepatitis B virus.4 In this study, we investigated whether MHC class I-unrestricted noncytolytic 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.3 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-μm 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.6 CD8+ T-cell–mediated antiviral activity has also been demonstrated in controlling other lentiviruses such as SIV and FIV.2,3 Furthermore, CD8+ depletion in vivo dramatically increased plasma viral loads in SIV-infected macaques.3 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 noncytolytic 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 of 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\(^+\) PBMCs by a semipermeable membrane filter (trans-well). Although less efficient than CD8\(^+\) T cells in cocultures, these cells could suppress HTLV-I replication even when direct cell-to-cell contact, which is essential for class I-restricted CTL response, was prohibited (Table 2). Furthermore, the percentage viabilities of the control cells and the cells in trans-well cultures were similar (88% ± 8% and 90% ± 9%, respectively), and HTLV-I proviral DNA was detected in trans-well cultures at levels comparable to CD8\(^+\) PBMC cultures that yielded substantial amounts of p19 Ag (data not shown). These results suggest that class I-unrestricted noncytolytic response of CD8\(^+\) T cells may play a role in controlling HTLV-I infection.

A previous study demonstrated the inverse relationship between numbers of peripheral CD8\(^+\) T cells and levels of gamma interferon production and HTLV-I expression. Furthermore, it has also been reported that persons with asymptomatic HTLV-I infection have heightened immune reactivity, whereas those with ATLL do not, suggesting that there may be immune regulation of HTLV-I infection. Recently, HTLV-I–specific CTLs have been found in healthy carriers or patients with HAM/TSP, and it has been described that HTLV-I is not latent and is actively replicating in infected individuals, and that the chief determinants of the equilibrium viral load of HTLV-I are virus-specific CTL response. In this study, we identified anti–HTLV-I activity other than CTL response. The significance of the non-CTL response in vivo warrants further investigation.

### Table 1. HTLV-I replication in unfractonated 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>
<th>auto CD8(^+) cells (9:1)</th>
<th>CD8(^+) PBMCs (4:1)</th>
<th>auto CD8(^+) cells (9:1)</th>
<th>CD8(^+) PBMCs (4:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>212</td>
<td>115 (46%)‡</td>
<td>25 (88%)</td>
<td>180 (15%)</td>
<td>77 (64%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>465</td>
<td>190 (59%)†</td>
<td>28 (94%)</td>
<td>358 (23%)</td>
<td>228 (51%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>52</td>
<td>1120</td>
<td>512 (54%)†</td>
<td>60 (95%)</td>
<td>2540 (—)</td>
<td>602 (46%)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>&lt;25</td>
<td>240</td>
<td>152 (37%)†</td>
<td>35 (85%)</td>
<td>140 (42%)</td>
<td>70 (71%)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>527</td>
<td>320</td>
<td>529 (—)</td>
<td>272 (48%)</td>
<td>393 (25%)</td>
<td>312 (41%)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>91</td>
<td>493</td>
<td>480 (3%)</td>
<td>111 (78%)</td>
<td>530 (—)</td>
<td>242 (51%)</td>
<td></td>
</tr>
</tbody>
</table>

HTLV-I = human T-cell lymphotrophic virus type I; PBMCs = peripheral blood mononuclear cells; unfract = unfractonated. PBMCs were obtained from 3 pairs of HTLV-I carriers (1–6). Three million unfractonated 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 (trans-well)</th>
<th>auto CD8(^+) cells (coculture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1280</td>
<td>2200 (96%)</td>
<td>71 (49%)</td>
</tr>
<tr>
<td>2</td>
<td>4080</td>
<td>100 (92%)</td>
<td>360 (91%)</td>
</tr>
<tr>
<td>3</td>
<td>2490</td>
<td>100 (92%)</td>
<td>1101 (79%)</td>
</tr>
</tbody>
</table>

See Table 1 for abbreviations.

Two million CD8\(^+\) PBMCs derived from healthy HTLV-I carriers were cultured alone, reconstituted with a half million autologous CD8\(^+\) T cells (coculture), or separated from one million of autologous CD8\(^+\) T cells by a semipermeable membrane filter (trans-well).

### References


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