Cutting Edge: Epitope-Dependent Effect of Nef-Mediated HLA Class I Down-Regulation on Ability of HIV-1-Specific CTLs to Suppress HIV-1 Replication

Hiroko Tomiyama, Mamoru Fujiwara, Shinichi Oka and Masafumi Takiguchi

*J Immunol* 2005; 174:36-40; doi: 10.4049/jimmunol.174.1.36
http://www.jimmunol.org/content/174/1/36

**References**
This article cites 15 articles, 5 of which you can access for free at:
http://www.jimmunol.org/content/174/1/36.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
It is believed that Nef-mediated HLA class I down-regulation is one of the mechanisms that allow HIV-1-infected cells to escape from being killed by HIV-1-specific human CTLs. In this study, we show that the effect of Nef-mediated HLA class I down-regulation on the ability of HIV-1-specific CTLs to suppress HIV-1 replication is epitope dependent. The CTLs specific for two Pol epitopes presented by HLA-B*5101, one of the HLA alleles associated with slow progression to AIDS, effectively killed HIV-1-infected CD8+ T cells and suppressed HIV-1 replication. In contrast, those specific for the other four epitopes failed to kill HIV-1-infected CD4+ T cells and partially or hardly suppressed HIV-1 replication. The difference in the ability among these two types of CTLs may result from the difference of the number of HLA class I epitope complex on the surface of NL4-3-infected CD4+ T cells. The Journal of Immunology, 2005, 174: 36–40.

HIV-1-specific CD8+ T cells occurs during acute and chronic phases of HIV-1 infections, although the mechanisms of the HIV-1 escape still remain unclear. Previously, various investigators proposed several hypotheses concerning mechanisms of HIV-1 escape from the host immune system such as mutations of immunodominant epitopes (1), reduction in the number of HIV-1-specific CTLs by apoptosis of CD8+ T cells via Fas and TNF (2), skewed maturation of HIV-1-specific CD8+ T cells (3), and impaired cytolytic activity of HIV-1-specific CTL toward HIV-1-infected CD4+ T cells by Nef-mediated down-regulation of HLA class I molecules (4).

Nef down-regulates the surface expression of both HLA-A and -B molecules in HIV-1-infected cells because of internalization of these molecules from the cell surface by endocytosis in the presence of Nef (5). A previous study showed that the expression of HLA-A2 molecules on Nef-positive (Nef+) HIV-1-infected primary CD4+ T cells was 200- to 300-fold lower than that on Nef-defective (Nef−) HIV-1-infected ones (6). These observations suggested that the Nef-mediated HLA class I down-regulation may decrease the recognition of HIV-infected cells by HIV-1-specific CTLs. In fact, it was shown that HLA-A*0201-restricted HIV-1-specific CTLs failed to kill Nef+ HIV-1-infected CD4+ T cells but effectively killed Nef− HIV-1-infected ones (4). This was further confirmed by a study using two HLA-B*3501-restricted, HIV-1-specific CTL clones (7). The ability of HIV-1-specific CTLs to suppress HIV-1 replication was also impaired by Nef-mediated HLA class I down-regulation (7, 8). These studies strongly suggest that Nef-mediated HLA class I down-regulation is one of the major mechanisms by which HIV-1 escapes from HIV-1-specific CTLs. However, because only a very restricted number of HIV-1-specific CTLs has been tested for their ability to kill Nef+ and Nef− HIV-1-infected CD4+ T cells and to suppress the HIV-1 replication, it still remains uncertain whether Nef-mediated HLA class I down-regulation affects the killing ability of all HIV-1-specific CTLs.

HLA-B57, -B51, and -B27 alleles are associated with slow progression to AIDS (9). It has been speculated that long-term nonprogressors (LTNP) and slow progressors carry CTLs specific for conserved and dominant epitopes whose recognition is not affected by Nef-mediated HLA class I down-regulation. However, no study has yet investigated this hypothesis. To clarify the effect of Nef-mediated HLA class I down-regulation on CTLs specific for HIV-1 epitopes presented by HLA alleles that are associated with or not associated with slow progression to AIDS, we investigated the ability of both HLA-B*5101-restricted and HLA-A*3303-restricted HIV-1-specific CTLs to recognize HIV-1-infected CD4+ T cells. In this study, we show that HIV-1-specific CD8+ T cells have various ranges of ability to kill HIV-1-infected CD4+ T cells and to suppress the replication of HIV-1.

Materials and Methods

HIV-1-specific CTL clones and lines

HIV-1-specific CTL clones and CTL lines were generated previously (10–12). All CTLs were cultured in R10 medium supplemented with 200 U/ml recombinant human IL-2 and stimulated weekly with irradiated target cells prepsed with the appropriate HIV-1-derived peptide.

*Division of Viral Immunology, Center for AIDS Research, Kumamoto University, Kumamoto, and †AIDS Clinical Center, International Medical Center of Japan, Tokyo, Japan.

Received for publication August 18, 2004. Accepted for publication October 27, 2004.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This research was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Science, Sport and Culture; a Grant-in Aid for Scientific Research from the Ministry of Health, Labour and Welfare; the government of Japan; and a grant from Japan Health Science Foundation.

2 Address correspondence and reprint requests to Dr. Masafumi Takiguchi, Division of Viral Immunology, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan. E-mail address: masafumi@kajju.medic.kumamoto-u.ac.jp

3 Abbreviation used in this paper: LTNP, long-term nonprogressor.
**HIV-1 clones**

An infectious proviral clone of HIV-1, pNL-432, and its mutant, pNL-M20A (containing a substitution of Ala for Met at residue 20 of Nef), were reported previously (13).

**Infection of CD4+ T cells with HIV-1**

CD4+ T cells were purified from PBMCs of HIV-1-seronegative individuals with HLA-B*5101 or HLA-A*3303 by means of anti-human CD4 mAb-coated magnetic beads (MACS beads; Miltenyi Biotec). The purified CD4+ T cells were cultured and infected with HIV-1 clones as previously shown (7).

**CTL assay**

The cytotoxicity of CTL clones for cultured CD4+ T cells infected with HIV-1 (>40% p24 Ag-positive cells) was determined by a standard 31Cr release assay as shown previously (7).

**Flow cytometric analysis**

To assess HLA class I expression in HIV-1-infected CD4+ T cells, the cells were stained with anti-B5 mAb 4D12 following staining with allophycocyanin-labeled anti-mouse Ig (BD Pharmingen), and thereafter were fixed and permeabilized for intracellular HIV-1 p24 staining with FITC-labeled anti-p24 mAb (BD Pharmingen), and allophycocyanin-labeled anti-human TNF-α mAb.

**Suppression of HIV-1 replication by HIV-1-specific CTLs**

The ability of HIV-1-specific CTLs to suppress HIV-1 replication was examined as previously described (7). After CD4+ T cells had been incubated with the indicated HIV-1 clone following a 4-h incubation at 37°C in the presence of TNF-α, the cells were washed three times with R10 medium. HIV-1-infected CD4+ T cells were cocultured with HIV-1-specific CTLs. From days 2 to 7 postinfection, 10 µl of culture supernatant was collected, and the concentration of p24 Ag in the supernatant was measured by enzyme immunoassay (HIV-1 p24 Ag ELISA kit; ZeptoMetrix). On days 3, 4, and 5 postinfection, the percentage of intracellular p24 Ag-positive cells in the CD4+ T cell population was determined by flow cytometry.

**Peptide binding assay**

Binding of HIV-1 epitope peptides to HLA-B*5101 was examined by a peptide stabilization assay using RMA-S-B*5101 cells as previously described (10).

**Results and Discussion**

Ability of HIV-1-specific CTLs to suppress HIV-1 replication in HIV-1-infected CD4+ T cells

To investigate the ability of HIV-1-specific CTLs to suppress HIV-1 replication, we selected the CTLs specific for four HLA-B*5101 epitopes and two HLA-A*3303 epitopes, whose sequence are found in the NL-432 clone. We measured the ability of seven CTL clones or lines specific for these epitopes to suppress HIV-1 replication in primary CD4+ T cells infected with either HIV-1 clone NL-432 or its mutant NL-M20A, in which 1 aa of Nef has mutated and which has the ability to down-regulate cell surface expression of CD4 but not that of HLA class I molecules (13). The surface expression of HLA-B*5101 was indeed down-regulated in NL-432-infected CD4+ T cells but not in NL-M20A-infected ones (Fig. 1A). CD4+ T cells infected with the HIV-1 clones were cocultured with or without the HIV-1-specific CTLs, p24-positive CD4+ T cells were not detected in the cultures of NL-M20A-infected CD4+ T cells with the SF2-Pol283-8-specific CTL line, SF2-Pol743-9-51 CTL clone, or SF2-Gag327-9-249 CTL clone. They were also undetected in the cultures of NL-432-infected CD4+ T cells with the SF2-Pol283-8-specific CTL line or SF2-Pol743-9-51 CTL clone, whereas the number of the p24-positive CD4+ T cells was reduced by approximately one-half in the cultures with the SF2-Gag327-9-249 CTL clone. In contrast, the number of the p24-positive CD4+ T cells was not reduced in the cultures of NL-432-infected and NL-M20A-infected CD4+ T cells with HLA-mismatched HIV-1 Nef-specific CTL clones, SF2-6-218 and SF2-6-219 (Fig. 1B). These results suggest that SF2-Pol283-8-specific CTL line and SF2-Pol743-9-51 CTL clone completely suppressed Nef+ HIV-1 replication and that SF2-Gag327-9-249 CTL clone only partially suppressed it. Two HLA-A*3303-restricted CTLs, the SF2-Gag144-152-10 clone and the SF2-Env697-706 line, gave the same results as the latter clones (data not shown).

The enzyme immunoassay analysis confirmed the results of the flow cytometric analysis (Fig. 1C). The SF2-Pol283-8 line and SF2-Pol743-9-51 clone completely suppressed replication of both NL-M20A and NL-432, whereas two CTL clones, SF2-Gag144-152-10 and SF2-Gag327-9-249, as well as one CTL line, SF2-Env697-706, partially suppressed NL-432 replication (21.7–44.0%) and effectively suppressed NL-M20A replication (82.4–89.9%). These results taken together suggest that the recognition by the latter CTLs was affected by Nef-mediated HLA class I down-regulation but that by the former ones was not.

To compare quantitatively the ability of these CTLs to suppress NL-432 replication, we tested the ability of the SF2-Pol283-8 or SF2-Pol743-9-51 at various E:T ratios to suppress NL-432 replication (Fig. 1D). Approximately 50% suppression of NL-432 replication was found when SF2-Gag144-152-10 and SF2-Gag327-9-249 CTL clones were tested at an E:T ratio of 1:1, whereas both SF2-Pol283-8 and SF2-Pol743-9-51 CTL clones showed ~50% suppression at an E:T ratio of 0.001:1, indicating that these CTLs have 1000-fold stronger ability to suppress NL-432 replication than SF2-Gag144-152-10 and SF2-Gag327-9-249 CTL clones.

The number of p24-positive CD4+ T cells was not reduced in the culture of NL-432-infected CD4+ T cells with the SF2-Rev71-11-55 clone, whereas it was partially reduced in that of NL-M20A-infected CD4+ T cells with the same clone (data not shown). This clone also failed to suppress NL-432 replication but partially suppressed NL-M20A replication (Fig 1C). These results suggest that this CTL clone can weakly recognize NL-M20A-infected CD4+ T cells but not NL-432-infected CD4+ T cells.

**Ability of HIV-1-specific CTLs to kill HIV-1-infected CD4+ T cells and to produce cytokines by stimulation with HIV-1-infected CD4+ T cells**

To clarify the mechanism by which HIV-1-specific CTLs suppress HIV-1 replication, we investigated the activity of the HIV-1-specific CTL clones and lines to kill HIV-1-infected CD4+ T cells and to produce cytokines when stimulated with HIV-1-infected CD4+ T cells. SF2-Pol743-9-51 CTL clone and SF2-Pol283-8-specific CTL line, which showed strong suppression of NL-432 replication, effectively killed CD4+ T cells infected with either NL-432 or NL-M20A. The result for the SF2-Pol743-9-51 clone was also confirmed by using the SF2-Pol743-9-specific CTL line (Fig. 2A). The cytolytic activity of these two CTLs for HLA-B*5101 CD4+ T cells infected with NL-432 was almost identical with that of those infected with NL-M20A at any E:T ratios (Fig. 2B). These results...
indicate that Nef-mediated HLA class I down-regulation does not affect the ability of these CTLs to kill HIV-1-infected CD4\(^+\) T cells. In contrast, the three HIV-1-specific CTLs (SF2-Gag144-152-10 and SF2-Gag327-9-249 CTL clones, and SF2-Env697-706 CTL line) killed NL-M20A-infected CD4\(^+\)/H11001 T cells but failed to kill NL-432-infected CD4\(^+\)/H11001 T cells (Fig. 2A), suggesting that Nef-mediated HLA class I down-regulation affected the ability of these CTLs to kill HIV-1-infected CD4\(^+\) T cells. These results are consistent with those of a previous study showing that 2 HLA-B*5101-restricted CTL clones killed NL-M20A-infected CD4\(^+\) T cells but failed to kill NL-432-infected CD4\(^+\) T cells (7).

Next, we investigated the ability of these CTLs to produce IFN-γ and TNF-α after having been stimulated with HIV-1-infected CD4\(^+\) T cells (Fig. 2C). The total percentages of IFN-γ- and TNF-α-producing cells were ~2–5% and 4–9% in the HIV-1 Pol-specific CTLs stimulated with NL-432-infected and NL-M20A-infected ones, respectively. In contrast, the total percentages of IFN-γ- and TNF-α-producing cells were ~4 and 4–6% in the HIV-1 Gag- and Env-specific CTLs stimulated with CD4\(^+\) T cells infected with HIV-1 NL-432 and NL-M20A, respectively. Thus, there was no difference in the number of cytokine-producing cells between these two groups of HIV-1-specific CTLs. These results suggest that the difference in the ability to suppress HIV-1 replication between the two groups results from that in cytolytic activity between them, and that cytokines secreted from the CTLs are partially involved in the suppression of HIV-1 replication.

The SF2-Rev71-11-55 clone failed to produce cytokines after stimulation with either CD4\(^+\) T cells infected with NL-432 or those infected with NL-M20A (Fig. 2C). This result together with that of suppression of HIV-1 replication was calculated. D. Comparison of the ability of HIV-1-specific CTLs to strongly suppress Nef\(^+\) HIV-1 replication. CD4\(^+\) T cells isolated from donor U-13 were infected with NL-432 and then cocultured with the HIV-1-specific CTLs at various E:T ratios. Percentage of suppression of HIV-1 replication was calculated.
of TCR to recognize the epitope and by the amount of the epitope presented on the surface of HIV-1-infected cells. We investigated the ability of TCR to recognize the epitope among four HLA-B*5101-restricted CTLs. We measured the ability of the peptides to bind to HLA-B*5101 molecules (BL50) by an HLA-B*5101 stabilization assay, and also measured the ability of CTLs to kill epitope peptide-pulsed cells (LL50, peptide concentration providing a half of maximum percent specific lysis; Table I). A high BL50/LL50 ratio indicates a high ability of TCR to recognize the epitope.

In contrast, BL50 values of Pol743-9-51 and Pol283-8-specific CTLs showed lower BL50/LL50 ratios than SF2-Gag327-9-249 and SF2-Rev71-11-55 CTLs (Table I). These results indicate that the ability of TCR of the former CTLs to recognize the epitope was much lower than that of the latter ones. Both Pol743-9-51 and Pol283-8-specific CTLs effectively killed NL-432-infected CD4+ T cells, whereas SF2-Gag327-9-249 and SF2-Rev71-11-55 CTLs failed to kill them. These findings together suggest that the peptide had higher ability to bind to HLA-B*5101 than the latter ones. In contrast, LL50 values of Pol743-9-51 and SF2-Rev71-11-55 CTLs were 6- and 20-fold lower than those of SF2-Pol283-8 and SF2-Gag327-9-249 CTLs, respectively. Thus, the Pol743-9-51 and Pol283-8-specific CTLs showed lower BL50/LL50 ratio than SF2-Gag327-9-249 and SF2-Rev71-11-55 CTLs (Table I). These results indicate that the ability of TCR of the former CTLs to recognize the epitope was much lower than that of the latter ones. Both Pol743-9-51 and Pol283-8-specific CTLs effectively killed NL-432-infected CD4+ T cells, whereas SF2-Gag327-9-249 and SF2-Rev71-11-55 CTLs failed to kill them. These findings together suggest that the

### Table I. Ability of HLA-B*5101-restricted CTLs to recognize the epitopes

<table>
<thead>
<tr>
<th>CTLs</th>
<th>Epitope</th>
<th>Sequence</th>
<th>(A) Binding Ability of Peptide (BL50)</th>
<th>(B) Cytolytic Activity for Peptide-Pulsed Cells (LL50)</th>
<th>(A)/(B) Ability of TCR to Recognize the Epitope</th>
<th>(C) Cytolytic Activity for NL-432-Infected Cells (% specific lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol743-9-51</td>
<td>Pol743-9</td>
<td>LPPYVAKEI</td>
<td>6.1 x 10^{-6} M</td>
<td>5.0 x 10^{-7} M</td>
<td>1.220</td>
<td>36.0</td>
</tr>
<tr>
<td>Pol283-8</td>
<td>Pol283-8</td>
<td>TAFTIPSI</td>
<td>6.8 x 10^{-6} M</td>
<td>3.0 x 10^{-8} M</td>
<td>227</td>
<td>28.7</td>
</tr>
<tr>
<td>Gag327-9-249</td>
<td>Gag327-9</td>
<td>NANPDCKTI</td>
<td>4.0 x 10^{-6} M</td>
<td>1.0 x 10^{-9} M</td>
<td>4.000</td>
<td>8.0</td>
</tr>
<tr>
<td>Rev71-11-55</td>
<td>Rev71-11</td>
<td>VPLQPLPER</td>
<td>5.0 x 10^{-5} M</td>
<td>5.0 x 10^{-7} M</td>
<td>10.000</td>
<td>0</td>
</tr>
</tbody>
</table>

*LL50, Peptide concentration providing a half of maximum percent specific lysis.*
difference in the ability between these CTLs to kill NL432-infected CD4\(^+\) T cells is due to that in the number of epitopes presented by HLA-B*5101 on the surface of NL-432-infected CD4\(^+\) T cells rather than that in the ability of TCR to recognize the epitope. A recent study also showed that the abilities of HIV-1-specific CTLs to kill cell lines infected with Nef-defective HIV-1 IIIB clone and to suppress replication of this clone were associated with specificity of the CTLs but not with functional avidity of the CTLs (14). Thus, the number of HLA-epitope complex presented on HIV-1-infected CD4\(^+\) T cells may be critical for recognition of HIV-1-specific CTLs.

HLA-B57 and -B27 alleles are well-known factors associated with slow progression to AIDS (10). A recent study revealed that HIV-1-specific CD8\(^+\) T cells have a high proliferation capacity that is coupled to perforin expression in HLA-B*5701 + LTNP but not in HLA-B*5701\(^-\) or HLA-B*5701\(^+\) progressors (15), suggesting that HIV-1-specific CD8\(^+\) T cells, which have a high proliferation capacity and effector function, control HIV-1 replication in HLA-B*5701 + LTNP. However, the mechanism of the association of these HLA alleles with slow progression to AIDS still remains unclear. The present study revealed that the CTLs specific for the two Pol epitopes presented by one of the HLA class I molecules associated with slow progression to AIDS, HLA-B*5101, completely suppressed HIV-1 replication and killed HIV-1-infected CD4\(^+\) T cells, implying that these cells effectively control HIV-1 replication in vivo. Because we investigated a limited number of CTLs restricted by HLA alleles that are not associated with slow progression of AIDS in the present and previous studies (8), it still remains unclear that the existence of these CTLs is associated with slow progression of AIDS. Further analysis of HIV-1-specific CTLs restricted by various HLA alleles will clarify the mechanism of the association of these HLA alleles with slow progression to AIDS.

In the present study, we showed that the effect of Nef-mediated HLA class I down-regulation on recognition by HIV-1-specific CD8\(^+\) T cells of HIV-1-infected CD4\(^+\) T cells vary in epitopes, and particularly demonstrated the existence of HIV-1-specific CTLs that could completely suppress Nef\(^+\) HIV-1 replication and effectively kill primary CD4\(^+\) T cells infected with Nef\(^+\) HIV-1. These CTLs are expected to suppress HIV-1 replication in vivo.

Acknowledgments
We thank Dr. Adachi for the generous gift of the NL-M20A clone; Sachi Doki for technical assistance; and Sachiko Sakai for secretarial assistance.

References