Title
Natural suppression of human immunodeficiency virus type 1 replication is mediated by transitional memory CD8+ T cells.

Permalink
https://escholarship.org/uc/item/8wt6w8hp

Journal
Journal of virology, 85(4)

ISSN
1098-5514

Authors
Killian, M Scott
Johnson, Carl
Teque, Fernando
et al.

Publication Date
2011-02-08

Peer reviewed
Natural Suppression of Human Immunodeficiency Virus Type 1 Replication Is Mediated by Transitional Memory CD8⁺ T Cells

M. Scott Killian, Carl Johnson, Fernando Teque, Sue Fujimura, and Jay A. Levy*  
Department of Medicine, University of California San Francisco, San Francisco, California 94143

Received 25 May 2010/Accepted 19 November 2010

HIV replication is suppressed in vitro by a CD8⁺ cell noncytotoxic antiviral response (CNAR). This activity directly correlates with an asymptomatic clinical state. The objective of this study was to identify the phenotype of CD8⁺ cell subsets having strong CNAR activity. CD8⁺ cell subset frequencies and CNAR levels were measured for human immunodeficiency virus (HIV)-uninfected individuals and three groups of HIV type 1 (HIV-1)-infected individuals: asymptomatic individuals with low-level viremia (vHIV), antiretroviral-drug-treated subjects with undetectable virus levels (TxHIV), and therapy-naïve aviremic elite controllers (EC). CD8⁺ cells from the vHIV individuals exhibited the highest HIV-suppressing activity and had elevated frequencies of CD45RA⁻CD27⁺ and PD-1⁺ (CD279⁺) cells. Functional assessments of CD8⁺ cells sorted into distinct subsets established that maximal CNAR activity was mediated by CD45RA⁻CCR7⁻CD27⁺ and PD-1⁺CD8⁺ cells. T cell receptor (TCR) repertoire profiles of CD8⁺ cell subsets having strong CNAR activity exhibited increased perturbations in comparison to those of inactive subsets. Together, these studies suggest that CNAR is driven by HIV replication and that this antiviral activity is associated with oligoclonally expanded activated CD8⁺ cells expressing PD-1 and having a transitional memory cell phenotype. The findings better describe the identity of CD8⁺ cells showing CNAR and should facilitate the evaluation of this important immune response in studies of HIV pathogenesis, resistance to infection, and vaccine development.

MATERIALS AND METHODS

Human subjects. HIV-1-infected (n = 100) and uninfected (HIV⁻) (n = 19) subjects were selected from participants in ongoing studies at the University of California San Francisco (UCSF). Among the HIV-1-infected subjects, all of whom had been infected for more than 5 years, three groups were studied: (i) individuals on antiretroviral therapy with very low viral loads (TxHIV⁺) (n = 44), (ii) elite controllers (EC) (n = 15) who had been infected with HIV-1 for at least 10 years without exhibiting AIDS-defining symptoms and had undetectable plasma viral loads (<50 copies HIV RNA/ml) and normal CD4⁺ T cell counts (>400 CD4⁺ T cells/µl) in the absence of antiretroviral therapy, and (iii) viremic individuals (HV⁻) (n = 41) who were asymptomatic, had viral loads ranging from 3.6- to 4.8-log RNA copies per ml, and were not receiving antiretroviral therapy. Each subject signed informed consent forms, and the study received approval from the Committee for Human Research at UCSF. Salient features of the study population are provided in Table 1.

Clinical measures. Complete differential blood cell counts (CBCs) to determine erythrocyte numbers, hemoglobin levels, and levels of total leukocytes, granulocytes, lymphocytes, monocytes, platelets, and T cell subsets were determined by the UCSF clinical laboratories. Measurements of plasma HIV RNA levels were performed using a branched-DNA (bDNA) assay (Siemens Diagnostics, Emeryville, CA) or were self-reported.

Cell specimens. Whole blood was collected in evacuated tubes (BD) containing EDTA and sodium heparin for immunophenotyping and functional studies, respectively. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood collected in evacuated tubes containing EDTA (BD Biosciences) by density gradient separation over Ficoll (Sigma). From the PBMC of each study subject, CD4⁺ and CD8⁺ cells were serially isolated by immunomagnetic bead separation (Miltenyi) prior to cell sorting. Purities of the isolated cells were >95%, as measured by flow cytometry.
TABLE 1. Demographic and immunologic characteristics of the study subjects

<table>
<thead>
<tr>
<th>Status of study subjects</th>
<th>Gender (no. of males/females)</th>
<th>Age (yr)</th>
<th>No. of lymphocytes/μl (× 1,000)</th>
<th>No. of CD4+ T cells/μl</th>
<th>% CD4+ T cells</th>
<th>No. of CD8+ T cells/μl</th>
<th>% CD8+ T cells</th>
<th>CD4/CD8 ratio</th>
<th>HIV-1 RNA log no. of copies/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV−</td>
<td>16/3</td>
<td>43 (33–56)</td>
<td>1.7 (1.4–2.0)</td>
<td>835 (650–1,209)</td>
<td>40 (37–48)</td>
<td>580 (513–647)</td>
<td>26 (23–30)</td>
<td>1.6 (1.2–1.8)</td>
<td></td>
</tr>
<tr>
<td>Thx HIV+</td>
<td>44/0</td>
<td>50 (40–56)</td>
<td>2.1 (1.8–2.4)</td>
<td>497 (312–662)</td>
<td>29 (20–35)</td>
<td>802 (588–1,160)</td>
<td>47 (39–55)</td>
<td>0.6 (0.4–0.9)</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>EC</td>
<td>15/0</td>
<td>51 (48–53)</td>
<td>2.1 (1.7–2.4)</td>
<td>690 (638–830)</td>
<td>43 (35–49)</td>
<td>693 (554–890)</td>
<td>37 (32–42)</td>
<td>1.1 (0.9–1.5)</td>
<td>&lt;1.7</td>
</tr>
<tr>
<td>vHIV+</td>
<td>39/2</td>
<td>48 (39–56)</td>
<td>2.3 (1.6–2.8)</td>
<td>447 (357–633)</td>
<td>27 (20–33)</td>
<td>901 (687–1,350)</td>
<td>52 (44–60)</td>
<td>0.5 (0.4–0.8)</td>
<td>3.9 (3.6–4.8)</td>
</tr>
</tbody>
</table>

* Except for gender, numbers provided are median values, with interquartile ranges in parentheses. Tx, treated.
duced or no CNAR activity in comparison to the CNAR activity in asymptomatic HIV-infected individuals (see the introduction). In the present study, we evaluated unstimulated \textit{(ex vivo)} CD8\(^+\)/H11001 cells from healthy uninfected blood donors (HIV\(^-\)/H11002), elite controllers (EC), viremic HIV-infected individuals (vHIV), and HIV-infected subjects receiving antiretroviral therapy (TxHIV) (Fig. 1 and Table 1).

We observed the following trend of CNAR activity among CD8\(^+\)/H11001 cells from individuals in these groups: vHIV > EC > TxHIV > HIV\(^-\) (Fig. 1A).

To identify potential subsets of cells that mediate strong CNAR activity, we measured the whole-blood frequencies of CD8\(^+\)/H11001 cell subsets in the aforementioned groups (Table 2). In comparison to the HIV\(^-\) and EC subjects, vHIV individuals exhibited elevated frequencies \((P < 0.05)\) of CD45RA\(^-\), CD11b\(^-\), HLA-DR\(^-\), and CD27\(^+\)/H11001 cells. vHIV subjects also exhibited the highest frequencies of CD45RA\(^-\)/CD27\(^+\), CD57\(^+\)/CD28\(^-\), and PD-1\(^+\)/CD8\(^+\) cells (Fig. 1B, C, and D, respectively). A direct correlation \((r^2 = 0.62)\) was observed between CNAR activity and the frequency of CD45RA\(^-\)/CD27\(^+\)/CD28\(^-\) cells within the CD8\(^+\) cell compartment (Fig. 1D).

These cross-sectional comparisons of CD8\(^+\)/H11001 cells from aviremic and viremic individuals demonstrate an association between low-level HIV replication, frequencies of distinct CD8\(^+\)/H11001 cell subsets, and CNAR activity.

**Maximal CNAR activity is mediated by activated CD8\(^+\) T cells.** Having identified correlations between the heightened CNAR activity of bulk CD8\(^+\) cells and the frequencies of selected CD8\(^+\) cell subsets (e.g., CD45RA\(^-\)/CD27\(^+\) cells) measured by fluorescence-activated cell sorting (FACS), we...
CD8⁺ T cells, as they encounter environmental stimuli, become “activated” and newly express a variety of surface antigens. To investigate their association with CNAR, cells differing in their levels of expression of several activation markers (e.g., the CD8 beta chain [CD8β], HLA-DR, and CD38, IL-25, CD28, C1.7, CD95, CD11b, and PD-1) were evaluated. To investigate the differentiation state of CNAR-mediating cells, CD8⁺ cells differing in their levels of coexpression of CD45RA CD62L, CD45RA CCR7, CD11b CD57, CD11b CD28, CD25 CD28, and CD45RA CD28 CD28 were assessed (Fig. 3). First, we compared the CNAR activities of CD8⁺ cells that differed in their levels of coexpression of CD45RA and CD62L or CCR7 (Fig. 3A and B). In comparison to naive (CD45RA⁺ CD62L⁺ or CD45RA⁺ CCR7⁺) cells, the more immunologically mature CD8⁺ cells (CD45RA⁻ CD62L⁻ or CD45RA⁻ CCR7⁻) exhibited superior CNAR activity (e.g., >90% suppression versus <50% suppression when the 0.5:1 cell input values were compared). Next, we evaluated the CNAR activity of CD8⁺ cells that differed in their levels of coexpression of CD11b and CD57 or CD28 (Fig. 3C and D). CD11b⁺ cells were observed to be mostly CD57 negative, whereas these cells were heterogeneous for expression of CD28. Maximal suppression was associated with a CD57⁻ phenotype, while both CD11b⁺ CD28⁺ and CD11b⁻ CD28⁻ cells exhibited strong CNAR activity. Then, we evaluated the CNAR activity of CD8⁺ cells that varied in their levels of coexpression of CD28 and CD57 or CD28 (Fig. 3E and F). Maximal suppression was exhibited by CD57⁻ CD28⁻ and CD27⁺ CD28⁺ cells. Finally, among six CD8⁺ cell subsets that differentially express CD45RA, CD27, and CD28, the two CD45RA⁻ CD27⁺ subsets were found to most potently suppress HIV replication (Fig. 3G). Therefore, the CD8⁺ cells that exhibited maximal suppression of HIV repli-

**TABLE 2. CD8⁺ cell subset frequencies in HIV-infected and uninfected individuals**

<table>
<thead>
<tr>
<th>Cell subset</th>
<th>HIV⁻ (n = 19)</th>
<th>TxHIV⁺ (n = 12)</th>
<th>EC (n = 15)</th>
<th>sHV⁺ (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD38⁺</td>
<td>64 (50–69)</td>
<td>62 (49–69)</td>
<td>60 (55–70)</td>
<td>70 (59–76)</td>
</tr>
<tr>
<td>HLA-DR⁺</td>
<td>15 (10–22)</td>
<td>31 (22–46)</td>
<td>33 (25–46)</td>
<td>49⁺ (36–62)</td>
</tr>
<tr>
<td>CD27⁺</td>
<td>22 (19–26)</td>
<td>26 (20–31)</td>
<td>21 (17–23)</td>
<td>31⁺ (25–37)</td>
</tr>
<tr>
<td>CD28⁺</td>
<td>57 (49–71)</td>
<td>47 (38–53)</td>
<td>40 (33–56)</td>
<td>35⁺ (27–46)</td>
</tr>
<tr>
<td>CD25⁺</td>
<td>4 (3–6)</td>
<td>5 (3–6)</td>
<td>3 (2–4)</td>
<td>2⁺ (1–4)</td>
</tr>
<tr>
<td>CD122⁺</td>
<td>31 (19–44)</td>
<td>16 (11–25)</td>
<td>21 (18–29)</td>
<td>17⁺ (13–26)</td>
</tr>
<tr>
<td>CD45RO⁺</td>
<td>35 (28–46)</td>
<td>50 (43–54)</td>
<td>42 (34–48)</td>
<td>47⁺ (38–58)</td>
</tr>
<tr>
<td>CD45RA⁺</td>
<td>69 (59–83)</td>
<td>67 (57–72)</td>
<td>73 (64–78)</td>
<td>59⁺ (52–71)</td>
</tr>
<tr>
<td>CD57⁺</td>
<td>19 (12–22)</td>
<td>43 (30–54)</td>
<td>36 (32–41)</td>
<td>41⁺ (34–55)</td>
</tr>
<tr>
<td>CD62L⁺</td>
<td>62 (54–71)</td>
<td>50 (45–57)</td>
<td>52 (43–58)</td>
<td>42⁺ (36–49)</td>
</tr>
<tr>
<td>CD11b⁺</td>
<td>31 (19–41)</td>
<td>15 (7–27)</td>
<td>17 (11–21)</td>
<td>10⁺ (6–16)</td>
</tr>
<tr>
<td>PD–1⁺</td>
<td>9 (6–15)</td>
<td>ND</td>
<td>18 (15–23)</td>
<td>44⁺ (34–51)</td>
</tr>
</tbody>
</table>

*Significantly different from the value for HIV⁻ subjects (P < 0.05).
*Significantly different from the value for EC subjects (P < 0.05).
*Significantly different from the value for TxHIV⁺ subjects (P < 0.05).
*Values are medians (interquartile ranges). ND, no data.
cation were CD45RA$^+$ CD27$^+$ CD28$^+$ cells, although appreciable anti-HIV activity was also mediated by CD45RA$^+$ CD27$^+$ CD28$^+$ cells. Comparisons of bulk CD8$^+$ cells, which were unstained or stained with antibodies, revealed no substantial differences in CNAR activity (data not shown). Thus, activation of the cells and/or blocking of the surface antigens due to antibody binding was not involved. These results show that CNAR is associated with memory CD8$^+$ cells, particularly those with a transitional memory phenotype.

**CNAR is associated with oligoclonal CD8$^+$ cell populations.**

To investigate the diversity of T cell receptor (TCR) usage among CD8$^+$ cell subsets having strong CNAR activity, TCR profiling was performed (Fig. 4). Specifically, bulk CD8$^+$ cells (Fig. 4C), CD45RA$^-$ CD27$^-$ CD28$^-$, CD45RA$^+$ CD27$^+$ CD28$^+$, CD45RA$^+$ CD27$^+$ CD28$^+$, CD45RA$^+$ CD27$^+$ CD28$^+$ (Fig. 4B), CD45RA$^+$ CD27$^+$ CD28$^+$ (Fig. 4A), CD45RA$^+$ CD27$^+$ CD28$^+$, and CD45RA$^-$ CD27$^-$ CD28$^-$ CD8$^+$ cells were analyzed from 3 HIV-1-infected (vHIV$^+$) individuals. Among the subsets evaluated, the CD45RA$^+$ CD27$^+$ CD28$^+$ CD8$^+$ cells exhibited Gaussian-like distributions of CDR3 lengths in each TCRV$\beta$ family, indicating a lack of clonal dominance within this subset. As described above (for Fig. 3G), this population exhibited poor CNAR activity. In contrast, TCRV$\beta$ families within the CD45RA$^+$ CD27$^+$ CD28$^+$ CD8$^+$ cell subset, a population with robust CNAR activity (Fig. 3G), exhibited a striking degree of perturbation (Fig. 4B). These data provide evidence that the CNAR is associated with CD8$^+$ cell subsets having a biased (i.e., a non-Gaussian-like distribution) T cell receptor usage.

CD8$^+$ cells with strong CNAR activity do not exhibit classical cytotoxic T lymphocyte (CTL) activity. Past studies have shown that CNAR does not involve cell killing (28). To confirm those findings in the present studies, the CD8$^+$ cells were removed after 3 days of cocultivation with the HIV-infected CD4$^+$ cells (Fig. 5). As shown previously (50, 51), HIV-infected cells persist in the presence of CNAR activity. HIV levels rapidly increased in the cell culture supernatants upon removal of the CD8$^+$ cells (Fig. 5A).

In separate experiments, CD8$^+$ cells were evaluated for degranulation upon their exposure to HIV peptides or HIV-
FIG. 3. Transitional memory CD8+ cells exhibit maximal CNAR activity. Shown are staining profiles (left) and anti-HIV activities (right) of CD8+ cells that were separated into distinct populations based on the expression of CD45RA and CD62L (A), CD45RA and CCR7 (B), CD57 and CD11b (C), CD28 and CD11b (D), CD57 and CD28 (E), CD27 and CD28 (F), and CD45RA, CD27, and CD28 (G). Suppression data are shown for CD8+ cell/infected CD4+ cell coculture ratios of 0.25:1 (G only), 0.5:1, and 1:1 (left to right). Results are representative of at least 2 independent experiments with different CD8+ cell sources.
infected heterologous CD4+ cells in CD107 mobilization assays. The mobilization of lysosome-associated membrane glycoproteins (LAMPS), including CD107a (LAMP-1) and CD107b (LAMP-2), to the cell surfaces of CD8+ cells is directly associated with CTL activity (4). CD8+ cells that were expanded in vitro with HIV-specific (Fig. 5B, left) or CMV-specific (data not shown) antigens and then incubated in the presence of their cognate peptides underwent marked degranulation. In comparison, CD8+ cells did not degranulate when placed into culture with heterologous CD4+ cells that had been infected for 3 days and were producing substantial levels of HIV (Fig. 5B, right). Also, the levels of CD107 expression on CD8+ cells were not found to differ between CD8+ cells cultured alone and those cocultured with acutely infected CD4+ cells (data not shown). Notably, the CD8+ cells that were stimulated with HIV peptides were able to suppress HIV replication in acutely infected heterologous CD4+ cells poststimulation.

In another series of experiments, we assessed the ability of various subsets of CD8+ cells to produce soluble factors having anti-HIV activity. In two independent experiments (Fig. 5C), conditioned medium from the CD57+ PD-1+ CD8+ cells exhibited increased anti-HIV activity in comparison to conditioned medium from bulk CD8+ cells or those lacking a CD57+ PD-1+ cell phenotype. Similarly, CD57− PD-1− cells suppressed HIV replication by >50% when assessed in transwell assays (data not shown). These results demonstrate that HIV-suppressing CD8+ cells do not eliminate HIV-infected cells and do not exhibit detectable degranulation in the presence of HIV-infected CD4+ cells. Moreover, CD8+ cell subsets with strong CNAR activity do suppress HIV replication via the secretion of a soluble factor(s). Thus, they are unlike classical CTL in function.

**DISCUSSION**

In previous studies, we observed that CD8+ cells from HIV-infected individuals vary in their abilities to suppress HIV replication in primary CD4+ cells; CD8+ cells from asymptomatic persons have the highest CD8+ cell noncytotoxic antiviral response (CNAR) (3, 7, 14, 21, 25, 35). To further characterize this anti-HIV activity, we systematically compared the whole-blood frequencies (Table 1) and HIV-suppressing activities of various CD8+ cell subsets (Fig. 2 and 3) among HIV-infected and uninfected individuals.

In comparison to CD8+ cells from aviremic (EC and TxHIV) HIV-infected individuals, those from asymptomatic subjects with low-level viremia (vHIV) were found to exhibit the strongest CNAR activity (Fig. 1). These findings support past results showing that when viral loads are below detectable levels, as is characteristic of elite controllers and subjects treated with antiretroviral therapy, the CNAR is generally low or not detectable (44). This observation indicates that CNAR is activated upon HIV replication. Those elite controllers who exhibited some CNAR activity (Fig. 1) most likely had blips of virus replication that were sufficient to sustain this response (16). Importantly, the viremic individuals in this study were healthy long-term survivors of HIV infection and exhibited low viral loads (median, 3.9 logs) (Table 1). Indeed, our findings are consistent with those of previous studies establishing this anti-HIV response as a characteristic of CD8+ cells from asymptomatic long-term survivors with low-level viremia (3, 7, 14, 25, 35).

Cross-sectional analyses of whole blood revealed increased frequencies of CD45RA+ CD27+ and CD57+ CD28− CD8+ cells in the vHIV group (Fig. 1). In addition, we observed a direct correlation between CNAR activity and the frequency of CD45RA+ CD27+ CD8+ cells. These observations link CNAR activity with transitional memory cells (see below). The finding of decreased frequencies of CD45RA− CD27− CD8+ cells in patients receiving antiretroviral therapy (Fig. 1A and B) provides an explanation for the previously observed loss of CNAR activity in CD8+ cells from these subjects (22, 44). Still, differences in CD8+ cell subset frequencies, as measured by flow cytometry, do not necessarily account for a loss or gain of antiviral function. Therefore, cell sorting experiments were performed to evaluate directly the antiviral activities of CD8+ cell subsets that change in frequency with HIV infection.

In assessing cell function, our experiments demonstrate that CD8+ cell subsets (without prior in vitro stimulation) exhibit differential abilities to suppress HIV replication. With respect to CD8+ cells of distinct hematopoietic lineages, CD3+ (T) cells expressing CD8β have strong CNAR activity, whereas...
CD8⁺ (NK) cells do not (Fig. 2). Of note, CD8β is not expressed by circulating CD8⁺ γ/δ T cells (30), thus excluding γ/δ T cells from being part of CNAR.

In previous investigations with mitogen-stimulated CD8⁺ cells, we observed that noncytotoxic anti-HIV activity was highest among HLA-DR⁺, CD11b⁺, and VCAM⁺ cells (11, 25). Similarly, in this study of peripheral blood CD8⁺ cells that were not mitogen stimulated, CNAR activity was found to be mediated by CD8⁺ cells having activated phenotypes (Fig. 2). Specifically, CD8β⁺, HLA-DR⁺, CD95⁺, C1.7⁺, and PD-1⁺ CD8⁺ cells exhibited maximal CNAR activity. CD8β is down-modulated upon activation, and the frequency of this down-modulation in CD8β⁺ cells is increased in HIV-infected individuals (43). CD8β⁺ cells also suppress virus replication in feline immunodeficiency virus (FIV)-infected cats (13).

Moreover, as noted in other studies of CD8⁺ cells that were activated in vitro, CNAR was mediated chiefly by CD57⁺ CD8⁺ cells (2). In the present studies of CD8⁺ cells not stimulated in vitro, both CD57⁺ CD28⁺ and CD57⁻ CD28⁻ subsets were able to suppress HIV replication, although the CD28⁺ cells showed superior CNAR activity. At the time of the earlier study (2), CD28 and CD57 were believed to be mutually exclusive antigens on CD8⁺ cells and the depletion of CD57⁺ cells was thought to yield relatively pure populations of CD28⁺ cells. However, as described in this study (Fig. 1C), HIV-infected persons can harbor appreciable numbers of CD57⁻ CD28⁻ CD8⁺ cells. Furthermore, CD8⁺ cells have now been shown to downmodulate CD28 expression during the process of immunologic maturation (46).

Further phenotypic analyses of CD8⁺ cells that potently suppress HIV replication provided insight into the differentiation state of cells mediating CNAR. Circulating CD8⁺ cells can be classified into the following subsets: naïve cells (CD45RA⁺ CD27⁻ CD28⁺), central memory cells (CD45RA⁺ CCR7⁻ CD62L⁻), transitional memory cells (CD45RA⁻ CD27⁺ CCR7⁻), and effector cells (CD45RA⁻ CD27⁻ CCR7⁻) (15, 42). Memory cells express high surface levels of CD95 and exhibit little cytolytic activity in the absence of in vitro prestimulation. Effector cells express high levels of CD11b (10) and have high cytolytic activity without in vitro prestimulation. As noted above, we found that CNAR activity is mediated by CD8⁺ memory cells (Fig. 3), chiefly of the transitional memory (CD45RA⁻ CD27⁺ CCR7⁻) phenotype. Supportive evidence that CNAR activity is mediated by memory CD8⁺ cells is our observation that the CD8⁺ cell population having strong CNAR activity exhibits skewed T cell receptor usage (Fig. 4). Additional studies are needed to establish the overall contribution of clonally expanded

**FIG. 5.** Nonlytotoxic features of HIV-suppressing CD8⁺ cells. (A) HIV replication levels were evaluated in cultures from which CD8⁺ cells were removed following suppression of HIV replication. Shown are reverse transcriptase (RT) levels in the supernatants of HIV-infected cells cultured alone (■), in the presence of HIV-suppressing CD8⁺ cells (○), and upon removal of the HIV-suppressing CD8⁺ cells after 4 days of coculture (*). (B) CD107a⁺/CD107b levels were measured in HIV-specific CD8⁺ cells (left) and bulk CD8⁺ cells (right) upon exposure to cognate antigen and heterologous HIV-infected CD4⁺ cells, respectively. (C) CD8⁺ cells from a viremic HIV-infected individual were sorted into 2 subsets: CD57⁻ PD1⁺ cells and those lacking this phenotype (not CD57⁻ PD1⁻). Conditioned medium collected from cultures containing these CD8⁺ cell subsets was replaced onto CD4⁺ cells that were acutely infected with HIV. Shown are the RT levels in each culture at the time of peak virus replication in the control. Data in each panel are representative of at least 2 separate experiments.
CD8+ cells to CNAR. In this regard, CD8+ cell clones isolated from HIV-infected individuals can exhibit CNAR activity without HIV-specific CTL activity (18, 47). Also, our findings are consistent with the very recent report that memory CD8+ cells, particularly CD45RA+CD27+ cells, effectively suppress HIV replication (12). However, our studies used a primary HIV-1 isolate and included biologic assessments of the antiviral function of CD8+ cells from HIV-1-infected individuals. Thus, we were able to distinguish the CNAR from classical CTL activity (see below).

HIV-specific (tetramer-positive) CD8+ cells have been described to predominantly exhibit a CD45RA+CD27+CD57-CCR7- phenotype characteristic of transitional memory cells (8). In contrast, strong CTL responses have traditionally been associated with a CD45RA-CD27-CD28- effector cell phenotype (46). In comparison to bulk CD8+ cells or those that are specific for other viruses (e.g., CMV), HIV-specific CD8+ cells contain substantially lower levels of perforin, a protein required for granule-mediated cytolysis (1). These observations have led to speculation that HIV-specific CD8+ cells are defective killers (29, 49). Indeed, CD8+ CD45RA-CD27+CD28+ cells exhibit very little lytic activity in CD3 monoclonal antibody (MAb)-mediated redirected cytotoxicity assays (15). This finding is consistent with our past and present observations that HIV-suppressing CD8+ cells do not eliminate HIV-infected cells and that their antiviral effect is rapidly reversible (Fig. 5A) (50, 51). Moreover, CD8+ cells exhibiting strong CNAR activity do not degranulate in the presence of HIV-infected CD4+ cells (Fig 5B) yet do secrete a soluble antiviral factor (Fig 5C). Therefore, we propose that CD8+ cells having a CD45RA-CD27+ phenotype, likely including some that are HIV-specific, are noncytotoxic HIV-suppressing cells (8).

Considerable attention has been given to the role of PD-1-expressing CD8+ cells in HIV infection. PD-1 is a member of the CD28 family, which has immunoregulatory functions (40), and this antigen is frequently expressed on HIV-specific CD8+ cells having a transitional memory phenotype (41). In agreement with other studies (48), we observed that PD-1 expression on CD8+ cells is increased in the context of HIV infection (Table 2). However, in those studies the HIV-specific CD8+ cells exhibiting high levels of PD-1 expression were found to be functionally defective (48). In contrast, our studies demonstrate that PD-1+ CD8+ cells have a previously unappreciated anti-HIV activity (Fig. 2F).

In summary, maximal CNAR activity is associated with CD8+ T cells having a CD3+CD8+dimCD11b+CD57-CD95+c1.7+PD-1+ cell phenotype. These markers, along with HLA-DR (25), indicate that the CNAR is mediated by activated CD8+ cells. Furthermore, strong suppression of HIV replication is associated with CD8+ cells having a CD45RA+CD27+CD28-CCR7+ phenotype that is characteristic of transitional memory cells. Notably as well, PD-1+ CD8+ cells, previously considered dysfunctional, exhibit strong CNAR activity. Overall, our immunophenotyping and functional studies indicate that fewer than 50% of CD8+ cells mediate greater than 90% of CNAR activity. These studies better distinguish the ex vivo phenotypes of CD8+ T cells that mediate CNAR and provide insight for why HIV-infected subjects can differ in their levels of this important antiviral activity. This information can be helpful in developing novel immunotherapeutic strategies and directing vaccine design.

ACKNOWLEDGMENTS

This research was supported by NIH grant 5R01AI056992-07 and California HIV/AIDS Research Program grant F05-SF-218. We thank Vernon Maino (BD Biosciences) for advice and some reagents used for this study.

REFERENCES


