HIV-Specific CD8\(^+\) T Cell Function in Children with Vertically Acquired HIV-1 Infection Is Critically Influenced by Age and the State of the CD4\(^+\) T Cell Compartment


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HIV-Specific CD8\(^+\) T Cell Function in Children with Vertically Acquired HIV-1 Infection Is Critically Influenced by Age and the State of the CD4\(^+\) T Cell Compartment\(^1\)


The immunology of vertical HIV transmission differs from that of adult infection in that the immune system of the infant is not fully matured, and the factors that influence the functionality of CD8\(^+\) T cell responses against HIV in children remain largely undefined. We have investigated CD8\(^+\) T cell responses in 65 pediatric subjects with vertically acquired HIV-1 infection. Vigorous, broad, and Ag dose-driven CD8\(^+\) T cell responses against HIV Ags were frequently observed in children who were older than 3 years of age and maintained CD4\(^+\) T cell counts >400 cells/\(\mu\)l. In contrast, younger age or a CD4\(^+\) T cell count <400 cells/\(\mu\)l was associated with poor CD8\(^+\) T cell responses and high HIV loads. Furthermore, subjects with a severely depleted and phenotypically altered CD4\(^+\) T cell compartment had circulating Gag-specific CD8\(^+\) T cells with impaired IFN-\(\gamma\) production. When viral load was not suppressed by antiviral treatment, subjects that fell below the putative age and CD4\(^+\) T cell count thresholds had significantly reduced CD8\(^+\) T cell responses and significantly higher viral loads. Thus, the data suggest that fully effective HIV-specific CD8\(^+\) T cell responses take years to develop despite an abundance of Ag in early life, and responses are further severely impaired, independent of age, in children who have a depleted or skewed CD4\(^+\) T cell compartment. The results are discussed in relation to differences between the neonatal and adult immune systems in the ability to respond to HIV infection. *The Journal of Immunology, 2003, 170: 4403–4410.*

Human immunodeficiency virus-1-specific CD8\(^+\) T cell responses are thought to play a substantial role in the containment and suppression of viremia during both primary and chronic phases of HIV infection in adults (reviewed in Ref. 1). These responses appear concurrently with early control of viremia during primary infection (2, 3), and loss of these cells has been linked to rapid disease progression (4). Long-term nonprogressive infection is associated with strong HIV-specific responses in both CD8\(^+\) T cells and CD4\(^+\) T cells (4–8), and immune pressure exerted by CD8\(^+\) T cells can lead to selection of viral epitope escape mutants in vivo (9–12). In addition, an inverse correlation has been observed between numbers of circulating HIV Gag-specific CD8\(^+\) T cells and HIV load, suggesting immune control of the virus (13). However, subsequent studies have shown either no correlation (14, 15) or a direct positive correlation between CD8\(^+\) T cell responses and viral load (16), and the basis for these differences is unclear.

All of the studies mentioned were performed in adults, and little is known about the correlation between CD8\(^+\) T cell responses, control of virus, and disease progression in children. HIV-specific CD8\(^+\) T cell-mediated cytolysis can be detected in children, although weaker than in adults and rarely before 6 mo of age (17, 18). Recently, HIV-specific pediatric CD8\(^+\) T cell responses have also been demonstrated using HLA tetramer and IFN-\(\gamma\) ELISPOT assays (19, 20). In adults, primary infection is marked by an initial burst in viremia that is usually contained within 3–4 mo resulting in lower viral burden, resolution of clinical symptoms, and the establishment of a viral set point that is predictive of the time course to disease outcome (21, 22). The dynamics of HIV-1 infection in vertically infected children are different compared with adults, in that neonates often experience a greater initial viral burst and viral loads decrease only slowly over time (23, 24). A high thymic output and a high number of circulating CD4\(^+\) T cell targets may explain the greater viral burst in neonates (25–28). The relationship between HIV-specific cellular immunity and viral dynamics in neonates and young children is not known.

The recombinant vaccinia virus IFN-\(\gamma\) ELISPOT assay is a sensitive and reliable method for quantifying CD8\(^+\) T cell IFN-\(\gamma\) secretion in response to viral Ags, and is particularly useful when the number of blood cells available for study is limited (29). Using this assay in conjunction with HLA tetramers and six-color flow cytometry, we investigated how the strength and breadth of the CD8\(^+\) T cell response against HIV-1 in infected children is influenced by age and CD4\(^+\) T cell phenotype and counts. Furthermore, we have evaluated the relationship between CD8\(^+\) T cell responses against HIV-1 and the circulating viral load. We find that the

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CD8+ T cell response is influenced, quantitatively and qualitatively, by subject age and CD4+ T cell phenotype and counts. Pediatric subjects apparently need to be older than 3 years of age and stay above a minimum number of circulating CD4+ T cells to mount strong and Ag-driven CD8+ T cell responses against HIV. When these conditions are not met, CD8+ T cell responses are poor and HIV load is high. These observations are discussed in relation to neonatal tolerance induction and to development of the immune system in the infant. In addition, our data suggest a model for the relationship between the CD8+ T cell responses against HIV and viral load that may help reconcile apparently conflicting observations in the literature.

Materials and Methods

Patient samples and viral load measurements

HIV-1-infected pediatric subjects were treated and followed at the Jacobi Medical Center (Bronx, New York). Heparinized whole-blood samples were obtained from 65 subjects after informed consent, based on local Institutional Review Board-approved protocols. All subjects included in the study were infected by mother-to-child transmission at birth. Sample size ranged from 2 to 5 ml per bleed. PBMCs were isolated by ficoll-paque plus density gradient centrifugation (Amersham Pharmacia Biotech, Uppsala, Sweden). Plasma HIV-1 RNA was measured with the Amplicor HIV-1 monitor with a lower limit of quantification at 400 copies of RNA/ml (Roche Diagnostic Systems, Branchburg, NJ).

IFN-γ ELISPOT assay

HIV-specific CD8+ T cell responses were measured using the recombinant vaccinia IFN-γ ELISPOT assay (29). Each well of a sterile multiscience 96-well filtration plate (MAHAS#510, Millipore, Bedford, MA) was coated with 50 μl of anti-INF-γ mAb (Mabtech, Stockholm, Sweden) at a concentration of 10 μg/ml in 1M sodium bicarbonate buffer (pH 9.5). After an overnight incubation at 4°C, each well was washed four times with PBS (Cellgro, Herndon, VA) and blocked with 50 μl of 5% pooled human serum in RPMI (Cellgro) for 1 h at 37°C. PBMC (1.5 × 106) were added to triplicate wells, and recombinant vaccinia viruses expressing HIV-1 IIIB Pol, Nef, Gag, Env, or Rev (Therion Biologics, Cambridge, MA) were added at a multiplicity of infection of 2:1 directly to the cell solution. Vaccinia strain TK− was used as negative control, and Staphylococcus enterotoxin B (Sigma-Aldrich, St. Louis, MO) was used as a positive control. After incubation overnight at 37°C, plates were washed four times using PBS with 0.05% Tween 20 (Fisher Biotech, Fair Lawn, NJ). Bio- tinylated anti-INF-γ mAb 7-B6-1 (Mabtech) was added as 1 μg/ml in 100 μl of PBS, and the plate was incubated for 2 h at 37°C. Next, plates were washed four times using 0.1% Tween 20 in PBS and then treated with avidin-conjugated HRP H (Vector Laboratories, Burlingame, CA). After 1 h, plates were washed four times with 0.1% Tween 20 in PBS. Fifty microliters of stable diaminobenzidine tetrahydrochloride substrate (Re- search Genetics, Huntsville, AL) were added to each well for 5 min and then washed away with water. IFN-γ spot-forming cells (SFC) were visualized and counted using an AID ELISPOT reader system (Autoimmun Diagnostika, Strassberg, Germany). Raw counts were standardized to express SFC per million CD8+ T cells. Background frequencies obtained with vaccinia strain TK− were subtracted from Ag-specific frequencies to obtain the final count. A reading of >20 SFC per million CD8+ T cells after subtraction of background was considered positive.

Six-color flow cytometry, mAbs, HLA-A2 tetramers, and cytokine flow cytometry

CD8+ T cells specific for HIV-1 Gag and Pol Ags were identified and enumerated using PE-conjugated HLA-A2 tetrameric complexes refolded with the Gag77–86 and Pol epitope SLYNTVATL or the Pol76–84 epitope ILKEPVHGV (Coulter Immunotech, Marseilles, France). The phenotype of HIV-1 Gag77–86-specific CD8+ T cells was investigated using mAbs against CD3, CD8, CD27, CD45RA, CD45RO, CD62L, and CCR7. Anti-CD27 FITC, anti-CD62L FITC, purified anti-CCR7 IgM, anti-IgM biotin, and streptavidin-allophycocyanin conjugate were purchased from BD PharMingen (San Jose, CA). PE-Texas Red-conjugated mAbs against CD3, CD8, CD45RA and streptavidin-allophycocyanin conjugate were purchased from BD PharMingen. IFN-γ was measured using the ELISPOT assay (29). Each well of a sterile multiscreen plate was coated with 50 μl of stable diaminobenzidine tetrahydrochloride substrate (Research Genetics, Huntsville, AL) were added to each well for 5 min and then washed away with water. IFN-γ spot-forming cells (SFC) were visualized and counted using an AID ELISPOT reader system (Autoimmun Diagnostika, Strassberg, Germany). Raw counts were standardized to express SFC per million CD8+ T cells. Background frequencies obtained with vaccinia strain TK− were subtracted from Ag-specific frequencies to obtain the final count. A reading of >20 SFC per million CD8+ T cells after subtraction of background was considered positive.

Statistical analysis

The ELISPOT and flow cytometry data obtained were analyzed by descriptive statistics, linear regression, t test, and the Mann-Whitney rank sum test, as appropriate, using SigmaStat software (SPSS, Chicago, IL).

Results

Patient cohort characteristics

We have analyzed peripheral blood samples from 65 children with vertically acquired HIV-1 infection (Table I). We anticipated that this study of 65 HIV-infected children would provide the power to determine the influence of the clinical parameters of viral load, age, and CD4+ T cell counts on the CD8+ T cell responses against HIV. The subjects ranged in age from 1 to 16 years old; 55% were female, and 49% were of Hispanic and 51% were of non-Hispanic black ethnic origin. Fifty-seven of the 65 subjects were on antiretroviral treatment at the time of the study. Forty-five subjects were on highly active antiretroviral treatment (HAART) including protease inhibitor, and 26 of those were on a salvage regimen because of previous treatment failure. Two were receiving a triple drug regimen without protease inhibitor, 10 were treated with dual antiviral drugs, and 3 were treated with a single drug. Symptomatic HIV disease, plasma viremia, and CD4+ T cell counts varied widely in the treated subjects, indirectly reflecting the problem of adherence to complex antiretroviral drug regimens in pediatric patients and the cumulative effect of drug resistance, viral diversification, and duration of infection on CD4+ T cell depletion and disease progression.

CD8+ T cell responses against HIV-1 Ags

The recombinant vaccinia virus-based ELISPOT assay allows assessment of the strength of CD8+ T cell responses against the gene products of viruses and is particularly useful when the number of PBMCs available for study is limited. We used vaccinia constructs

CD8+ T CELL FUNCTION IN PEDIATRIC HIV INFECTION

Table I. Characteristics of pediatric HIV-infected patient cohort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Age</td>
<td>1–16 years (6.8 ± 3.9)†</td>
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<tr>
<td>Sex</td>
<td>55% female, 45% male</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>49% Hispanic, 51% non-Hispanic black</td>
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<tr>
<td>Viral load</td>
<td>400–7.5 × 105 copies/ml (89.117 ± 188.264)</td>
</tr>
<tr>
<td>CD4+ T cell count</td>
<td>20–2,136 cells/μl (962 ± 551)</td>
</tr>
<tr>
<td>CD4+ T cell percentage</td>
<td>1–47% (29.5 ± 11.3)</td>
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† Mean and SD.
expressing HIV-1 Gag, Pol, Env, Nef, and Rev to measure CD8⁺ T cell activity in the peripheral blood of our pediatric cohort. Responses against at least one of the HIV Ags were detected in 55 of the 65 (85%) children. Pol was the most frequently recognized Ag, followed by Env and Gag (Fig. 1A). Nef was recognized in only 26% of the subjects (Fig. 1A). Responses against the HIV-1 Rev Ag were assessed when sufficient cell numbers were available. When present, Rev-specific responses were weak (<50 SFC per million PBMC) and detected in only 3 of the 20 subjects tested (data not shown and not included in the further analysis). In the patients who had detectable responses against the respective Ags, responses against Pol and Gag tended to be the strongest followed by responses against Nef, whereas responses against Env appeared somewhat weaker, although these differences did not reach statistical significance (Fig. 1B). Thus, CD8⁺ T cell responses against HIV Ags were frequently detected, and Pol was the most frequently recognized Ag in the cohort.

**Strength and breadth of CD8⁺ T cell responses against HIV are influenced by age**

We were next interested in the relationship between different clinical parameters and the CD8⁺ T cell response against HIV. Analysis of the strength of responses vs age, CD4⁺ T cell count, and viral load revealed no clear linear relationships (not shown). However, the total anti-HIV CD8⁺ T cell responses (the sum of responses against the four Ags) of children younger than 4 years of age were significantly weaker compared with those of children older than 4 years (Figs. 2A and 3A). Seven of 14 subjects in this age group had no detectable responses, and responses in all but 1 subject were considered very weak. In contrast, 31 of 32 subjects in the age group of 4–9 years had CD8⁺ T cell responses against HIV, and responses were significantly stronger than those in the younger subjects (p < 0.001) (Fig. 3A). The CD8⁺ T cell activity in the younger age group was weak not only in strength but also in breadth as compared with the subjects aged 4–9 years (p = 0.004) (Fig. 3B). There was no significant difference in viral load between the age groups (Fig. 3C). We next assessed the circulating frequencies of CD8⁺ T cells specific for HLA-A2-restricted epitopes Gag 77–85 and Pol 476–484 using the corresponding HLA-A2 tetramers. Forty-nine HLA-A2-positive subjects, of whom 11 were 0–3 years old and 38 were older than 3 years of age, were identified in an expanded cohort of 85 pediatric patients. In the younger age group, only 1 of 11 had detectable numbers of CD8⁺ T cells specific for the Gag epitope and 2 of 11 had cells specific for the Pol epitope (Fig. 3D). In contrast, in the older age group, 11 of 38 subjects had circulating CD8⁺ T cells specific for the Gag epitope and 9 of 38 had cells specific for the Pol epitope. The circulating frequencies detected in the older subjects were considerably higher than those detected in the younger age group (Fig. 3D). Together, these data show a difference in the strength of HIV-specific CD8⁺ T cell responses in the subgroup of younger children as compared with the older subgroup, and suggest the presence of an age threshold for strong CD8⁺ T cell responses against HIV-1 in pediatric subjects with vertically acquired HIV infection.

**Anti-HIV CD8⁺ T cell activity depends on a preserved CD4⁺ T cell compartment**

We next analyzed the correlation of total anti-HIV CD8⁺ T cell responses with absolute CD4⁺ T cell counts (Fig. 2B). There was a trend toward stronger HIV-specific CD8⁺ T cell responses in subjects with CD4⁺ T cell counts in the 400-1500 cells/μl span, as compared with subjects with CD4⁺ T cell counts <400 cells/μl or >1500 cells/μl (p = 0.07) (Fig. 4A). The breadth of HIV-specific
CD8⁺ T cell responses was similar irrespective of CD4⁺ T cell count (Fig. 4B). In support of the significance of the poor responses in subjects with low CD4⁺ T cell counts, viral load was significantly higher in this group \( p < 0.003 \) (Fig. 4C). As expected, there was a strong inverse correlation between age and CD4⁺ T cell counts in the entire cohort of 65 patients \( r = 0.62; p < 0.001 \) (Fig. 2C). This indicated that the poor anti-HIV CD8⁺ T cell activity in subjects with >1500 CD4⁺ T cells/µl was linked to the young age of these subjects, whereas the weakness in responses in patients with <400 CD4⁺ T cells/µl was not.

These results support a requirement for CD4⁺ T cell help for the maintenance of strong anti-HIV CD8⁺ T cell responses in pediatric subjects. However, more experiments were needed to address the function of HIV-specific CD8⁺ T cells in subjects with high and low CD4⁺ T cell counts. The function of HIV Gag₇₇₋₈₅-specific CD8⁺ T cells detectable by HLA-A2/Gag₇₇₋₈₅ tetramer were analyzed in eight subjects with circulating frequencies 0.15% of CD8⁺ T cells, and where sample availability allowed a careful functional analysis (Table II). These eight subjects were between 5 and 16 years old. IFN-γ ELISPOT responses against Gag-expressing recombinant vaccinia were detected in six of the eight subjects, while no such responses were seen in subjects P37 and P59. Similarly, no IFN-γ was detected in these two subjects by intracellular cytokine flow cytometry after stimulation with the Gag₇₇₋₈₅ synthetic peptide. Subjects P37 and P59 both had absolute CD4⁺ T cell counts >200 cells/µl, whereas the other six had counts between 462 and 1333 cells/µl. Thus, the lack of IFN-γ production in Gag-specific CD8⁺ T cells in subjects P37 and P59.
supports the role of CD4+ T cells in sustaining the function of anti-HIV CD8+ T cell responses in the pediatric patients.

Ag-driven CD8+ T cell responses against HIV in pediatric subjects who pass the age and CD4+ T cell requirements

Having identified age and circulating CD4+ T cell numbers as important parameters influencing anti-HIV CD8+ T cell responses in the pediatric HIV-infected subjects, we were interested in the influence of antiviral treatment and viral replication on these responses. HIV load might affect the HIV-specific CD8+ T cell frequency and function in at least two ways: 1) as the cause of CD4+ T cell destruction and the consequent lack of T cell help, and 2) as the source of stimulating Ag. Only 14 of the 57 subjects that were on HAART or treated with dual or triple antiviral drugs had viral loads consistently suppressed (>6 mo) below the limit of detection (400 copies/ml). These 14 subjects tended to have weaker CD8+ T cell responses than the 43 subjects that experienced incomplete or incomplete suppression of the virus, although this difference did not reach statistical significance (p = 0.19) (Fig. 5A). However, in view of our results of a negative influence of young age and low CD4+ T cell counts, we repeated the analysis in the subgroup of subjects older than 3 years of age and with CD4+ T cell counts >400 cells/μl (n = 37). Within this group, CD8+ T cell responses were significantly stronger in subjects with incomplete viral control (p = 0.03) (Fig. 5B). To analyze this further, we broke the group of subjects with unsuppressed HIV (n=43) down into two groups, the first comprising subjects that passed the putative age and CD4+ T cell count requirements (n=25) (group 1), and a

FIGURE 5. Ag dose-driven CD8+ T cell responses in subjects that pass the age and CD4+ T cell count requirements for strong responses. A, Strength of total CD8+ T cell responses against HIV Env, Gag, Pol, and Nef as measured by IFN-γ ELISPOT in subjects receiving antiretroviral treatment. Comparison between subjects with consistently suppressed HIV (n=14) and subjects with inconsistent or partially controlled virus (n=43). B, CD8+ T cell responses in subjects older than 3 years of age and with CD4+ T cell counts >400 cells/μl. C, CD8+ T cell responses in subjects with uncontrolled or partially controlled viremia. Group 1, Subjects that clear the age and CD4+ T cell count requirements for strong responses (n=25). Group 2, Subjects that fall below the age and CD4+ T cell count requirements for strong responses (n=18). D, Viral load in Group 1 and Group 2. Data are expressed as mean and SE. *, p < 0.05; **, p < 0.005 as determined by the Mann-Whitney rank sum test.

FIGURE 6. Six-color flow cytometric assessment of circulating Gag-specific CD8+ T cells. PBMC from subject P59 stained with HLA-A2 Gag77–85 tetramer together with mAbs against CD3, CD8, CD45RA, CCR7, and CD62L (left panel), or with mAbs against CD3, CD8, CD45RO, CCR7, and CD27 (right panel), and analyzed with six-color flow cytometry. One representative experiment with PBMC from subject P59 is shown.
second group of those that did not (n = 18) (group 2). Group 1 displayed CD8+ T cell responses against HIV that were ~4.5 times higher than responses in Group 2 (p < 0.001) (Fig. 5C), and had significantly lower viral loads (p < 0.001) (Fig. 5D). These data indicate that the strength of anti-HIV CD8+ T cell responses, in subjects that pass the putative age and CD4+ T cell requirements for functional responses, is determined by the availability of HIV Ag. Furthermore, the data may suggest a correlation between the strength of CD8+ T cell responses and partial control of HIV in pediatric subjects experiencing treatment failure.

Circulating Gag-specific CD8+ T cells display an effector memory phenotype

We next hypothesized that the poor IFN-γ production observed in Gag-specific CD8+ T cells could be associated with a change in memory and effector cell markers. PBMC from subjects with HLA-A2/Gag77-85 tetramer-positive cells were analyzed for expression of CD3, CD8, CD27, CD45RA, CD45RO, CD62L, and CCR7 by six-color flow cytometry. Surprisingly, tetramer-positive CD3+ CD8+ T cells from all donors had a similar predominantly CD45RA+RO−, CD27−, CD62L−, and CCR7− phenotype (Fig. 6). Thus, circulating CD8+ T cells specific for the HIV-1 Gag77-85 epitope display an activated, non-lymph node-homing, effector memory phenotype (31) irrespective of the capacity to produce IFN-γ upon recognition of Ag.

Loss of IFN-γ production in Gag-specific CD8+ T cells is associated with a skewed CD4+ T cell phenotype

The results shown in Fig. 4 and Table II suggest that low numbers of circulating CD4+ T cells are associated with both a poor overall CD8+ T cell response against HIV, and with impaired ability of Gag77-85-specific CD8+ T cells to produce IFN-γ. We next investigated the phenotype of CD4+ T cells in subjects with detectable Gag77-85-specific cells (Table II). In particular, the expression of CCR7 and CD45RA, which defines naive and central and effector memory populations of CD4+ T cells in the model by Salustro et al. (31), was investigated. The CCR7 and CD45RA expression pattern of circulating CD4+ T cells in subjects P37 and P59 was markedly different from that observed for the six subjects with detectable IFN-γ production against HIV-1 Gag (Fig. 7). CD4+ T cells with a naive CCR7+CD45RA+ phenotype were reduced in subjects P37 and P59 (p = 0.004), whereas CCR7−CD45RA− effector memory cells were increased (p = 0.036). Numbers of CCR7+CD45RA− central memory CD4+ T cells did not differ significantly between the two groups. However, a CCR7−CD45RA+ population of CD4+ T cells was significantly increased in frequency (p = 0.003). These data suggest that the dysfunction of Gag-specific CD8+ T cells in subjects P37 and P39 is associated with not only a quantitative CD4+ T cell deficiency but also a shift in the distribution of naive, memory and effector CD4+ T cell populations toward a late-stage phenotype.

Discussion

The present study aimed at investigating the influence of characteristics such as age, CD4+ T cell phenotype and cell counts, and HIV-1 viral load on CD8+ T cell-mediated immunity in pediatric HIV infection. This could be expected to be a very complex issue, and the potential answers to be equally complex. We have found that subjects younger than 4 years of age have, for the most part, very poor CD8+ T cell responses against HIV Ags as compared with the older subjects, measured both as the magnitude and the breadth of responses. Our results also in two ways support the importance of the CD4+ T cell compartment in maintaining good CD8+ T cell responsiveness. First, subjects with <400 CD4+ T cells/μl tended to have weak anti-HIV responses, as compared with subjects who had between 400 and 1500 CD4+ T cells/μl. The relatively strong responses in the latter group were paired with significantly lower viral loads, suggesting that HIV-specific CD8+ T cells contribute to the containment of viremia in these pediatric subjects. Of note, the breadth of CD8+ T cell responses was no different in subjects with low CD4+ T cell counts, indicating that the breadth of responses is preserved even as the magnitude declined in these subjects. Second, loss of Ag responsiveness was observed in two of eight subjects with circulating CD8+ T cells specific for the HLA-A2-restricted Gag77-85 epitope. These two subjects were characterized by CD4+ T cell counts of <200 cells/μl, while the six subjects with responsive CD8+ T cells against Gag had CD4+ T cell counts between 462 and 1333 cells/μl. We have previously described one pediatric patient with similarly unresponsive Pol-specific CD8+ T cells and CD4+ T cell counts <200 cells/μl (32). Interestingly, a gradual loss of an IFN-γ expression in HIV-specific CD8+ T cells was recently observed in adults progressing to AIDS (33), and a similar persistence of CD8+ T cells with deficient IFN-γ expression was previously observed in murine chronic lymphocytic choriomeningitis virus infection under conditions of CD4+ T cell depletion (34). It is tempting to speculate that this murine CD8+ T cell dysfunction may be mechanistically similar to the impairment of CD8+ T cell function that we have observed in the present study. The subjects with functionally impaired Gag77-85-specific CD8+ T cells displayed a phenotypically altered CD4+ T cell compartment, as compared with the subjects with responsive Gag77-85-specific CD8+ T cells. A significantly lower fraction of CD4+ T cells in these subjects were naive (CCR7+CD45RA−), and larger fraction were CCR7−CD45RA+ effector memory CD4+ T cells. In addition, there was an increase in CCR7−CD45RA+ CD4+ T cells that are not found in healthy control subjects and may be end-stage differentiated (31, 35).
The strong CD8+ T cell activity against HIV in subjects who pass our postulated age and CD4+ T cell count requirements for strong anti-HIV responses is associated with lower viral loads, as compared with subjects who do not pass these requirements and have weak responses and high viral loads. Thus, the CD8+ T cell responses against HIV appear to be beneficial. However, we also observe that subjects with unsuppressed viremia have stronger CD8+ T cell responses than subjects with consistently suppressed (<400 copies/ml) virus, indicating that the responses are Ag driven. These two observations may at first appear to be contradictory, but may be explained by the following model: with the lack of CD4+ T cell help, or under the influence of as-yet-undefined young age-dependent negative factors, CD8+ T cells are unable to respond efficiently to HIV and cannot contribute to lowering of viral burden. In contrast, under circumstances where CD4+ T cell help is present, and the negative age factor is absent, HIV-specific CD8+ T cells can respond in an Ag dose-dependent manner and contribute to eliminating virus and lowering HIV load. This may also help reconcile the apparent inconsistencies in the literature where some studies have described that strong CD8+ T cell responses are associated with lower viral load (13), whereas others have observed the opposite correlation or no correlation at all between anti-HIV CD8+ T cell immunity and viral burden (14–16).

What are the mechanisms behind the relatively poor HIV-specific CD8+ T cell response in subjects 3 years and younger, and what changes at ages 4–5 years? One factor that could potentially influence the effect of young age on CD8+ T cell responses to HIV is that combination antiretroviral drug treatment became available around 1996. Effective treatment would have led to reduced antigenic stimulation for the HIV-specific immune response (36). However, this age group had high viral loads indicating that lack of Ag was not the cause of the weak CD8+ T cell responses. In fact, the youngest children exhibited a viral-immune disconnect with high viral loads, high CD4+ T cell counts, and poor HIV-specific CD8+ T cell activity.

Following the landmark murine transplantation experiments of Medawar and colleagues in the early 1950s (37), it became generally accepted that neonatal T cells are uniquely susceptible to tolerance induction. More recent experiments have shown that neonatal Ag exposure does not induce absolute tolerance in most cases (reviewed in Ref. 38). However, T cells from infants or cord blood are less responsive to stimulation in vitro and often develop into Th2-type cells in culture (38, 39). In mice, neonates are prone to develop nonprotective Th2 responses against murine retrovirus at high viral doses, whereas low doses can result in generation of CTL (40). Also, both murine and human neonatal T cells may undergo activation-induced cell death in vitro under conditions in which adult T cells remain viable (41, 42). This may suggest that the weakness of the CD8+ T cell response against HIV that we (this study and Ref. 20) and others (43) have observed in infants is caused by high-dose Ag exhaustion and induction of Th2-like responses in early life. A previous report has indicated that diminished HIV-specific CTL activity is associated with decreased Th1-type and enhanced Th2-type responses to HIV during perinatal HIV infection (44). Interestingly, poor CD8+ T cell responses were observed in children infected with rotavirus or respiratory syncytial virus (45, 46). More studies are needed to clarify whether exhaustion and induction of Th2-like responses in early life can explain the weakness of CD8+ T cell responses, and what can be done to boost them.

Gag-specific CD8+ T cells identified with HLA-A2 tetramer complexes in 8 of the 32 HLA-A2-positive subjects displayed a largely homogenous phenotype, positive for CD27 and CD45RO, and negative for CD45RA, CD62L, and CCR7 (Fig. 6). The pattern of expression seems no different in the subjects with impaired IFN-γ production, and changes in these markers is therefore most probably not associated with this deficiency. This pattern is consistent with the dominant CD27+ CD45RA+ CCR7− phenotype of HIV-specific CD8+ T cells observed in adults (35, 47). It is also similar to the less biased, but still largely CD45RO+CD45RA− CD27+ phenotype of circulating CD8+ T cells specific for Epstein-Barr virus (48, 49), influenza virus (50), and CMV in HIV-negative individuals, although it should be stressed that, in particular, the CMV-specific cells display more diverse phenotypes in healthy subjects (30, 50, 51).

The majority of HIV infections in the preadolescent age group continue to be caused by vertical transmission from the mother to the infant. In the year 2001, an estimated 2.7 million children worldwide were living with HIV infection, and 600,000 died from AIDS (52). The vast majority of new pediatric infections are occurring in developing countries, primarily in sub-Saharan Africa but also in Latin America and Asia. Since the advent of HAART, children and adults in the Western world have experienced a significant delay in disease progression and have a longer life expectancy (53, 54). However, suppression of the virus is often incomplete or only temporary due to a multitude of factors such as adherence to medication, pharmacokinetics, pharmacogenetics, and viral resistance. These factors make it necessary to understand the dynamics between the virus, host factors, and the developing HIV-specific CD8+ T cell response. The determination of factors that influence HIV-specific immune responses in children remains an important and relatively understudied field in HIV research.

In summary, we have found that CD8+ T cell responses against HIV in infected children are critically influenced by age, CD4+ T cell counts and characteristics, and availability of Ag. Pediatric subjects apparently need to be older than 3 years of age and stay above a minimum of circulating CD4+ T cells to mount efficient CD8+ T cell responses against HIV Ags. When these conditions are met, CD8+ T cell immunity develops in an Ag-dependent manner. In contrast, when these conditions are not met, CD8+ T cell responses are poor and HIV load is high. The strong influence of age on CD8+ T cell responses show that the immunology of vertically acquired HIV infection is unique from that of adult infection, and the rules that govern immune responses in neonates and young children need to be further defined.

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References


