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Reconstitution of Virus-Specific CD4 Proliferative Responses in Pediatric HIV-1 Infection

Margaret E. Feeney,*‡ Rika Draenert,*† Kathleen A. Roosevelt,* Stephen I. Pelton,¶ Kenneth McIntosh,§ Sandra K. Burchett,‡ Charlotte Mao,§ Bruce D. Walker,*† and Philip J. R. Goulder*‡§

Gag-specific CD4 proliferative responses correlate inversely with HIV-1 RNA levels in infected adults, and robust responses are characteristic of long-term nonprogressive infection. However, strong responses are seldom detected in adult subjects with progressive infection and are not generally reconstituted on highly active antiretroviral therapy (HAART). To date, the role of HIV-1-specific Th responses in children has not been thoroughly examined. We characterized Gag-specific CD4 responses among 35 perinatally infected subjects, including 2 children who spontaneously control viremia without antiretroviral therapy, 21 children with viral loads (VL) of <400 on HAART, and 12 viremic children. Gag-specific Th activity was assessed by lymphoproliferative assay, and responses were mapped using overlapping Gag peptides in an IFN-γ ELISPOT. Robust proliferative responses were detected in the children exhibiting spontaneous control of viremia, and mapping of targeted Gag regions in one such subject identified multiple epitopes. Among children ≥5 years old, 14 of 17 subjects with VL of <400 on HAART demonstrated a significant p24 proliferative response (median p24 stimulation index, 20), in contrast with only 1 of 9 viremic children (median p24 stimulation index, 2.0; p = 0.0008). However, no subject younger than 5 years of age possessed a significant response, even when viremia was fully suppressed. When compared with adults with VL of <400 on HAART, Th responses among children with VL of <400 were both more frequent (p = 0.009) and of greater magnitude (p = 0.002). These data suggest that children may have a greater intrinsic capacity to reconstitute HIV-1-specific immunity than adults, and may be excellent candidates for immune-based therapies. The Journal of Immunology, 2003, 171: 6968–6975.

Studies of HIV-1-infected adults have demonstrated the critical importance of HIV-1-specific immune responses for the establishment and maintenance of effective immune control of viremia (1, 2). The magnitude of the Gag-specific CD4 proliferative response correlates inversely with plasma HIV-1 RNA levels in untreated adults, and robust Th cell activity is frequently detected in adults who spontaneously control viremia (3). Although HIV-specific CD4+ cells persist during chronic infection and continue to secrete IFN-γ, the frequency of IFN-γ-secreting CD4+ cells does not differ significantly between long-term nonprogressors and those with progressive disease (4–7). Rather, it is the proliferative capacity of the HIV-specific CD4+ cells that correlates with viral containment, and this capacity appears to be selectively impaired in most adults during the early stages of infection (3). To date, strong HIV-1-specific CD4+ proliferative activity has not been reported in children. However, this element of the antiviral immune response has not been well characterized in HIV-1-infected children whose viremia is controlled either spontaneously or on highly active antiretroviral therapy (HAART).

Studies of pediatric HIV-specific T cell responses have to date focused primarily on the CTL response mediated by CD8+ cells. Most such studies have concluded that, during the first years of life, the CTL response in perinatally infected children is diminished compared with that of adults (8–12). Perinatally acquired HIV-1 infection also differs from adult infection in terms of its clinical disease progression and viral kinetics. Rapid progression is much more common among untreated children than adults, with 25–30% of infants progressing to AIDS or death by 18 mo of age (13). Infected infants experience a prolonged high-level primary viremia, which only decreases to adult levels after 3–5 years (14, 15). It is not known whether these differences in the clinical manifestations of pediatric HIV infection are attributable to the observed deficiency in HIV-specific T cell responses during infancy. The reasons underlying differences in the cellular immune response between adults and young children are also poorly understood. Because the effectiveness of antiviral CTL responses is dependent on virus-specific CD4+ helper cells (16–21), it is possible that differences in the CD4 response may contribute to the observed deficiency of the infant CTL response.

Although the virus-specific immune response of children appears to be weaker in some respects than that of adults, the incomplete maturity of the developing immune system may provide some advantages in the setting of chronic viral infection. Thymic
output of naive T cells is robust during childhood and wanes with age as the thymus involutes, as manifested by the more rapid reconstitution of the CD4+ cell population (particularly those of naive CD45RA+ phenotype) following intensive chemotherapy in children (22). Analyses of TCR rearrangement excision circles confirm that the number of recent thymic emigrants decreases markedly with age (23, 24). The frequency of recent thymic emigrants is diminished in HIV-1-infected children, but is restored in those who achieve potent viral suppression on HAART (25, 26). Although initiation of HAART in both HIV-1-infected adults and children results in a prompt increase in the total number of circulating CD4+ cells, in children 75% of this increase is attributable to the naive CD45RA+ population (27), whereas in adults a CD45RO+ population predominates (28, 29). The predominance of naive cells during immune repopulation may give children an advantage in the generation of new Ag-specific responses while on HAART. Both children and adults who achieve potent viral suppression on HAART readily reconstitute CD4 proliferative responses to common recall Ags such as Candida and tetanus toxoid, but several studies indicate that HAART-treated adults fail to reconstitute responses to HIV-1 Ags (3, 28, 30). The ability of children to reconstitute HIV-1-specific CD4 responses following viral suppression on HAART has not been previously demonstrated. In this study, we present a detailed characterization of HIV-1-specific CD4 responses among children who control viremia spontaneously, and among children with suppressed viral replication on HAART.

Materials and Methods

Study subjects

Thirty-five perinatally HIV-1-infected subjects were recruited through the outpatient HIV clinics at Children’s Hospital in Boston, MA, and the Boston Medical Center, including 2 children who spontaneously control viremia without antiretroviral therapy, 21 children with viral loads (VL) of <400 on HAART, and 12 viremic children. Subjects ranged in age from 1.3 to 17 years. All subjects had CD4 percentages of >15%, and all had been vaccinated with tetanus toxoid (used as a positive control). Five HIV-1-exposed seronegative children (age, 3–9) were studied as control subjects. For comparison, 26 HIV-1-infected adults on HAART were recruited through the outpatient HIV clinics at Massachusetts General Hospital and the Shattuck Hospital in Boston, MA. This study was approved by the Institutional Review Board at each clinical site, and all subjects and/or legal guardians signed written informed consent before participation.

Isolation of PBMC

PBMC were isolated from fresh whole blood by Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO) density gradient centrifugation within 16 h of venipuncture.

Lymphoproliferative assay

PBMC were plated at 10^5 cells/well into 6 replicate wells of a 96-well plate containing the following Ags: baculovirus-expressed recombinant p24 protein (5 μg/ml; Protein Sciences, Meriden, CT), baculovirus control Ag (0.15 μg/ml; Protein Sciences), tetanus toxoid (2 μg/ml; Connaught Laboratory, Willowdale, Ontario, Canada), PHA (5 μg/ml), and medium alone (RPML 1640 (Sigma-Aldrich) supplemented with penicillin-streptomycin, HEPES buffer, t-glutamine, and 10% human AB serum). After 6 days, each well was pulsed with 1 mCi [3H]thymidine for 6 additional hours before harvesting of cells onto glass fiber filters using the Packard Filtermate Harvester. Incorporation was measured by TopCount scintillation counter (Packard Instruments, Meriden, CT). A stimulation index (SI) was calculated for each Ag as the cpm of stimulated wells divided by the cpm of control wells. For categorical analyses, an SI of ≥5 was considered significant. For the 28 subjects in whom replicate assays were performed at different time points, the intraclass correlation coefficient of variation was 0.30, and the average SI was used for analysis.

Synthetic HIV-1 peptides

Four hundred ten overlapping peptides (18-mers with 10-residue overlap) spanning the entire clade B HIV-1 consensus sequence (http://www.hiv.lanl.gov/content/hiv-db/mainpage.html) were synthesized at the Massachusetts General Hospital Peptide Core Facility by Fmoc chemistry on an automated peptide synthesizer (MBS 396; Advanced Chemtech, Louisville, KY).

CD8 depletion

CD8 depletion was performed by adding RosetteSep human CD8 depletion mixture (StemCell Technologies, Vancouver, British Columbia, Canada) to whole blood before Ficoll separation. This procedure consistently yielded >98% purity of CD4+ cells.

CD4 ELISPOT assay

Fresh CD8-depleted PBMC were plated at 50–100,000 cells/well in 96-well polyvinylidene difluoride-backed plates (Millipore, Bedford, MA) that had been precoated overnight with 0.5 μg/ml anti-IFN-γ mAb (Mabtech, Nacka, Sweden). Peptides were added at a final concentration of 10 μg/ml, and three control wells were included that contained cells but no peptide. Plates were then incubated for 36 h at 37°C and 5% CO_2 and developed as previously described (31). Individual IFN-γ-secreting cells were counted using the AID ELISpot Reader System (Autoimmun Diagnostika, Strassberg, Germany). Results are reported as the number of spot-forming cells (SFC) per million input cells (SFC/million) after subtraction of the background response (mean SFC of the no-Ag wells; in all cases, ≤30 SFC/million). A positive response was defined as ≥3 SDs above the average of the negative control wells (or ≥30 SFC/million if all controls were zero). Mapping of the Gag response was performed using 18-mer peptides (based on HIV-1 clade B consensus sequence (http://www.hiv.lanl.gov/content/immunology/index.html)) or 22-mer peptides (based on the HIV-1 p22 sequence); both sets overlap by 10 aa.

Comprehensive screening for CD8 responses

Subjects were screened by IFN-γ ELISPOT assay for responses to a panel of 410 overlapping peptides spanning all translated regions of HIV-1 using a matrix strategy followed by reconfirmation of individual peptide responses as previously described (31). The total magnitude of the CD8 response was defined as the sum of all individual peptide responses, excluding the lower magnitude response in cases where two adjacent peptides were recognized (to avoid the possibility of overestimation due to epitopes contained in the region of overlap).

Intracellular cytokine staining

Intracellular staining for IFN-γ was performed following stimulation with four pools of overlapping HIV-1 18-mer peptides spanning Gag (66 peptides), Env (133 peptides), Pol (113 peptides), and Nef, Rev, Tat, Vif, Vpr, and Vpu (99 peptides) as previously described (32). In brief, 0.5–1.0 × 10^6 PBMCs were incubated with peptide pools (1 μg/ml for each peptide) and 1 μg/ml each of anti-CD28 and anti-CD49d (BD Biosciences, Mountain View, CA) at 37°C and 5% CO_2 for 1 h, before addition of 10 μg/ml brefeldin A (Sigma-Aldrich). After an additional 5-h incubation (37°C and 5% CO_2), cells were washed and surface stained with anti-CD4-PE, anti-CD8-PE/Cy7, and anti-CD4-allophycocyanin (BD Biosciences) for 30 min. After washing, cells were fixed and permeabilized (Caltag Laboratories, Burlingame, CA) and anti-IFN-γ-PE/Cy7 (BD Biosciences) was added for an additional 30-min incubation. Cells were then washed and analyzed on a FACSCalibur four-color flow cytometer. Data was analyzed using CellQuest software (BD Biosciences). The background staining for IFN-γ was ≤0.02%. Responses of ≥0.05% were considered positive.

Statistical analysis

Statistical analysis was performed using Stata statistical software, release 8.0 (StataCorp, College Station, TX). The magnitude of the proliferative response was compared between groups using the Wilcoxon rank sum test. The frequency of significant proliferative responses was compared by Fisher’s exact test. Correlations were assessed using the Spearman rank correlation coefficient. All tests were two-tailed, with p < 0.05 considered significant.

Results

Strong Gag-specific T help in children who spontaneously control viremia

Long-term survival following perinatal HIV-1 infection has been previously reported (33, 34) and is increasingly common in the HAART era (35). However, to our knowledge, children who spontaneously control HIV-1 viremia below the limits of detection...
without antiretroviral therapy have not been described. Two such children were identified among perinatally HIV-1-infected clinical cohorts in Boston. The first (TCH-017) is a 12-year-old girl who has maintained an HIV-1 RNA level of <400 copies/ml for 3 years following discontinuation of all antiretroviral medications in May 2000, except for two viral blips not exceeding 1000 HIV-1 RNA copies/ml. This child has been clinically asymptomatic and has maintained a CD4 percentage of >25%. A lymphoproliferative assay using fresh cells from this child demonstrated strong proliferation in response to recombinant p24 Gag Ag at two different time points (SI, 28 and 25). To determine the proportion of the CD4 response targeting each HIV-1 protein, intracellular staining for IFN-γ was performed following stimulation with pools of overlapping HIV-1 peptides spanning all HIV-1 gene products. This comprehensive assessment of Th activity demonstrated that the CD4 response was highly focused on Gag, with 0.35% of CD4+ cells recognizing Gag peptides (Fig. 1). The response to all other HIV-1 proteins was ≤0.04%. A second spontaneous controller (BMC-033) displayed a similarly robust proliferative response to the p24 Ag (p24 SI, 25). This subject is a 13-year-old boy who has maintained a VL of <1000 HIV-1 RNA copies/ml for 22 mo after discontinuing antiretroviral therapy in June 2001 (most recent VL measurement, 442 HIV-1 RNA copies/ml). The presence of a vigorous CD4 proliferative response to p24 Ag in both of these clinical outliers suggests that this response may be a marker for spontaneous control of viremia in children, as it is in adults with long-term nonprogressive HIV-1 infection.

Strong p24-specific proliferation in children during prolonged treatment with HAART

To determine whether drug-mediated viral suppression is associated with the presence of HIV-1-specific CD4 proliferative responses in children, we assessed p24-specific proliferation in 33 perinatally infected children on HAART. Clinical data including age, HIV-1 RNA levels, CD4 counts, nadir CD4 counts, and antiretroviral regimen are shown in Table I. Subjects ranged in age from 1.3 to 17 years (median, 10 years). Twenty-one subjects (64%) had nondetectable viremia (<400 RNA copies/ml), whereas the remaining 12 had VL of >1,000 HIV-1 RNA copies/ml (median, 13,200). Lymphoproliferative responses were also assessed in 5 HIV-1-exposed seronegative control children (age, 3–9), and all lacked significant responses (p24 SI range, 0.8–1.2).

Strong proliferative responses to p24 were seen in the majority of children with nondetectable viremia on HAART. Among children ≥5 years old, 14 of 17 subjects with VL of <400 demonstrated a p24 SI of >5, compared with only 1 of 9 viremic subjects (p = 0.0008) (Fig. 2). All subjects with suppressed viremia had a p24 SI of >3. The median proliferative response among the children with nondetectable viremia was 20 (range, 3.6–69), which is significantly greater than among viremic children (median p24 SI, 2.0; p = 0.003).

In contrast, all seven subjects younger than 5 years of age lacked significant p24 proliferative activity, regardless of VL. These seven children ranged in age from 1.3 to 4.8 years. All were receiving HAART, which was begun at a median age of 4 mo (range, 7 wk to 24 mo; Table II). Four children had achieved potent viral suppression (VL, <400), and three of these subjects had consistently maintained a nondetectable VL since beginning HAART during the first 4 mo of life. Although the p24 SI in all subjects was <5, there was a progressive increase in responses with increasing age (r = 0.99; p < 0.0001), which paralleled a log-linear increase in the response to tetanus toxoid (Fig. 3). However, the response to p24 Gag was consistently much weaker than the tetanus response.

Several clinical parameters were tested as potential covariates of the p24-specific proliferative response. There was no correlation between the magnitude of the Th response and total CD4 count, nor were there stronger responses among those with low nadir CD4 values, as has been reported in adults (36). There was a trend toward stronger p24 proliferative responses in subjects with a higher total CD4 percentage (r = 0.48; p = 0.053). Among the subjects with VL of <400, there was no correlation between the duration of viral suppression (median, 30 mo; range, 13–56 mo) and the p24 SI. HIV-1 RNA measurements obtained before initiation of antiretroviral therapy (available for 12 subjects) showed no significant correlation with p24 SI, and strong responses were seen in children who began HAART with HIV-1 RNA levels as high as 657,000 copies/ml. Among children ≥5 years old, there was no correlation between age and p24 SI (r = −0.06; p = 0.83), suggesting that there may be a developmental threshold for generation of virus-specific Th responses.

Proliferative response to p24 among adults following prolonged viral suppression on HAART

Prior studies of HIV-1-infected adults suggest that strong proliferative responses to HIV-1 Ags are uncommon among individuals who begin HAART during the chronic phase of infection (30, 37, 38). However, because of the very high prevalence of such responses among children on HAART, we assessed whether prolonged viral suppression may lead to development of HIV-1-specific proliferative responses in adults, or whether their presence is unique to children. Lymphoproliferative responses to p24 Gag were measured in 26 adults on HAART with VL of <400 RNA copies/ml. Significant p24-specific proliferation (p24 SI, >5) was detected in 11 of these 26 subjects (42%). Although this frequency of response is higher than in most previously published studies of chronically infected adults, it is nonetheless significantly lower.
Table I. Subjects ≥5 years old

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<th>Current CD4 No</th>
<th>Current CD4%</th>
<th>Nadir CD4 No</th>
<th>Nadir CD4%</th>
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<th>Duration of VL &lt;400 (mo)</th>
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*Age, HIV RNA (copies per milliliter), and CD4 data at the time the lymphoproliferative assay was performed.

ARV, Antiretroviral; NA, not applicable.

Relationship of the CD4 proliferative response to HIV-1-specific CTL response

CD4+ Th cells are thought to contribute to the control of viral infections in part by fostering the development of CTL responses and the persistence of CTL effector function (16–21). Among adults with untreated HIV-1 infection, a positive correlation has been demonstrated between the p24-specific proliferative response and levels of Gag-specific CTL precursors (39), but no correlation was found in a second adult cohort which assessed IFN-γ production by CD4+ cells rather than proliferative activity (7). To investigate whether p24-specific proliferation correlates with either Gag-specific or total HIV-1-specific CTL activity in children, we measured CTL responses to a set of overlapping peptides spanning all translated HIV-1 gene products in 17 randomly selected subjects. The frequency of Gag-specific IFN-γ-secreting CD8+ cells ranged from 0 to 3.2% (data not shown) (31). The total frequency of CD8+ cells specific for all HIV-1 proteins ranged from 0.1 to 6.3%. Neither the total HIV-1-specific nor Gag-specific CTL magnitude correlated with the p24-specific proliferative activity among children in this cohort (r = 0.09, p = 0.76; and r = 0.31, p = 0.31, respectively). However, because the magnitude of the virus-specific CTL response is known to decline with time on HAART (40), it is possible that the use of antiviral therapy in this cohort may have impacted CTL magnitude and obscured this relationship.

The Gag-specific CD4+ IFN-γ response is strong and broadly directed in a spontaneous controller but weak in children on HAART

To determine the breadth and specificity of the Gag-specific CD4+ response, mapping of the targeted regions was performed by IFN-γ

![FIGURE 2](image-url). Gag-specific proliferation is influenced by age and presence of viremia. The p24 SI is displayed separately for subjects with VL of <400 (●) and VL of >400 (▲), with subjects <5 years old on the left and subjects ≥5 years old on the right.
Table II. Subjects <5 years old

<table>
<thead>
<tr>
<th>ID</th>
<th>Current Age (years)</th>
<th>Age HAART Begun (mo)</th>
<th>HIV RNA</th>
<th>Duration of VL &lt;400 (mo)*</th>
<th>CD4 No.</th>
<th>CD4%</th>
<th>p24 SI</th>
<th>T tox SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCH-72</td>
<td>1.3</td>
<td>6.5</td>
<td>24,000</td>
<td>NA</td>
<td>1,923</td>
<td>26</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>BMC-41</td>
<td>1.8</td>
<td>6</td>
<td>322</td>
<td>1</td>
<td>2,662</td>
<td>23</td>
<td>1.4</td>
<td>3.9</td>
</tr>
<tr>
<td>TCH-76</td>
<td>2.8</td>
<td>1.5</td>
<td>&lt;50</td>
<td>32</td>
<td>1,018</td>
<td>39</td>
<td>2.4</td>
<td>10.5</td>
</tr>
<tr>
<td>TCH-75</td>
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<td>3</td>
<td>&lt;50</td>
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<td>752</td>
<td>40</td>
<td>2.4</td>
<td>10.4</td>
</tr>
<tr>
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<td>2,113</td>
<td>38</td>
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<td>1.3</td>
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<td>NA</td>
<td>964</td>
<td>33</td>
<td>3.0</td>
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<tr>
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<td>4</td>
<td>&lt;75</td>
<td>54</td>
<td>963</td>
<td>40</td>
<td>3.9</td>
<td>111.5</td>
</tr>
</tbody>
</table>

* Age, HIV RNA (copies per milliliter), and CD4 data at the time lymphoproliferative assay was performed. T tox, Tetanus toxoid; NA, not applicable.

ELISPOT. The frequency of IFN-γ-secreting CD4+ cells was measured in one spontaneous controller (TCH-017) and nine children with suppressed viremia on HAART. A strong and broadly directed Gag-specific IFN-γ response was detected in the one child exhibiting spontaneous control of viremia. This subject recognized 22 of the 66 overlapping Gag peptides (Fig. 5A), yielding a summed magnitude of 3580 SFC/million CD4+ cells, or 0.36% of the total CD4+ population. In contrast, the frequency of IFN-γ-secreting CD4+ cells was low among children on HAART, despite robust p24-specific proliferation in all subjects tested (p24 SI range, 6.3–47). Among these subjects, responses were narrowly directed, with a median of two peptides recognized per subject (data from a representative subject are displayed in Fig. 5B). The median total response was 145 SFC/million, and only one subject had a total response exceeding 500 SFC/million (Fig. 5C). Neither the magnitude nor the breadth of the Gag-specific IFN-γ response correlated with the p24 SI. The low frequency of responding cells in these children on HAART is consistent with the described diminution of Gag-specific IFN-γ-secreting CD4+ cells seen in adults following effective viral suppression (41).

Although most of the Gag peptides were infrequently targeted, we identified one immunodominant region of Gag that was recognized by 5 of the 10 subjects and was the dominant response in 4 subjects (PVGEIYKRWIILGNKIV; p24, 259–276). This region lies within the highly conserved α helix that forms the interface between the two subunits of the p24 homodimer. Fig. 6 demonstrates fine-mapping of this response for subject TCH-065, in whom the response was highly immunodominant with 0.15% of CD4+ cells responding (1470 SFC/million CD4+ cells). There was no HLA class II allele shared among the subjects who exhibited this response.

Discussion

Our results demonstrate that vigorous Gag-specific CD4 proliferative responses are present in the vast majority of children who have achieved potent viral suppression on HAART. These responses are significantly stronger and more prevalent than those of adults treated with HAART for a similar period of time. However, strong responses were not detected in children younger than 5 years of age, suggesting that there may be a developmental threshold for the generation of these responses. Despite robust Gag-specific proliferative activity, the frequency of IFN-γ-secreting Gag-specific CD4+ cells was low in most HAART-treated children. In contrast, very strong and broadly directed IFN-γ responses to Gag peptides were detected in one child spontaneously controlling viremia. Taken together, our data suggest that children may have the ability to reconstitute HIV-1-specific CD4 proliferative responses once viral replication is effectively suppressed by HAART.

The frequent detection of p24 proliferative responses among children on HAART raises the intriguing possibility that children may have a greater intrinsic capacity for immune reconstitution of virus-specific immunity. Although the use of HAART has dramatically altered the natural history of HIV-1 disease and now allows for reconstitution of pathogen-specific immune responses even in those with severely damaged immune systems (42), it has unfortunately not led to reconstitution of HIV-1-specific immunity in the majority of adults. Early studies of HAART-treated adults showed that, although proliferative responses to opportunistic pathogens could be reconstituted (28, 43, 44), responses to HIV-1 Ags are seldom restored in adults who begin HAART during the chronic phase of infection (30, 37, 38). Among children, reconstitution of HIV-1-specific CD4 responses has not previously been assessed in detail. We found that a large majority (82%) of children who achieve potent viral suppression on HAART demonstrate robust Gag-specific Th responses. Because of the cross-sectional nature of this study, it cannot be known with certainty

![FIGURE 3](http://www.jimmunol.org) Proliferative responses among children <5 years of age. SIs for tetanus toxoid (left) and p24 Gag (right) are displayed on a logarithmic scale by age for the seven subjects <5 years old.

![FIGURE 4](http://www.jimmunol.org) Significant p24-specific proliferation is more frequent in children than adults. The p24 SI is displayed separately for children (○) and adults (●). All subjects were on HAART with VL of <400. Solid bars indicate median values, and the dotted line indicates the significance threshold of SI = 5.
whether these responses were present before the initiation of HAART, or whether a period of viral suppression allowed for the development of a strong CD4 response. Longitudinal studies will be needed to definitively determine whether these responses represent true immune reconstitution. However, the absence of HIV-1-specific Th activity in all but one of the viremic children tested strongly suggests that, in most cases, the Th response only emerged once viral replication was effectively suppressed.

Our data suggest that the prevalence of Th responses among HAART-treated adults may be substantially higher than previously thought, consistent with one recent report of Th activity in adults who had been treated with HAART for a duration similar to that of our cohort (45). It is possible that viral suppression must be maintained for several years in order for such responses to be generated, although no correlation between duration of viral suppression and p24 proliferative activity could be demonstrated in our study sample. Nonetheless, HIV-1-specific Th responses were significantly more frequent and of greater magnitude among children than among adults in our cohort despite similar durations of viral suppression, suggesting that children may have a greater intrinsic capacity for immune reconstitution, perhaps due to their greater thymic output of naive cells.

The lack of significant Gag-specific Th activity in the <5-year-old cohort is particularly striking for its contrast to prior observations of acutely HIV-1-infected adults. Adults diagnosed with acute symptomatic HIV-1 infection who are promptly started on HAART consistently develop a Gag-specific CD4 proliferative response (3, 46,

**FIGURE 5.** Mapping of the Gag-specific CD4+ IFN-γ response. Negatively selected CD4+ cells were tested for recognition of 66 overlapping peptides spanning Gag in an IFN-γ ELISPOT assay. Gag peptides are displayed across the x-axis, and the y-axis displays the number of SFC per million CD4+ cells. A, Subject TCH-017, who controls viremia spontaneously, demonstrated a strong response to multiple epitopes, with 22 of 66 peptides recognized for a total response of 3580 SFC/million. B, Subject TCH-015, a representative child on HAART with p24 SI of 23, recognized 7 peptides for a total response of 470 SFC/million. C, Subject TCH-065 was the only child on HAART with a strong CD4+ response (total, 1690 SFC/million). This response was highly focused on one Gag epitope.

**FIGURE 6.** Fine-mapping of the dominant CD4+ IFN-γ response in TCH-065. Negatively selected CD4+ cells from TCH-065 were expanded in vitro for 10 days with recombinant p24 and IL-2, and then plated at 25,000 cells/well with progressive truncations of Gag-36 in an ELISPOT assay. Results are reported as SFC/million after subtraction of background. The minimal recognized peptide was the 12-mer GEIYKRWIIILGL.
47), whereas those in whom treatment is delayed until the chronic phase of infection generally lack this response. The precise opposite was observed among the vertically infected infants in our cohort. Several of these young subjects began HAART in the first few weeks following infection and are therefore analogous to adults who are treated during acute HIV-1 infection, yet CD4 proliferative responses were universally absent in this group. The lack of CD4 proliferative responses among these HAART-treated infants may be due to insufficient antigenic stimulation during a critical period (48), or it may represent a more general developmental difference in the capacity of infants and young children to mount CD4-mediated immune responses. Longitudinal studies of HIV-1-infected infants are needed to more clearly define the progressive maturation of the antiviral cellular immune response.

Although Gag-specific proliferation of CD4+ cells was present in nearly all children with HAART-mediated viral suppression, Gag-specific IFN-γ-secreting cells were of very low frequency in most of these subjects. This discordance between the proliferative capacity and IFN-γ secretion by CD4+ cells has been noted previously in several adult cohorts (4, 45, 49) and more recently in children (50). In adults, the frequency of Gag-specific CD4 cells that secrete IFN-γ has been observed to decline progressively with time on suppressive antiviral therapy, falling from a median of 0.12 to 0.03% in one cohort (41). Therefore, prolonged viral suppression may account for the very low frequency of IFN-γ-producing cells among the children we studied. Most studies assessing IFN-γ production by CD4 cells have found a very poor correlation between the frequency of these cells and viral control or nonprogressor status (4–6). Rather, the ability of CD4 cells to proliferate upon restimulation with HIV-1 Ags seems to uniquely correlate with viral control and long-term nonprogressor status (3). Perhaps newer flow-cytometric assays based on CFSE staining or IL-2 production will prove to be more convenient and quantifiable measures of relevant CD4 antiviral function (5).

The high prevalence of vigorous Gag-specific CD4 responses following suppressive HAART treatment in these children may have important implications for treatment strategies. These responses may enable children to contain viremia immunologically after cessation of antiviral therapy, or following structured treatment interruptions to boost HIV-1-specific CTL function. Such interruptions in antiviral therapy have led to successful drug-free control of viremia in some adults who were treated during acute HIV-1 infection and maintain HIV-1-specific Th function (46), although results have been disappointing among those treated during chronic infection, who generally lack T help (51, 52). Future studies involving closely supervised treatment interruptions in children could also yield important information about the functionality of the CD4 Th response and the clinical significance of this preserved proliferative capacity. Perhaps most importantly, if further studies confirm the ability of HIV-1-infected children to reconstitute HIV-1-specific immunity, infants and children may be excellent candidates for therapeutic immunization and other immunomodulatory therapies.

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References


