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HIV-1 Replication Increases HIV-Specific CD4+ T Cell Frequencies but Limits Proliferative Capacity in Chronically Infected Children

Zachary A. Scott,*‡ Coreen M. Beaumier,* Mark Sharkey,† Mario Stevenson,‡ and Katherine Luzuriaga2†‡

This study investigated the relationship between HIV-1 replication and virus (HIV-1; CMV)-specific CD4+ T cell frequency and function in HIV-1-infected children. HIV-1 gag p55-specific CD4+ T cell IFN-γ responses were detected in the majority of children studied. p55-specific responses were detected less commonly and at lower frequencies in children with <50 copies/ml plasma HIV-1 RNA than in children with active HIV-1 replication. In children with <50 copies/ml plasma HIV-1, p55-specific responses were detected only in children with evidence of ongoing HIV-1 replication, indicating a direct relationship between HIV-1 replication and HIV-specific CD4+ T cell frequencies. In contrast, p55-specific proliferative responses were detected more frequently in children with <50 copies/ml plasma HIV-1. CMV-specific CD4+ responses were more commonly detected and at higher frequencies in CMV-infected children with suppressed HIV-1 replication. The lack of HIV-specific CD4+ proliferative responses, along with the preservation of CMV-specific CD4+ responses in children with controlled HIV-1 replication, suggests that viral replication may have deleterious effects on HIV-1 and other virus-specific CD4+ responses. Vaccination to stimulate HIV-specific CD4+ T cell responses in these children may synergize with antiretroviral therapy to improve the long-term control of viral replication, and may perhaps allow the eventual discontinuation of antiretroviral therapy. The Journal of Immunology, 2003, 170: 5786–5792.

The use of combination antiretroviral therapies (ART) has markedly reduced the morbidity and mortality associated with pediatric HIV-1 infection (1, 2). However, several factors may limit the longevity of the benefits provided by ART. Combination therapy regimens are often complex, and even occasional lapses in adherence may allow the emergence of viruses resistant to ART regimens. Toxocities have also limited the use of ART in some children. Thus, there is growing interest in combining ART with measures to induce HIV-1-specific immune responses in the hope of providing better long-term control of HIV-1 replication. A better understanding of the relationship between HIV-1 replication and virus-specific immune responses is necessary for the successful development of these treatment strategies. Greater insight into virus-specific CD4+ T cells may be particularly important, because CD4+ T cells serve as a major substrate for HIV-1 replication, as well as provide antiviral helper or effector functions (3, 4).

Many studies investigating HIV-specific CD4+ T cell responses have failed to detect vigorous HIV-specific CD4+ T cell prolifer-
screen for HIV-1 infection. Many of the children in the cohort were diagnosed younger than 18 mo of age, in which case the PCR was used to detect provirus in PBMC. Viral isolation was used to confirm infection in all children.

Written informed consent was obtained from the legal guardian of all study participants in accordance with the Institutional Review Board of the University of Massachusetts Medical School. The guidelines of the U.S. Department of Health and Human Services governing experimentation in humans were followed.

**Quantification of plasma HIV-1 RNA copy number by RT-PCR**

HIV-1 RNA was quantified in 200 μl of EDTA-anticoagulated plasma (stored at −70°C within 6 h following phlebotomy) by PCR after reverse transcription (Amplitaq; Roche Diagnostics, Branchburg, NJ). Plasma samples with values below the detection limit of the standard assay (<400 copies/ml) were subsequently tested using 450 μl of plasma and a modified assay with a detection limit of 50 HIV-1 RNA copies/ml. All assays were performed in a single laboratory that participates in an ongoing quality certification program for HIV-1 RNA quantitation sponsored by the National Institutes of Health.

**CMV serology**

CMV infection status was determined using ELISA for the presence of anti-CMV IgG Ab in serum samples taken at the time of study. Serological analysis was performed by the University of Massachusetts Memorial Health Care Serology Laboratory (Worcester, MA).

**Enumeration of lymphocyte subsets in the peripheral blood**

The relative percentages of CD3+CD4+ and CD3+CD8+ lymphocytes in the peripheral blood were enumerated by direct immunofluorescence with FITC- or PE-conjugated mouse mAbs (BD Immunocytometry Systems, San Jose, CA). Samples were analyzed by flow cytometry using CellQuest software (FACScan; BD Immunocytometry Systems).

**Intracellular cytokine staining assay (ICS)**

Ag-specific CD4+ T cell responses were detected using an ICS (Fig. 1). Briefly, anti-CD28 and anti-CD49d mAbs (3 μg/ml; BD PharMingen, San Diego, CA) were added to 0.5 ml of fresh heparinized whole-blood aliquots from each study subject and incubated with the following protein Ags: HIV-1 S downward 5′-p55 and CMV pp65 (5 μg/ml; Austral Biologicals, San Ramon, CA). Two additional whole-blood aliquots (both containing the anti-costimulatory Abs) were set up as negative (no Ag) and positive controls (Staphylococcus enterotoxin B (4 μg/ml; Toxin Technology, Sarasota, FL)) for each study subject. Samples were incubated at a 5′ slant for 6 h at 37°C and 5% CO2, Brefeldin A (BD PharMingen) was added for the final 4 h of incubation. After incubation, EDTA was added to each tube, and the samples were fixed (FACS lysis solution; BD Immunocytometry Systems) and permeabilized (FACS permeabilizing solution; BD Immunocytometry Systems) according to the manufacturer’s instructions. Each sample was stained with the following conjugated Abs: PerCP-anti-CD4, FITC-anti-CD45RO, PE-anti-CD69, and allophycocyanin-anti-IFN-γ (BD Immunocytometry Systems and BD PharMingen). Flow cytometry analysis was performed within 24 h of sample preparation. Samples were gated on CD4+CD45RO+ small lymphocytes and analyzed for CD69 and IFN-γ expression. Results are expressed as the percentage of CD4+CD45RO+ small lymphocytes expressing CD69 and IFN-γ Ag-specific CD4 T cells were not detected in the CD45RO+ population.

All experimental samples exhibited strong CD4+ T cell IFN-γ responses to Staphylococcus enterotoxin B. Responses in the absence of Ag (no Ag controls) ranged from 0 to 0.05% (median, 0.02%). Experimental responses were considered positive if they exceeded 0.06% (2 SDs above the mean negative control value).

After stimulation with HIV-1 p55 Ag, CD4+ T cell IFN-γ responses in HIV-1-uninfected adults (median, 0.01%; range, 0.01–0.03%) did not differ from background responses (no Ag control; median, 0.01%; range, 0–0.04%). Similarly, CMV pp65-specific CD4 T cells were detected in two HIV-1-uninfected, CMV-seropositive donors, but were not detected in CMV-seronegative donors.

**Lymphoproliferative assays (LPA)**

LPA were performed as previously described (5, 10, 12) with the following modifications. PBMC were separated from fresh whole blood by Ficoll-Paque (Amer sham Pharmacia Biotech, Piscataway, NJ) density centrifugation and were incubated with HIV-1 S downward 5′ and CMV pp65 protein Ags at a concentration of 10 μg/ml (Austral Biologicals). PBMC were incubated in triplicate wells (10 cells/well) for 7 days before harvesting. Cells incubated with medium alone or pokeweed mitogen (10 μg/ml; Sigma-Aldrich, St. Louis, MO) served as negative and positive controls, respectively. Plates were analyzed (Wallac 1450 MicroBeta TriLux; PerkinElmer Wallac, Gaithersburg, MD) and stimulation indices (SI) were defined as the ratio of the mean cpm of the stimulated well to the mean cpm of the negative control wells. Assays were considered valid only if the cpm measured in each of the negative control wells were <1000. SI of >3 were considered positive responses.

**Detection of HIV-1 2-long terminal repeat (LTR) circles in PBMC**

PBMC pellets were resuspended in buffer P1 and extrachromosomal DNA was purified using a QIAprep spin miniprep kit (Qiagen, Valencia, CA) using the modifications recommended by the manufacturer for the isolation of low copy number plasmids. Final elution of the purified DNA was in 120 μl of elution buffer preheated to 70°C. Two-long terminal repeat junction sequences were amplified from 30 μl of extrachromosomal DNA in a 60-μl reaction containing 1× HotStarTag buffer, 200 μM deoxyribonucleotide triphosphates, 500 nM each primer, 200 nM fluorescent probe, and 2 U of HotStarTag (Qiagen). The reverse and forward primers were 5′-TAGAC CAGATCTGAGCCGTGGGA-3′ and 5′-GTAGTTCTGCCAATCAGGGA AG-3′, respectively, which annealed to nt 13–34 (HIV-1 LTR R region) and nt 8770–8749 (HIV-1 LTR U3 region) of HIV-1 LTR (GenBank accession no. K02013). The fluorescent probe (5′-AGCCTCAATAGAC TTGCGTTAGTGCC-3′) was complementary to nt 67–93 of HIV-1 LTR and was modified with 6-FAM reporter dye on the 5′ end and 6-TAMRA quencher dye on the 3′ end. After an initial denaturation/enzyme activation step (95°C, 10 min), PCR amplification proceeded for 45 cycles (95°C, 15s; 62°C, 1 min). To control for the effect of sequence polymorphisms at primer binding sites and to allow for the quantitation of total viral genomes, amplifications were performed using primers that were reversed in orientation to those listed above and a different probe (5′-6-FAM-AGTTGCG GGAGCTTTAGGTGCG-6-TAMRA-3′) which annealed to nt 9103–9081 of HIV-1 LTR. Copy numbers were determined from a standard curve generated by the quantitation software of the Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). The limit of detection of the assay was one copy.

**Statistical analysis**

All data sets were tested for statistical differences using the Wilcoxon rank sum (univariate) or Kruskal-Wallis rank sum (multivariate) nonparametric tests for non-Gaussian distributed data. All tests were two-tailed and p values ≤0.05 were considered significant. All analyses were performed using Axum 7.0 software (MathSoft, Cambridge, MA).

**Results**

**Study population characteristics**

Peripheral whole blood samples were acquired from 44 children with established HIV-1 infection. Characteristics of the study cohort are presented in Table I. Study children were placed into groups based on plasma HIV-1 load and ART history. Children in group A (n = 16) were on combination ART and had <50 copies/ml plasma HIV-1 RNA. Children in group B (n = 22) were also on combination ART but had >50 copies/ml plasma HIV-1 RNA (median, 1,316 copies/ml; range, 99–57,901 copies/ml). Age at study entry, median CD4+ T cell counts, and median duration of ART before study enrollment did not differ significantly between these two groups. Several children (group C, n = 6) were either not receiving ART or not adherent to a prescribed therapy (median plasma HIV-1 RNA, 60,603 copies/ml; range, 11,427–259,350 copies/ml). Finally, 10 HIV-uninfected adults (group D) served as negative control donors.

**HIV-1 gag p55-specific CD4+ T cells are commonly detected in children with established HIV-1 infection**

Using an ICS (Fig. 1), HIV-1 p55-specific CD4+ T cell responses were detected in 38 (86%) of 44 study children at a median frequency of 0.34% (range, 0.02–2.88%) of CD4+ CD45RO+ small lymphocytes. No relationships between age, CD4+ T cell percentage or count, and the detection or frequency of p55-specific CD4+ T cell responses were noted.
To confirm the HIV-1 specificity of the detectable CD4⁺ T cell IFN-γ responses, blood samples from 10 HIV-1-uninfected adults donors were tested (group D). HIV-1 p55-specific CD4⁺ T cell IFN-γ responses were not detected in the peripheral blood samples of any individuals in this group.

HIV-1 gag p55-specific CD4⁺ T cells are detected more commonly and at higher frequencies in children with ongoing HIV-1 replication.

Interestingly, 5 of the 6 children without detectable HIV-1 p55-specific CD4⁺ T cell responses also had <50 copies/ml plasma HIV-1 RNA. Therefore, we went on to examine the relationship between plasma HIV-1 load and HIV-1 p55-specific CD4⁺ T cells. HIV-1 p55-specific CD4⁺ T cell responses were less commonly detected in children with <50 copies/ml plasma HIV-1 RNA than in children with ongoing replication (Table II). HIV-1 p55-specific CD4⁺ T cell responses were detected in 11 (69%) of 16 children with undetectable HIV-1 plasma loads, whereas HIV-1 p55-specific CD4⁺ T cell responses were detected in 27 (96%) of 28 children with >50 copies/ml plasma HIV-1 RNA. In addition, higher frequencies of HIV-1 p55-specific CD4⁺ T cells were detected in children with active viral replication than in children with suppressed viral replication. As shown in Fig. 2A, HIV-1 p55-specific CD4⁺ T cell responses were detected at higher frequencies between group A and group B, with a median difference in HIV-1 p55-specific CD4⁺ T cell frequencies of 0.7% (range, 0.04–2.88%) and in group C (median, 0.3%). As shown in Fig. 2A, HIV-1 p55-specific CD4⁺ T cell responses were detected at higher frequencies between group A and group B, with a median difference in HIV-1 p55-specific CD4⁺ T cell frequencies of 0.7% (range, 0.04–2.88%) and in group C (median, 0.3%).
groups A and C was not statistically significant. The latter may be due to alteration of the relationship between viral replication and CD4+ T cell IFN-γ responses by CD4+ T cell depletion.

Cross-clade stimulation of HIV-1 gag p55-specific CD4+ T cell responses

The use of a clade B-derived (SF2 isolate) p55 protein Ag raised the concern that HIV-1 p55-specific CD4+ T cell responses may not be detected in children infected with other HIV-1 subtypes. Five children were born to women who acquired HIV-1 in areas in which non-clade B viruses predominate (P-1209 and P-1326, Zimbabwe; P-1210, South Africa; P-1211, Cambodia; and P-1318, Ivory Coast). Responses were detected in two children (P-1210 and P-1326), born to mothers most likely infected with HIV-1 clade C viruses. Importantly, both responders had detectable plasma HIV-1 RNA, whereas each of three children lacking detectable p55-specific CD4+ T cell responses presented with <50 copies/ml plasma HIV-1 RNA.

CMV pp65-specific CD4+ T cells are detected at higher frequencies in children with <50 copies/ml plasma HIV-1 load

To further our understanding of virus-specific CD4+ T cells in HIV-infected children, CMV tegument pp65-specific CD4+ T cell frequencies were also measured in 19 HIV-1- and CMV-coinfected children. CMV pp65-specific CD4+ T cell IFN-γ responses were detected in 13 (68%) of 19 children at a median frequency of 0.18% (range, 0–4.37%). As presented in Table II, CMV-specific CD4+ T cell responses were detected more frequently in children with <50 copies/ml plasma HIV-1 RNA (5/5, 100%) than in those children with active HIV-1 replication (8/14, 57%). The difference in the proportion of responders did not reach statistical significance, but suggested a trend toward an inverse relationship between active HIV-1 replication and the presence of CMV-specific CD4+ T cell responses. To further investigate this relationship, the frequencies of CMV pp65-specific CD4+ T cell IFN-γ responses were plotted by group. As illustrated in Fig. 2B, all 5 children in group A had detectable pp65-specific CD4+ T cell responses at a median frequency of 1.07% (range, 0.1–4.37%). Conversely, only 5 (50%) of 10 children in group B had detectable pp65-specific CD4+ T cell IFN-γ responses (median, 0.07%; range, 0–0.68%). These responses were significantly lower than those detected in group A (Wilcoxon rank sum, p = 0.028). CMV pp65-specific

Table II. Proportion of children with undetectable or active HIV-1 replication with detectable HIV-1- or CMV-specific CD4+ T cell responses

<table>
<thead>
<tr>
<th>Proportion of Responders with HIV-1 Replication Status</th>
<th>HIV-1 p55</th>
<th>CMV pp65</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 copies/ml (%)</td>
<td>11/16 (69)</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>&gt;50 copies/ml (%)</td>
<td>27/28 (96)</td>
<td>8/14 (57)</td>
</tr>
<tr>
<td>p value (Fisher’s exact test)</td>
<td>0.0185</td>
<td>0.128</td>
</tr>
</tbody>
</table>

* Forty-four children infected with HIV-1 were analyzed for the presence of HIV-1- and CMV-specific CD4+ T cell IFN-γ responses. The proportion of children with plasma HIV-1 loads of <50 copies/ml (undetectable; group A) or >50 copies/ml (groups B and C) with positive CD4+ T cells responses against HIV-1 p55 and CMV pp65 Ags is listed.

* Only children coinfected with CMV were considered in this analysis. CMV-specific responses were not studied in all coinfected children due to insufficient specimen samples.
CD4+ T cell IFN-γ responses were detected in 3 (75%) of 4 children in group C at intensities similar to those found in group B (median, 0.016%; range, 0.03–0.25%).

To confirm the specificity of the detectable CD4+ T cell IFN-γ responses, blood samples from two HIV-1- and CMV-uninfected adults donors were tested (group D). No CMV pp65-specific CD4+ T cell IFN-γ responses were detected in these individuals.

Low frequency virus-specific CD4+ T cell IFN-γ responses in children with depleted CD4+ T cells

CD4+ T cell percentages of <15% are associated with higher mortality in HIV-1-infected children (13). Within the cohort, only two patients in group C, P-1017 and P-1325, presented with severely depressed CD4+ T cell percentages (<15%) and counts (<150 cells/μl). Despite high-level viremia in both children (>10^5 copies/ml), HIV-1 pp65-specific CD4+ T cell IFN-γ responses (Fig. 2A) were either undetectable (P-1017) or low frequency (P-1325; 0.04%). Similarly, CMV-specific IFN-γ responses (Fig. 2B) were either undetectable (P-1325) or low frequency (P-1017; 0.08%). These data suggest that CD4+ T cell depletion may alter the previously observed relationship between plasma HIV-1 load and the intensity of pp65-specific CD4+ T cell IFN-γ responses.

Ongoing HIV-1 replication is associated with detectable HIV-specific CD4+ T cell responses

Despite undetectable HIV-1 viral loads, some variability was observed in HIV-1 pp55-specific CD4+ T cell IFN-γ responses in group A. HIV-1 2-LTR DNA circles were measured in the PBMC obtained from 14 children in group A at the same time points used in the ICS data. Limited PBMC sample volumes precluded further study of the remaining 2 children (P-1033 and P-1035). Two-long terminal repeat DNA circles are abortive products of HIV-1 reverse transcription that occur when proviral DNA fails to integrate into the host genome (14). The presence of 2-LTR DNA circles within peripheral blood lymphocytes represents ongoing HIV-1 replication. Nine children in whom HIV-1 pp55-specific CD4+ T cell IFN-γ responses (median frequency, 0.22%; range, 0.09–0.70%) were detected were defined as responders. As illustrated in Fig. 3, HIV-1 2-LTR DNA circles were detected in all 9 responder children, at a median frequency of 3.5 copies/10^6 PBMC. By contrast, 2-LTR DNA circles were detected at a frequency of <1 copy/10^6 PBMC in all 5 nonresponder children. The difference in the median frequencies of 2-LTR DNA circles between the two groups proved to be significant (Wilcoxon rank sum, p = 0.04).

Changes in HIV-1 gag pp55-specific CD4+ T cell frequencies are temporally associated with changes in plasma HIV-1 load

Because our data suggested an association between HIV-1 replication and HIV-1 pp55-specific CD4+ T cell IFN-γ responses, follow-up ICS assays were performed in several children who returned to the clinics. In most cases, plasma HIV RNA loads and HIV-1 pp55-specific CD4+ T cell frequencies were stable over time (data not shown). However, changes in plasma HIV-1 RNA levels were accompanied by changes in pp55-specific CD4+ T cell frequencies (Fig. 4). For example, patient P-1320 presented with plasma HIV-1 RNA of 24,514 copies/ml and an HIV-1 pp55-specific CD4+ T cell IFN-γ response of 1.80%. Following a change in therapy and a reduction of viral load by >1 log, the pp55-specific CD4+ T cell frequency fell to 0.57%. Conversely, child P-1207 presented with plasma HIV-1 RNA <50 copies/ml plasma HIV-1 load and a weak HIV-1 pp55-specific CD4+ T cell IFN-γ response (0.09%). Over the course of 2 mo, HIV-1 RNA became detectable in this child’s plasma (~1100 copies/ml at two time points) and the frequency of HIV-1 pp55-specific CD4+ T cell responses rose to 0.90%. The temporal association between plasma HIV-1 load and the frequency of pp55-specific CD4+ T cell IFN-γ responses again illustrates the dependency of HIV-specific CD4+ T cell responses on the availability of HIV-1 Ag.

Diminished HIV-specific in vitro lymphoproliferative responses in children with active HIV-1 viral replication

Nineteen children studied using the ICS assay (8 from group A and 9 from group B) were concurrently tested for the presence of lymphoproliferative responses (LPA) against HIV-1 p55 (Fig. 5); HIV-1 pp55-specific IFN-γ secretion was detected in 16 (84%) of these 19 children. By contrast, HIV-1 pp55-specific lymphoproliferative responses were detected in only 6 (32%) of 19 children tested. LPA responses were detected in 4 (50%) of 8 children with <50 copies/ml plasma HIV-1 load (group A), but were detected in only 2 children (P-1012 in group B and P-1107 in group C) with plasma HIV-1 RNA of >50 copies/ml CD4+ T cell counts were similar in both groups, suggesting that the observed differences in HIV-1 pp55-specific LPA responses were due to differences in viral load. HIV-1 pp55-specific LPA responses were not detected in 6 HIV-uninfected donors (group D). Interestingly, there was no apparent relationship between the presence of pp55-specific LPA responses and the frequency of CD4+ T cell IFN-γ responses (data not shown), although concurrent pp55-specific CD4+ T cell IFN-γ responses (responder median, 0.68%; range, 0.13–0.86%) were detected in 5 (83%) of the 6 LPA responders. HIV-1 pp55-specific
CD4+ T cell IFN-γ responses were also detected in 11 (85%) of 13 LPA nonresponders.

Discussion

The past 5 years have seen marked progress in the study of HIV-specific CD4+ T cell populations and understanding how the frequency and function of these cells relate to HIV-1 replication. A cross-sectional study of HIV-1 p24-specific CD4+ T cell proliferative responses found an inverse relationship between the presence of proliferative responses and plasma HIV-1 RNA in therapy-naive, chronically infected adults (5). The absence of HIV-specific CD4+ T cell proliferation in viremic individuals suggested the loss or dysfunction of these cells as a consequence of concurrent HIV-1 replication. Later, using a flow-based ICS, HIV-specific CD4+ T cells producing IFN-γ were detected ex vivo in adults with progressive HIV-1 infection (7). HIV-1 gag-specific CD4+ T cells were more commonly detected and were detected at higher frequencies in HIV-1 progressors than in individuals with undetectable plasma HIV-1 loads, although no significant relationship between plasma HIV-1 RNA and the frequency of HIV-specific CD4+ T cell responses was observed. These data demonstrated that HIV-specific CD4+ T cells are not depleted from the circulation of individuals with long-standing HIV-1 replication. Recently, at least two groups have described the detection of IFN-γ-producing HIV-specific CD4+ T cells but diminished in vitro proliferative responses in individuals with ongoing HIV-1 replication, clarifying the seemingly discordant previous findings (10, 11). In long-term nonprogressors (LTNP) with controlled HIV-1 replication, HIV-specific CD4+ T cell IFN-γ responses were found concurrently with HIV-1 proliferative responses. The lack of proliferative responses in adults with progressive HIV-1 infection suggested a specific functional defect in HIV-specific CD4+ T cells.

Available data suggest the selective impairment of HIV-specific T cell responses in early vertical infection. We have previously demonstrated the delayed generation of HIV-specific CD8+ T cells in young infants (15, 16) and that HIV-specific CD4+ and CD8+ T cell responses are infrequently detected in the circulation of children who receive potent combination ART in the first few months of life (12). Until recently, however, most children did not receive early suppressive ART, and persistently high plasma HIV-1 loads have been documented over the first several years of life (17). Robust viral replication in the setting of a developing immune system may dramatically alter the establishment and maintenance of HIV-specific CD4+ and CD8+ T cell effector and memory cell populations.

Data from the present study demonstrate that ex vivo HIV-1- and CMV-specific CD4+ T cell IFN-γ responses are commonly detected in children with established HIV-1 infection, despite the diverse therapeutic, clinical, and virological backgrounds of these children. Responses were more commonly detected and were detected at higher frequencies in the circulation of children with plasma HIV-1 RNA of >50 copies/ml than in children with plasma HIV-1 RNA of <50 copies/ml. Conversely, CMV pp65-specific CD4+ T cell IFN-γ responses were more commonly detected and were detected at higher frequencies in children with undetectable plasma HIV-1 load. These data suggest that CD4+ T cell IFN-γ responses can be primed and maintained in vertically infected children. Furthermore, the detection of HIV-specific CD4+ T cells in most children, at frequencies comparable to those found in adults, indicates that the generation and maintenance of these cells are not appreciably altered by long-standing HIV-1 replication. However, the weak or undetectable HIV-1-specific CD4+ T cell responses detected in two children with severely depleted CD4+ T cell percentages suggests the importance of immune competence in maintaining these responses over time. Our findings are compatible with studies in chronically infected adults that demonstrate that HIV-1 and other virus-specific CD4+ T cells are not deleted following prolonged viremia (7, 8, 10, 11).

In children with <50 copies/ml plasma HIV-1 RNA, HIV-1 p55-specific CD4+ T cell IFN-γ responses were detected only in the peripheral blood of children in whom 2-LTR DNA circles were detected using a real-time PCR-based assay. Although some groups have questioned the validity of 2-LTR circles as a marker of ongoing viral replication (18, 19), the correlation between the detection of HIV-1 p55-specific CD4+ T cell IFN-γ responses and the presence of 2-LTR DNA circles underscores their usefulness as a surrogate of covert viral replication. These data illustrate the ability of ongoing HIV-1 replication to drive and maintain HIV-specific CD4+ T cell IFN-γ responses and show that low-level HIV-1 replication, undetectable by ultrasensitive quantitation assays, is sufficient to maintain HIV-specific CD4+ T cell frequencies comparable to those found in children with significantly higher viral burdens.

Most prior studies have not documented a strong relationship between CD4+ T cell responses and viral load (7, 8, 10, 11, 20). Pitcher et al. (7) documented a difference in the frequency of HIV-specific CD4+ T cell IFN-γ responses between adults with active replication and those with long-term suppressed HIV-1 replication, but this difference was not statistically significant. We feel that there are several reasons why such a relationship was not observed in previous reports. First, several studies focused on HIV-1-infected LTNP and compared the frequency of HIV-specific CD4+ T cells in these individuals to those found in active HIV-1 progressors (8, 10, 20). Several analyses have presented evidence of ongoing HIV-1 replication in LTNP despite undetectable plasma HIV-1 loads by standard quantitation assays (21, 22). The study of LTNP may have minimized the differences in observed HIV-specific CD4+ T cell frequencies when compared with individuals with progressive infection. Second, some studies used routine (limit of detection, 400 RNA copies/ml) rather than ultrasensitive (limit of detection, 50 RNA copies/ml) assays for the quantitation of plasma HIV-1 RNA, thereby making it likely that low levels of HIV-1 replication were not detected (7, 8, 11). Finally, not all plasma HIV-1 RNA is replication competent, and it could be that HIV-specific CD4+ T cell frequencies correlate better with measures of recent replication events. Future studies that use high-resolution analysis of recent replication events or plasma HIV-1 load may continue to clarify the relationship between ongoing HIV-1 replication and the frequency of HIV-specific CD4+ T cells IFN-γ responses.
Aside from LTNP, many studies have described weak or absent HIV-specific CD4+ T cell proliferative responses in viremic adults who did not receive ART during acute infection (5, 6, 10, 23). By contrast, the present study detected HIV-specific CD4+ T cell proliferative responses in children who did not receive ART during acute HIV-1 infection. Responses were primarily detected in children with plasma HIV-1 RNA of <50 copies/ml. These data suggest that the major factor associated with reduced in vitro proliferative capacity is the presence of vigorous ongoing viral replication and that control of viral replication may allow the reversal of the proliferative defect. In this regard, the limited proliferative capacity of HIV-specific CD4+ T cells may be similar to those described in other virus-specific CD4+ T cell responses during acute infection (24).

There are several potential mechanisms to explain diminished in vitro p55-specific CD4+ T cell proliferation in children with ongoing HIV-1 replication. The recent demonstration by Douek et al. (25) that HIV-specific CD4+ T cells are preferentially infected raises the possibility that the absence of proliferative responses in viremic individuals may be a direct consequence of HIV-1 infection. Sieg et al. (26) have reported that diminished CD4+ T cell proliferation in HIV-infected individuals is associated with reduced expression of several cell cycle proteins, resulting in early G1 arrest. Observed imbalances in T cell nucleoside pools in HIV-1-infected individuals may inhibit normal cellular proliferation (27). Finally, continuous exposure of HIV-specific CD4+ T cells in chronically viremic persons may allow clonal exhaustion that precludes the ability of these cells to proliferate in vitro and possibly in vivo.

In summary, HIV-specific CD4+ T cell IFN-γ responses were commonly detected in a large cohort of children with established HIV-1 infection. A close relationship between the presence and frequency of HIV-specific CD4+ T cells and plasma HIV-1 load was observed. The majority of children with active HIV-1 replication failed to mount appreciable HIV-specific proliferative responses, despite displaying high frequencies of IFN-γ-producing cells. These data support the uninterrupted use of combination ART in HIV-1-infected children to preserve HIV-1 and other virus-specific CD4+ T cell function by controlling HIV-1 replication. Vaccination to stimulate HIV-specific CD4+ T cell responses under continued ART may improve the long-term control of viral replication and perhaps allow the eventual discontinuation of ART.

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References