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Differential Restoration of Myeloid and Plasmacytoid Dendritic Cells in HIV-1-Infected Children after Treatment with Highly Active Antiretroviral Therapy

Zheng Zhang,† Junliang Fu,‡ Qingxia Zhao,† Yun He,† Lei Jin,* Hui Zhang,* Jinxia Yao,* Linqi Zhang,¶§ and Fu-Sheng Wang***

Numerical and functional deficits in myeloid (mDC) and plasmacytoid dendritic cell (pDC) subsets have been found in both adult and pediatric HIV-1 carriers. Whether these impaired DC subsets can be restored after treatment with highly active antiretroviral therapy (HAART) is currently unknown, especially in HIV-1-infected children. In this report, we characterized mDC and pDC subsets in 18 HIV-1-infected children who received HAART treatment and compared them with those in 6 untreated HIV-1-infected children and 27 HIV-1-uninfected healthy children. Among children treated with HAART, 11 were found to suppress HIV-1 replication successfully below the detection limit (HAART-suppressed group) while the remaining 7 failed (HAART-failure group). In HAART-suppressed children, a gradual and complete restoration of the frequency and function of mDCs was observed while the recovery of pDCs was only partial. However, mDC and pDC subsets in HAART failure children were indistinguishable from the HAART-naive infected children. We also found that mDC frequency and IFN-α-releasing capacity of pDC positively correlated with CD4 T cell percentages in all HIV-1-infected children. In HAART-naive children, the mDC frequency correlated the HIV-1-specific CTL frequency. Our findings suggest that HAART has a differential impact on the restoration of mDC and pDC subsets. These findings may help guide the development of HIV-1-specific immune therapy aimed at fully restoring host immune function in chronically HIV-1-infected children.


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4 Abbreviations used in this paper: HAART, highly active antiretroviral therapy; DC, dendritic cell; mDC, myeloid DC; pDC, plasmacytoid DC; HS, HAART suppressed; HF, HAART failure; IN, HAART naive; HC, healthy control.
Subjects. Based on characterization of 24 HIV-1-infected children in comparison to 27 uninfected healthy children, we found that HAART can successfully restore the frequency and function of mDCs, but these parameters were only partially recovered in pDCs. Our findings indicate that the persistent impairment of pDCs may result in a sustained defect in DC-mediated immunity and incomplete host immune reconstitution in HIV-1-infected children following HAART treatment.

Materials and Methods

Subjects

Twenty-four HIV-1-infected children and 27 age- and sex-matched, HIV-uninfected healthy children were enrolled in this study. Informed consent was obtained from their parents or legal guardians, in accordance with institutional review board guidelines for the protection of human subjects. Blood samples were evaluated for viral load, CD4 T cell counts, and CD4 T cell percentage as part of routine clinical monitoring. Of the 24 HIV-1-positive patients, 6 were female and 18 were male. Fifteen patients were infected with HIV-1 by contaminated blood transfusion and 9 by vertical transmission. Among the 24 HIV-1-infected children, 15 received two nucleoside reverse transcriptase inhibitors plus a non-nucleoside reverse transcriptase inhibitor (nevirapine or efavirenz), 3 received one non-nucleoside reverse transcriptase inhibitor plus a protease inhibitor (indinavir), and the remaining 6 had not been treated at the time of analysis. Twelve patients were evaluated one to three times at intervals of 2–8 mo within a 12-mo follow-up period.

The 24 patients initially enrolled had the comparable clinical conditions such as age, duration of infection, number of circulating CD4 T cells (<100 cells/μl), and serum HIV viral loads (ranging from 10^5 to 10^10 copies/ml). They were classified into three groups on the basis of their treatment status and plasma HIV-1 RNA levels. The first group (n = 11) was HAART-suppressed (HS) children who were defined as those receiving HAART, with the plasma HIV-1 RNA level below the detection limit (<500 copies/ml) according to our home-based assay. The second group (n = 6) was HAART-failure (HF) children who received HAART but failed to suppress viral replication (HIV-1 RNA level ≥2000 copies/ml). The third group (n = 7) was HAART-naïve (HN) children who had never received HAART before the time of evaluation and had active plasma viral replication (plasma HIV-1 RNA level ≥2000 copies/ml). Baseline clinical data are shown in Table I. Duration of infection and age did not differ among the three groups of patients. It is worth noting that the median duration of HAART in the HS children (96 wk; range, 30–139 wk) was longer than that in the HF children (median, 35 wk; range, 7–104 wk; Table I).

FACS analysis of peripheral DCs

For analysis of DC subsets, fresh heparinized peripheral blood was incubated with a lineage mixture of FITC-conjugated mAbs (including anti-CD3, anti-CD14, anti-CD16, anti-CD20, and anti-CD56 mAbs) and PerCP-conjugated anti-HLA-DR mAb, PE-conjugated anti-CD11c, anti-CD123, or anti-isotype mAbs (BD Pharmingen) were added separately to each tube and incubated for 20 min at 4°C. The RBCs were then lysed with FACS lysing solution (BD Pharmingen) and removed by extensive washing. The residual blood cells were fixed before analysis by the three-color flow cytometry using FACScalibur (BD Biosciences). Circulating mDCs were defined as Lin−1 "HLA-DR+ CD11c+", and pDCs were defined as Lin−1 "HLA-DR− CD123+" (see Fig. 2 and Ref. 22).

Cytokine assays

Freshly prepared PBMC populations (2 × 10^6 cells/0.5 ml/well) were stimulated in 24-well plates with medium alone, 50 μg/ml poly(I:C) (Amerham), or 6 μg/ml CpG 2216 (Sangon) for 24 h at 37°C in the presence of penicillin, streptomycin, t-glutamine, and 10% FCS (23). Cell-free supernatants were harvested and tested in duplicate for IFN-α or IL-12 by use of a commercial ELISA kit according to the manufacturer’s instructions (BioSource International). Absorbance was measured on an automatic plate reader. Sensitivity of the assays was ~10 pg/ml. Recombinant cytokine standards were used in ELISA. Per cell IFN-α or IL-12 production was calculated by dividing the amount of IFN-α or IL-12 produced in each tube by the number of CD123+ pDCs or CD11c+ mDCs present in the tube, using the formula IFN-α/(pDC% × 4 × 10^4) or IL-12/(mDC% × 4 × 10^4).

Tetramer peptide-MHC complexes and HLA class I genetic typing

Four tetramer peptide-MHC complexes composed of three major HLA-A0201, A1101, and A2402 alleles were produced because higher frequencies of these alleles are found among Chinese populations (24–26). Two of the MHC-peptide complexes were formed between two HIV-1-immunodominant epitopes and HLA-A0201: one in the p17 gag protein (SL9, SLYNTVAL) and the other in reverse transcriptase (IV9, ILKEPVHVG). The other two MHC-peptide complexes were formed between HLA-A1101 and a nef epitope (QK10, QVPLRPMTVK) and between HLA-A2402 and another nef epitope (RL9, RYLKDGQLL). The sequences of HIV-1 protein epitopes used in the study correspond to the regions of HIV-1 genotype B that are the most dominant strains identified in our patient populations (27). All tetramer peptide-MHC complexes were purchased commercially (Proimmune). HLA genotyping was performed according to previous protocols (28, 29). The cut-off value was defined as 0.03%.

Plasma HIV-1 RNA monitoring

The 7900HT Sequence Detection System (Applied Biosystems) was used to quantify HIV-1 RNA levels in plasma samples in our laboratory. The cut-off value was 500 copies/ml.

Statistical analysis

All data were analyzed using SPSS software. Data are summarized as medians and ranges. Nonparametric Kruskal-Wallis test was used for primary comparisons among different groups and given an overall p value. The secondary comparisons between the groups of healthy controls (HC) and HIV-1-infected patients were performed using the Mann-Whitney U test. The correlations between variables were evaluated by use of the Spearman rank correlation test. For all tests, two-sided p < 0.05 was considered to be significant.

Results

Restoration of CD4 T cell percentages in infected children is dependent on the effectiveness of HAART treatment

Studies with humans have shown that increases in CD4 T cell counts correlate with full HIV-1 suppression (12–17, 30, 31). In the study, the median CD4 T cell percentages were 27%, 16%, and 3%, in the HC, HS, and HF groups, respectively. There was no significant difference in the CD4 T cell percentages among the three groups. The median CD4 T cell counts in the HC, HS, and HF groups were 1335, 812, and 395 cells/μl, respectively. There was no significant difference in the CD4 T cell counts among the three groups. The median CD4 T cell percentages of the children receiving HAART were higher than those of the children not receiving HAART. The median CD4 T cell percentages of the children receiving HAART were higher than those of the children not receiving HAART. The median CD4 T cell counts of the children receiving HAART were higher than those of the children not receiving HAART. The median CD4 T cell counts of the children receiving HAART were higher than those of the children not receiving HAART. The median CD4 T cell counts of the children receiving HAART were higher than those of the children not receiving HAART. The median CD4 T cell counts of the children receiving HAART were higher than those of the children not receiving HAART.

Table I. Characteristics of pediatric populations in the study

<table>
<thead>
<tr>
<th>Cases</th>
<th>HC</th>
<th>HS</th>
<th>HF</th>
<th>HN</th>
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<td>11</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>9.0 (8–14)</td>
<td>12.0 (9–15)</td>
<td>9.5 (5–11)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
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<td>6/5</td>
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<td>6/0</td>
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<tr>
<td>Infection route (b/v)</td>
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<td>5/2</td>
<td>2/4</td>
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<tr>
<td>Infection time (year)</td>
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<td>9.0 (8–9)</td>
<td>8.0 (5–9)</td>
</tr>
<tr>
<td>HLA-A gene type (A02/A11/A24)</td>
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<td>2/4/2</td>
<td>3/3/0</td>
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<tr>
<td>HAART duration (wk)</td>
<td>NA</td>
<td>96.0 (30–139)</td>
<td>35.0 (7–104)</td>
<td>NA</td>
</tr>
</tbody>
</table>

* M, Male; F, Female; b, blood transfusion; v, vertical transmission.

* NA, Not applicable; data are median (range).

* Weeks after initiation of HAART.

* p < 0.05, compared with HS children.
our study, the CD4 T cell percentage of all lymphocytes was used as a prognostic indicator for children as recommended by the Working Group on Antiretroviral Therapy (www.aidsinfo.nih.gov/guidelines) and the HIV-1 Pediatric Prognostic Markers Collaborative Study Group (30). Consistent with early reports, the CD4 T cell percentage significantly increased in HIV-1-infected children with full viral suppression by HAART treatment (HS group). Fig. 1 summarizes the CD4 percentages and viral load in all three groups of infected children relative to that of healthy normal controls. As shown in Fig. 1A, all three HIV-1-infected groups (HS, HF, and HN) had reduced CD4 T cell percentages to differing degrees compared with those of the HC group (median, 32.1%; range, 26.2–43.4%). However, the HS children exhibited a CD4 T cell percentage (median, 23.6%; range, 13.9–37.0%) that was markedly greater than those of HF children (median, 8.4%; range, 0.6–23.0%) and HN children (median, 9.1%; range, 3.0–13.1%). The degree of CD4 T cell percentage recovery in the three groups was significantly inversely correlated with the levels of plasma HIV-1 RNA ($r = -0.687$, $p < 0.001$, Spearman rank correlation test), with the HS group having undetectable levels (<500 copies/ml), a median $5.20 \times 10^4$ copies/ml in the HF group, and $6.68 \times 10^5$ copies/ml in the HN group (Fig. 1B).

Restoration of circulating impaired mDCs and pDCs correlated with viral suppression by successful HAART

To study the impact of HAART on DC subsets, we analyzed frequencies and functions of circulating mDCs and pDCs in HIV-1-infected children before and after treatment with HAART and compared them to those of uninfected HC. mDCs were defined as Lin$^-$/CD11c$^+$/HLA-DR$^+$ cell population based on flow cytometry (Fig. 2A). As shown in Fig. 2B, the mDC and pDC frequencies were significantly lower ($p < 0.05$) in the HN than in the HC groups, suggesting HIV-1 infection causes significant reductions for both DC subsets. Following treatment with HAART, there appeared to be differential restoration for mDCs and pDCs. In the HS group, mDCs were restored to levels (median, 0.60%; range, 0.28–1.11%) similar to those observed in the HC group (median, 0.58%; range, 0.09–1.39%), whereas the pDCs recovery was only partial (HS group median, 0.14%; range, 0.03–0.46%, and the HC group median, 0.34%; range, 0.11–1.19%). In the HS group, mDC and pDC frequencies were higher than their counterparts in both the HF and HN groups; this difference was particularly pronounced for the mDC population (Fig. 2B). Furthermore, the degree of mDC and pDC recovery was inversely correlated with degree of viral loads (Fig. 2C). Also, there is significant positive correlation or correlative trends between mDC or pDC frequency and CD4 T cell percentages, respectively (Fig. 2D).

We also simultaneously investigated whether restoration of mDC and pDC frequencies after HAART treatment correlate with their function in cytokine release. Freshly collected PBMC were stimulated with one of the following TLR ligands: TLR3L, poly(I:C) or TLR9L CpG oligodeoxynucleotides (23, 32–34). Poly(I:C) stimulates mDCs to secrete IL-12 (23, 33), whereas CpG stimulates pDCs to secrete IFN-$\alpha$ secretion (23, 34). Our previous demonstrations that depletion of BDCA-1$^+$ cells (90% of mDC subset) and BDCA-4$^+$ cells (90% of pDC subset) from the total PBMC population markedly reduced poly(I:C)-induced IL-12 production and CpG-induced IFN-$\alpha$ production by almost 90%, respectively, indicates that we can use stimulated PBMC cytokine release as surrogate markers of mDC and pDC function. As shown in Fig. 2B, the amount of poly(I:C)-induced IL-12 production and CpG-induced IFN-$\alpha$ production was significantly reduced in the HN group (median, 120.2 pg/ml; range, 78.6–265.1 pg/ml and median, 10.7 pg/ml; range, <10–64.8 pg/ml, respectively) compared with that in the HC groups (IL-12 median, 478.5 pg/ml; range, 213.8–1375.2 pg/ml and IFN-$\alpha$ median, 605.7 pg/ml; range, 104.3–1508.3 pg/ml, respectively). After treatment with HAART, poly(I:C)-induced IL-12 production was restored for both the HS (median, 350.7 pg/ml; range, 69.6–875.1 pg/ml) and HF (median, 105 pg/ml; range, <10–105.7 pg/ml) and HF group children (median, 254.3 pg/ml; range, <10–513.4 pg/ml), although the former experienced significantly higher recovery than the latter (Fig. 2B). In addition, partial restoration of CpG-induced IFN-$\alpha$ production was also found for both the HS (median, 85.8 pg/ml; range, 10–455.9 pg/ml) and HF (median, 10.1 pg/ml; range, <10–68.4 pg/ml) groups of children, despite the degree of pDC functional recovery being far less in the HF compared to that in the HS group, or compared to that of IL-12 production by mDCs after poly(I:C) stimulation. Furthermore, the degree of restoration for both mDCs and pDCs is clearly correlated inversely with plasma viral loads (Fig. 2C). Also, there is a significantly positive correlation or correlative trends between pDC-associated IFN-$\alpha$ production or mDC-associated IL-12 production and CD4 T cell percentages, respectively (Fig. 2D).

We proceeded to estimate the amount of IL-12 or IFN-$\alpha$ production per individual mDC or pDC before and after HAART treatment by correcting the input number of each DC subset before stimulation. In the HC group, we found that each mDC produced $\sim34.7$ fg of IL-12, and a single pDC produced $\sim65.8$ fg of IFN-$\alpha$. In the HN group, however, the levels of production of IL-12 and IFN-$\alpha$ were reduced to 12.7 and 3.2 fg, respectively. After HAART treatment, IL-12 production per mDC was greater in the HS group (median, 28.4 fg), than in the HF group (median, 14.8 fg). Indeed mDC IL-12 production in the HS group did not differ from that of the HC group. We also found that HAART treatment resulted in increased IFN-$\alpha$ production per pDC in the HS group (median, 11.4 fg), relative to the HF group (median, 6.3 fg). In the case of IFN-$\alpha$, however, the increase was not sufficient to bring the

FIGURE 1. Distribution of CD4 T cell percentage and plasma HIV-1 RNA loads in HIV-1-infected children. A. Distribution of CD4 T cell percentage by group. B. The distribution of plasma HIV-1 RNA load by group. Boxes represent the 5th, 25th, 75th, and 95th percentiles and the median value (solid line) of each subset. Overall $p < 0.001$, Kruskal-Wallis test; *, $p < 0.05$, Mann-Whitney $U$ test.
HS group value up to the level of the HC group. Differential restoration of mDCs and pDCs after HAART treatment is therefore not only observed in terms of the quantities present in the peripheral blood, but also in their cytokine release capabilities. Such a dichotomy in the response to HAART treatment between DC subsets is not yet understood; however, it may be associated with

FIGURE 2. Restoration of circulating impaired mDCs and pDCs correlated with viral suppression by HAART in HIV-1-infected children. A, Identification of mDCs and pDCs using flow cytometric analysis. Fresh PBMC were gated from total peripheral leukocytes based on their forward and side scatter (R1). FITC lineage-negative cells (R2) from PBMC were identified as either mDCs or pDCs; the PerCP-HLA-DR- and PE-CD11c+ subpopulation (R3) was identified as mDCs, whereas the PerCP-HLA-DR+ and PE-CD123+ subpopulation (R4) was identified as pDCs. B, Distribution of DC subset frequency and cytokine-releasing capacity among the groups. Boxes represent 5th, 25th, 75th, and 95th percentile and the median value (solid line) of each subset. Overall $p = 0.003, <0.001, = 0.007, and <0.001$ for mDC%, pDC%, IL-12 concentration, and IFN-α concentration, respectively; Kruskal-Wallis test; *, $p < 0.05$, Mann-Whitney $U$ test. C, The analysis of correlations between HIV-1 RNA loads and circulating DC subset frequency or cytokine-releasing capacity. D, The analysis of correlations between CD4 T cell percentages and circulating DC subset frequency or cytokine-releasing capacity. □, HS children; ○, HF children; Δ, HN children; solid line, linear growth trend. $r$, correlative coefficient. Values of $p$ are shown.
differing susceptibility of these two subsets to infection by R5 viruses (18, 19).

Sequential changes in mDCs and pDCs after treatment with HAART and their relationships with CD4 T cell, HIV-1-specific CTL frequency, and viral load

In addition to the cross-sectional studies performed above, we also conducted longitudinal studies on the changes of mDCs and pDCs in the HS group of children over the course of HAART treatment. As shown in Fig. 3A, before initiation of HAART, mDC (median, 0.35%; range, 0.06–0.54%) and pDC frequencies (mean, 0.12%; range, 0.04–0.22%) were significantly lower than those in the HC group. After HAART treatment, both circulating mDC and pDC frequencies showed a steady increase and reached to a median value of 0.50 and 0.26%, respectively, after >100 wk of treatment. However, only the mDC frequency returned to levels similar to those found in HC children (median, 0.58%;  \( p > 0.05 \) vs HC group). The pDC frequency, in contrast, restored ~63% of normal levels found in HC children (median, 0.41%;  \( p < 0.05 \) vs HC group). The differential response of mDCs and pDCs to HAART treatment has therefore been confirmed by our sequential studies.

Over the course of HAART treatment, we found positive changes in CD4 T cell percentages and reverse changes in HIV-1-specific CTL responses that were in accordance with the DC frequency changes described above. CD8 T cell percentages and reverse changes were measured by tetramer labeling of the most

**FIGURE 3.** Sequential changes of mDCs and pDCs after successful HAART treatment and their associations with CD4 T cells, HIV-1-specific CTL frequency, and viral loads. A, Long-term alterations of DC subset, CD4 T cell, HIV-1-specific CTL frequencies, and plasma HIV-1 RNA levels in virally suppressed children. Boxes represent 5th, 25th, 75th, and 95th percentile and the median value (solid line) of each subset. N, numbers of cases. B, Analysis of correlation between the duration of HAART and DC subset, CD4 T cell, HIV-1-specific CTL frequency, and HIV-1 RNA levels in virally suppressed children. □, HS children; △, HN children; solid line, linear growth trend. \( r \), correlative coefficient. Overall \( p < 0.001, p < 0.001, p = 0.029, p < 0.001, \) and \( p < 0.001 \) for CD4 T cell percentage, plasma viral loads, mDC%, pDC%, and tetramer% among different groups by weeks, Kruskal-Wallis test; \( p \) values are shown, Mann-Whitney U test.
prevalent HLA alleles among the Chinese population (HLA-A2, -A11, -A24) along with gag (SL9), pol (IV9), or nef (QK10 and RL9) epitopes (Fig. 3A). In this longitudinal study, HAART treatment resulted in steady increases in CD4 T cell percentages, which reached a median value of 22.8% from a pretreatment median of 6.45%. Conversely, tetramer-positive CD8 T cell frequencies were found decreased along with the increases of mDC, pDC, and CD4 percentages over the course of treatment. This decrease correlated positively with viral load reduction in plasma after HAART treatment (r = 0.720, p < 0.01, Spearman rank correlation test; Fig. 3A). To further confirm the positive correlation between HIV-1-specific CTL response and plasma viral load, we measured tetramer-positive CD8 T cells. We arbitrarily chose total frequencies of tetramer-binding cells for those patients with two or more epitopes detected simultaneously as values for CTL responses. Fig. 4A summarizes the frequencies of tetramer-positive CD8 T cells for all samples collected. When these samples were further categorized according to their treatment status, HF and HN groups of children were found to have significantly higher tetramer-positive CD8 T cells than those of the HS group (Fig. 4B). This finding is in agreement with what we have found for the sequential samples: HIV-1-specific CD8 T cell response was positively correlated with viral load, but inversely with frequencies for both mDCs and pDCs in HAART-treated patients.

Tetramer-positive CD8 T cells were found to correlate positively with mDCs and to have a positively correlated trend with pDCs (r = 0.7211, p < 0.05 for mDCs; r = 0.359, p > 0.05 for pDCs) in all HN patients (Fig. 4C). These results suggest that DC subsets and HIV-1-specific CTLs are tightly linked although the cause-and-effect relationship between the two is not clear at this point.

Furthermore, we analyzed the correlation between the duration of HAART treatment and aforementioned immune parameters (Fig. 3B). We observed positive correlations between the duration of HAART and DC subset frequencies or CD4 T cell percentages (r = 0.3190, 0.452, and 0.608 for mDC, pDC, and CD4 T cells, respectively; all p < 0.05) and negative correlations between the duration of HAART and viral loads or CTL frequency (r = −0.7460 and −0.7051, respectively; all p < 0.05) in these children. These data suggest that duration of HAART treatment is a potential factor affecting recovery of DC subsets and the host immune reconstitution.

For comparison, we also analyzed longitudinal alteration of the DC subsets, CD4 T cells, HIV-1-specific CTL frequencies and HIV-1 viral loads in HF children. Our results showed that both CD4 T cell percentage and DC subset frequencies appeared to be slightly increased or unchanged after the median 35-wk duration of HAART treatment. The HIV-specific CTL frequency and plasma RNA levels decreased or were unaltered in this group of children (data not shown).

**Discussion**

Although previous research has shown that HIV-1-infected individuals have reduced mDC and pDC levels (14, 15, 17, 21), the characterization of blood DC subsets and their effect on immune reconstitution in HAART-treated HIV pediatric patients has not yet been well defined. In this report, we characterized blood DC subsets in HIV-1-infected children before and after treatment with HAART. We have found that the frequency and function of circulating mDCs and pDCs were impaired in HIV-1-infected patients, and their responses to HAART treatment were significantly different. We found that HAART treatment could completely restore mDCs, but that recovery for pDCs appeared to be incomplete. Thus, we observed a differential restoration of DC subsets in children with full viral suppression. Our data indicating that the pDC IFN-α-releasing function is still impaired, despite long-term successful HAART treatment, supports the notion that pDCs are profoundly impaired in pediatric patients with HIV-1 infection (21). The mechanism underlying this persistent deficit remains unclear.

The present study is partially but not completely consistent with a recent report that pDC frequency, but not IFN-α-secreting ability, could recover to normal levels (21). Differences in duration of HAART, existence of HIV-1 viral resistance, drug toxicity, and poor patient compliance to medication may account for the discrepancy (5, 6). The age of patients undergoing HAART in our study (8–15 years), relative to previous research that examined HAART effects in infants (21), may be an important factor in the differences between the results. Our longitudinal studies based on the exact duration of HAART therapy indicate that there was a

**FIGURE 4.** Circulating HIV-1-specific CTL responses to HAART treatment in HIV-1-infected children and their relations with DC subsets. A. The frequency of tetramer-positive CD8 T cells specific for the HLA-A2, -A11, and -A24-restricted epitopes such as SL9 (p17 gag protein, SLYNTVAL), IV9 (reverse transcriptase, ILKPEVHG), QK10 (nef protein, QVPLR-PMTYK), and RL9 (nef protein, RYLKDQQL). B. The distribution of tetramer-positive CD8 T cell frequency by group. ○, one sample; dashed line, cut-off value of tetramer is defined as 0.03%. Boxes represent 5th, 25th, 75th, and 95th percentile and the median value (solid line) of each subset. Overall p = 0.043 for tetramer; *, p < 0.05, Mann-Whitney U test. C and D. Correlation analysis of HIV-1-specific CTL frequency with mDC or pDC frequency in HN children. △, HN children; solid line, linear growth trend. r, correlative coefficient. Values of p are shown.
Differential recovery of DC subsets in HIV-1 children after HAART

gradual but incomplete increase in pDC frequency. The significant positive correlation between pDC frequency and duration of HAART suggests that an extended period of antiviral therapy might be indicated to better restore circulating pDCs. It is noteworthy that recovery of pDCs was slower in our examined subjects than in adults in previous reports (15, 35, 36). This might be explained, in part, by differences in immune system plasticity between adults and children.

In concordance with studies conducted in adult populations (12–15), both mDC percentages and their capability for IL-12 production were reduced in viremic children, indicating that this DC subset is also depleted in HIV-1 infection. Long-term HAART could suppress viral load and result in complete recovery of mDC frequency and function. The functional impairment of mDCs could completely recover in HS children. This differential restoration of mDCs and pDCs in HIV-1-infected children after HAART might be explained in part by differences in HIV susceptibility, variable sensitivities to HAART treatment, and selective interaction of HIV-1 with mDCs and pDCs (18, 19, 37).

In agreement with other data (38–42), HIV-1–specific CTL frequency declined as mDCs and pDCs increased in HAART-treated and virally suppressed children. However, in HIV patients, there is a positive correlation between mDC frequency and HIV-1–specific CTL frequency, suggesting that HIV-1–specific CTL responses are partially dependent on overall levels of DC subsets (especially mDCs), as previously reported (43–45). However, the cause-and-effect relationship between the DC and CTL is currently unknown and warrant further studies. Furthermore, significant positive correlations were observed between CD4 T cell percentages and mDC frequency or IFN-α production by pDCs. Our results indicate that the full recovery of mDC percentages occurred in parallel with CD4 T cell recovery in HS children. These results, in concordance with another report (20), suggest that restorations of DC subsets after HAART treatment are associated closely with the improvement of circulating CD4 T cell percentages in HIV-1-infected children.

Based on our examination of circulating DC subsets throughout the period of HAART treatment in HIV-1-infected children, we can draw the following conclusions: First, HIV-1 infection can lead to a decrease in circulating DC subset frequency and DC functional impairment. Impaired functions include the IL-12 and IFN-α release capacities of mDCs and pDCs, respectively. Second, long-term antiviral therapy can successfully control HIV-1 replication; mDC frequency and function can be completely recovered, whereas pDC frequency and function can recover partially. The duration of HAART treatment may influence the degree of recovery of functionally impaired pDCs in HAART-treated children. Third, recovery of the DC subsets closely correlated with improvement of CD4 T cell percentages in HIV-1-infected children. Thus, cellular immune responses that involve both CD4 Th cells may be induced, coordinated, and regulated by DCs.

Ag-nonspecific innate immunity and Ag-specific adaptive immunity cooperate to eradicate invading pathogens through the actions of immune cells and their effector proteins (46). Taken together with previous findings (14, 15, 21, 35, 36), the present findings suggest that HAART has a differential impact on the restoration of mDC and pDC subsets, which is simultaneously in accordance with the process of host immune reconstitution via augmentation of both the innate and adaptive immune responses. Thus, HAART treatment may establish a novel balance between the innate and adaptive immune responses, such that HAART-mediated recovery of innate immune function probably facilitates the increase of CD4 T cell percentage; however, further study needs to uncover the causative relationship between the recovery of DC subsets and the improvement of CD4 T cell and CTL response. These results further indicate that both mDCs and pDCs actively participate in the immunopathogenesis of pediatric HIV-1 disease. These findings may help guide the development of HIV-1–specific immune therapy aimed at fully restoring immune function in chronically HIV-1–infected children.

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Disclosures

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References


