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We studied changes in 60 immunological parameters after the administration of highly active antiretroviral therapy (HAART) in 192 clinically stable antiretroviral drug–experienced HIV-1–infected children 4 months–17 years old. The studied immunological parameters included standard lymphocyte subsets and lymphocyte surface markers of maturation and activation. The most significant changes during the 48-week study period were seen for $CD8^+$, $CD8^+CD62L^+CD45RA^+$, $CD8^+CD38^+HLA\text{-}DR^+$, and $CD4^+$ T cell percentages ($P < .0001$ for all parameters). These changes suggest that significant decreases in the expression of activation markers and increases in the expression of naive markers in the $CD8^+$ T cell population may be related to better virologic control in these HIV-1–infected children, who had relatively stable immune function at the initiation of HAART. At week 44 of HAART, the major immunological parameters in these HIV-1–infected children moved from baseline values to about halfway to two-thirds of the way toward the values in healthy, uninfected children.

Present antiretroviral drug treatment guidelines for children with HIV-1 infection were influenced greatly by Pediatric AIDS Clinical Trials Group (PACTG) Protocol 338 [1, 2]. That study established the superiority of protease inhibitor (PI)–containing regimens, often referred to as highly active antiretroviral therapy (HAART), in reducing viral loads to undetectable levels (<400 HIV-1 RNA copies/mL) in >50% of HIV-1–infected children 2–17 years old. The companion follow-up study, PACTG 377, compared 4 different HAART regimens in a similar population of HIV-1–infected children 4 months–17 years old. PACTG 377 demonstrated a similar reduction in viral loads in 44%–69% of children at week 12 of therapy [3, 4].

The pathogenesis of AIDS is presently thought to be a consequence of much more than $CD4^+$ T cell lymphocytopenia and the direct cytopathic effects of HIV-1 [5]. Numerous studies of adults and children have revealed that HIV-1 infection is associated with evidence of marked activation, apoptotic cell death, differentiation, and turnover of $CD8^+$ T cells, as well as diminished thymic output and reduced numbers of naive T cells [6–15]. The marked reductions in viral load observed with successful HAART would be expected to result in improvement of the immunological parameters of T cell maturation, activation, and function. Numerous studies of adults have reported the beneficial effects that successful HAART has on activation and

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activation markers on both $CD4^+$ and $CD8^+$ T cell subsets, as well as the recovery of immune function [16, 17]. In addition, several small studies of the effects of HAART in children have demonstrated significant changes in maturation markers [18–20]; the activation markers CD38, HLA-DR, and CD95 [20, 21]; and the functional markers of cytokine production, lymphoproliferative responses, and T cell clonality [20–22]. Indeed, in PACTG 338, an analysis of trends in a set of 40 immunological parameters for 101 children receiving HAART showed evidence of change in a large number of parameters. The most significant changes were seen for markers on $CD8^+$ T cells, activation markers (CD38 and HLA-DR), and the apoptosis-associated surface marker CD95 on $CD8^+$ T cells [23]. Taken together, the results of these studies suggest significant similarities and differences in the mechanisms and overall functional potential of immune reconstitution between children and adults.

The present study of the changes in immunological parameters observed in PACTG 377 extended the investigations of changes in the immunological parameters in children in PACTG 338 to a larger number of concomitantly analyzed parameters in a larger group of children. The PACTG 377 study population represented a greater range of ages in the children, including some children as young as 4 months old, than was found in PACTG 338, and we used methodological techniques identical to those used in PACTG 338 to examine changes in the PACTG 377 study population's immunological parameters. We also compared changes in the immunological parameters observed in PACTG 377 with the corresponding values in a very large population of healthy, HIV-uninfected children who were similar in age, socioeconomic status, and sex. Temporal changes in 60 immunological parameters for the 192 children enrolled in the study were monitored for 48 weeks. The studied immunological parameters included the standard lymphocyte subsets, lymphocyte surface markers of maturation and activation, and in vitro lymphoproliferation to HIV and non-HIV antigens. We aimed to determine which were the most important immunological parameters, as measured by change after the initiation of HAART, in children who were naive to HAART. Independent confirmation of the key immunological parameters identified in PACTG 338 will permit identification of a small number that should be studied intensively and measured routinely for management of this patient population. We also studied the key immunological parameters with respect to their association with the age of the child, HIV-1 RNA load at week 48, and corresponding values in healthy children.

SUBJECTS, MATERIALS, AND METHODS

Study design and patient population. PACTG 377 was a multicenter, randomized clinical trial that compared values for viral load in children receiving nucleoside analogue therapy to values after the administration of 1 of 4 stavudine (d4T)–containing regimens: nevirapine and ritonavir; lamivudine (3TC) and nelfinavir; nevirapine and nelfinavir; and lamivudine, nevirapine, and nelfinavir. All children were infected with HIV-1, were 4 months–17 years old, had stable $CD4^+$ T cell counts or percentages, remained in Centers for Disease Control and Prevention Immune Category 1 or 2 during the 4 months before study entry [24], and had been receiving the same antiretroviral therapy during the 16 weeks before study entry. All children were naive to d4T, 3TC, PIs, and nonnucleoside reverse transcriptase inhibitors. Exclusion criteria included grade 3 or 4 (severe or life-threatening) laboratory test abnormalities (judged on the basis of protocol-specified, standard pediatric toxicity criteria), active opportunistic and/or serious bacterial infection, and diagnosis of malignancy or pregnancy. A total of 193 children from 50 sites entered the study between December 1997 and September 1998. One child did not start treatment and was excluded from this analysis. The duration of study treatment for each child was initially planned to be 48 weeks, but it was extended to 96 weeks for children who were still receiving their initial study treatment at week 48 (see Wiznia et al. [3] and Krogstad et al. [4] for additional information). The institutional review boards of all the sites participating in PACTG 377 approved this study. Written, informed consent was obtained from all children or their legal guardians.

Lymphocyte subset measurements. Lymphocyte surface markers (CD3, CD4, CD8, and CD19) were measured by 2- or 3-color flow cytometry, and complete blood counts and differentials were determined using standard methods in local laboratories at the enrolling sites. All laboratories participated in the National Institute of Allergy and Infectious Diseases (NIAID) Flow Cytometry Quality Assessment Program [25]. Basic lymphocyte subsets were evaluated at screening; entry; study weeks 4, 8, 12, 16, and 24; and every 12 weeks thereafter.

Three-color flow-cytometric measurements. Lymphocyte phenotyping was performed using a panel of fluorochrome-conjugated monoclonal antibodies formulated and pretitered by the supplier for these studies. Flow cytometry was performed in 7 PACTG Core Immunology Laboratories using a consensus whole-blood lysis protocol and common lots of pretitered monoclonal reagents produced commercially (Pharmingen). The extended 3-color flow panel consisted of the following combination of markers: CD4/CD45RA/CD62L, CD4/CD38/HLA-DR, CD4/CD28/CD95, CD8/CD45RA/CD62L, CD8/CD38/HLA-DR, and CD8/CD28/CD95. After staining and processing EDTA-anticoagulated blood in accordance with the consensus protocol [23], cells were analyzed using 3-color flow cytometers, and instrument setup, analysis gating, and reporting were performed in accordance with the guidelines established in the consensus protocol. After anchor gating on either $CD4^+$ or $CD8^+$ T cells, a dual-parameter analysis of the remaining 2 fluorochromes in each tube

was performed. These extended 3-color flow panels were studied at entry, week 16, and week 44.

Lymphoproliferative assay measurements. Lymphoproliferative responses were measured using pokeweed mitogen, candida antigen, tetanus toxoid, HIV p24/25/gag, and HIV rgp120SF antigens. However, none of the lymphoproliferative responses to pokeweed mitogen, candida antigen, tetanus toxoid, or HIV-specific antigens showed significant changes over time (data not shown). Although this lack of response may have been due in part to the relatively well-preserved immune status of this group of children at baseline, there may also have been some technical difficulties associated with damage to the samples during shipping, suboptimal in vitro culture conditions, or suboptimal doses of antigens.

Viral load. Blood samples for quantification of HIV-1 RNA were obtained at screening; entry; study weeks 4, 8, 12, 24, 36, 44, and 48; and every 12 weeks thereafter. HIV-1 RNA load was assessed using the Roche Amplicor Monitor HIV-1 Assay (Roche Diagnostics Corporation) [26] at a single laboratory at Johns Hopkins University (Baltimore, MD) that had been certified as proficient in the use of this assay by the NIAID Virology Quality Assurance Program [27]. Samples obtained at screening through week 12 were assayed for HIV-1 RNA load in batched fashion; subsequent samples were assayed at the specified individual time points. The lower limit of quantification for HIV-1 RNA was 400 copies/mL.

Statistical analysis. For a comparison among groups, Fisher's exact test was used for categorical variables and the Wilcoxon/Kruskal-Wallis test was used for continuous variables [28]. The baseline HIV-1 RNA load was defined as the geometric mean of the screening and the entry HIV-1 RNA loads. All *P* values were 2-sided and were not adjusted for multiple comparisons. Because 60 immunological parameters were evaluated in this analysis, caution should be exercised in the interpretation of the *P* values. A conservative solution to the problem of multiple comparisons is the Bonferroni method, which multiplies the nominal *P* value by the overall number of statistical tests [29]. If the result is still $<.05$, then the result is clearly statistically significant. When the Bonferroni method is used for this study, a *P* value between .0008 (.05/60) and .05 should be interpreted as being suggestive of significance but does not necessarily definitively show significance. $P<.0008$ should be considered to be clear evidence of statistical significance. For the purposes of determining the week 16 and week 44 values, observations within an 8-week window were used. Variations in the number of available test results by immunological parameter and time point were primarily due to differences in site funding for special immunological studies and, to a much lesser extent, to missed visits or patients leaving the study.

The magnitude of change over time in the values for the immunological parameters was evaluated using the level of significance of the time parameter in a mixed-effects model [30] and a "measure of discrimination." The measure of discrimination was the proportion of week 44 values that were within the interquartile ranges for both the baseline and the week 44 time points [23]. This measure of discrimination ranges from 0% to ~50%. A measure of discrimination of 0% indicates a major shift in values between baseline and week 44, which shows very good discrimination. At the other extreme, if no real change occurred between baseline and week 44, then the measure of discrimination would yield values of ~50%, indicating no discrimination. Results for the measure of discrimination could be marginally higher than 50%, because of ties at the 25th and 75th percentile values. It can be argued that the degree of statistical significance (*P* value) is not the ideal measure for evaluating the importance of immunological parameters in this situation, because a comparison of values may be highly statistically significant but may also be associated with a considerable overlap in values. Because the eventual goal is to establish criteria for the classification of patients with respect to immunological response, the measure of discrimination is valuable in this setting.

As a nested substudy, 32 children received diphtheria-tetanus toxoids–acellular pertussis (DTaP) vaccine at week 16 or 36. Because of the effect of DTaP vaccine on lymphoproliferative responses to tetanus and certain activation markers, for those assays, these 32 children were excluded from the analysis of changes.

RESULTS

Study population. For the 192 children in this study, the median age was 6.2 years, the median $CD4^+$ T cell count was 697 cells/μL, and the median HIV-1 RNA load was 4.48 $\log_{10}$ copies/mL. Overall, 63% of the children were black and also non-Hispanic, 55% were female, 60% had received prior treatment with a combination of zidovudine and didanosine, and 35% had received prior treatment with didanosine alone. Baseline patient characteristics were well balanced among the treatment groups.

Lymphocyte subset measurements. The $CD8^+$ T cell percentage decreased significantly from a baseline median of 42% to 33% at week 44 ($P<.0001$) (table 1). Over this same time period, the $CD4^+$ T cell percentage increased from a median of 28% to 35%, the $CD4^+$ T cell count increased from a median of 676 to 955 cells/μL, and the $CD4^+$ T cell percentage:$CD8^+$ T cell percentage increased from a median of 0.7 to 1.1 ($P<.0001$ for all 3 parameters). For the lymphocyte subset measurements, the greatest discrimination from baseline to week 44 values was seen for the $CD8^+$ T cell percentage and the $CD4^+$ T cell percentage: 27% of the week 44 values were within the interquartile ranges at both baseline and week 44. Trends over time for the $CD4^+$ T cell count, $CD4^+$ T cell percentage, $CD8^+$ T cell percentage, and $CD4^+$ T cell percentage:$CD8^+$ T cell

Table 1. Changes in lymphocyte subset parameters in children treated with highly active antiretroviral therapy.

Parameter	Baseline (n = 181–188)	Week 16 (n = 187)	Week 44 (n = 169)	P value for trend[a]	Measure of discrimination, %
$CD4^+$ T cells					
Count, cells/μL	676 (463–1073)	900 (632–1251)	955 (649–1324)	<.0001	34
Percentage	28 (22–35)	33 (27–39)	35 (28–40)	<.0001	27
$CD8^+$ T cells					
Count, cells/μL	1053 (780–1477)	1081 (774–1340)	989 (681–1280)	.0016	42
Percentage	42 (33–49)	36 (30–44)	33 (28–41)	<.0001	27
$CD19^+$ T cells					
Count, cells/μL	391 (250–612)	470 (303–759)	474 (309–716)	.0034	39
Percentage	16 (12–23)	18 (13–22)	18 (12–23)	.0083	51
$CD4^+$ T cell percentage:$CD8^+$ T cell percentage	0.7 (0.5–1.1)	0.9 (0.6–1.3)	1.1 (0.7–1.4)	<.0001	28
White blood cells, $\times$ 1000/mm^3	5.6 (4.1–7.1)	6.0 (4.8–7.7)	5.8 (4.8–7.8)	<.0001	39
Lymphocytes, %	50 (40–58)	50 (42–59)	50 (41–58)	.28	49

NOTE. Data are median (25th–75th percentiles), unless otherwise indicated.

[a] Level of significance of the time parameter in a mixed-effects model was used to assess the change in values over time.

percentage are shown in figure 1. The majority of the changes were seen within the first 24 weeks.

Three-color flow-cytometric measurements. For the 3-color flow-cytometric measurements, the trends from baseline to week 44 were highly significant ($P < .0001$) for 21 immunological parameters (table 2). Between baseline and week 44 values, the greatest discrimination was seen for $CD4^+CD45RA^-$ T cell count (17%), $CD8^+CD38^+HLA\text{-}DR^+$ T cell percentage (18%), and $CD8^+CD62L^+CD45RA^+$ T cell percentage (22%). Figure 2 shows the trends in time for these parameters and also for the $CD8^+HLA\text{-}DR^+$ T cell percentage, for which the trend over time was highly significant.

Most significant immunological parameters. The impact of the immunological parameters was measured by both the most significant trend over time (the F statistic associated with the time parameter of the mixed-effects model) and the greatest discrimination. Ranks of all 60 immunological parameters were determined for both measures of impact. Although the $CD4^+$ T cell percentage:$CD8^+$ T cell percentage had the most significant trend over time, 8 other immunological parameters had greater discrimination. Similarly, although the $CD4^+CD45RA^-$ T cell count had the greatest discrimination, 10 other immunological parameters had more significant trends. For each of the 60 immunological parameters, the ranks associated with these 2 measures were added together, and the immunological parameters with the smallest total rank sum were declared to be the most significant. By use of this approach, the $CD8^+$, $CD8^+CD62L^+CD45RA^+$, $CD8^+CD38^+HLA\text{-}DR^+$, and $CD4^+$ T cell percentages were identified as the immunological parameters with the most significant change between baseline and week 44 (data not shown).

Age-related differences. Figures 1 and 2 show the trends over time by age group for the 7 most significant immunological parameters, as determined by the rank sum approach, and the $CD4^+$ T cell count. The decline in $CD8^+$ T cell percentage and the increase in $CD4^+$ T cell percentage over time were greater for the older children.

Relationship between the expression of markers and viral load at week 48. Table 3 illustrates the relationship between viral load and the expression of several maturation, activation, and apoptosis markers at week 48. Children with $CD4^+$ T cell percentages in the fourth quarter (>40%) were much more likely than children with CD4 T^+ cell percentages in the first quarter ($\leq$28%) to have an HIV-1 RNA load $\leq$400 copies/mL (77% vs. 28%).

Comparison versus healthy children. A comparison of values for the key cell surface markers in HIV-1–infected children in this study at baseline and at week 44 after HAART with the corresponding values in healthy children in a recently published study [31] is given in table 4. In general, values in HIV-1–infected children at week 44 of treatment with HAART moved about halfway to two-thirds of the way toward those in healthy, uninfected children.

DISCUSSION

HAART is now capable of reducing viral load to below the level of detection in >50% of children and has virtually eliminated the occurrence of opportunistic infections and death in children with stable or early HIV disease [2, 4, 32]. As a result, immunological parameters are going to play an increasingly important role in the evaluation and future direction of therapy for HIV-1–infected children. The present study (PACTG 377) and PACTG 338 [23], the only other large multicenter pediatric study of several immunological parameters, aimed to identify which immunological parameters should play key roles in such

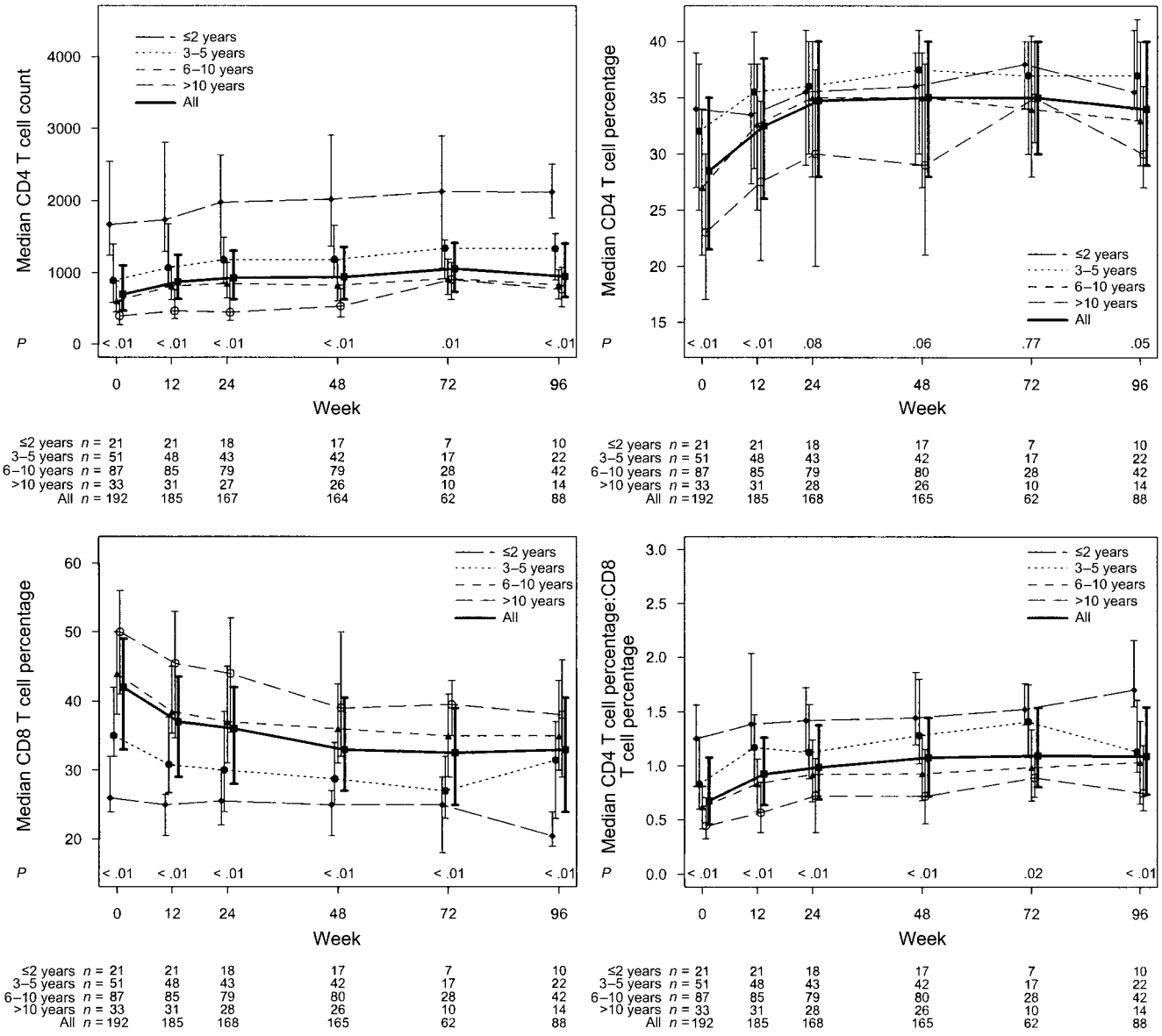

Figure 1. Changes in lymphocyte subset parameters and $CD4^+$ T cell percentage:$CD8^+$ T cell percentage over time for all ages combined and by age group. Bars indicate the 25th and 75th percentiles. *P* values are for comparisons of age groups.

an evaluation. The particular marker combinations were selected for their reported utility in distinguishing functional attributes of T cell subsets. For example, CD38 and HLA-DR are important activation markers on $CD4^+$ and $CD8^+$ T cells. Expression of the CD45RA isoform alone or in combination with the important adhesion and homing molecule CD62L marks naive cytotoxic and helper T cells, respectively. Up-regulation of CD28 facilitates an important costimulatory signal for T cell receptor–mediated effector function and proliferative expansion of antigen-primed T cells. Finally, CD95 expression is a marker for activation, and, in some circumstances, its up-regulation increases the susceptibility of lymphocytes to Fas-mediated apoptosis, which is a primary mechanism involved in the regulation of lymphocyte subsets. Although both PACTG 338 and PACTG 377 involved the investigation, by use of 3-color flow-cytometric data, of a large number of immunological parameters (40 and 60, respectively) and a relatively large number of HIV-1–infected children (101 and 143, respectively), because of the natural variation in the identification process, the results of neither study should be considered to be definitive with respect to the identification of the key immunological parameters. However, because the 2 studies present the results of data from 2 separate groups of children, the extent to which they identify the same parameters provides important independent confirmation of the identification of the key ones.

Results from the study of immunological parameters in

Table 2. Changes in 3-color flow-cytometric parameters in children treated with highly active antiretroviral therapy.

Parameter	Baseline (n = 116–143)	Week 16 (n = 67–85)	Week 44 (n = 100–125)	P value for trend[a]	Measure of discrimination, %
$CD4^+$ T cell percentage					
$CD4^+CD45RA^-$	30 (22–40)	35 (24–45)	31 (24–39)	.038	49
$CD4^+CD45RA^-$[b]	8 (6–11)	10 (7–14)	11 (8–13)	<.0001	26
$CD4^+CD28^+$	98 (96–99)	98 (96–99)	98 (96–99)	.33	61
$CD4^+CD95^+$	35 (25–46)	37 (27–49)	32 (26–45)	.55	49
$CD4^+CD62L^+CD45RA^+$	68 (56–76)	63 (52–73)	67 (60–74)	.18	50
$CD4^+CD62L^+CD45RA^+$[b]	19 (12–25)	21 (14–26)	24 (18–30)	<.0001	32
$CD4^+CD38^+$HLA-DR^+	8 (5–13)	6 (4–10)	5 (4–8)	<.0001	29
$CD4^+CD95^+CD28^-$	1 (0–2)	1 (1–2)	1 (0–2)	.57	55
$CD4^+CD95^+CD28^+$	34 (25–45)	34 (26–45)	30 (25–42)	.60	49
$CD4^+CD95^-CD28^+$	63 (52–73)	62 (50–72)	66 (53–73)	.29	49
$CD8^+$ T cell percentage					
$CD8^+CD45RA^-$	38 (27–50)	33 (22–43)	31 (21–41)	<.0001	33
$CD8^+CD45RA^-$[b]	15 (10–24)	11 (7–17)	10 (5–15)	<.0001	26
$CD8^+CD28^+$	35 (26–48)	46 (36–56)	49 (37–64)	<.0001	23
$CD8^+CD95^+$	59 (48–71)	54 (41–69)	54 (35–68)	.0007	30
$CD8^+CD38^+$	80 (66–90)	71 (55–82)	74 (64–82)	.011	46
$CD8^+$HLA-DR^+	47 (33–59)	36 (29–47)	32 (20–42)	<.0001	24
$CD8^+CD62L^+CD45RA^+$	38 (28–50)	45 (35–54)	50 (40–61)	<.0001	22
$CD8^+CD62L^+CD45RA^+$[b]	16 (12–19)	16 (14–20)	17 (13–20)	.10	42
$CD8^+CD62L^-CD45RA^+$	20 (12–32)	21 (14–29)	17 (10–23)	<.0001	42
$CD8^+CD38^+$HLA-DR^+	36 (25–47)	25 (14–34)	21 (14–32)	<.0001	18
$CD8^+CD38^-$HLA-DR^+	6 (2–15)	8 (6–16)	7 (4–11)	.32	46
$CD8^+CD95^+CD28^-$	45 (31–55)	35 (22–50)	31 (18–47)	<.0001	23
$CD8^+CD95^+CD28^+$	15 (10–19)	16 (11–22)	16 (11–23)	.036	34
$CD8^+CD95^-CD28^+$	19 (11–30)	28 (16–38)	30 (20–43)	<.0001	23
$CD4^+$ T cell count, cells/μL					
$CD4^+CD45RA^-$	197 (141–282)	256 (179–380)	307 (230–390)	<.0001	17
$CD4^+CD28^+$	637 (429–1002)	788 (499–1087)	947 (658–1302)	<.0001	30
$CD4^+CD95^+$	234 (163–333)	297 (204–413)	334 (244–423)	<.0001	24
$CD4^+CD62L^+CD45RA^+$	445 (239–755)	528 (314–754)	623 (415–935)	.0043	37
$CD4^+CD38^+$HLA-DR^+	51 (32–79)	49 (34–75)	57 (37–81)	.14	48
$CD4^+CD95^+CD28^-$	5 (0–14)	8 (5–21)	8 (0–17)	.67	40
$CD4^+CD95^+CD28^+$	220 (153–320)	280 (191–401)	323 (232–415)	<.0001	24
$CD4^+CD95^-CD28^+$	425 (226–698)	493 (286–744)	582 (378–910)	.0004	34
$CD8^+$ T cell count, cells/μL					
$CD8^+CD45RA^-$	389 (253–545)	315 (193–450)	279 (153–456)	<.0001	30
$CD8^+CD28^+$	373 (222–546)	410 (274–569)	431 (313–636)	.096	41
$CD8^+CD95^+$	580 (357–922)	499 (321–754)	466 (249–693)	.0002	36
$CD8^+CD38^+$	814 (548–1239)	672 (419–983)	713 (530–885)	.0003	47
$CD8^+$HLA-DR^+	460 (288–723)	343 (223–568)	282 (171–475)	<.0001	24
$CD8^+CD62L^+CD45RA^+$	388 (229–537)	409 (306–570)	437 (318–639)	.11	38
$CD8^+CD62L^-CD45RA^+$	195 (106–346)	189 (105–325)	159 (85–231)	<.0001	42
$CD8^+CD38^+$HLA-DR^+	337 (228–605)	227 (119–378)	221 (117–305)	<.0001	24
$CD8^+CD38^-$HLA-DR^+	62 (28–152)	84 (47–173)	67 (34–118)	.17	51
$CD8^+CD95^+CD28^-$	432 (247–721)	327 (158–543)	292 (135–523)	<.0001	32
$CD8^+CD95^+CD28^+$	138 (88–221)	145 (106–236)	153 (103–227)	.73	48
$CD8^+CD95^-CD28^+$	205 (110–309)	234 (137–369)	252 (160–408)	.056	36

NOTE. Data are medians (25th–75th percentiles) of anchor marker ($CD4^+$ or $CD8^+$ T cell) values, unless otherwise indicated.

[a] Level of significance of the time parameter in a mixed-effects model was used to assess the change in values over time.

[b] Data are calculated percentages (25th–75th percentiles) of total lymphocytes.

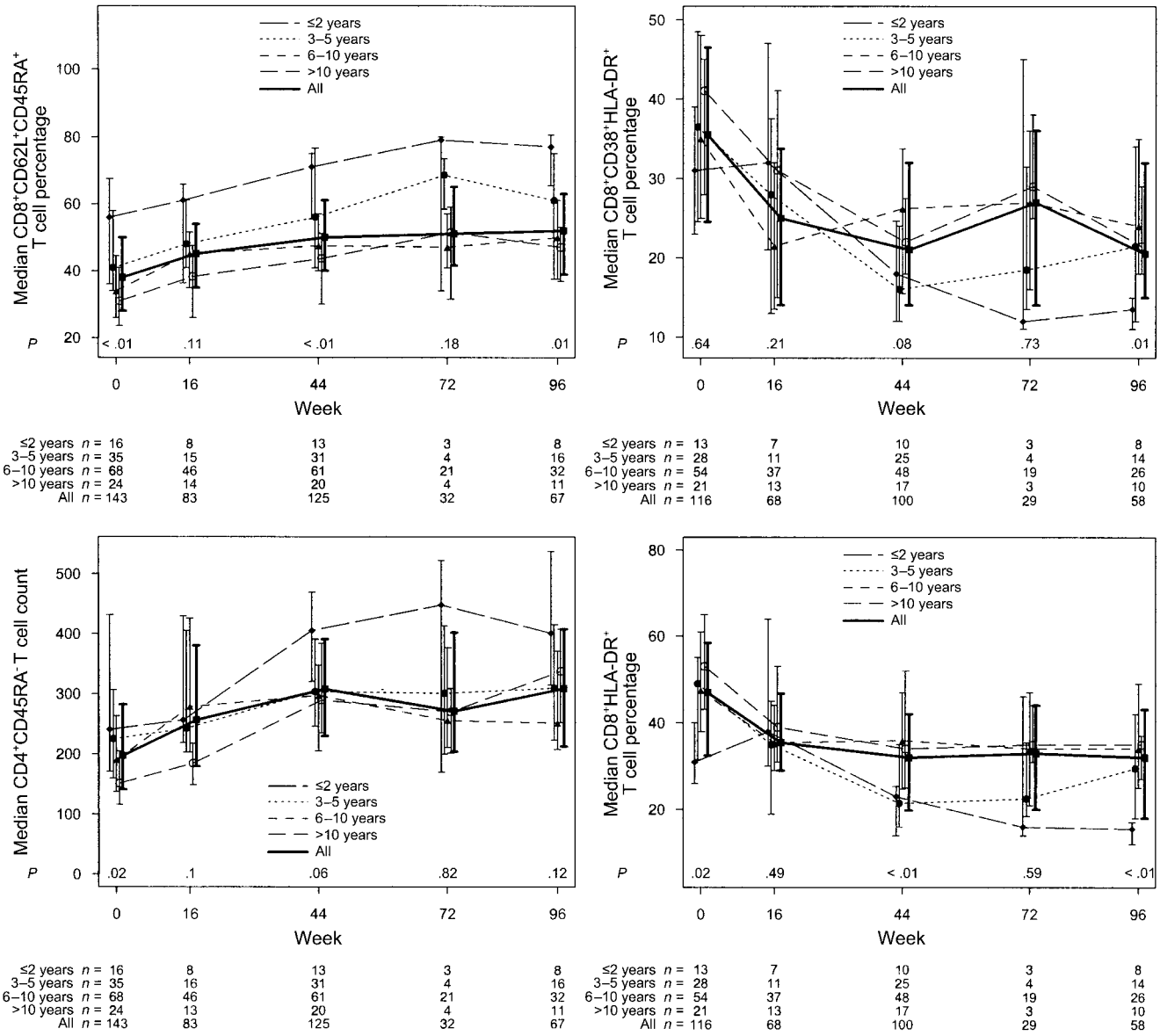

Figure 2. Changes in major maturation and activation markers on $CD4^+$ and $CD8^+$ T cells over time for all ages combined and by age group. Bars indicate the 25th and 75th percentiles. *P* values are for comparisons of age groups.

PACTG 377 extended and corroborated those of PACTG 338. Two of the top 3 immunological parameters that had the greatest discrimination and significant trends in PACTG 338 were also 2 of the top 3 immunological parameters in PACTG 377. Thus, this finding provides independent confirmation that decreases in the $CD8^+$ T cell percentage and the $CD8^+CD38^+HLA\text{-}DR^+$ T cell percentage occur in children who have successful responses to HAART. The $CD8^+CD38^+HLA\text{-}DR^+$ T cell percentage decreased from a median of 36% at baseline to 21% at week 44, and the total percentage of $CD8^+HLA\text{-}DR^+$ T cells decreased from 47% to 32%. This decrease was not only due to the overall decrease in the total number of $CD8^+$ T cells but might be explained, in part, by a decrease in the number of circulating activated cytotoxic T cells with specificity to HIV antigens. This change is encouraging, because the presence of high levels of HLA-DR and CD38 on $CD8^+$ T cells has been associated with progression to AIDS [7, 8]. Although the clinical impact of a reduction in $CD8^+$ T cell activation markers has not yet been confirmed in large, long-term studies of children, the reductions observed in $CD8^+$ and $CD8^+HLA\text{-}DR^+CD38^+$ T cell percentages appear to be worthy of additional evaluation.

Both the range of values of immunological parameters and the extent of responses after the initiation of HAART depended on the age of the child (figures 1 and 2). For example, the median $CD8^+$ T cell percentages were ~50%, 44%, 35%, and 26% for the age groups >10 years, 6–10 years, 3–5 years, and

Table 3. Association between immunological parameters and viral load at week 48 of highly active antiretroviral therapy.

Parameter	First quarter	Second quarter	Third quarter	Fourth quarter	P^a
$CD8^+$ T cell percentage	≤27.0[b]	27.0[b]–33.0[c]	33.0[c]–40.5[d]	>40.5[d]	<.0001
HIV-1 RNA load, ≤400 copies/mL	83 (33/40)	63 (24/38)	46 (21/46)	30 (12/40)	
HIV-1 RNA load, 401–<10,000 copies/mL	10 (4/40)	32 (12/38)	26 (12/46)	33 (13/40)	
HIV-1 RNA load, ≥10,000 copies/mL	8 (3/40)	5 (2/38)	28 (13/46)	38 (15/40)	
$CD8^+CD38^+HLA\text{-}DR^+$ T cell percentage	≤14.0[b]	14.0[b]–21.0[c]	21.0[c]–32.0[d]	>32.0[d]	<.0001
HIV-1 RNA load, ≤400 copies/mL	78 (21/27)	76 (19/25)	54 (13/24)	22 (5/23)	
HIV-1 RNA load, 401–<10,000 copies/mL	22 (6/27)	16 (4/25)	25 (6/24)	39 (9/23)	
HIV-1 RNA load, ≥10,000 copies/mL	0 (0/27)	8 (2/25)	21 (5/24)	39 (9/23)	
$CD8^+CD62L^+CD45RA^+$ T cell percentage	<40.0[b]	40.0[b]–50.3[c]	50.3[c]–61.2[d]	>61.2[d]	<.0001
HIV-1 RNA load, ≤400 copies/mL	37 (13/35)	59 (16/27)	71 (22/31)	81 (25/31)	
HIV-1 RNA load, 401–<10,000 copies/mL	31 (11/35)	22 (6/27)	19 (6/31)	19 (6/31)	
HIV-1 RNA load, ≥10,000 copies/mL	31 (11/35)	19 (5/27)	10 (3/31)	0 (0/31)	
$CD4^+$ T cell percentage	≤28.0[b]	28.0[b]–35.0[c]	35.0[c]–40.0[d]	>40.0[d]	<.0001
HIV-1 RNA load, ≤400 copies/mL	28 (11/39)	53 (20/38)	59 (26/44)	77 (33/43)	
HIV-1 RNA load, 401–<10,000 copies/mL	26 (10/39)	29 (11/38)	30 (13/44)	16 (7/43)	
HIV-1 RNA load, ≥10,000 copies/mL	46 (18/39)	18 (7/38)	11 (5/44)	7 (3/43)	

NOTE. Data are % (no./total no. of patients), unless otherwise indicated.

[a] Wilcoxon exact *P* value.
[b] First quartile (25th percentile).
[c] Second quartile (median).
[d] Third quartile (75th percentile)

≤2 years, respectively, at baseline. Although there was a >10% decrease in the median $CD8^+$ T cell percentage by week 48 for those >10 years old, there was little, if any, decrease by week 48 for those ≤2 years old. Although this result suggests an age-associated response differential, it may have been due to the greater potential for a decrease in these values in the older age group, or it may be associated with the fact that the children in this study had stable HIV disease and that long-term stable disease may be more treatment sensitive than is short-term stable disease. It also should be noted that some parameters, such as $CD4^+$ T cell count and $CD8^+$ T cell percentage, change substantially as children age. Such age-dependent changes may dampen or enhance observed changes in parameters over time if children are studied over long periods.

There was a clear association between immunological parameters at week 48 and HIV-1 RNA load at week 48 (table 3). For example, 83% of children with a $CD8^+$ T cell percentage <27% (the 25th percentile) had an undetectable HIV-1 RNA load (≤400 copies/mL), compared with 30% of children with a $CD8^+$ T cell percentage >40.5% (the 75th percentile). Although it is not possible to ascribe a causative association between a change in an immunological parameter and HIV-1 RNA load, it may be a beneficial strategy to aim to induce both undetectable levels of HIV-1 RNA and a desirable change in an immunological parameter, such as a $CD8^+$ T cell percentage <27%. Furthermore, tracking early trends in immunological parameters might serve to identify individuals who are likely to continue to respond to HAART or have virologic failure.

Our findings have implications for the development of future strategies for the treatment of HIV-1–infected children. We identified the immunological parameters that were the most sensitive to HAART and showed that it could move the values of these parameters halfway to two-thirds of the way toward those in healthy, uninfected children. A significant body of data from both children and adults suggests that changes in both $CD4^+$ and $CD8^+$ T cell compartments result from a complex interaction between new thymic output and activation-induced expansion and apoptosis [18–22]. The net affect on a particular marker combination in any individual appears to be related to many factors, including age, the degree of immunological damage before HAART, and the extent and duration of virologic control.

Although HAART is effective in reducing viral load, considerable additional therapeutic progress is needed before HIV-1–infected children's immune systems can be restored so that their immunological parameters are at the levels found in healthy children. Future studies to identify improvement in cell-mediated as well as humoral immune function should focus on HIV-1–specific immunity, general immune recognition, and memory for other microbial antigens. Therapeutic strategies that include not only suppression of viral load but also optimization of immunological parameters need to be developed for HIV-1–infected children.

Table 4. Comparison of key immunological parameters in children with HIV-1 infection with those in healthy children.

	2–6 years old				6–12 years old			
Parameter	HIV+ baseline[a]	HIV+ HAART[b]	Healthy children[c]	HAART return[d]	HIV+ baseline[a]	HIV+ HAART[b]	Healthy children[c]	HAART return[d]
$CD4^+$ T cell percentage	29 (16–42)	35 (24–45)	38 (28–47)	0.67	27 (18–40)	35 (23–45)	37 (31–47)	0.80
$CD8^+$ T cell percentage	41 (26–50)	32 (22–43)	23 (16–30)	0.50	45 (32–58)	37 (25–51)	25 (18–35)	0.40
$CD8^+CD62L^+CD45RA^+$ T cell percentage	40 (22–63)	56 (34–81)	64 (42–81)	0.67	33 (20–50)	48 (29–63)	58 (39–73)	0.60
$CD8^+CD38^+$HLA-DR^+ T cell percentage	35 (19–59)	21 (9–38)	13 (5–29)	0.64	33 (19–56)	21 (12–42)	9 (2–20)	0.50
$CD8^+$HLA-DR^+ T cell percentage	50 (24–70)	30 (14–45)	16 (7–37)	0.59	48 (23–68)	34 (17–58)	12 (6–29)	0.39

NOTE. HAART, highly active antiretroviral therapy; HIV+, children with HIV-1 infection.

[a] Data are medians (10th–90th percentiles) of children with HIV-1 infection at baseline.

[b] Data are medians (10th–90th percentiles) of children with HIV-1 infection at week 44 of HAART.

[c] Data are medians (10th–90th percentiles) of healthy children with no HIV infection (see Shearer et al. [31]).

[d] Fraction of the difference between baseline values in children with HIV-1 infection and values in healthy children that was restored at week 44 of HAART, calculated as (HIV+ HAART value − HIV+ baseline value) / (value in healthy children − HIV+ baseline value).

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