Comparison of CD8+ T-cell subsets in HIV-infected rapid progressor children versus non–rapid progressor children

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Background: CD8+ T-cell subsets have not been adequately described in HIV-infected (HIV+) children classified with respect to disease progression as rapid-progressors (RPs) and non–rapid progressors (non-RPs).
Objective: The purpose of this investigation was to determine the distribution of CD8+ T-cell subsets in HIV+ children and correlate the findings with degree of immunosuppression and HIV viral burden.
Methods: By means of 3-color flow cytometry, percentages of CD38+DR+, CD28+, and CD57+ CD8+ T-cell subsets were examined in RP (n = 15) and non-RP (n = 36) HIV+ children and in HIV-exposed but uninfected (n = 11) and HIV-unexposed (n = 8) children. The CD8+ T-cell subsets were correlated with mean CD4+ T-cell percentages and HIV RNA levels. Analysis of covariance was used for group comparisons for the control of the covariate of age.
Results: The HIV-exposed and HIV-unexposed controls were not different from each other in CD8+ T-cell subset percentages, except that the DR−CD38+CD8+ T-cell percentages were higher in the exposed controls than in the unexposed controls. RPs had a higher mean percentage of DR+CD38+CD8+ T cells than non-RPs and both control groups, and RPs had higher viremia than non-RPs. CD38+CD8+ T-cell percentages did not correlate with viral burden as it has been seen to do in HIV+ adults. Percentages of CD28+CD8+ T cells were lower in HIV-infected children than in controls. There was a positive correlation of percentage of CD28+CD57−CD8+ T cells with CD4+ T-cell percentages in each HIV-infected group.
Conclusion: CD8+ T cells become activated (dual expression of DR and CD38) and lose CD28, some acquiring CD57, in relation to rapidity of disease progression in pediatric HIV infection. (J Allergy Clin Immunol 2001;108:258-64.)

Key words: *Pediatric HIV-1 infection, CD4+ T cells, CD8+ T cells, CD8+ T-cell subsets, HIV disease progression*

Abbreviations used
Cy-5: Cyanine-5
CTL: Cytotoxic T lymphocyte
HAART: Highly active antiretroviral therapy
HIV+: HIV-infected
LIP: Lymphoid interstitial pneumonitis
non-RP: Non–rapid progressor
NRTI: Nucleoside analog reverse transcriptase inhibitor
PE: Phycoerythrin
RP: Rapid progressor

Before the use of highly active antiretroviral therapy (HAART), distinct patterns of disease progression were seen in perinatally infected children.[1-3] Some infants became rapid progressors (RPs), whereas others maintained immunologic control of their infection and had years of slow decline before the development of AIDS.

Cytotoxic T lymphocyte (CTL) responses, mediated by HIV-specific CD8+ T cells, are critical in initial containment of viremia in HIV-infected (HIV+) adults.[4-6] This function is lost late in infection.[7,8] Adult nonprogressors, in comparison with progressors, have been shown to have more effective CD8+ T-cell function.[9-12] HIV+ children who showed more favorable patterns of clonal expansion in the CD8+ T-cell receptor Vβ repertoire had less loss of CD4+ T cells and less CD8+ T-cell activation.[13]

The virus-induced cytotoxic response is associated with an increase in CD8+ T cells with high levels of activation antigens such as CD38 and HLA-DR.[14,15] High CD38 expression in the early phase of HIV infection in adults has strong predictive value for disease progression.[16,17] Increases in proportions of CD8+ T cells expressing CD38 and expressing HLA-DR have been described in HIV+ children as well.[18-20] However, studies evaluating the use of CD38 as a marker for progression of disease in children have shown conflicting results.[20,21]

Engagement of the CD28 molecule on CD8+ T lymphocytes in HIV+ individuals during activation has been reported to increase CD8+ cell proliferation and differentiation and prevent apoptosis.[22-24] Stimulation via the CD28 molecule enhances and stabilizes IL-2 production and IL-2R expression on the cell surface.[25] Loss of CD28 expression has been observed on CD8+ T cells in HIV+ adults,[24] and loss of responsiveness to anti-CD28 stimulation might be associated with progression to AIDS in adults.[26] HIV+ children have been shown to have lower-than-normal CD28 expression on CD8+ T cells as well,[27,28] and the loss of CD28 correlates with loss of CD4+ T cells.[28]

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TABLE I. Clinical characteristics of children

	Control patients*		HIV-infected patients*		
	Exp	Unexp	RP	Non-RP	Differences among groups
Total number	11	8	15	36	—
Age: mean ± SD (y)	1.7 ± 0.7	6.4 ± 4.3	3.1 ± 2.7	6.7 ± 3.5	$P = .0001$ (Non-RP value is greater than Exp and RP values; Unexp value is greater than Exp value)
CD4%: age-adjusted mean ± SD	31.7 ± 3.9	37.2 ± 8.3	18.1 ± 12.8	27.9 ± 2.1	$P = .0001$ (RP value is lower than Exp value [$P = .002$]; RP value is lower than Non-RP value [$P = .003$])
Viral load: mean ± SD	—	—	67,511 ± 98,464 (n = 11)	11,967 ± 12,420 (n = 32)	$P = .04$
Antiretroviral therapy	—	—	—	2 (none)	—
Single NRTI	—	—	8	26	—
2 NRTI	—	—	7	6	—
HAART	—	—	0	1	—

Exp, Exposed to HIV; *Unexp,* not exposed to HIV; *RP,* rapid progressors; *Non-RP,* non–rapid progressors; *NRTI,* nucleoside analog reverse transcriptase inhibitor; *HAART,* highly active antiretroviral therapy.
*HIV disease progression groups (RP and non-RP) are defined in the text.

$CD8^+$ T-cell subsets have been associated with specific functional properties. Cytolytic function of $CD8^+$ cells has been linked to cells with a $CD28^-$ and $CD57^+$ phenotype.[29,30] $CD28^-CD57^+CD8^+$ T lymphocytes have been shown to contain clonal expansions of virus-specific memory CTLs in subjects chronically infected with viruses, including HIV and cytomegaloviruses.[30]

We began investigating $CD8^+$ T-lymphocyte subsets in children before the HAART era and subsequently studied them in children whose stable clinical courses did not merit HAART or whose parents declined HAART. Consequently, our patient cohorts were grouped by pattern of disease progression to determine the relationship between $CD4^+$ T-cell numbers, HIV RNA, and the proportions of $CD28^+$, $CD38^+DR^+$ (activated), and clonally driven ($CD57^+$) $CD8^+$ T cells during the natural course of HIV infection.

METHODS

Patient cohort

Fifty-one children with vertically acquired HIV infection and 19 control children who received care at the Texas Children's Hospital Allergy and Immunology Clinic were enrolled. Some of the control children were HIV-exposed but HIV-uninfected (n = 11); others were HIV-unexposed (n = 8). The protocol was reviewed and approved by the Human-Subjects Research Committee of Baylor College of Medicine, and the parents or guardians of all children gave written informed consent for the study. A 1-time blood draw was performed, and a short questionnaire detailing medications and clinical wellness at the time of the blood draw was administered.

Definition of HIV disease progression

We classified subjects into 2 groups on the basis of the pattern of HIV disease progression. The RPs were those subjects who had previously had an AIDS-defining clinical condition other than lymphoid interstitial pneumonitis/pulmonary lymphoid hyperplasia or who had reached Centers for Disease Control–defined category 3 immune suppression before their second birthday. The comparison group of HIV^+ subjects (non–rapid progressors [non-RPs]) were 36 children who did not meet the criteria of rapid disease progression; they are described in Table I.

Three-color immunophenotyping

Immunophenotyping was performed according to published procedures.[31] Briefly, heparinized peripheral blood was ficoll-separated on Histopaque gradients (Sigma Chemical Company, St Louis, Mo) designed for small blood volumes. The cells were washed twice and resuspended to 2×10^7/mL in PBS containing 2% FBS for staining. One million cells were stained on ice for 20 minutes with 2- and 3-color combinations of the following antibodies: CD45-FITC, CD14-phycoerythrin (PE), CD3-FITC, CD19-PE, CD4-PE, CD8-PE, CD8-biotin with avidin cyanine-5 (Cy-5), CD38-PE, CD57-FITC, CD28-PE, and DR-FITC (Becton Dickinson Immunocytometry Systems, San Jose, Calif). Each sample tube was washed and fixed in 1% paraformaldehyde. The sample analysis was performed on an EPICS 753 (Coulter Corporation, Hialeah, Fla) with Cicero acquisition (Cytomation, Inc, Ft Collins, Colo); this was equipped with an argon laser (488 nm) at 50 mW and a HeNe laser (635 nm) at 32 mW to excite FITC/PE and Cy-5, respectively. Emission filters included a 457-502 argon laser blocker, a 550 dichroic long-pass filter to bend the FITC emission to a 525 band-pass, and a 615 dichroic long-pass filter to bend the PE emission to a 575 band-pass and send the Cy-5 emission to a 680 band-pass. All color combinations were corrected for spectral overlap through use of single- and double-color controls. The maximum compensation necessary was 40% on the Cy-5 signal to subtract PE overlap. The CD45/CD14 combination was used as a quality-control tube. The gated cell population had to be at least 85% $CD45^+$ for inclusion of the specimen in the analysis; most cells were greater than 98% $CD45^+CD14^-$. In addition, samples that had high numbers of B cells or natural killer cells were not included in the study when $CD3^+$ lymphocytes were <40%.

Viremia measurements

Plasma samples anticoagulated in acid citrate dextrose solution were analyzed for HIV RNA copy number through use of the Diagene assay, as previously reported by the Baylor College of Medicine Center for AIDS Research Virology Laboratory.[32]

TABLE II. Characteristics of CD8+ T cells in HIV-infected children: CD38 and DR subsets

	Percent (mean)*	SE	*P* value†
$CD8^+DR^-CD38^-$			
RP	17.7	3.2	—
Non-RP	17.8	2.0	—
Unexp control	25.8	4.1	$P = .2$
Exp control	(15.7)	(3.5)	($P = .9$)
$CD8^+DR^+CD38^-$			
RP	5.4	1.5	—
Non-RP	4.0	0.9	—
Unexp control	3.9	1.9	$P = .7$
Exp control	(1.8)	(1.6)	($P = .2$)
$CD8^+DR^+CD38^+$			All values differ; $P = .001$
RP	33.5	3.6	—
Non-RP	21.8	2.2	—
Unexp control	9.5	4.6	—
Exp control	(8.9)	(4.1)	($P = .0001$)
$CD8^+DR^-CD38^+$			Unexp and Non-RP values are higher than RP values; $P = .04$
RP	43.5	4.6	—
Non-RP	56.6	2.8	—
Unexp control	60.8	5.8	—
Exp control	(73.7)	(5.1)	(All values differ; $P = .0001$)

Exp, Exposed to HIV; *Unexp,* not exposed to HIV; *RP,* rapid progressors; *Non-RP,* non–rapid progressors.
*The mean percentages have been age-adjusted. The HIV-exposed control percentages are shown in parentheses. The age-adjusted mean percentages of the RP and non-RP HIV-infected groups used for comparison with the HIV-unexposed control group are shown in this table. The age-adjusted mean percentages of the RP and non-RP HIV-infected groups used for comparison with the HIV-exposed control group were slightly different from those shown in the table because of age differences between the HIV-exposed and HIV-unexposed controls.
†HIV-infected study groups compared with each control group. HIV-exposed control group comparison *P* values are shown in parentheses.

Statistical analysis

Analysis of variance followed by Bonferroni testing was used to compare continuous variables among the subject groups. These analyses were followed by Pearson product-moment correlation analysis to investigate the relationship of CD8+ T cells with age, CD4+ T cells, and viremia. Analysis of covariance was performed for all patient groups in comparison with the control groups to examine differences in CD8+ T cells and CD4+ T cells while age differences were controlled for.

RESULTS

Clinical characteristics of study cohort

The subjects' clinical characteristics, including ages, CD4+ T-cell percentages, viral RNA levels, and antiretroviral treatment, are presented in Table I. The controls had a mean age of 3.7 years; they ranged in age from 0.6 to 15.8 years. The HIV-exposed controls (n = 11) were similar to the RP group in age, and the HIV-unexposed controls (n = 8) were similar to the non-RP HIV+ group in age. Age differences being controlled for, the CD8+ T-cell subsets differed between control groups only for the $DR^-CD38^+CD8^+$ and the $CD57^+CD28^+CD8^+$ subsets. The mean $DR^-CD38^+CD8^+$ value was higher in the HIV-exposed control group (80.6 ± 4.0) than in the HIV-unexposed control group (62.2 ± 4.8; $P = .02$). The $CD57^+CD28^+CD8^+$ subset, which was present in very low percentages, was higher in the HIV-unexposed group (2.0 ± 0.5 for HIV-unexposed controls versus 0.4 ± 0.4 for HIV-exposed controls; $P = .04$). The level of viremia was significantly higher for the RP group than for the non-RP group. The mean percentages of CD4+ T cells were significantly lower in the patient groups than in the control groups. Sixty-seven percent of the HIV-infected subjects were receiving only single-agent nucleoside analog reverse transcriptase inhibitor (NRTI) therapy; 25% of the subjects received dual NRTI therapy.

CD8+ T lymphocytes: CD38 and DR subsets

CD38 is expressed on a large percentage of CD8+ T cells from young children and is probably on newly released thymic emigrants. The $DR^-CD38^+CD8^+$ subset thus might represent naive CD8+ T cells in children.[33] Expected percentages of this subset include higher percentages in the younger subjects and lower percentages in the older subjects, inasmuch as CD38 expression is normally lost over time. Therefore, all analyses were performed with the analysis of covariance for the control of the covariate of age. Interestingly, the HIV-exposed control group had a higher percentage of the $DR^-CD38^+CD8^+$ subset than did the HIV-unexposed control group. The percentages of $CD38^+CD8^+$ T cells did not differ between the infected and uninfected groups. However, the RP group had a higher percentage of the $DR^+CD38^+CD8^+$ subset than the non-RP HIV+ group or either of the control groups (Table II). The non-RP HIV+ group also had a higher percentage of the $DR^+CD38^+CD8^+$ subset than either of the control groups.

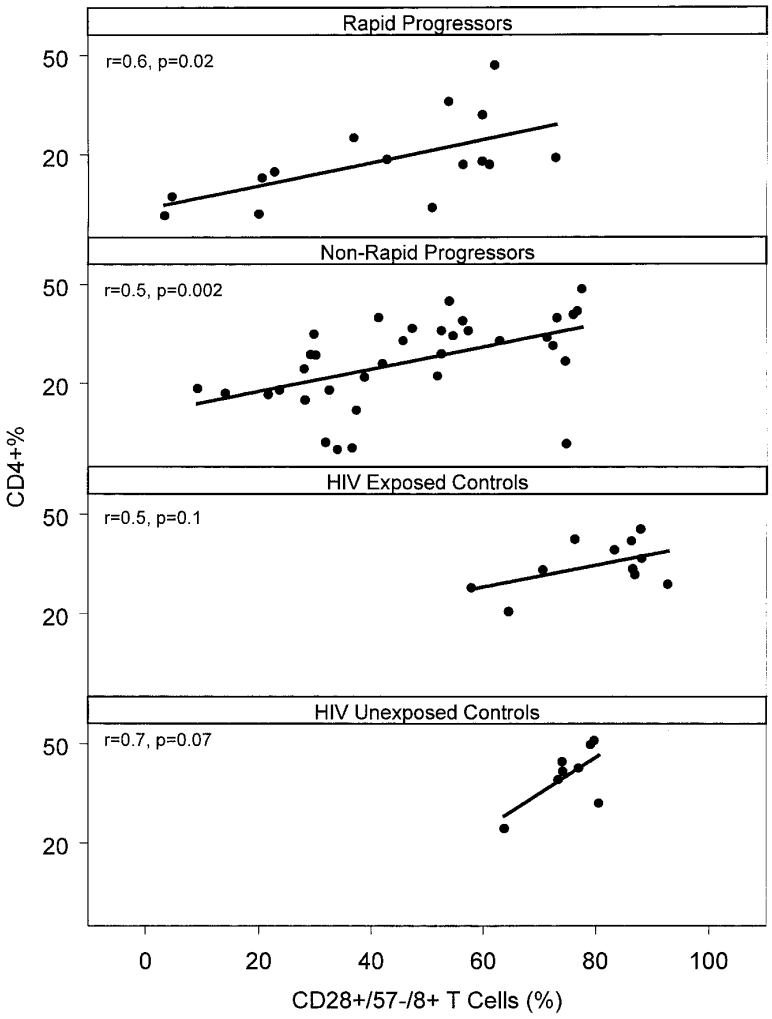

FIG 1. Increases in percentage of $CD4^+$ T cells correlate with increases in percentage of the $CD28^+CD57^-CD8^+$ T-lymphocyte subset for each group of HIV^+ subjects but not for control subjects.

$CD8^+$ T lymphocytes: CD28 and CD57 subsets

Both HIV^+ groups had significantly lower percentages of $CD28^+CD8^+$ T cells than either of the control groups (Table III). For each group of HIV^+ subjects, a higher percentage of $CD28^+CD8^+$ T cells positively correlated with $CD4^+$ T-cell percentage (Fig 1). This correlation was not significant in the controls. The RP group had a higher percentage of $CD57^-CD28^-CD8^+$ T cells than the non-RP HIV^+ group or either of the control groups. For the RP group, $\log_{10}$ virus load correlated with a higher percentage of $CD28^+CD8^+$ T cells (Fig 2).

DISCUSSION

CD38 is thought of as both an activation marker and a marker of immaturity, the former because of its expression on activated T and B lymphoblasts and the latter because of its presence on bone marrow precursors.[33,34] Like others, we have found that $DR^+CD38^+CD8^+$ (activated) T-cell percentages are higher in HIV-infected children than in controls. Our data also show that overall percentages of $CD38^+CD8^+$ T lymphocytes were not higher in these HIV^+ children.

The expression of CD38 on $CD8^+$ T lymphocytes has been shown to be a predictor of disease progression in adults.[35,36] In this study, the rapidly progressing group had the most $DR^+CD38^+CD8^+$ T cells and the highest level of viremia. Other investigators have found that level of viremia correlates with numbers of $CD38^+CD8^+$ T cells.[35,37] In our study, however, both for the HIV^+ group as a whole and for the subgroups, the percentages of $CD38^+CD8^+$ T cells did not correlate with level of viremia. We conclude that because $CD38^+CD8^+$ T-cell percentages are normally higher for infants and children than for adults and because in children CD38 is a marker for naive $CD8^+$ T cells, the percentage of cells expressing CD38 without DR is not predictive of disease progression. Conversely, the $DR^+CD38^+CD8^+$ T-cell subset was more elevated in RPs; therefore, percentages of the $DR^+CD38^+CD8^+$ T-cell sub-

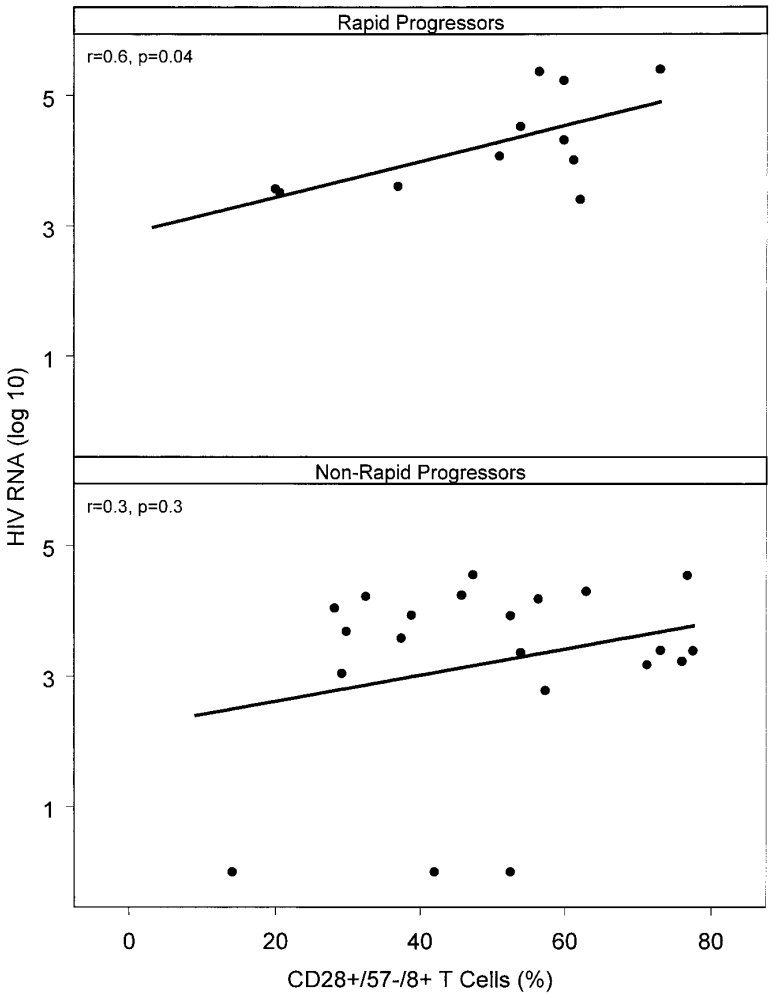

FIG 2. Increases in $\log_{10}$ viral load correlate with increases in percentage of the $CD28^+CD57^-CD8^+$ T-lymphocyte subset for the RP group only.

set might be useful as a prognostic marker. Longitudinal study is needed to confirm this finding.

HIV-exposed control subjects had a higher percentage of the $DR^+CD38^+CD8^+$ T-cell subset than HIV-unexposed controls, suggesting that $CD8^+$ T cells are more activated in uninfected HIV-exposed infants than in normal infants. Other studies have shown more activated $CD8^+$ T cells in HIV-exposed infants than in HIV-unexposed infants.[38,39] This might be related to studies showing that some uninfected HIV-exposed children have evidence of HIV-specific immune responses.[39]

In this study, all HIV^+ subjects had lower percentages of $CD28^+CD8^+$ T cells than controls. Other investigators have reported a disease stage–dependent loss of CD28 expression on T lymphocytes in HIV-infected children.[27] McCloskey et al[40] recently found that activated lymphocytes with a memory phenotype lacking CD28 expression were especially prone to apoptosis in children. In our subjects, only 1 of whom was treated with HAART, the percentage of $CD4^+$ T lymphocytes correlated positively with the percentage of $CD28^+CD8^+$ T lymphocytes.

All $CD8^+$ T lymphocytes express CD28 at birth, and the expression of CD28 is lost over time. This loss is accelerated in HIV infection. Multiple investigators have shown that the loss of CD28 on $CD8^+$ T lymphocytes indicates functional differentiation to cytotoxic memory cells.[41,42] All HIV^+ children had significantly higher percentages of $CD28^-CD8^+$ T lymphocytes than controls, and this loss of CD28 was most marked in the RP group. This change might be due to the cytotoxic T-cell response in these children. We also found a trend toward greater percentages of $CD57^+CD28^-CD8^+$ T lymphocytes in the non-RP HIV^+ group than in the controls. $CD57^+CD28^-CD8^+$ T lymphocytes have been shown to contain clonal expansions of virus-specific memory CTLs in subjects chronically infected with viruses, including HIV and cytomegaloviruses.[30,42] Larger study groups might more clearly show more $CD57^+CD28^-CD8^+$ T lymphocytes in HIV^+ children.

The RP group has more CD4 loss for age, more viremia, and more activated $DR^+CD38^+CD8^+$ T lymphocytes than the non-RP group. We also found that more viremia correlated with higher $CD28^+CD8^+$ T-

TABLE III. Characteristics of CD8+ T cells in HIV-infected children: CD28 and CD57 subsets

	Percent (mean)*	SE	*P* value†
$CD8^+CD57^-CD28^-$			All values differ; $P = .001$
RP	36.9	3.7	—
Non-RP	26.4	2.3	—
Unexp control	13.4	4.8	—
Exp control	(15.0)	(4.4)	(RP values higher than Non-RP and Exp values; $P = .0004$)
$CD8^+CD57^+CD28^-$			
RP	22.8	4.4	—
Non-RP	23.3	2.7	—
Unexp control	8.6	5.6	$P = .06$
Exp control	(10.2)	(5.1)	($P = .1$)
$CD8^+CD57^+CD28^+$			
RP	2.4	1.0	—
Non-RP	3.0	0.6	—
Unexp control	1.8	1.3	$P = .7$
Exp control	(0.5)	(1.1)	($P = .2$)
$CD8^+CD57^-CD28^+$			Unexp values higher than RP and Non-RP values; $P = .0001$
RP	37.7	5.2	—
Non-RP	48.4	3.2	—
Unexp control	76.2	6.6	—
Exp control	(74.1)	(6.2)	(Exp values higher than RP and Non-RP values; $P = .001$)

Exp, Exposed to HIV; *Unexp,* not exposed to HIV; *RP,* rapid progressors; *Non-RP,* non–rapid progressors.
*The mean percentages have been age-adjusted. The HIV-exposed control percentages are shown in parentheses. The age-adjusted mean percentages of the RP and non-RP HIV-infected groups used for comparison with the HIV-unexposed control group are shown in this table. The age-adjusted mean percentages of the RP and non-RP HIV-infected groups used for comparison with the HIV-exposed control group were slightly different from those shown in the table because of age differences between the HIV-exposed and HIV-unexposed controls.
†HIV-infected study groups compared with each control group. HIV-exposed control group comparison *P* values are shown in parentheses.

lymphocyte percentages for the RP group alone. This correlation of viremia with CD28 expression has been described previously in adults and was felt to reflect more recently activated lymphocytes possibly recirculating from the lymph nodes, where $CD8^+$ T cells remained predominantly $CD28^+$ during HIV infection.[43,44] It is likely that some of the RPs failed to establish an effective CTL response, which would result in a loss of $CD28^+CD8^+$ T cells and then conversion to memory $CD28^-CD8^+$ T cells accompanied by reduced viremia.

In summary, our findings suggest that as HIV^+ children move through stages of HIV disease progression, their $CD8^+$ T cells acquire activation markers (dual expression of DR and CD38), lose CD28, and acquire CD57—all of these being indicative of sequential changes induced by HIV infection.

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