Characteristics of lymphocyte subsets in HIV-infected, long-term nonprogressor, and healthy Asian children through 12 years of age

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Background: There are limited data on the immune profiles of HIV-positive children compared with healthy controls, and no such data for Asian children.
Objectives: To immunophenotype HIV-positive Asian children, including long-term nonprogressors (LTNPs), compared with age-matched healthy controls.
Methods: We used flow cytometry to analyze 13 lymphocyte and monocyte subsets from 222 untreated, HIV-positive children with 15% to 24% $CD4^+$ T cells and no AIDS-related illnesses and 142 healthy children (controls). Data were compared among age categories. Profiles from LTNPs (n = 50), defined as children ≥8 years old with $CD4^+$ T-cell counts ≥350 cells/mm^3, were compared with data from age-matched non-LTNPs (n = 17) and controls (n = 53).
Results: Compared with controls, HIV-positive children had lower values (cell count per mm^3 and percent distribution) for T_H cells and higher values for cytotoxic T cells, with reductions in populations of naive T_H and cytotoxic T cells, B cells, and natural killer (NK) cells. HIV-positive children had high values for activated T_H and cytotoxic T cells. Compared with non-LTNPs, LTNPs had higher values of T_H and cytotoxic T cells, naive and memory T-cell subsets, and B and NK cells. Surprisingly, counts of activated T_H and cytotoxic T cells were also higher among LTNPs. LNTPs were more frequently male.
Conclusion: Untreated, HIV-infected Asian children have immune profiles that differ from those of controls, characterized by low values for T_H cells, naive T cells, B cells, and NK cells but high values for cytotoxic, activated T_H, and cytotoxic T cells. The higher values for activated T cells observed in LTNPs require confirmation in longitudinal studies. (J Allergy Clin Immunol 2010;126:1294-301.)

Key words: *HIV, children, lymphocyte, monocyte, phenotyping, long-term nonprogressors, antiretroviral therapy, Asia, disease progression, pediatric AIDS*

HIV-positive children are generally infected with HIV at birth, from their mothers; about one third progress to AIDS within the first year of life without antiretroviral therapy (ART),[1] and most become ill from HIV by age 5 years.[2] Less than 10% remain healthy by age 8 years without ART. They are called HIV long-term nonprogressors (LTNPs).[3]

Hallmarks of HIV infection are high turnover of $CD4^+$ T cells, activation of polyclonal B cells, and persistent immune activation.[4,5] Immune activation is characterized by expression of activation markers on T cells, which is associated with decreases in numbers of $CD4^+$ T cells, increases in HIV RNA, and progression of HIV disease. ART partially reverses these defects. $CD4^+$ T-cell regeneration after ART is better in children than adults because of children's actively functioning thymic glands.[6]

It is not clear why LTNPs can control HIV. Lessons can be learned from so-called *elite controllers*, who account for <1% of the HIV population and keep HIV at less than 50 copies/mL without ART.[7] Elite controllers have a robust, HIV-specific $CD8^+$ T-cell response (particularly against the HIV protein gag), preserve the naive T-cell population that mediates responses to new antigens, generate polyfunctional T cells, and have low-level immune

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Abbreviations used
ART: Antiretroviral therapy
CDC: Centers for Disease Control and Prevention
IQR: Interquartile range
LTNP: Long-term nonprogressor
NK: Natural killer
OR: Odds ratio
PREDICT: Pediatric Randomized to Early versus Deferred Initiation in Cambodia and Thailand

activation. Some also have mutations in the chemokine coreceptor CCR5, HLA haplotypes that can select immunogenic HIV epitopes, or infections with defective forms of HIV that replicate poorly.[8-11] These natural states of immune control of HIV are not seen in the general HIV-infected population, despite the success of ART.[12]

Several studies have reported low counts of $CD4^+$ T cells, high counts of $CD8^+$ T cells, and high counts of activated $CD8^+$ T cells in children with HIV[4,13]; characterizations of other cell subsets and comparisons with age-matched healthy controls have not been systematically performed. Studies in healthy controls in the United States and Africa have shown that lymphocyte counts, particularly of $CD4^+$ T cells, can be affected by age, sex, and ethnicity.[14,15]

This study is the first in Asia to evaluate lymphocyte and monocyte subsets in HIV-positive children and age-matched healthy controls. We also examined the immunologic profile of pediatric LTNPs compared with non-LTNPs and healthy controls. The results might be used to better characterize the immune profile of pediatric HIV and to understand the immunologic profile of LTNPs.

METHODS

We used data from first visits with 222 HIV-positive children from 7 sites in Thailand and 2 sites in Cambodia who enrolled in the Pediatric Randomized to Early versus Deferred Initiation in Cambodia and Thailand (PREDICT) study (http://www.clinicaltrials.gov/ct/show/NCT00234091). These children were 1 to 12 years old and never treated with ART except as part of prevention of mother-to-child HIV transmission, and had percentages of $CD4^+$ T cells in the moderate immune suppression range ($CD4^+$ T cells 15% to 24%) without severe symptoms of HIV infection (Centers for Disease Control and Prevention [CDC] class C). The age-matched healthy controls were enrolled at the Well-Child Care clinic at Chulalongkorn University hospital in Bangkok, one of the PREDICT study sites. Healthy controls were excluded for abnormal growth (below 3rd or above 97th percentile, according to the Thai growth chart), febrile illness, respiratory and other infections at screening, or medical illnesses that might result in abnormal immunity such as HIV infection or exposure and allergic conditions. Medical histories were provided for groups, which each underwent physical examinations and blood sample collection. HIV RNA levels were measured in blood samples from HIV-positive children. Our case definition for LTNP was ≥8 years in age with no indication for ART ($CD4^+$ T cells ≥ 350 cells/mm^3). Because age affects lymphocyte subsets, we chose children of similar ages as controls for LTNPs: the first group was HIV-positive children ≥8 years old with CD4 <350 cells/mm^3 (non-LTNPs); the second group was healthy children ≥8 years old. Each study was approved by national and local institutional review boards. All caregivers consented to the study, and healthy children ≥7 years old also gave assent.

Flow-cytometer set-up, gating, and marker placement in each laboratory were dictated by guidelines established by the National Institute of Allergy and Infectious Diseases—Division of AIDS and the advanced flow cytometry working group of the Pediatric AIDS Clinical Trials Group, in collaboration with the technical divisions of the manufacturers of the flow reagents.[16] All laboratories were approved by the National Institute of Allergy and Infectious Diseases—Division of AIDS and passed the annual quality assurance programs of the United Kingdom National External Quality Assessment Service and Mahidol University, Bangkok, Thailand. By convention, we use CD numbers only when necessary in the text and elsewhere when the presence and absence of markers is necessary. More details are available in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analyses

Analyses were performed with Stata 11 (College Station, Tex). Demographic and baseline data were described according to HIV status or as healthy controls. For descriptive analyses, median interquartile range (IQR) and percentage distribution were used; the *t* test and Mann-Whitney *U* test were used to compare differences between groups including distributions of T-cell subsets; the χ^2 test was performed for categorical variables. We classified children according to whether they were LTNPs and used Kruskal-Wallis tests or Mann Whitney *U* tests to make comparisons among LTNPs, non-LTNPs, and healthy controls. More details are provided in the Statistical analyses section of this article's Online Repository.

We compared counts and percentages of T-cell subsets between healthy controls and HIV-positive children by using regression models stratified by 4 age categories and adjusted for sex. Separate regressions were performed for each age group. Additional regression models were run with age expressed first as a categorical covariate, then as a continuous covariate adjusted for HIV status and sex. Counts and percentages of T-cell subsets were transformed by $\log_{10}$ or Box-Cox power transformations to distribute residual errors more normally. Logistic regression analysis assessed whether T-cell subset distributions predicted which children would be LTNPs. Odds ratios (ORs) and 95% CIs were reported relative to the LTNP group.

RESULTS

We enrolled 222 untreated HIV-positive children and 147 age-matched healthy controls (Table I). The HIV-positive group included Thai and Cambodian children, whereas the HIV-negative group included only Thai children. The median age was approximately 6.5 years; healthy controls were slightly older. Children with HIV were mainly mildly symptomatic (CDC class A). The median number of $CD4^+$ T cells was lower among HIV-positive children; the median amount of HIV RNA was 4.8 $\log_{10}$ copies/mL.

We compared counts and percentages of cell subsets between HIV-positive children and healthy controls in 4 age categories (<2-3 years, 4-5 years, 6-8 years, 9-12 years). Within these groups, sex was generally balanced between HIV-positive children and healthy controls (Table I). HIV-positive children had significantly lower total counts of T cells in the age group 9 to 12 years and lower total percentages of T cells across all age groups than healthy controls (Fig 1, *A*). Counts and percentages of T_H cells and naive and memory cell subsets differed significantly between HIV-infected children and healthy controls across all age groups. HIV-positive children had lower counts and percentages of T_H cells (Fig 1, *B*) and higher counts and percentages of cytotoxic T cells (Fig 1, *C*). Counts and percentages of B cells (Fig 1, *D*) and natural killer (NK) cells (Fig 1, *E*) were significantly lower in some age groups of HIV-positive children. The HIV-positive children had lower counts of naive T_H cells (see this article's Fig E1 in the Online Repository at www.jacionline.org) and memory T_H cells (see this article's Fig E2 in the Online Repository at www.jacionline.org) but higher counts and percentages of activated T_H cells (see this article's Fig E3 in the Online Repository at www.jacionline.org). There were lower counts and percentages

TABLE I. Demographics of HIV-positive and healthy children

Characteristic	Overall (n = 364)	HIV-positive (n = 222)	Healthy controls (n = 142)	*P* value
Mean age (y) (SD)	6.6 (2.9)	6.3 (2.8)	7.0 (2.9)	.03
Median age (y) (IQR)	6.5 (4.3-8.7)	6.4 (4.1-8.4)	6.8 (4.6-9.2)	.04
Age categories, n (%)				.12
<2-3 y	79 (21.7)	54 (24.3)	25 (17.6)	
4-5 y	74 (20.3)	47 (21.2)	27 (19.0)	
6-8 y	135 (37.1)	83 (37.4)	52 (36.6)	
9-12 y	76 (20.9)	38 (17.1)	38 (26.8)	
Males, n (%)				
<2-3 y	37 (46.84)	30 (55.56)	7 (28.00)	.01
4-5 y	32 (43.24)	22 (46.81)	10 (37.04)	.35
6-8 y	57 (42.22)	35 (42.17)	22 (42.31)	.94
9-12 y	22 (28.95)	8 (21.05)	14 (36.84)	.01
Sex, M:F, n (%)	148:216 (41:59)	95:127 (43:57)	53:89 (37:63)	.30
Nationality, n (%)				<.001
Thai	264 (72.5)	122 (55.0)	142 (100.0)	
Cambodian	100 (27.5)	100 (45.0)	0 (0.0)	
CDC class N:A:B (%)		1:62:37		
Median CD4 % (IQR)	24.5 (18.6-32.3)	20.2 (16.5-23.5)	34.8 (30.2-38.6)	<.001
Median CD4 count (cells/mm^3) (IQR)	835 (578-1130)	631 (434-887)	1099 (895-1452)	<.001
Median HIV RNA $\log_{10}$copies/mL (IQR)	—	4.8 (4.3-5.0)	—	—

F, Female; *M,* male; —, not applicable.

of naive cytotoxic T cells (see this article's Fig E4 in the Online Repository at www.jacionline.org) and higher counts and percentages of memory cytotoxic T cells (see this article's Fig E5 in the Online Repository at www.jacionline.org) and activated cytotoxic T cells (see this article's Fig E6 in the Online Repository at www.jacionline.org) in HIV-positive children compared with healthy controls. Numbers of activated and perivascular monocytes did not differ between HIV-positive children and healthy controls (data not shown).

In the regression analyses (with age as either categorical or continuous values), age had a significant impact on percentages and counts of T_H cells, memory T_H cells, B cells, NK cells, activated monocytes, and perivascular monocytes, but only affected cell counts for the other subsets. Analysis by sex did not affect cell subsets, except in a univariate analysis, girls had higher percentages of $CD4^+$ T cells (OR, 2.17; 95% CI, 0.27-4.08; P = .025) and lower percentages of memory cytotoxic T cells (OR, –0.07; 95% CI, 0-15; P = .043) than boys.

The characteristics of children ≥8 years old categorized as LTNPs (n = 50), non-LTNPs (n = 17), and healthy controls (n = 53) generally matched (see this article's Table E1 in the Online Repository at www.jacionline.org). Counts of cell subsets were significantly different in LTNPs compared with non-LTNPs and healthy controls (see this article's Table E2 in the Online Repository at www.jacionline.org). In general, values for cell subset counts of LTNPs were between those of non-LTNPs and healthy controls. Importantly, the LTNPs had higher counts of T_H and cytotoxic T cells, as well as naive and memory cell subsets, and higher counts of B cells and NK cells, than non-LTNPs, but values for LTNPs were lower than those of healthy controls, except for counts of total cytotoxic and memory cytotoxic T cells. The LTNPs had higher counts of activated T_H and cytotoxic T cells than the healthy controls, and surprisingly, higher counts than the non-LTNPs. Counts of the 2 monocyte subsets did not differ between groups. There were fewer significant differences between LTNPs and non-LTNPs in percentages of cell subsets (see this article's Table E3 in the Online Repository at www.jacionline.org). Only the total percentage of T_H cells was significantly higher in LTNPs, whereas the percentage of activated monocytes was lower among LTNPs, compared with non-LTNPs. There were, however, significant differences between LTNPs and healthy controls in percentages of all subsets except the 2 monocyte subsets.

Data were further analyzed by using more stringent criteria for LTNPs: using $CD4^+$ T-cell counts ≥500 cells/mm^3 (instead of CD4 ≥ 350 cells/mm^3) and removing from the analysis data from children with CDC B classification (mainly history of pneumonia). These datasets included 29 LTNPs, 38 non-LTNPs, and 53 healthy controls in the first analysis and 35 LTNPs, 12 non-LTNPs, and 53 healthy controls in the second analysis. Findings from each analysis were similar to data derived by using the original definition of LTNP (data not shown).

We examined whether sex, CDC category, HIV RNA level, and lymphocyte cell-subset values could identify which children were LTNPs (Table II). Factors that identified LTNPs, by univariate and multivariate analyses, were male sex; higher counts of total T cells, T_H cells, cytotoxic T cells, and their naive and memory subsets; and higher counts of B cells. Surprisingly, higher counts of activated T_H cells also identified LTNPs, whereas there were no statistically significant differences in counts of activated cytotoxic T cells between LTNPs and non-LTNPs. Levels of HIV RNA and CDC category (N, A, or B) did not identify LTNPs.

DISCUSSION

This is the first study to show that the immunologic profile of untreated, HIV-positive Asian children differs significantly from that of age-matched healthy controls. Children with HIV had lower counts and percentages of T_H cells and naive and memory T-cell subsets. Values for cytotoxic T cells and the memory subset were higher in HIV-infected children than in healthy controls.

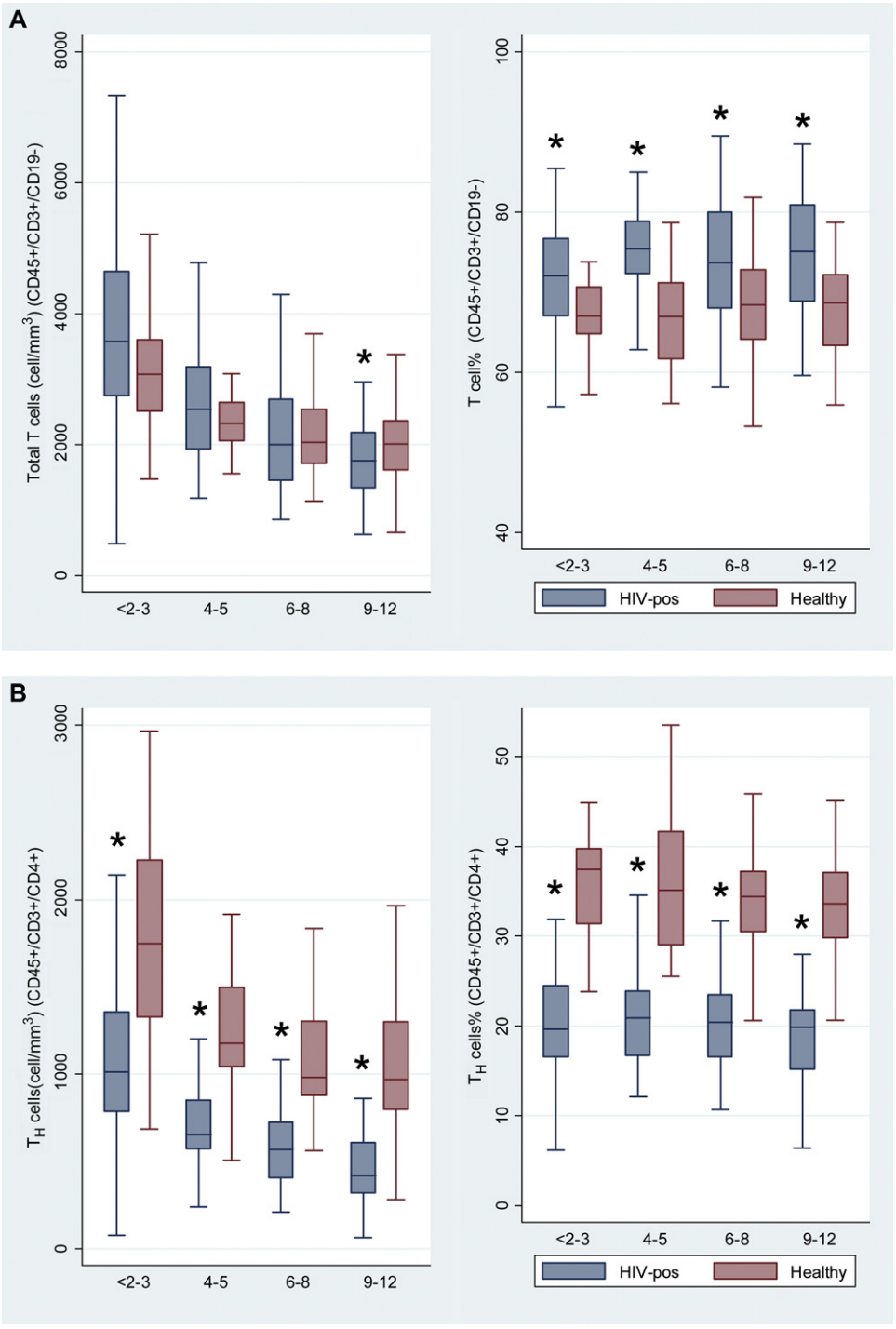


FIG 1. Comparison of cell subsets among 4 age groups of HIV-positive and healthy children. **A,** $CD45^+/CD3^+/CD19^-$ (total T cells). **B,** $CD45^+/CD3^+/CD4^+$ (T_H cells). **C,** $CD45^+/CD3^+/CD8^+$ (cytotoxic T cells). **D,** $CD45^+/CD3^-/CD19^+$ (B cells). **E,** $CD45^+/CD3^-/CD16^+/CD56^+$ (NK cells). *$P < .05$. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

Notably, HIV-positive children had lower counts and percentages of naive cytotoxic T cells and higher counts and percentages of activated T_H and cytotoxic T cells than controls.

Several studies have described the immunologic profile of HIV-positive children but did not include extensive flow-cytometry analyses of lymphocyte subsets, using more than 2 fluorochromes, or age-matched healthy controls, as this study has.[13,17,18] We found that the profile of HIV-infected children is characterized by low values for T_H cells and high values for cytotoxic T cells, as well as a reduced naive T-cell population. After birth, there is an expansion of the naive T-cell pool from T-cell proliferation. HIV infection leads to continuous activation of

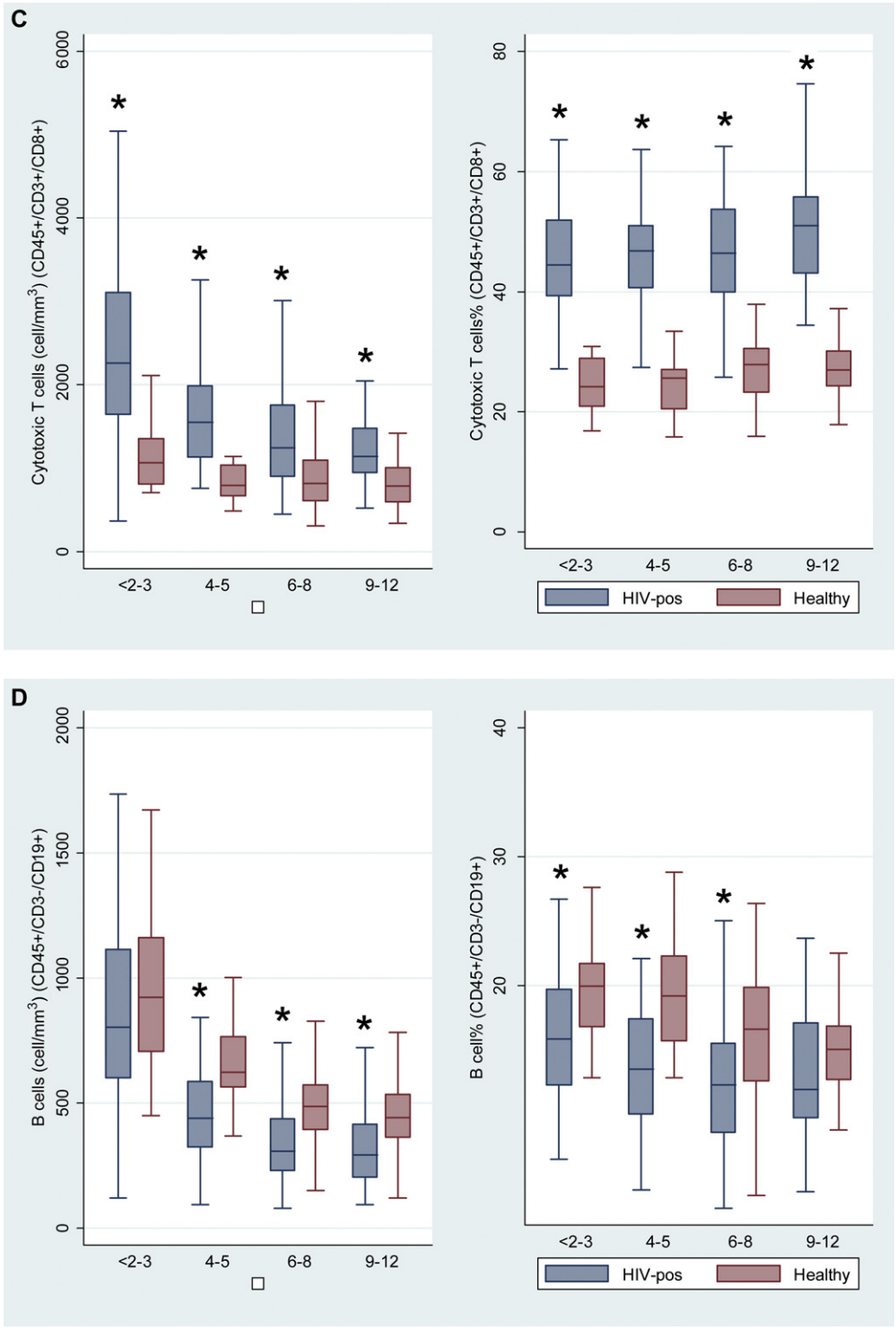


FIG 1. *(Continued)*

naive T cells and loss of this population.[19] Even neonates who have been exposed to HIV but not infected have lower values of naive T cells than HIV-unexposed neonates.[20] The HIV-infected children also had increased values for memory $CD8^+$ T cells. Other studies have shown this to be associated with HIV viremia and decreases in $CD4^+$ T cells.[17,21]

The HIV-positive children in this study had a remarkable increase in activation of T_H and cytotoxic T cells compared with controls. Persistent immune activation is associated with unfavorable clinical outcome from HIV. It is largely driven by HIV viremia, which explains the high numbers of activated T cells observed in untreated HIV-positive children in this study.[18,22]

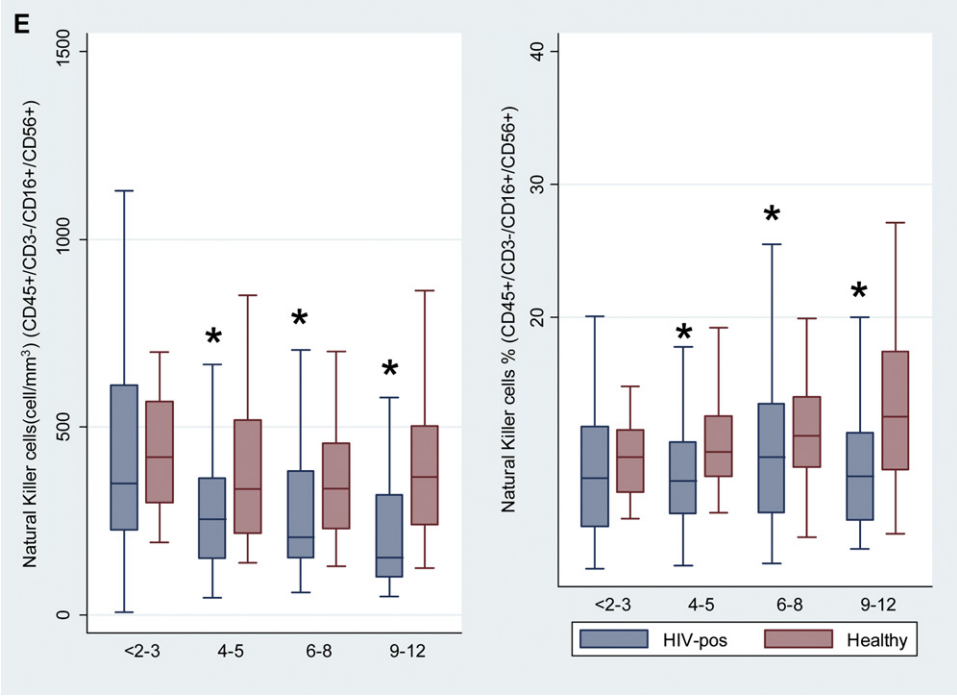


FIG 1. *(Continued)*

The T-cell activation marker CD38 DR (on cytotoxic T cells) correlated with high HIV RNA and the rate of HIV disease progression in children and adults.[13,17,18,23] The lower values for B cells observed in the HIV-positive children might arise during HIV replication, which causes lysis of B cells in germinal centers of lymph nodes.[5,24] NK cells are important for the lysis of HIV-infected cells; numbers of these cells were also reduced in the HIV-positive children compared with controls.[23] Low concentrations of activated monocytes and perivascular monocytes exist in the peripheral blood and have been identified in children and adults with HIV-associated neurologic diseases.[25,26] The HIV-positive children in this study did not have detectable neurologic disease, which could explain the lack of differences observed in monocyte subsets compared with healthy controls. The effects of age, sex, and ethnicity on lymphocyte counts have been evaluated in several studies of healthy individuals.[14,27,28] Counts of lymphocytes—particularly of T_H cells, B cells, and NK cells—tend to peak in early childhood, then decrease with age, whereas percentages of lymphocyte vary less with age[15,27-30]; we also observed this effect in our study population. Similar to this study, girls in Malawi had higher counts of $CD4^+$ T cells than boys.[15] Flow cytometry patterns of the HIV-positive Asian children in this study were similar to those reported in non-Asians.[3,13,17,21,22] A comparison of flow cytometry patterns between healthy Asian children and those of other ethnic groups is an important topic for future study.

There are few studies of pediatric LTNPs.[31,32] The Women and Infant Transmission Study followed 137 HIV-positive children from birth and found 10 to be LTNPs, defined as children with >25% $CD4^+$ T cells and >500 $CD4^+$ T cells/mm^3 $CD4^+$ T cells at 8 years of age. The investigators found that activated cytotoxic T cells, levels of CD8 DR below 5%, and levels of CD8 and CD38 below 25% at 2 months of age predicted which children would become LTNPs.[3] Children who are slow progressors have been found to have lower percentages of memory $CD4^+$ T cells and higher percentage of naive $CD4^+$ T cells.[32] Overall, the LTNPs in this study had counts and percentages of cell subsets that were closer to those of healthy controls than of the non-LTNPs. Compared with the non-LTNPs, the LTNPs had higher counts of T_H and cytotoxic T cells, naive and memory cell subsets, and B and NK cells. The higher counts of memory and NK cells observed in the LTNPs in this study contradict previous reports.[21,32] More surprisingly, the LTNPs had higher counts, but not percentages, of activated T cells, particularly of T_H cells, than the non-LTNPs. This is in contrast to studies in children and adults from other ethnic groups.[3,7,13,32,33] The reasons for the differences are unclear but could include greater variation in cell counts, compared with cell percentages, and the cross-sectional measurement, which can vary with time. Being male was significantly associated with being an LTNP in this study; this finding differs from other pediatric LTNP studies[3,31] and requires confirmation in larger studies with longer periods of observation. We did not associate levels of HIV RNA with being an LTNP, although HIV RNA levels generally associated with decreases in $CD4^+$ T cells.[17] Another difference in our study was that CDC class did not predict being an long-term nonprogression, likely because none of the children in this study had severe HIV disease. Longitudinal analyses of cell subsets are underway in the PREDICT study; these might identify lymphocyte subsets associated with long-term nonprogression in children in the deferred arm of the study.

This study differs from other studies of pediatric HIV in the large number of HIV-positive children without severe immune suppression, the inclusion of healthy controls, the detailed examination of lymphocyte and monocyte subsets, and the Asian ethnicity of all children. Importantly, we were able to characterize the immunologic profile of a relatively large number of LTNPs in comparison with non-LTNPs and healthy controls. The study is

TABLE II. Univariate and multivariate regression analyses of predicting factors for LTNPs

	Univariate			Multivariate		
Variables	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Sex						
Female	1			1		
Male	4.6	0.9-22.4	.032	10.2	1.1-96	.021
CDC stage						
N/A	1					
B	1.0	0.31-3.4	.963			
Log HIV RNA at baseline	0.4	0.1-1.2	.081	2.1	5.8-472.8	.361
Total T cells						
<1412	1			1		
≥1412	37.4	8.2-170.3	<.001	67.1	9.3-485	<.001
Naive T_H cells						
<128	1			1		
≥128	21.6	5.4-87.0	<.001	32.2	5.9-175.3	<.001
Memory T_H cells						
<138	1			1		
≥138	21.6	5.4-87.0	<.001	26.5	5-139.7	<.001
Activated T_H cells						
<35.5	1			1		
≥35.5	4.0	1.2-13.4	.0219	3.6	1-12.9	.047
Cytotoxic T cells						
<954	1			1		
≥954	8.8	2.5-30.7	.0004	9.7	2.4,38.9	.001
Naive cytotoxic T cells						
<176	1			1		
176-292	4.6	1.1-20.4		5.3	1.1-26.5	
≥292	14.3	3.1-66.0	<.001	16	3-84.9	.002
Memory cytotoxic T cells						
<411	1			1		
≥411	5.9	1.8-19.9	.0037	6.7	1.8-25.5	.004
Activated cytotoxic T cells						
<379	1			1	1	
≥379	2.8	0.9-9.2	.0930	2.7	0.7-9.8	.135
B cells						
<216	1			1		
216-285	3.4	0.83-14.2		5.5	1.1-29	
≥286	22.1	3.9-124.3	<.001	30.7	4.4-214.5	<.001
NK cells						
<108	1			1		
≥108	1.9	0.6-6.4	.2873	1.5	0.4-5.5	.569

LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 ≥350 cells/mm^3. Non-LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 <350 cells/mm^3. Healthy controls are defined as healthy children age ≥8 years. For lymphocyte subsets, values are expressed in cell count (cells/mm^3)

limited by possible variations in percentages and counts of cell subsets between time points. A longitudinal examination of cell subsets would allow groups to be compared more accurately and LTNPs to be compared with non-LTNPs.

This study used immunophenotype analysis to characterize the immunologic profile of HIV-positive Asian children compared with their age-matched healthy controls. The longitudinal phase of the PREDICT study could identify lymphocyte subsets that predict HIV disease progression. Such knowledge could have a significant public health impact in identifying children who require early ART and those for whom ART could be deferred.

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Clinical implications: The distinct immunologic profile of LTNPs might identify lymphocyte subsets associated with HIV disease progression.

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METHODS

For the HIV-positive children, flow cytometry was performed at 4 central laboratories (3 in Thailand and 1 in Cambodia) by dual-platform flow cytometry with FACS Calibur (Becton Dickinson, San Jose, Calif) at 3 sites and Coulter (Beckman Coulter, Miami, Fla) at 1 site. For the healthy controls, cytometry was performed at 1 site in Thailand using FACS Calibur. Flow analysis was performed with a standardized 3-color analysis protocol that gated on cells stained with 1 fluorochrome (generally peridinin chlorophyll protein or CyChrome conjugated to CD45, CD4, or CD8), followed by 2-color analysis of cells stained with the prescribed remaining fluorochromes (fluorescein isothiocyanate and phycoerythrin). The relative proportions of each subset were expressed as the percentage of the anchor gated population. For both groups, the following subsets were performed: $CD45^+/3^+/19^-$ (total T cells), $CD45^+/3^+/4^+$ (T_H cells), $CD4^+/45RA^+/62L^+$ (naive T_H cells), $CD3^+/4^+/45RO^+$ (memory T_H cells), $CD4^+/DR^+/38^+$ (activated T_H cells), $CD45^+/3^+/8^+$ (cytotoxic T cells), $CD8^+/45RA^+/62L^+$ (naive cytotoxic T cells), $CD3^+/4^-/45RO^+$ (memory cytotoxic T cells), $CD8^+/DR^+/38^+$ (activated cytotoxic T cells), $CD45^+/3^-/19^+$ (B cells), $CD45^+/3^-/16^+/56^+$ (NK cells), $CD14^+/16^+/DR^+$ (activated monocytes), and $CD14^+/16^+/163^+$ (perivascular monocytes believed to be precursors of brain perivascular macrophages).[E1]

Statistical analysis

Histograms were plotted and examined, and the Shapiro-Wilk test was performed to test normality and to select the best transformation method to be used in each regression. Most of cell subset counts and percentages were transformed by Box-Cox power transformation except for activated T_H-cell percentage, activated monocyte percentage, and activated cytotoxic T-cell counts, for which distributions were better with a $\log_{10}$ transformation. Untransformed data were used in regressions for activated cytotoxic T-cell percentage and memory T_H-cell percentage.

We first categorized the cell subset counts and percentages into 4 groups by their quartiles. After examining the distribution by LTNP status in contingency tables together with their ORs, we collapsed adjacent quartiles if this was supported by the distribution. Covariates that were assessed to predict LTNP included sex, CDC classification, baseline log HIV RNA, and cell subsets. In the logistic regression models, covariates with $P \leq .1$ in univariate models were adjusted for in the multivariate models. For example, the distribution by cytotoxic T-cell percentage quartiles was 59, 29, 6, 6 for LTNPs and 14, 24, 32, 30 for non-LTNPs, which corresponded to OR (95% CI) for LTNP of 1 (reference), 3.4 (0.8-14.2), 22.8 (2.4-214.6), and 22.4 (2.3-201.9), respectively. Quartiles 3 and 4 were therefore combined.

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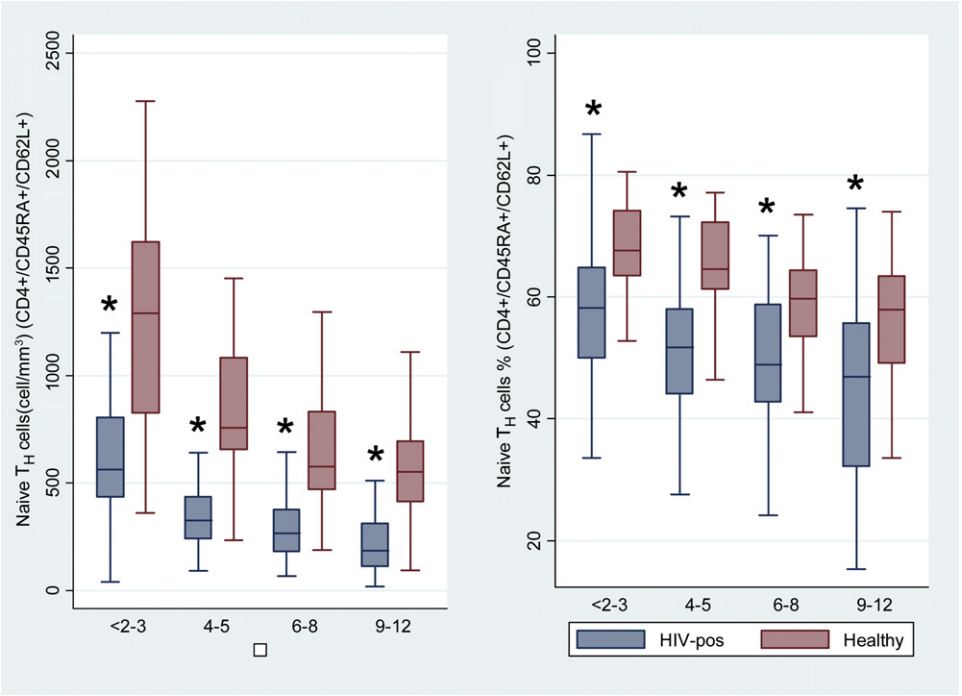


FIG E1. Comparison of cell subsets between HIV-positive and healthy children for 4 age groups. $CD4^+/45RA^+/62L^+$ (naive T_H cells). **P* < .05. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

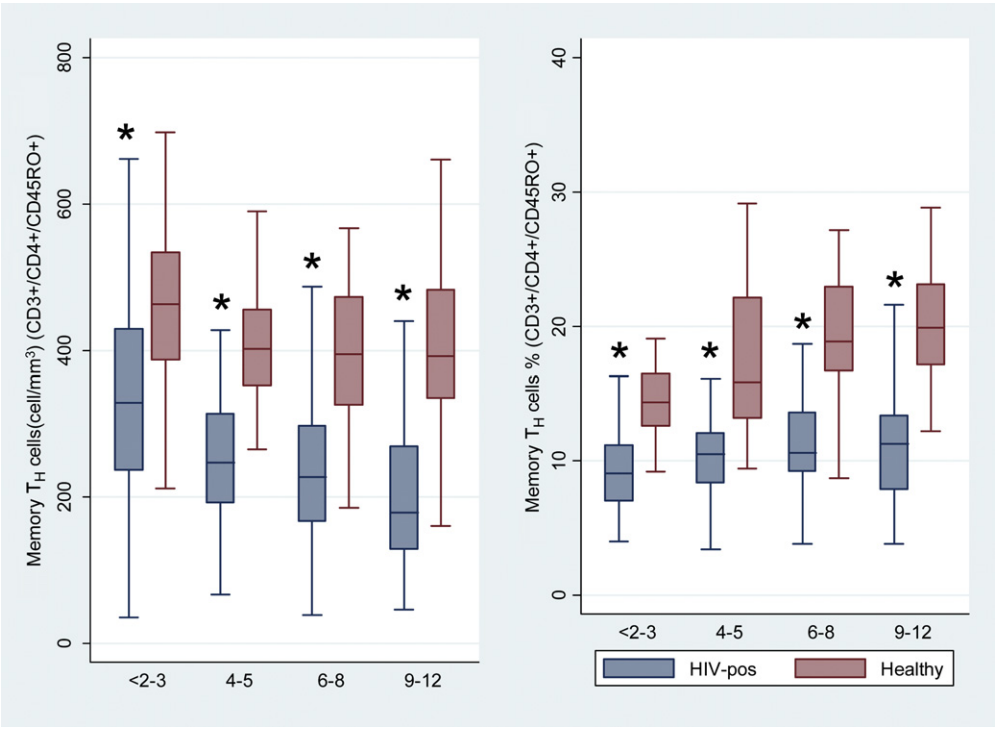


FIG E2. Comparison of cell subsets between HIV-positive and healthy children for 4 age groups. $CD3^+/4^+/45RO^+$ (memory T_H cells). *$P < .05$. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

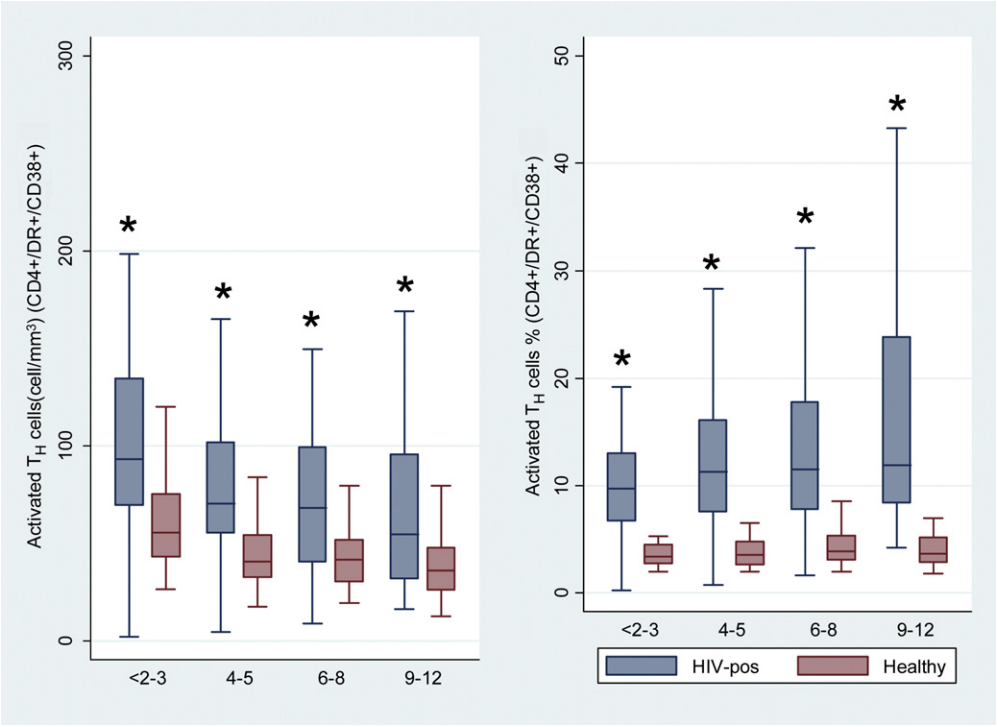


FIG E3. Comparison of cell subsets between HIV-positive and healthy children for 4 age groups. $CD4^+/DR^+/38^+$ (activated T_H cells). *$P < .05$. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

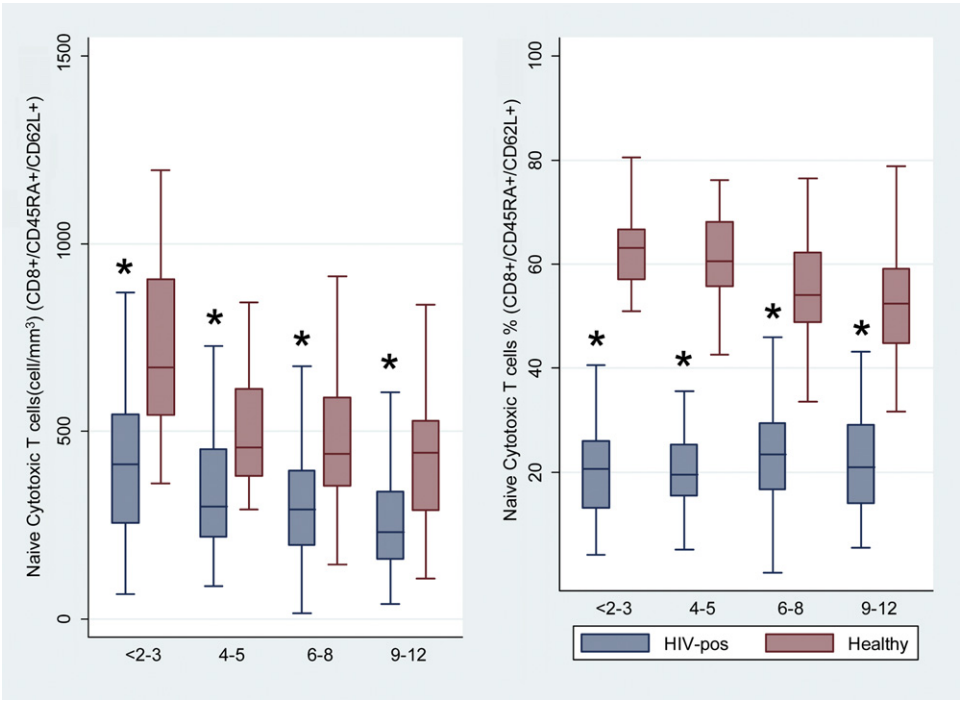


FIG E4. Comparison of cell subsets between HIV-positive and healthy children for 4 age groups. $CD8^+/45RA^+/62L^+$ (naive cytotoxic T cells). **P* < .05. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

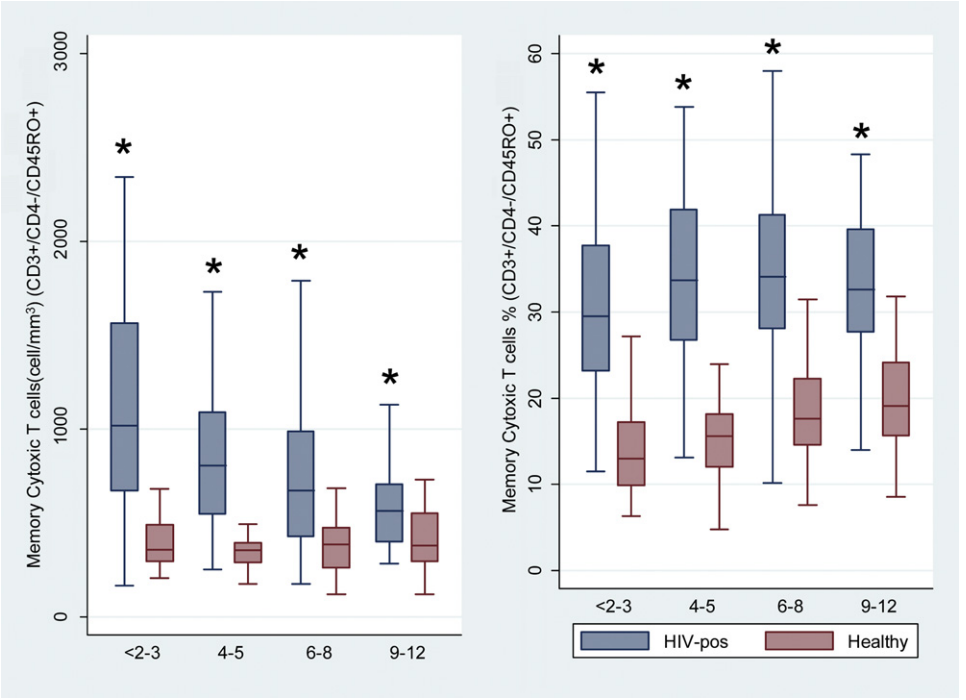


FIG E5. Comparison of cell subsets between HIV-positive and healthy children for 4 age groups. $CD3^{+}/4^{-}/45RO^{+}$ (memory cytotoxic T cells). *$P < .05$. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

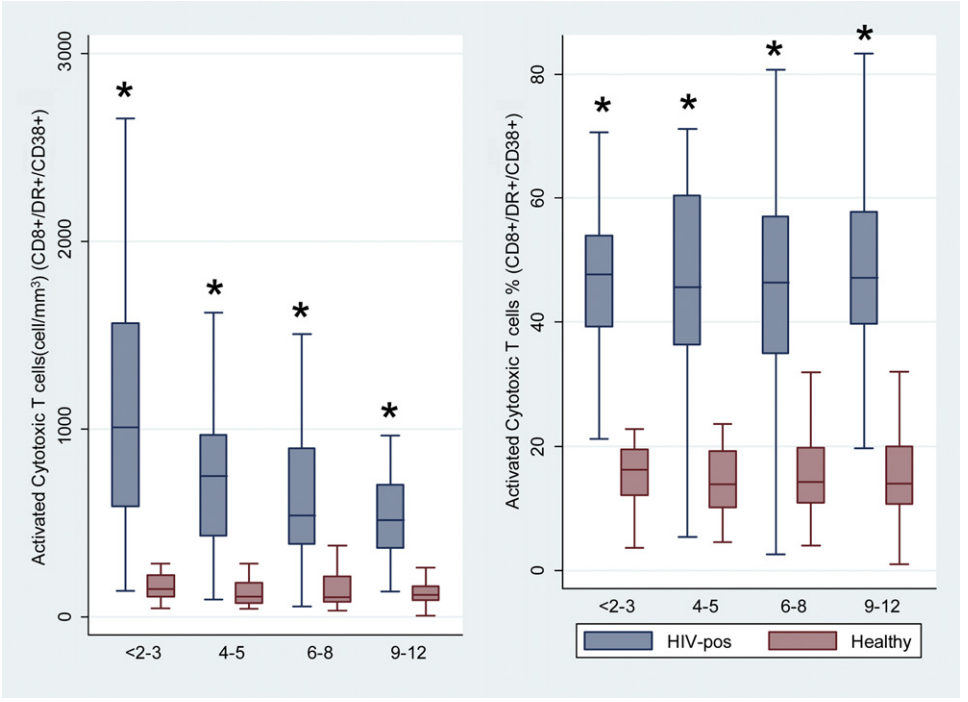


FIG E6. Comparison of cell subsets between HIV-positive and healthy children for 4 age groups. $CD8^+/DR^+/38^+$ (activated cytotoxic T cells). *$P < .05$. No. of children: <2 to 3 years (n = 79), 4 to 5 years (n = 74), 6 to 8 years (n = 135), 9 to 12 years (n = 76). *Pos,* Positive.

TABLE E1. Demographics of LTNPs, non-LTNPs, and healthy controls

Characteristic	LTNPs (n = 50)	Non-LTNPs (n = 17)	Healthy controls (n = 53)
Mean age (y) (SD)	9.7 (1.5)	9.9 (1.1)	10.1 (1.4)
Median age (y) (IQR)	9.0 (8.4-11.0)	9.8 (9.0-10.3)	10.0 (8.8-11.1)
Sex M:F, n (%)	19:31 (38:62)	2:15 (11.8:88.2)	20:33 (37.7:62.3)
Nationality, n (%)			
Thai	42 (84.0)	11 (64.7)	53 (100.0)
Cambodian	8 (16.0)	6 (35.3)	0 (0.0)
CDC class N:A:B (%)	4:66:30	0:71:29	—
Median HIV RNA $\log_{10}$copies/mL (IQR)	4.4 (3.9-4.7)	4.7 (4.4-4.9)	—

F, Female; *M,* male; —, not applicable.

LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 ≥350 cells/mm^3. Non-LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 <350 cells/mm^3. Healthy controls are defined as healthy children age ≥8 years.

TABLE E2. Comparison of cell subset counts among LTNPs, non-LTNPs, and healthy controls

Cell subset	LTNPs (n = 50) (cells/mm^3), median (IQR)	Non-LTNPs (n = 17) (cells/mm^3), median (IQR)	Healthy controls (n = 53) (cells/mm^3), median (IQR)	*P* value (3 gr.)	*P* value (LTNPs vs non-LTNPs)	*P* value (LTNPs vs healthy controls)
Total T cells	2032 (1663-2597)	1186 (1094-1360)	1986 (1616-2367)	<.001	<.001	.4208
T_H cells	575 (417-757)	244 (223-312)	970 (800-1301)	<.001	<.001	<.001
Naive T_H cells	266 (189-417)	107 (70-128)	553 (438-726)	<.001	<.001	<.001
Memory T_H cells	245 (177-290)	129 (90-146)	401 (336-459)	<.001	<.001	<.001
Activated T_H cells	76 (41-115)	36 (26-44)	36 (27-48)	<.001	.0002	<.001
Cytotoxic T cells	1283 (1151-1759)	901 (736-997)	767 (588-1006)	<.001	<.001	<.001
Naive cytotoxic T cells	306 (212-408)	158 (91-192)	419 (290-506)	<.001	.0005	.0063
Memory cytotoxic T cells	734 (543-988)	411 (350-558)	363 (238-530)	<.001	.0004	<.001
Activated cytotoxic T cells	577 (441-895)	416 (362-558)	114 (81-168)	<.001	.0228	<.001
B cells	326 (256-441)	166 (146-231)	432 (363-534)	<.001	<.001	.0005
NK cells	224 (137-411)	137 (95-181)	337 (227-481)	<.001	.0074	.0045
Activated monocytes	33 (15-66) (n = 24)	49 (26-137) (n = 12)	31 (21-50)	.140	.1001	.8863
Perivascular monocytes	16 (6-46) (n = 24)	25 (13-73) (n = 12)	16 (12-23)	.239	.3305	.7167

3 gr., The *P* value signifies any difference between the 3 groups (LTNP, non LTNP and healthy controls). LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 ≥350 cells/mm^3. Non-LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 <350 cells/mm^3. Healthy controls are defined as healthy children age ≥8 years.

TABLE E3. Comparison of cell subset percentages among LTNPs, non-LTNPs, and healthy controls

Cell subset	LTNPs (n = 50) (%), Median (IQR)	Non-LTNPs (n = 17) (%), Median (IQR)	Healthy controls (n = 53) (%), Median (IQR)	*P* value (3 gr.)	*P* value (LTNPs vs non-LTNPs)	*P* value (LTNP vs healthy controls)
Total T cells	74 (68-82)	76 (71-80)	68 (65-72)	<.001	.7458	.0002
T_H cells	20 (17-24)	15 (14-17)	34 (30-39)	<.001	.0002	<.001
Naive T_H cells	49 (40-56)	47 (29-55)	58 (50-63)	<.001	.0751	.0003
Memory T_H cells	12 (9-14)	10 (7-12)	20 (17-23)	<.001	.0705	<.001
Activated T_H cells	12 (7-17)	13 (11-20)	3 (3-5)	<.001	.1710	<.001
Cytotoxic T cells	50 (42-55)	53 (50-56)	27 (23-30)	<.001	.1537	<.001
Naive cytotoxic T cells	24 (15-29)	18 (13-22)	53 (46-60)	<.001	.1622	<.001
Memory cytotoxic T cells	34 (30-40)	32 (26-44)	19 (15-23)	<.001	.6396	<.001
Activated cytotoxic T cells	46 (37-54)	56 (40-69)	14 (11-21)	<.001	.0851	<.001
B cells	12 (9-17)	10 (9-16)	15 (13-17)	<.001	.4322	.0012
NK cells	7 (5-12)	9 (5-10)	12 (9-15)	.005	.9483	.0026
Activated monocytes	7 (5-11) (n=24)	11 (9-18) (n=12)	8 (6-13)	.026	.0149	.1263
Perivascular monocytes	4 (2-8) (n = 24)	6 (3-12) (n = 12)	4 (3-6)	.408	.2021	.9737

3 gr., The *P* value signifies any difference between the 3 groups (LTNP, non LTNP and healthy controls). LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 ≥350 cells/mm^3. Non-LTNPs are defined as antiretroviral-naive, HIV-positive children age ≥8 years with CD4 <350 cells/mm^3. Healthy controls are defined as healthy children age ≥8 years.