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

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Abstract

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 non-progressors (LTNPs), compared with age-matched healthy controls.

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A complete list of the PREDICT Study Team can be found in the Online Repository.

Methods—We used flow cytometry to analyze 13 lymphocyte and monocyte subsets from 222 untreated, HIV-positive children with 15%–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 helper T cells and higher values for cytotoxic T cells, with reductions in populations of naïve helper and cytotoxic T cells, B cells, and natural killer (NK) cells. HIV-positive children had high values for activated helper and cytotoxic T cells. Compared with non-LTNPs, LTNPs had higher values of helper and cytotoxic T cells, naïve and memory T-cell subsets, and B and NK cells. Surprisingly, counts of activated helper and cytotoxic T cells were also higher among LTNPs. LNTPs were more frequently male.

Conclusions—Untreated, HIV-infected Asian children have immune profiles that differ from those of controls, characterized by low values for helper T cells, naive T cells, B cells, and NK cells but high values for cytotoxic, activated helper, and cytotoxic T cells. The higher values for activated T cells observed in LTNPs require confirmation in longitudinal studies.

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

Keywords

HIV; children; lymphocyte; monocyte; phenotyping; long-term non-progressors; antiretroviral therapy; Asia; disease progression; pediatric AIDS

Introduction

HIV-positive children were 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[2]. Less than 10% remain healthy by age 8 without ART—they are called HIV long-term non-progressors (LTNPs)[3].

Hallmarks of HIV infection are high turnover of CD4+ T cells and 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 following 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 naïve T-cell population that mediates responses to new antigens, generate polyfunctional T cells, and have low-level immune activation. Some also have mutations in the chemokine co-receptor 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 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 6 sites in Thailand and 2 sites in Cambodia who enrolled in the Pediatric Randomized to Early vs. Deferred Initiation in Cambodia and Thailand Study (PREDICT, http://www.clinicaltrials.gov/ct/show/NCT00234091). These children were 1–12 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 cell 15%–24%) without severe symptoms of HIV infection (Center 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 (NIAID-DAIDS) 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 NIAID-DAIDS and passed the annual quality assurance programs of the United Kingdom National External Quality Assessment Service and Mahidol University, Bangkok, Thailand. By convention, we used clusters of designation (CD) numbers only when necessary in the text and elsewhere when presence and absence of markers is necessary. More details are in the Online Repository.

Statistical analyses

Analyses were performed with Stata 11 (College Station, Tx, USA). 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; T-test and Mann-Whitney-U test were used to compare differences between groups including distributions of T-cell subsets; the Chi-square $\chi 2$ 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 in the Online Repository.

We compared counts and percentages of T-cell subsets between healthy controls and HIV–positive children 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, and 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 more normally distribute residual errors. Logistic regression analysis assessed whether T-cell subset distributions predicted which children would be LTNPs. Odds ratios (OR) and 95% confidence intervals (95% CI) 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 where as 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.

Figures 1A to 1E and Figures E1 to E6- (Online Repository)

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 9–12-year age group, and lower total percentages of T cells across all age groups than healthy controls (Figure 1A). Counts and percentages of helper T 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 helper T cells (Figure 1B) and higher counts and percentages of cytotoxic T cells (Figure 1C). Counts and percentages of B cells (Figure 1D) and natural killer (NK) cells (Figure 1E) were significantly lower in some age groups of HIV-positive children. The HIV-positive children had lower counts of naive helper T cells (Online Repository Figure E1) and memory helper T cells (Online Repository Figure E2), but higher counts and percentages of activated helper T cells (Online Repository Figure E3). There were lower counts and percentages of naive cytotoxic T cells (Online Repository Figure E4), and higher counts and percentages of memory cytotoxic T cells (Online Repository Figure E5) and activated cytotoxic T cells (Online Repository Figure E6) 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 helper T cells, memory helper T 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% confidence intervals [CI] 0.27–4.08, P=0.025) and lower percentages of memory cytotoxic T cells (−0.07, 95% CI 0-15, P=0.043) 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 (Online Repository Table E1).

Counts of cell subsets were significantly different in LTNPs compared with non-LTNPs and to healthy controls (Online Repository Table E2). 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 helper 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 helper and cytotoxic T cells than the healthy controls, and surprisingly, also 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 (Online Repository Table E3). Only the total percentage of helper T 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 using more stringent criteria for LTNPs: using CD4+ T cell counts ≥ 500 T cells/mm^3 (instead of CD4 ≥ 350 T 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 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, helper T cells, cytotoxic T cells and their naive and memory subsets; and higher counts of B cells. Surprisingly, higher counts of activated helper T cells also identified LTNPs, where as 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 helper T 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 Notably, HIV-positive children had lower counts and percentages of naive cytotoxic T cells and higher counts and percentages of activated helper 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 helper T 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 naive T cells and loss of this population[19]. Even neonates that have been exposed to HIV but not infected have lower values of naive T cells, compared with 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 helper 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] 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 helper T cells, B cells, and NK cells—tend to peak in early childhood then decrease with age, where as 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% and >500 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 helper and cytotoxic T cells, of naive and memory cell subsets, and B and NK cells. The higher counts of memory cell 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 helper T 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 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 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 long-term non-progression, 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 non-progression 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 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 more accurately compared and for LTNPs to be compared with non-LTNPs.

This study used immunophenotype analysis to characterize the immunologic profile of HIV-positive Asian children, compared to 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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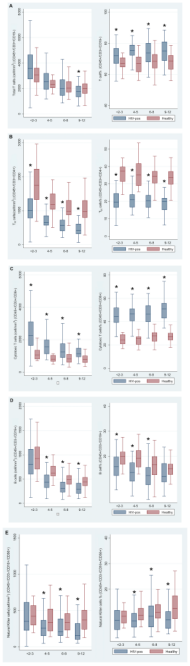
Abbreviations

ART	Antiretroviral therapy
CDC	Center for Disease Control and Prevention
HIV	Human Immunodeficiency Virus
LTNP	Long term non progressor
WHO	World Health Organization



Figures 1A to 1E. Comparison of cell subsets among 4 age groups of HIV-negative and HIV-positive children

Figure 1A: CD45+/3+/19- (Total T cells), Figure 1B: CD45+/3+/4+ (Helper T cells), Figure 1C: CD45+/3+/8+ (Cytotoxic T cells), Figure 1D: CD45+/3-/19+ (B cells), Figure 1E: CD45+/3-/16+/56+ (Natural killer cells).

Asterisk * denotes P value < 0.05.

Number of children: <2-3 years (n=79), 4-5 years (n=74), 6-8 years (n=135), 9-12 years (n=76).

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, years (SD)	6.6 (2.9)	6.3 (2.8)	7.0 (2.9)	0.03
Median age, years (IQR)	6.5 (4.3-8.7)	6.4 (4.1-8.4)	6.8 (4.6-9.2)	0.04
Age categories, n (%)				0.12
<2-3yrs	79 (21.7)	54 (24.3)	25 (17.6)	
4-5 yrs	74 (20.3)	47 (21.2)	27 (19.0)	
6-8 yrs	135 (37.1)	83 (37.4)	52 (36.6)	
9-12 yrs	76 (20.9)	38 (17.1)	38 (26.8)	
% male in age groups, n (%)				
<2-3yrs	37 (46.84)	30 (55.56)	7 (28.00)	0.01
4-5 yrs	32 (43.24)	22 (46.81)	10 (37.04)	0.35
6-8 yrs	57 (42.22)	35 (42.17)	22 (42.31)	0.94
9-12 yrs	22 (28.95)	8 (21.05)	14 (36.84)	0.01
Sex M:F	148: 216 (41: 59)	95: 127 (43: 57)	53:89 (37:63)	0.30
Nationality, n (%)				<0.001
Thai	264 (72.5)	122 (55.0)	142 (100.0)	
Cambodia	100 (27.5)	100 (45.0)	0 (0.0)	
%CDC 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)	<0.001
Median CD4 count, cells/mm^3 (IQR)	835 (578-1130)	631 (434-887)	1099 (895-1452)	<0.001
Median HIV RNA log^{10} copies/ml (IQR)	-	4.8 (4.3-5.0)	-	-

Table II

Univariate and multivariate regression analyses of predicting factors for long-term non-progressors (LTNPs)

	Univariate			Multivariate		
Variables	**OR**	**95%CI**	**P-value**	**OR**	**95%CI**	**P-value**
Gender						
Female	1			1		
Male	4.6	0.9, 22.4	**0.032**	10.2	1.1, 96	**0.021**
CDC stage						
N/A	1					
B	1.0	0.31-3.4	0.963			
Log HIV-RNA at baseline	0.4	0.1- 1.2	0.081	2.1	5.8, 472.8	0.361
Total T cells						
< 1412	1			1		
≥1412	37.4	8.2-170.3	**<0.001**	67.1	9.3,485	**<0.001**
Naïve helper T cells						
< 128	1			1		
≥128	21.6	5.4-87.0	**<0.001**	32.2	5.9,175.3	**<0.001**
Memory helper T cells						
< 138	1			1		
≥138	21.6	5.4-87.0	**<0.001**	26.5	5,139.7	**<0.001**
Activated helper T cells						
<35.5	1			1		
≥35.5	4.0	1.2-13.4	**0.0219**	3.6	1,12.9	**0.047**
Cytotoxic T cells						
<954	1			1		
≥954	8.8	2.5-30.7	**0.0004**	9.7	2.4,38.9	**0.001**
Naive cytotoxic T cells						

	Univariate			Multivariate		
Variables	**OR**	**95%CI**	**P-value**	**OR**	**95%CI**	**P-value**
<176	1			1		
176-292	4.6	1.1-20.4		5.3	1.1,26.5	
≥292	14.3	3.1-66.0	**<0.001**	16	3,84.9	**0.002**
Memory cytotoxic T cells						
<411	1			1		
≥411	5.9	1.8-19.9	**0.0037**	6.7	1.8,25.5	**0.004**
Activated cytotoxic T cells						
<379	1			1	1	
≥379	2.8	0.9-9.2	0.0930	2.7	0.7,9.8	0.135
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	**<0.001**	30.7	4.4,214.5	**<0.001**
Natural killer cells						
<108	1			1		
≥108	1.9	(0.6-6.4)	**0.2873**	1.5	0.4,5.5	0.569

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