0031-3998/95/3803-0390$03.00/0
PEDIATRIC RESEARCH

Vol. 38, No. 3, 1995
Printed in U.S.A.

Increased Expression of Activation Markers on CD8 Lymphocytes in Children with Human Immunodeficiency Virus-1 Infection

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ABSTRACT

The aims of the present study were to analyze the impact of perinatal human immunodeficiency virus (HIV)-1 infection on lymphocyte maturation in children, to determine the expression of activation markers on $CD8^+$ cells, and to define predictors of survival in HIV-infected children. Seventy-one children presenting HIV-related symptoms were included in the study; 29 were less than 2 y old and 42 were 2 to 12 y of age. Results were compared with those obtained in normal children of a similar age. In HIV-infected children the proportion of $CD4^+$ and $CD8^+CD45RA^+$ cells was significantly decreased, whereas that of $CD8^+$, $CD8^+CD38^+$, and $CD8^+CD45RO^+$ cells was strikingly increased compared with controls. In children less than 2 y old the absolute number of $CD4^+$ and $CD8^+CD45RA^+$ cells decreased, and the number of $CD8^+CD45RO^+$ cells increased significantly, whereas the number of $CD8^+$ and $CD8^+CD38^+$ cells did not change. The absolute number of $CD4^+$ T cells declined with age both among controls and among HIV-infected children. In contrast, the absolute number of $CD8^+$ cells and CD8 subsets decreased with age only in controls but not in infected children. In HIV-1-infected children the expression of the CD38 and CD45RO markers on $CD8^+$ cells was significantly correlated, indicating that these were activated cells. The survival of less than 2-y-old children with AIDS symptoms was positively correlated with the total number of CD8 cells and $CD8^+CD38^+$ cells at time of entry into the study. Most of the children who died by the end of the study had a CD8 count of less than $750/mm^3$ and a $CD38^+CD8^+$ count of less than $600/mm^3$ when first seen, whereas most of those who were alive had higher counts. (***Pediatr Res* 38: 390–396, 1995**)

Abbreviations

HIV, human immunodeficiency virus
PBL, peripheral blood lymphocytes
NK, natural killer

Only a relatively small fraction of the offspring of HIV-1-infected mothers become infected by the virus (1, 2). Among the maternal factors which determine transmission of HIV-1 to the fetus are elevated p-24 antigenemia (2) and absence of high affinity antibodies to the neutralizing domain of gp120 (3). Neonates and young children seem to be relatively resistant to HIV infection and to progression of HIV disease, although it is not clear as yet what factors are involved. Among HIV-1-infected hemophilic patients the rate of progression to AIDS is much lower in children less than 13 y old than in adults (4, 5). The lower rate of development of AIDS in HIV-1-infected children compared with infected adults may be associated with differences in lymphocyte populations (5). An age-dependent cofactor or immune alteration in children may either control viral replication or limit its damage in the later phase of HIV disease (4).

In addition to marked impairment of the $CD4^+$ T cell population, in HIV-1-infected adults alterations occur in other lymphocyte subsets and in the serum levels of soluble immune activation products (6, 7). $CD8^+$ cells seem to be vital for suppression of the replication of HIV-1 (8). After HIV-1 infection the proportion of CD8 cells among PBL increases, and these cells show signs of activation, such as enhanced expression of CD38 (9–12) and elevated serum levels of soluble CD8 molecules (6, 7). The majority of $CD8^+$ cells in HIV-infected adults display the CD45RO antigen (13), the expression of which is enhanced in T cells after activation, concomitantly with diminished expression of CD45RA (14). The CD45RA marker is typical for "naive" lymphocytes whereas the CD45RO marker is typical for "memory" cells (15). Among $CD4^+$ lymphocytes the $CD45RA^+$ cells induce

Received February 28, 1994; accepted February 21, 1995.
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Supported by National Institutes of Health-NIAID Grant IUOI-AI 27667 and by the T. J. Martel Foundation for Leukemia, Cancer and AIDS Research.

immune suppression, whereas $CD45RO^+$ cells function as helper-inducers (15, 16).

A number of studies have addressed the changes taking place in the immune system of children infected perinatally with HIV-1 (17–20). In previous studies of this laboratory various aspects of activation of the $CD8^+$ T cell population were studied in adult HIV-infected individuals (10, 11, 13). The aim of the present study was to analyze the lymphocyte phenotypes of children with perinatally acquired HIV-1 infection who varied in the time of appearance of AIDS-related clinical symptoms. The expression of activation markers on $CD8^+$ cells of HIV-infected children was determined and the role of these cells in HIV disease progression was assessed.

METHODS

Study population. The subjects in this study included 71 HIV seropositive (HIV^+) children seen at the pediatric infectious disease clinics of the Mount Sinai Medical Center and of the Beth Israel hospital, New York City. Of these, 29 HIV-infected children were less than 2 y old, and 42 children were 2 to 12 y of age. With the exception of three children who were infected by neonatal blood transfusion, the children were infected perinatally by their HIV^+ mother. All of the children in the study had AIDS-related symptoms at the time of their first visit in the clinic. Clinical evaluation of the HIV^+ children was according to the guidelines of the Center for Disease Control. Blood was obtained from all of the HIV-infected individuals at the time of their first visit. For each of the children in the study the presence of HIV-1 antibodies was determined by an ELISA and by HIV-1 demonstration in viral cultures. HIV-1 infection was confirmed by Western blot analysis. In addition, blood was obtained from a group of 41 healthy control children of ages similar to those of the HIV-infected children. The controls consisted of children with various noninfectious conditions seen at the pediatric outpatient clinic of the Mount Sinai Medical Center. The time of follow-up between the first visit of the children in the Clinic and definition of whether they were alive or dead at the end of the study was 3 y.

This study was carried out in adherence to guidelines of human experimentation as set forth by the Mount Sinai Medical Center, New York City and State, and the U.S. Department of Health and Human Services.

Immunophenotyping of lymphocyte subsets. Peripheral blood was taken by venipuncture from each subject using vacuum tubes with preservative-free heparin. Immunofluorescent staining was carried out by the whole blood technique, in which 150 μL of blood were incubated with 20 μL of specific pairs of MAb for 20 min at 25°C followed by the addition of 2 mL of fluorescene-activated cell sorter lysing solution (Becton Dickinson Immunocytometry Systems) and further incubation for 10 min at 25°C. Cells were washed twice with 0.01 M phosphate buffer in 0.85% saline (PBS) and fixed in 1% paraformaldehyde in PBS, pH 7.4. The MAb used were FITC-conjugated MAb against CD45, CD3, CD4, CD8, CD19, and CD45RA, and phycoerythrin-conjugated antibodies against CD8, CD14, CD16, CD38, CD56, and CD45RO. All the conjugated MAb were obtained from Becton Dickinson Immunocytometry Systems, San Jose, CA. Flow cytometric analysis was performed, as was described previously (10, 11) using a FACStar flow cytometer (Becton Dickinson Immunocytometry Systems) calibrated with CaliBrite beads and glutaraldehyde-fixed chicken red blood cells. Simulset software was used for the exclusion of dead cells, platelets, erythrocytes and granulocytes. The dual parameter contour program was used for analysis. In accordance with National Institutes of Health guidelines for flow cytometric immunophenotyping (20), the level of CD4 and $CD8^+$ T cells was assessed by determining the proportion of cells that were either $CD3^+CD4^+$ or $CD3^+CD8^+$ double positive. The level of NK cells was assessed by determining the proportion of cells that reacted with a mixture of phycoerythrin-labeled MAb against CD16 and CD56, but failed to react with FITC-labeled MAb against CD3.

Statistical analysis. The significance of differences of mean results in different groups was evaluated using a t test. Regression analyses were computed in STAVEIW-II of Macintosh to explore the association between parameters. The significance of differences in proportions between groups was evaluated either by the χ^2 test or by Fisher's exact probabilities, by using the software of Epidemiology Information Version 5.01 which was recommended by the Communicable Disease Center and World Health Organization Global Program on AIDS.

RESULTS

Lymphocyte subsets in AIDS patients less than 2 y old. The lymphocyte populations in HIV-infected children who were less than 2 y old when first seen in the clinic were compared with those found in control children of similar age. In children less than 2 y old, the percentage and absolute number of lymphocytes was not significantly different between controls and HIV-infected children (Tables 1 and 2). There were no differences in the proportion (Table 1) and absolute number (Table 2) of B-lymphocytes ($CD19^+$ cells) and in the absolute number of NK cells ($CD16^+$ and $CD56^+$ cells) between the two groups of children. The proportion of T-lymphocytes ($CD3^+$ cells) was slightly lower among HIV-infected children than among controls, whereas the absolute T cell number was significantly decreased ($p = 0.03$). The level of $CD4^+$ cells was significantly lower among HIV-infected children than among controls in terms of their percentage ($p = 0.0001$) and their absolute number ($p = 0.0008$). The proportion of $CD8^+$ cells was higher among HIV-infected children than among controls ($p = 0.0003$), although their absolute number did not differ significantly. The expression of two activation markers on $CD8^+$ lymphocytes was assessed. The proportion of $CD8^+CD38^+$ cells was higher in HIV-infected children less than 2 y of age than in healthy controls ($p = 0.02$). The absolute number of $CD8^+CD38^+$ cells was, however, similar in the two groups of children. The data shown in Tables 1 and 2 indicate that in both the infected and the control group the majority of the $CD8^+$ cells displayed the CD38 antigen. Among young HIV-infected children the percentage of $CD8^+CD45RO^+$ cells was strikingly higher than in normal controls ($p = 0.0001$), whereas that of $CD8^+45RA^+$ was reduced ($p = 0.008$). The number of $CD8^+$ cells expressing

Table 1. *The proportion of PBL expressing various surface markers among normal and HIV-infected children*

Phenotype	Less than 2-y-old children			2- to 12-y-old children		
	Controls (23)	HIV-infected (29)	p*	Control (18)	HIV-infected (42)	p*
Lymphocytes (%)	58 ± 13†	51 ± 18	NS	31 ± 7	44 ± 17	0.003
$CD3^+$	66 ± 7	61 ± 13	NS	70 ± 9	70 ± 16	NS
$CD4^+CD3^+$	44 ± 8	25 ± 15	0.0001	39 ± 8	20 ± 12	0.0001
$CD8^+CDD3^+$	22 ± 5	35 ± 15	0.0003	29 ± 5	47 ± 15	0.0001
$CD16^+$,$CD56^+$	13 ± 7	17 ± 7	0.04	14 ± 8	18 ± 14	NS
$CD19^+$	22 ± 8	21 ± 11	NS	19 ± 5	17 ± 7	NS
$CD8^+CD38^+$	20 ± 7	29 ± 15	0.02	24 ± 7	40 ± 17	0.0003
$CD8^+CD45RO^+$	12 ± 7	36 ± 21	0.0001	10 ± 1	46 ± 20	0.0001
$CD8^+CD45RA^+$	17 ± 7	12 ± 3	0.008	25 ± 7	12 ± 3	0.0001

* Significance of difference between results in normal and in HIV-infected children of similar age group. NS = not significant.
† Mean ± SD.

Table 2. *The absolute number of PBL expressing various surface markers among normal and HIV-infected children*

Phenotype	Less than 2-y-old children			2- to 12-y-old children		
	Controls (23)	HIV-infected (29)	p*	Controls (18)	HIV-infected (42)	p*
Lymphocyte (no./mm^3)	5734 ± 2005†	4497 ± 2466	NS	2401 ± 777	2932 ± 1955	NS
$CD3^+$	3816 ± 1378	2787 ± 1694	0.03	1641 ± 467	2161 ± 1590	NS
$CD4^+CD3^+$	2495 ± 961	1415 ± 1138	0.0008	904 ± 235	597 ± 463	0.01
$CD8^+CD3^+$	1260 ± 523	1432 ± 922	NS	687 ± 266	1476 ± 1295	0.01
$CD16^+$, $CD56^+$	735 ± 470	736 ± 512	NS	360 ± 246	478 ± 519	NS
$CD19^+$	1230 ± 676	941 ± 544	NS	465 ± 236	490 ± 435	NS
$CD8^+CD38^+$	1147 ± 527	1098 ± 825	NS	590 ± 267	1260 ± 1151	0.02
$CD8^+CD45RO^+$	739 ± 526	1323 ± 959	0.01	229 ± 106	1479 ± 1408	0.009
$CD8^+CD45RA^+$	1043 ± 436	591 ± 368	0.001	505 ± 237	379 ± 262	NS

* Significance of difference between results in normal and in HIV-infected children of similar age group. NS = not significant.
† Mean ± SD.

the CD45RO activation marker was, likewise, higher than in the normal controls ($p = 0.01$), whereas the number of $CD8^+CD45RA^+$ cells was markedly lower ($p = 0.001$). The results shown in Tables 1 and 2 indicate that, although CD45RO was present on about half of the $CD8^+$ cells in the controls, this marker was expressed on all of the CD8 cells in HIV-infected children. Conversely, the CD45RA marker was present on the majority of $CD8^+$ cells in normal controls but only on less than half of the $CD8^+$ cells in HIV-infected children.

Lymphocyte subsets in 2- to 12-y-old AIDS patients. The proportion of lymphocytes was higher among HIV-infected 2- to 12-y-old children than among controls (Table 1, $p = 0.003$). The proportion of T cell subsets in 2- to 12-y-old HIV-infected children differed markedly from that found in controls of similar age (Table 1). The percentage of $CD4^+$ cells was strikingly decreased ($p = 0.0001$), whereas that of $CD8^+$ cells was increased ($p = 0.0001$). The proportion of $CD8^+CD38^+$ cells and of $CD8^+CD45RO^+$ cells was significantly higher in HIV-1-infected children than in controls ($p = 0.0003$ and 0.0001, respectively), whereas that of $CD8^+CD45RA^+$ cells was lower ($p = 0.0001$). The majority of $CD8^+$ cells expressed CD38 both in infected and in normal children. All of the CD8 cells in infected children expressed CD45RO, whereas in healthy controls the majority of the CD8 cells expressed CD45RA. There were no significant differences between controls and AIDS patients in the percentage of total T, B, or NK cells.

The lymphocyte count among 2- to 12-y-old HIV-infected children was nonsignificantly higher than in controls (Table 2). The mean number of total T, B, and NK cells was slightly higher in HIV-infected children than in controls. The number of $CD4^+$ cells was lower than in controls ($p = 0.01$). In contrast, the population of $CD8^+$ cells was expanded in HIV-infected children ($p = 0.01$) and so were the populations of $CD8^+CD45RO^+$ and $CD8^+CD38^+$ cells ($p = 0.009$, and 0.02, respectively).

Correlation of age with the level of lymphocyte populations. Marked changes occur in the lymphocyte system in children during their normal development (21–25). In the present study the absolute number of various lymphocyte subsets in controls and in children with AIDS was correlated with their age, expressed on a log scale.

In control children the decline of the absolute number of lymphocytes closely correlated with age (Fig. 1*A*, $r = 0.85$, $p = 0.0001$). An age-related decline of the lymphocyte number was also detectable among HIV-infected children, in spite of a larger variation than among controls (Fig. 1*A*) ($r = 0.33$, $p = 0.006$). The decline of the absolute number of $CD4^+$ T cells among controls was significantly correlated with age (Fig. 1*A*, $r = 0.83$, $p = 0.0001$). An age-related decline of $CD4^+$ T cells was also seen among HIV-infected children (Fig. 1*A*, $r = 0.32$, $p = 0.008$). The lower r values in the infected group reflected a larger variation of the results among infected than among control children. The curve fit for the number of $CD4^+$ cells in controls was $y = -0.4358 (\log x) + 1750$, whereas that for

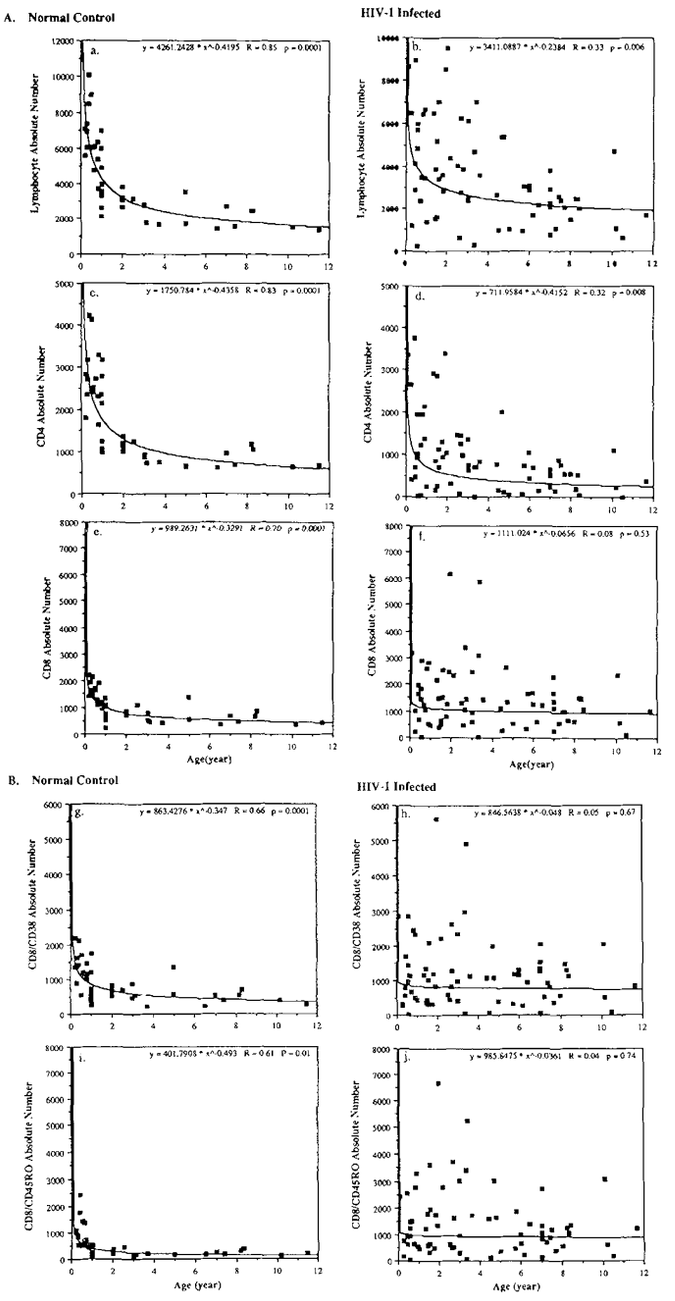

Figure 1. Correlation between age and the absolute numbers of various lymphocyte subsets in normal controls and in HIV-1-infected children. The absolute number of lymphocytes (per mm^3) of various subsets on a log scale is correlated with age (0 to 12 y) on an arithmetic scale. The left column depicts correlations obtained with the normal controls, whereas the right column depicts correlations obtained with the HIV-infected children. Correlation with age is shown for the following parameters: (*A*) (from top to bottom) 1) total number of lymphocytes, 2) number of $CD4^+$ cells, and 3) number of $CD8^+$ cells. (*B*) 1) number of $CD8^+CD38^+$ cells and 2) number of $CD8^+CD45RO^+$ cells.

HIV-infected children was $y = -0.4152 (\log x) + 712$. Thus for each age the average number of $CD4^+$ cells among HIV-infected children was less than half of that found among controls of similar age. Moreover, the rate of decline of the number of $CD4^+$ cells with age was similar in HIV-infected and control children.

The proportion of $CD8^+$ cells increased significantly with age in both controls ($r = 0.4$, $p = 0.003$) and in HIV-infected children ($r = 0.3$, $p = 0.005$, not shown). Among controls the absolute number of $CD8^+$ T cells was clearly seen to decrease with age. A striking correlation with age was noted in the controls not only for the total number of $CD8^+$ cells (Fig. 1*A*, $r = 0.70$, $p = 0.0001$) but also for the number of $CD8^+CD38^+$ ($r = 0.66$, $p = 0.0001$) (Fig. 1*B*) and $CD8^+CD45RO^+$ cells ($r = 0.61$, $p = 0.01$) (Fig. 1*B*). In contrast, in HIV-infected children no correlation between age and the absolute number of either $CD8^+$, $CD8^+CD38^+$, or $CD8^+CD45RO^+$ cells could be seen (Fig. 1*B*).

Correlation between various lymphocyte subsets. A strong correlation was noted between the expression of the CD38 and CD45RO markers on $CD8^+$ cells in individual HIV-infected children. The proportions of $CD8^+CD38^+$ and of $CD8^+CD45RO^+$ lymphocytes were closely correlated ($r = 0.9$, $p < 0.0001$) (Fig. 2*A*), as were the absolute numbers of $CD8^+CD38^+$ and of $CD8^+CD45RO^+$ cells ($r = 0.9$, $p < 0.0001$). The same strong correlation between expression of CD38 and expression of CD45RO on $CD8^+$ cells was also seen when HIV-infected children who were either younger than 2 y of age or older were analyzed separately (not shown). In contrast to the results obtained with HIV-infected children, no correlation was found between the proportion of CD8 cells expressing the CD38 and CD45RO markers in controls (not shown). No correlation was seen between the proportion of $CD8^+CD38^+$ cells and the proportion of $CD8^+CD45RA^+$ cells either among HIV-infected children (Fig. 2*B*) or among control children. A strong inverse correlation was noted between the percentage of $CD8^+$, $CD8^+CD38^+$ (Fig. 2*C*) or $CD8^+CD45RO^+$ cells, and the percentage of $CD4^+$ cells ($r =$

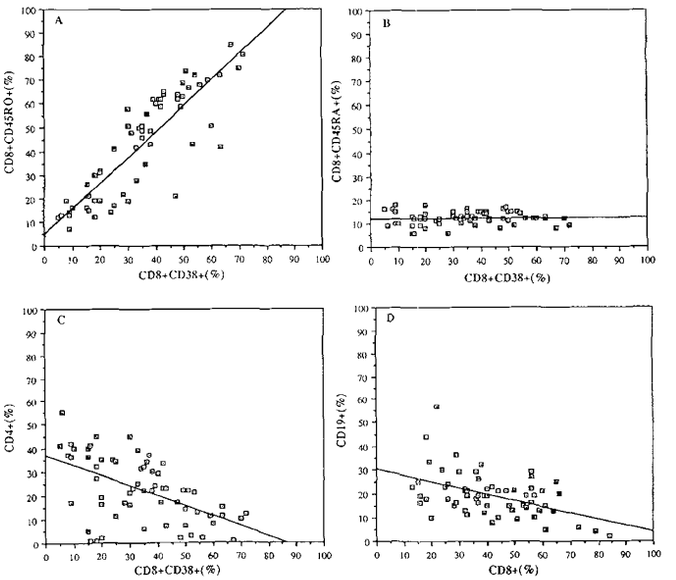

Figure 2. Correlation between various phenotypic markers in HIV-1-infected children. (*A*) Correlation between the proportion of $CD8^+CD45RO^+$ and $CD8^+CD38^+$ cells. (*B*) Correlation between the proportion of $CD8^+CD45RA^+$ and $CD8^+CD38^+$ cells. (*C*) Correlation between the proportion of $CD8^+CD38^+$ and $CD4^+$ cells. (*D*) Correlation between the proportion of $CD8^+$ and $CD19^+$ cells.

-0.4, $p = 0.0002$, $r = -0.6$, $p = 0.0001$, $r = -0.6$, $p = 0.0001$, respectively). There also was a striking inverse correlation between the proportion of $CD8^+$ (Fig. 2*D*), $CD8^+38^+$ and $CD8^+CD45RO^+$ cells and the proportion of $CD19^+$ cells ($r = -0.5$ $p = 0.0001$ for correlation with $CD8^+$ cells, and $r = -0.4$, $p = 0.005$ for correlation with either $CD8^+CD38^+$ or $CD8^+CD45RO^+$ cells).

Correlation between initial phenotype and clinical course. The phenotype of HIV-infected children, determined during their first visit, was correlated with their subsequent clinical course. The number of lymphocytes in various subsets and the survival of the children were correlated separately for HIV-infected children of the two age groups studied (Table 3). In children below the age of 2 y there was a strong correlation between low counts of $CD4^+$ cells ($<500/mm^3$) at the time of the first visit and mortality by the end of the study ($p < 0.00005$). In children less than 2 y old there also was a significant correlation between mortality and low counts of $CD8^+$ cells ($<750/mm^3$) ($p = 0.01$) and of $CD8^+CD38^+$ cells ($<600/mm^3$) ($p = 0.04$). The proportion of HIV-infected children with high counts of $CD8^+CD45RO^+$ cells ($>650/mm^3$) was higher among the survivors. There was, however, no significant correlation between the counts of this subset and mortality.

Among 2- to 12-y-old children the only statistically significant difference seen between the phenotypes of those children that remained alive throughout the study and those who had died was the number of $CD4^+$ cells at the first visit ($p = 0.009$). Pooling of the results obtained in HIV-infected children of all ages indicated a highly significant association between survival and high counts of $CD4^+$ cells, and to a lesser extent of $CD8^+$ cells.

Table 3. *Correlation of lymphocyte counts at first visit and survival*

Age at first visit (y)	Marker tested	Individuals with marker who at end of study were: Alive	Dead	Total	Significance of difference*
≤2 ($n = 21$)	$CD4^+$				
	$<500/mm^3$	0	5	5	0.00005
	$\geq 500/mm^3$	16	0	16	
	$CD8^+$				
	$<750/mm^3$	2	4	6	0.01
	$\geq 750/mm^3$	14	1	15	
	$CD8^+CD38^+$				
	$<600/mm^3$	4	4	8	0.04
	$\geq 600/mm^3$	12	1	13	
	$CD8^+CD45RO^+$				
	$<650/mm^3$	3	3	6	0.1
	$\geq 650/mm^3$	13	2	15	
>2 ($n = 29$)	$CD4^+$				
	$<500/mm^3$	6	7	13	0.009
	$\geq 500/mm^3$	15	1	16	
	$CD8^+$				
	$<750/mm^3$	6	3	9	0.6
	$\geq 750/mm^3$	15	5	20	
	$CD8^+CD38^+$				
	$<600/mm^3$	6	3	9	0.7
	$\geq 600/mm^3$	15	5	20	
	$CD8^+CD45RO^+$				
	$<650/mm^3$	7	3	10	1.0
	$\geq 650/mm^3$	14	5	19	
All ($n = 50$)	$CD4^+$				
	$<500/mm^3$	6	12	18	0.000002
	$\geq 500/mm^3$	31	1	32	
	$CD8^+$				
	$<750/mm^3$	8	7	15	0.04
	$\geq 750/mm^3$	29	6	35	
	$CD8^+CD38^+$				
	$<600/mm^3$	10	7	17	0.09
	$\geq 600/mm^3$	27	6	33	
	$CD8^+CD45RO^+$				
	$<650/mm^3$	10	6	16	0.3
	$\geq 650/mm^3$	27	7	34	

* The significance of the differences was calculated with Fisher's exact probability test.

DISCUSSION

Striking changes of the total number of lymphocytes and of various lymphocyte subsets occur during normal differentiation of the lymphoid system in children (21–25). In the present study the impact of neonatal HIV infection on the development of lymphocyte subsets was studied. After perinatal HIV infection the level of CD4 lymphocytes was already reduced within the first 2 y of life. The slope of the decline of the number of $CD4^+$ cells with age was, however, similar in HIV-infected and in control children. A comparable observation was made on the rate of decline of the proportion of $CD4^+$ cells (19). Thus, the level of $CD4^+$ cells in HIV-infected children is determined not only by viral infection but also by a differentiation process responsible for the age-related decline of the numer of CD4 cells. The proportion of $CD8^+$ cells was elevated in HIV-infected children of all age groups studied. Although in controls the absolute number of $CD8^+$ cells showed a strong negative correlation with age, no such a correlation was found in HIV-infected children. It became clear, therefore, that the level of $CD8^+$ cells in infected children was predominantly determined by viral infection.

In normal adults the CD38 activation marker (26) is expressed only on a fraction of peripheral blood $CD8^+$ cells, varying from 13 to 55% (7, 11, 27, 28) of the CD8 population. In HIV-infected adults the proportion of $CD8^+CD38^+$ cells rises early (10), and with progression of the disease into overt AIDS most CD8 cells are $CD38^+$ (9–12, 27, 28). In normal cord blood (12) and in children less than 4 y old (19) the majority of the $CD8^+$ lymphocytes express CD38. In the present study, CD38 was expressed on the majority of CD8 cells both in normal children and in HIV-infected children. In older HIV-infected children the proportion and number of $CD8^+CD38^+$ cells was considerably increased.

Activation of T cells increases the expression of the CD45RO marker while diminishing that of CD45RA (14). The proportion of $CD4^+$ or $CD8^+$ cells which express CD45RO increases in normal children with age (29). Similar to adult HIV-infected individuals (13), in seropositive children an increased proportion of CD8 cells are $CD45RO^+$ (18) and $CD45RA^-$ (19). In HIV-infected children tested in the present study, practically all of the $CD8^+$ cells were $CD45RO^+$, whereas the proportion of $CD8^+CD45RA^+$ was decreased. A

considerable percentage of the CD8 cells displayed both CD45RA and CD45RO, indicating that they were in a transitional state. The proportion of $CD8^+$ cells expressing CD45RO was higher in the present study, in which all of the children tested had HIV-related symptoms, than in the study of Froebel *et al.* (18) in which most of the HIV^+ children were asymptomatic.

Many of the markers expressed on activated lymphocytes are also present on immature lymphocytes. CD45RO, a marker of activated or memory cells (14, 15), is present on immature, cortical thymocytes (14). Similarly, the CD38 marker which is displayed by the majority of human thymus cells (30), is an early T cell activation marker (26). $CD8^+CD38^+$ PBL appearing after HIV infection could, therefore, constitute either activated T cells or immature T cells, released prematurely. In normal adults the expression of CD38 in circulating T cells is more closely associated with immaturity than with activation (12, 31). In contrast, $CD8^+CD38^+$ cells in HIV-infected individuals seem to represent activated cells, because the cytotoxic T cell activity in HIV-infected adults is correlated with the proportion of $CD8^+CD38^+$ cells (32) and the $CD8^+$ cells which exert the strongest HIV-specific cytotoxic activity express the CD38 antigen (33). In the present study the percentages of $CD8^+CD45RO^+$ cells and of $CD8^+CD38^+$ cells were strongly correlated among HIV-infected children but not among normal controls. Thus, in HIV-infected children $CD8^+CD38^+$ cells co-express the CD45RO marker and therefore probably constitute activated cells.

In adult HIV-infected individuals the most powerful prognostic indicators of disease progression were the percentage of $CD4^+$ cells and the CD4/CD8 ratio (6). In addition, a positive correlation was noted between the level of $CD8^+CD38^+$ cells in HIV-infected adults at the time of the first test and progression of HIV disease (28). After adjusting for the level of $CD4^+$ cells, high levels of $CD8^+CD38^+$ cells were shown to constitute an additional poor prognostic sign (34).

The prognostic significance of numerous laboratory tests in HIV-infected children is similar to the situation in adults (1). Neonatally infected children who have early AIDS-related symptoms and poor survival (17) were more likely to have a low $CD4^+$ count at the onset of symptoms than the other group of neonatally infected children (35). The striking prognostic relevance of the number of $CD4^+$ cells in HIV infection of children is corroborated by the results of the present study. In addition, the present study demonstrated a significant correlation between the total number of CD8 cells and $CD8^+CD38^+$ cells and the survival of children with perinatal HIV infection who were less than 2 y old at the time of entry into the study. Most of the young HIV-infected children who remained alive by the end of the study had a CD8 count of more than $750/mm^3$ and a $CD38^+CD8^+$ count of more than $600/mm^3$ when first seen. Less than 2-y-old HIV-infected children who remained alive also were more likely to have a somewhat higher $CD8^+CD45RO^+$ count at the time of study entry than those children who died. Among 2- to 12-y-old children no significant association between survival and initial CD8 and $CD8^+CD38^+$ counts was seen. The CD8 lymphocyte population plays a crucial role in the response of the host to HIV infection. $CD8^+$ cells suppress the replication of HIV-1 in $CD4^+$ cells (8, 36), although with progression of HIV disease their anti-viral activity diminishes (37). The bad prognosis of HIV-infected adults with high levels of $CD38^+CD8^+$ cells may be brought about by a number of mechanisms (34). $CD38^+CD8^+$ cells may represent $CD8^+$ cells with promiscuous cytotoxic activity that could lyse $CD4^+$ cells and thereby accelerate the progression of HIV disease. Alternatively, the rise in the number of $CD38^+CD8^+$ cells could reflect either a vigorous response of CD8 cells to an overwhelming viral load or a futile attempt to resist HIV infection by cells that have lost their anti-viral activity (36). Moreover, the expression of activation markers on T cells may accompany their transition to a state of increased susceptibility to programmed cell death. Akbar *et al.* (38) showed that $CD45RO^+$ T cells express reduced levels of bcl-2, and are more susceptible to apoptosis. Indeed, in HIV-infected individuals the augmented expression of activation markers on $CD8^+$ cells is correlated with increased propensity for apoptotic death of $CD8^+$ cells (39).

The finding that, unlike in the adult, low numbers of $CD8^+$ cells and $CD38^+CD8^+$ cells are inversely related to the survival of HIV-infected children less than 2 y of age suggests that in young children a high number of these cells may be advantageous for mounting an early and effective response to HIV infection. Indeed, the possibility now arises that the relative resistance of children to the progression of HIV infection (4, 5) may in part reflect the fact that they have high numbers of CD8 cells expressing the CD38 activation marker even before HIV infection, so that they do not have to mobilize these cells.

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