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Reference Values of Lymphocyte Subsets in Healthy, HIV-Negative Children in Cameroon▽

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Lymphocyte subset reference values used to monitor infectious diseases, including HIV/AIDS, tuberculosis, malaria, or other immunological disorders in healthy children in Cameroon, are lacking. Values for Caucasian cohorts are already being utilized for clinical decisions but could be inappropriate for African populations. We report here the immunological profile for children aged from birth through 6 years in Cameroon and also compare our values to data from other African and Caucasian populations. In a cohort of 352 healthy children, aged 0 to 6 years, the relative and absolute numbers of T-cell subsets, B cells, and NK lymphocytes were determined from peripheral blood collected in EDTA tubes. Samples were stained with BD Multitest reagents in Trucount tubes and analyzed by using CellQuest-Pro and FlowJo software. We evaluated about 23 different lymphocyte subsets in which the absolute number and percentage values differed significantly ($P < 0.05$) with age and peaked between 6 and 12 months. B-cell values were higher compared to reported values from developed countries. Differences in activated and differentiated T cells were observed in subjects between 1 and 6 years of age. The absolute $CD8^+$ T-cell count and the $CD4^+/CD8^+$ ratio seem to depend on gender. Normal lymphocyte subsets values among children from Cameroon differ from reported values in Caucasian and some African populations. The differences observed could be due to genetic and environmental factors coupled with the methodology used. These values could be used as initial national reference guidelines as more data are assembled.

Reference values of different lymphocyte subsets in a country where the HIV infection or other immune disease are widely spread are necessary for monitoring and predicting the immune status. In human immunodeficiency virus (HIV) infection, the flow cytometric determination of $CD4^+$ T lymphocytes has been one of the main immunological markers used to monitor the disease in HIV-infected children and adults.

Two-thirds of all people infected with HIV live in sub-Saharan Africa, although this region contains little more than 10% of the world's population (23). The prevalence of HIV-1 infection in Cameroon is ca. 5.5% in adults (2). In Cameroon, where many HIV-1 subtypes circulate (7, 11, 15, 21), vertical transmission of HIV contributes to a majority of the pediatric AIDS cases.

The mother-to-child transmission of HIV is ca. 19.1%, and the number of children (0 to 14 years old) living with HIV/AIDS was estimated at 45,000 in 2008. About 8,000 children are in need of antiretroviral drugs in Cameroon (2, 26). Clinical decisions are based on values published from western countries because immunological reference values in pediatric subjects are not available for the Cameroonian population. This highlights the need to obtain data on T-lymphocyte subsets among healthy HIV-uninfected children. It is well known that normal reference values differ among populations (6, 9, 12, 16), especially among children. Normally, the immune system is functionally immature at birth and undergoes a sequential development that is reflected in changing leukocyte values, a process that is genetically determined and additionally stimulated by antigen exposure (28). In the context of pediatric HIV infection, the principal immunologic change documented in large, multicentric, and longitudinal studies has been the CD4 T-lymphocyte count, which decreases rapidly in infected infants and children if compared to HIV-exposed (but uninfected) control subjects (1, 14, 19, 22). Documented evidence shows that reference values are given for the major lymphocyte subsets, including B cells, NK cells, and T cells, including both T-helper and cytotoxic T-cell subsets (8, 10, 17, 20). A smaller number of studies performed in the United States involve additional subsets, such as naive or effector T cells, with reports on activation markers being relatively rare (4, 18). Ours is the first study carried out in Africa that describes the cellular evolution, maturation, and activation of peripheral blood lymphocyte subsets in healthy children in Cameroon using a single-platform technology. The values obtained in this way could be used as a reference in other countries within this region with the same genetic and environmental factors to monitor HIV infection and other childhood diseases.

MATERIALS AND METHODS

Study design. The study was conducted in Yaounde, Cameroon. Children were enrolled at the Cite-Verte district hospital and the Mother-Child Hospital of Chantal BIYA Foundation between July 2009 and January 2010. Subjects were children from the township and were recruited for the study by the healthcare assistants at the vaccination unit and by the social assistants around the town.

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TABLE 1. Distribution of subjects in this study according to age and gender

Age of subject	No. of subjects		
	Female	Male	Total
Cord blood	22	16	38
0–3 mo	37	40	77
3–6 mo	29	24	53
6–12 mo	39	39	78
1–2 yr	27	28	55
2–6 yr	41	46	87

Since the children were below 6 years of age, only parental informed consent was obtained before assessing subjects for the study. Each individual was assessed clinically by a pediatrician or by a research physician for eligibility to take part in the study. A child was considered healthy when there was no history infectious diseases or hematological and immunological disorders. The parent or guardian of each participant completed a questionnaire. In particular, participants were assessed for signs of febrile illnesses, active infection, malnutrition, and clinical AIDS. The parent or legal guardian of each participant was questioned to determine whether the child was on any medication and whether the child had a history of any illness. An average of 60 subjects were recruited into each of 6 groups divided by sex according to age (Table 1). After parental informed consent, baseline demographic data, height, weight, and samples containing 2 to 3 ml of blood were obtained. HIV-infected children were referred to the Mother-Child Hospital of Chantal BIYA Foundation.

Exclusion criteria. Subject exclusion criteria were concurrent illness and/or medication, an axillary temperature of >38°C, malnutrition (weight for height of <70%), an unknown date of birth, and known HIV/AIDS infection. Blood samples were rejected if they were HIV infected or plasmodium positive or if the sample was clotted or rejected by the internal quality control of the FACSCalibur flow cytometry.

Investigations and flow cytometry. Samples from cord blood or peripheral blood was collected in Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA as an anticoagulant and analyzed within 6 h of collection at the Laboratory of Immunology of Chantal BIYA International Reference Centre (CIRCB).

The CIRCB algorithm for HIV detection was performed on samples from children aged 18 months and older. An HIV antibody rapid test (Determine HIV-1/2; Abbott Laboratories, Tokyo, Japan) was performed for children older than 18 months, and the Roche Amplicor v1.5 DNA PCR kit (Roche, Alameda, CA) was used for infants younger than 18 months. Confirmation was done by either PCR or serology.

A thick smear was prepared for the diagnosis of malaria and read by two experienced technicians. Table 2 shows the reagents used for sampling and the lymphocyte subsets parameters analyzed in the study. Reagents were kindly provided by BD Biosciences.

Determination of lymphocyte subsets (Table 2) was performed using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) using single-platform technology. Portions (50 μl) of whole blood were stained with 10 μl of Multitest reagent in Trucount tubes for 15 min (all from BD Biosciences). Red blood cells were lysed by using fluorescence-activated cell sorting lysing solution (BD Biosciences).

Sample data were acquired using CellQuest-Pro software (BD Biosciences) and analyzed with FlowJo software version 7.5.5 for Microsoft (TreeStar, San Carlos, CA).

Table 2 shows the lymphocytes analyzed on samples from cord blood and from peripheral blood. Since we used Multitest reagents with Trucount tubes, absolute subset count were obtained by using the formula: (the number of events in the region containing the cell population/the number of events in the absolute count bead region) × (the number of beads per tube/test volume).

Ethical approval. The study was approved by the CIRCB Ethics Committee (0024/08/CE/CIRCB) and submitted to the Ministry of Public Health who delivered an administrative authorization of research (authorization 631-11). Written informed consent was obtained from subjects' parents or legal guardian prior to blood collection.

Quality control. Weekly calibration and internal quality control of the instrument were performed using Calibrite beads (Calibrite 3 plus Calibrite APC) and FACSComp software (all from BD Biosciences). External quality control was performed on a regular basis using samples provided by UKNEQAS (United Kingdom National External Quality Assurance Scheme [United Kingdom]) and QASI (Quality Assessment Scheme for Immunology [Canada]).

Statistical methods. The effect of sex was evaluated by using a Mann-Whitney test. We used SPSS 17.0 to perform analyses. Medians and 10th and 90th percentiles were determined for all parameters within each age group. Kendall's nonparametric correlations were used to assess associations with age.

RESULTS

Study population characteristics. We recruited a total of 388 subjects, including 195 (50.3%) females and 193 (49.7%) males. The median age was 16.4 months (range, cord blood to 72 months). A total of 36 subject samples were excluded from

TABLE 2. BD reagents used and peripheral blood lymphocyte subsets measured in the study

Reagent (BD)	Subset	Anchor marker	Surface marker[a]	Subset
Multitest CD3/CD19/CD45/CD16 + 56	19	CD45	3^-/19	B cell
	16/56	CD45	3^-/natural killer	Natural killer cell
Multitest CD3/CD8/CD45/CD4	4	CD45	3/4	Helper T cell
	8	CD45	3/8	Cytotoxic T cell
	3	CD45		T cells
Multitest CD45RA/CD62L/CD3/CD4	4/45RA/62L	CD4	45RA/62L	Naive helper T cell ($CD62L^+$)
	4/45RA	CD4	$45RA/62L^+62L^-$	Naive helper T cell
Multitest CD45RA/CD62L/CD3/CD8	8/45RA/62L	CD8	45RA/62L	Naive cytotoxic T cell ($CD62L^+$)
	8/45RA	CD8	$45RA/62L^+62L^-$	Naive cytotoxic T cell
Multitest CD4/CD38/CD3/HLA-DR	4/DR/38	CD4	DR/38	Activated helper T cell
	4/DR	CD4	$DR/38^+38^-$	Activated helper T cell (DR^+)
		CD4	$38/DR^+DR^-$	Activated helper T cell ($CD38^+$)
Multitest CD8/CD38/CD3/HLA-DR	8/DR/38	CD8	DR/38	Activated cytotoxic T cell
	8/DR	CD8	$DR/38^+38^-$	Activated cytotoxic T cell (DR^+)
		CD8	$38/DR^+DR^-$	Activated cytotoxic T cell ($CD38^+$)
Multitest CD45RA/CD45RO/CD3/CD4	3/4/45RO	CD3	4/45RO	Memory helper T cells
	3/4/45RA	CD3	4/45RA	Naive helper T cells
Multitest CD45RA/CD45RO/CD3/CD8	3/8/45RO	CD3	8/45RO	Memory cytotoxic T cells
	3/8/RA	CD3	8/45RA	Naive helper T cells
αβ/γδ/CD3	γδ T cell	CD3	$\gamma\delta^+/\alpha\beta^-$	γδ T cell
	αβ T cell	CD3	$\gamma\delta^-/\alpha\beta^+$	αβ T cell

[a] The presence of a surface marker on a cell is assumed unless the marker is followed by a superscript minus sign. The presence of both plus and minus signs indicates a total cell population.

TABLE 3. Absolute lymphocyte subset concentration by age from peripheral blood from healthy children from Cameroon

Subset	Total no. of samples	Median lymphocyte subset concn (10th and 90th percentiles) for the indicated age group[a]						*P*	
		Cord blood	0–3 mo	3–6 mo	6–12 mo	12–24 mo	2–6 yr	Age	Sex
CD3	351	2,249 (430–2,977)	3,085 (2,352–4,776)	3,120 (1,123–4,378)	3,522 (2,039–5,024)	2,639 (794–3,307)	2,299 (1,159–3,242)	<0.00	0.95
CD4	351	1,552 (330–1,995)	2,001 (1,642–3,472)	2,133 (759–3,205)	2,252 (1,311–3,273)	1,667 (596–1,949)	1,289 (674–1,721)	<0.00	0.46
CD8	347	554 (140–1,188)	790 (570–1,714)	726 (298–1,413)	995 (563–1,796)	823 (194–1,165)	775 (308–1,249)	<0.00	0.34
CD19	349	552 (85–853)	1,525 (513–3,748)	1,940 (696–3,370)	1,858 (662–2,870)	1,526 (487–2,066)	965 (432–1,691)	<0.00	0.32
NK	349	496 (135–969)	546 (262–1,030)	450 (140–1,229)	504 (109–831)	397 (151–848)	333 (133–1,042)	0.05	0.32
4/DR/38	337	19 (7–79)	60 (39–137)	57 (25–126)	61 (38–185)	56 (21–110)	48 (23–91)	<0.00	0.28
4/DR	340	21 (9–82)	75 (56–164)	74 (29–155)	77 (45–209)	70 (29–127)	65 (34–122)	<0.00	0.4
4/38	343	1,496 (324–2,009)	1,882 (1,550–3,344)	1,987 (547–3,291)	2,065 (1,097–2,838)	1,169 (464–1,703)	1,043 (496–1,475)	<0.00	0.27
8/DR/38	339	6 (2–25)	31 (8–278)	45 (12–319)	99 (29–338)	71 (19–210)	61 (32–275)	<0.00	0.28
8/DR	339	6 (2–25)	34 (8–287)	45 (13–324)	102 (30–342)	81 (21–216)	77 (39–307)	<0.00	0.35
8/38	330	585 (160–1,399)	803 (454–2,305)	702 (259–1,410)	975 (461–1,841)	646 (169–1,132)	548 (249–983)	<0.00	0.21
3/4/RO	329	171 (44–873)	293 (183–688)	339 (205–538)	404 (298–615)	419 (218–634)	446 (260–663)	<0.00	0.21
3/4/RA	333	859 (109–1,459)	1,110 (589–2,230)	1,265 (306–2,271)	1,338 (479–2,285)	724 (288–1,474)	614 (365–1,078)	<0.00	0.24
4/RO	342	151 (52–321)	340 (166–480)	372 (239–611)	409 (308–752)	409 (231–653)	498 (306–718)	<0.00	0.03
4/RA	342	1,356 (934–1,941)	1,894 (968–3,087)	1,632 (451–2,796)	1,916 (994–2,931)	880 (359–2,010)	839 (482–1,657)	<0.00	0.38
3/8/RO	325	31 (8–141)	55 (26–194)	70 (17–343)	129 (71–371)	124 (38–221)	135 (57–344)	<0.00	0.64
3/8/RA	332	459 (63–1,283)	555 (436–1,189)	529 (210–1,007)	693 (362–1,469)	539 (175–899)	483 (206–910)	<0.00	0.08
8/RO	329	52 (13–133)	66 (17–228)	74 (18–320)	156 (38–395)	131 (52–438)	158 (63–390)	<0.00	0.44
8/RA	338	507 (279–1,139)	788 (413–1,531)	653 (376–1,374)	838 (443–1,515)	535 (223–1,222)	611 (250–1,046)	<0.00	0.19
4/RA/62L	281	1,201 (693–1,589)	1,392 (798–2,304)	1,304 (453–2,134)	1,541 (763–2,538)	708 (282–1,559)	665 (395–1,334)	<0.00	0.62
8/RA/62L	231	457 (91–826)	545 (272–809)	404 (219–757)	524 (236–1,042)	330 (120–555)	384 (156–641)	<0.00	0.64

[a] Values are medians (10th and 90th percentiles) obtained from BD absolute count with Tru-Count tube.

the analysis: 10 samples were infected with HIV-1, 21 showed malaria parasitemia, and 5 were coagulated. All subjects were well nourished (weigh for height of >80% with no edemas) and had no fever or diarrhea reported in the previous few days. Of the 352 remaining subjects included in the analysis, 180 (51.1%) were female and 172 were male. A small sex imbalance was present in some groups.

Variation of absolute lymphocyte count with age. In addition to median values, we tabulated the 10th and 90th percentiles. Twenty-one lymphocyte subset distributions by age are shown in Table 3. We observed that the numbers of B, NK, and T lymphocytes—both $CD4^+$ and $CD8^+$—increased from birth, peaked at between 6 and 12 months of age, and then decreased gradually by 2 to 6 years.

The absolute number of $CD3^+$ lymphocytes increased 1.6-fold from birth to between 6 to 12 months of age and decreased 1.5-fold between 2 and 6 years of age. The absolute number of $CD4^+$ T cells followed the same pattern; compared to other countries (Table 4), we observed some differences. A similar trend was observed among $CD19^+$ B lymphocytes, with a 3.3-fold increase during the first year of life and a 1.9-fold decrease occurring between 2 and 6 years of age. Activated helper T cells increased 2.5-fold from birth through 2 to 6 years of life, while activated cytotoxic T cells increased 10 times within the same period. The absolute number of helper and cytotoxic T cells increased 2.6- and 4.3-fold, respectively, from birth through 2 to 6 years of age, whereas the number of naive helper and cytotoxic T cells increased 1.5-fold during the first year and then decreased 1.8- and 1.4-fold, respectively, by 2 to 6 years.

Variation with age in lymphocyte subset percentages. Table 5 shows that children experience a gradual decrease in the percentage of both total $CD3^+$ T cells and $CD4^+$ T cells from medians of 65 and 44% at birth to medians of 59 and 35% between 2 to 6 years, respectively. The percentage of $CD8^+$ T cells increased from a median of 17% at birth to a median of 20% from 6 months to 6 years.

The percentage of B cells increased 2-fold from birth through 3 to 6 months of age and decrease gradually until 1.5-fold between 2 to 6 years of life. A different pattern was seen for the percentage of NK cells, which decreased 2-fold from birth through 3 months to 6 years of age. The $CD4^+$/$CD8^+$ ratio was 2.8 at birth and dropped to 1.8 in the following 6 years, most likely due to a larger expansion of $CD8^+$ T cells compared to $CD4^+$ T cells early in life. These results are similar to those of a study conducted in Europe (24). The γδ T

TABLE 4. Comparative values of CD4 absolute count from different countries

Country	CD4 absolute count (range) for the indicated age group[a]						Source or reference
	Cord blood	0–3 mo	3–6 mo	6–12 mo	12–24 mo	2–6 yr	
Cameroon	1,552 (330–1,995)	2,001 (1,642–3,472)	2,133 (759–3,205)	2,252 (1,311–3,273)	1,667 (596–1,949)	1,289 (674–1,721)	This study
United States		2,610 (1,600–4,000)	2,850 (1,800–4,000)	2,670 (1,400–4,300)	2,160 (1,300–3,400)	1,380 (700–2,200)	19
Netherlands		1,985 (1,294–4,012)*		2,721 (1,327–4,455)	2,073 (1,902–2,977)		25
Malawi		2,000 (1,300–4,200)*		2,200 (1,600–3,300)			10
Kenya		2,004 (1,515–2,493)			1,887 (1,429–2,345)		3
Zambia		2,131 (996–4,975)†			1,806 (1,035–3,531)‡		12
Uganda		1,693 (700–3,514)†			1,517 (733–2,943)‡		9
Saudi Arabia		3,050 (1,390–6,696)†			2,062 (607–3,854)		18
Ethiopia		1,816 (845–2,399)					23

[a] *, Values for children 0 to 6 months old; †, values for children less than 12 months old; ‡, values for children 1 to 6 years old.

TABLE 5. Percentage lymphocyte subset concentration by age from peripheral blood from healthy children in Cameroon

Subset	Total no. of samples	Median % lymphocyte concn (10th and 90th percentiles) for the indicated age group[a]						*P*	
		Cord blood	0–3 mo	3–6 mo	6–12 mo	12–24 mo	2–6 yr	Age[b]	Sex
CD3	352	65 (53–73)	60 (44–75)	52 (44–66)	59 (49–72)	57 (39–74)	59 (50–73)	NS	0.17
CD4	352	44 (34–57)	41 (32–57)	37 (25–51)	38 (28–48)	34 (21–46)	35 (26–44)	NS	0.71
CD8	352	17 (11–30)	16 (10–27)	14 (10–21)	20 (13–27)	18 (11–30)	20 (14–30)	NS	0.02
T4/T8	351	3 (2–5)	3 (2–5)	3 (2–5)	3 (2–4)	2 (1–4)	2 (2–3)	NS	0.04
CD19	349	16 (9–23)	27 (11–43)	34 (20–46)	30 (18–41)	29 (12–50)	25 (17–38)	NS	0.63
NK	349	16 (6–28)	10 (5–15)	8 (6–33)	8 (4–13)	8 (4–26)	8 (4–25)	NS	0.41
4/DR/38	338	2 (1–6)	3 (2–5)	3 (2–8)	3 (2–8)	4 (3–8)	4 (2–7)	NS	0.88
4/DR	337	2 (1–6)	4 (3–7)	4 (3–11)	4 (3–9)	5 (3–11)	5 (3–9)	NS	0.83
4/38	344	98 (95–99)	94 (89–97)	92 (77–96)	92 (83–95)	86 (71–90)	79 (71–84)	NS	0.44
8/DR/38	340	1 (1–4)	5 (2–32)	7 (2–29)	10 (6–32)	10 (6–33)	9 (6–23)	NS	0.75
8/DR	343	1 (1–7)	6 (2–32)	8 (2–29)	11 (6–32)	11 (6–34)	10 (8–33)	NS	0.66
8/38	345	95 (89–98)	97 (81–99)	95 (77–97)	93 (77–99)	84 (56–95)	79 (54–88)	NS	0.85
3/4/RO	333	10 (3–32)	11 (5–19)	12 (7–23)	12 (9–25)	16 (11–33)	19 (15–30)	NS	0.22
3/4/RA	334	41 (19–59)	41 (20–59)	36 (16–58)	38 (20–58)	29 (16–53)	27 (18–40)	NS	0.24
4/RA	342	90 (84–96)	85 (72–90)	81 (52–91)	80 (68–87)	67 (50–83)	64 (54–76)	NS	0.56
4/RO	342	10 (4–16)	15 (10–28)	19 (9–48)	20 (13–32)	33 (17–50)	36 (24–46	NS	0.56
3/8/RO	323	3 (1–6)	3 (1–12)	4 (1–14)	5 (3–19)	5 (3–13)	7 (4–16)	NS	0.7
3/8/RA	300	22 (11–40)	20 (14–49)	20 (15–25)	22 (14–54)	20 (15–69)	21 (16–49)	0.05	0.07
8/RA	329	91 (79–96)	93 (74–96)	88 (74–97)	84 (66–94)	75 (54–94)	80 (64–89)	NS	0.11
8/R0	338	9 (4–21)	7 (4–26)	12 (3–26)	16 (6–34)	26 (6–46)	20 (11–36)	NS	0.11
4/RA/62L	282	77 (62–85)	66 (53–78)	62 (43–72)	64 (52–77)	54 (38–68)	51 (42–64)	NS	0.97
8/RA/62L	236	75 (58–88)	71 (30–83)	62 (35-79)	53 (34–76)	49 (20–72)	48 (28–70)	NS	0.49
γδ	225	3 (2–5)	5 (2–6)	5 (3–8)	7 (4–15)	8 (5–18)	9 (6–16)	NS	0.47
αβ	224	94 (86–97)	91 (84–96)	90 (82–95)	89 (81–94)	88 (77–93)	88 (79–93)	NS	0.21

[a] Values are medians (10th and 90th percentiles) and are presented as percentages of the total lymphocyte concentration except for the ratio of CD4 T lymphocytes to $CD8^+$ T lymphocytes.
[b] NS, not significant.

cells in our cohort increased 3-fold, while in a Malawi population (10) the corresponding value was <2-fold.

The changes in distribution of $CD4^+$ and $CD8^+$ T-cell maturation subsets are equally compelling: the percentage of memory helper and cytotoxic T cells increased ~2-fold from birth to 6 years of age. The activated helper CD4/HLA-DR/CD38 T-cell populations increased 2-fold, while the activated cytotoxic CD8/HLA-DR/CD38 T-cell population increased 9-fold from birth to between 2 to 6 years of age. Table 6 shows comparative values for CD4 count percentages in African, Asian, and developed countries.

DISCUSSION

In this study, we assessed lymphocyte subsets from cord blood and from venous blood obtained from 352 healthy Cameroonian children, ranked by age and sex, not infected by HIV. Our study shows that changes in the absolute number of lymphocytes are not consistent with changes in their relative numbers. Lymphocyte subset concentrations peaked in the first year of life and decreased thereafter during childhood. We observed differences between studies conducted in developed countries but noted similarities to studies conducted in sub-Saharan Africa. The relative value of T lymphocytes in children 0 to 6 years old is higher in developed countries than in Cameroon and Malawi (10, 18). The mean T-lymphocyte concentration for children 0 to 12 months old in developed countries is higher, while the values are similar in African children between 2 and 6 years old (24). The B-lymphocyte concentration and relative frequency is higher in Cameroon children than in developed countries. The same has been observed in

TABLE 6. Comparative value of CD4 count percentages from different countries

Country	Comparative CD4 count percentage for the indicated age group[a]						Source or reference
	Cord blood	0–3 mo	3–6 mo	6–12 mo	12–24 mo	2–6 yr	
Cameroon	44 (34–57)	41 (32–57)	37 (25–51)	38 (28–48)	34 (21–46)	35 (26–44)	This study
United States		52 (35–64)	46 (35–56)	41 (32–51)	38 (28–47)	37 (31–47)	19
Netherlands	46.7 (40.2–61.9)	43.2 (37.6–46.4)*		43.5 (29.8–63.4)	42.4 (33.0–55.0)		25
Malawi		39 (29–49)*		36 (27–44)			10
Kenya		39.9 (37.8–42.2)			33.9 (31.7–36.1)		3
Zambia		36 (22–48)†			34 (23–49)‡		12
Uganda		31.9 (18.7–44.4)†			33.5 (18.8–45.8)‡		9
Saudi Arabia		45.6 (28–62)†			40.5 (29–50)		18
Ethiopia	46 (35–67)						23

[a] *, Values for children 0 to 6 months old; †, values for children less than 12 months old; ‡, values for children 1 to 6 years old.

other African countries (19), most likely reflecting the contribution of environmental or genetic factors.

The present study was undertaken to better understand the $CD4^+$ T-cell values as an important determinant for initiating antiretroviral drug treatment. Whereas in Cameroon the peak median $CD4^+$ T-cell concentration is lower than in developed countries (2.2×10^3cells/μl versus 2.85×10^3cell/μl) (18) (Table 5), the peak $CD8^+$ T-cell concentration is similar ($P > 0.05$) in both areas. Compared to studies in developed countries (4, 18), the $CD4^+$ T-lymphocyte percentages in Cameroonian children are lower in the first year of life and remained relatively stable in later life, with median values between 35 and 38%. Similar values have been observed in the Kenyan population (3), with a 39.9% median in the first 3 months of age and a 36.5% median at 4 to 12 months of age. In Ugandan children younger than 1 year old (Table 6), the median $CD4^+$ T-lymphocyte percentage was particularly low at 31.9% (9), whereas Nigerian children younger than 1 year old have a low $CD4^+$ T-lymphocyte count (percentage and concentration) directly comparable to that observed in other African countries (5). These studies indicate that the low $CD4^+$ T-lymphocyte percentages observed in Cameroonian children are not unusual for Africa.

In our study, the $CD4^+/CD8^+$ ratio varied between 2.75 and 1.76, while in the Malawian study (10), the value varied from 2 to 1.5. The discrepancy could be due to the lower value of $CD8^+$ T cells observed in Cameroonian children compared to Malawian children.

There are no reported data from Africa describing variations with age in activated, memory, and naive T-lymphocyte subsets. The trends on these subsets observed in Cameroon are similar to those described in developed countries. The median values for activated cytotoxic cells ($CD8/HLA\text{-}DR/CD38^{+/-}$), however, varied significantly ($P < 0.01$) from 92 to 79% in Cameroon children aged 6 months to 6 years and eventually from 95 to 87% in children of the same age in developed countries.

The present study shows—similar to studies performed in developed countries—that age is a highly significantly determinant in lymphocyte subset counting. A statistical significance was detected with all subsets investigated for both absolute and percent concentrations except for CD3/CD4/CD62L/CD45RA naive helper T cells.

Our findings have important implications for current guidelines regarding the immunologic assessment of HIV-infected children in Cameroon. Although the median and percentile values determined here were derived from healthy, HIV-negative children from Cameroon, the relatively low $CD4^+$ T-lymphocyte percentages in children younger than 1 year old means that 27% of these children would have met the World Health Organization (WHO) $CD4^+$ T-cell criterion for immunodeficiency in HIV-infected children (25); ca. 35% of Malawian children would meet this criterion (19). The data presented thus suggest that caution should be exercised when applying guidelines for the immunologic assessment of HIV-infected African children. A study completed in South Africa proposed to use a CD4/CD8 ratio of <1.0 as a parameter for HIV infection. The same has been suggested for early infant diagnosis of HIV in African children younger than 18 months old. This should be done where DNA PCR is not available and where the results of HIV rapid testing can be complicated by maternal antibodies (13, 29). These findings show that 4% of Cameroonian children younger than 18 months had a $CD4^+/CD8^+$ ratio of <1.0. This underlines the importance of using $CD4^+$ T-cell measurement in conjunction with clinical assessment and viral load. The most recent WHO guideline (27, 29) indicates that all children positive for HIV should be put on antiretroviral treatment without considering the number of $CD4^+$ T cells (27). Therefore, we do not suggest the use of a $CD4^+/CD8^+$ ratio of <1.0 for HIV infection diagnosis in children, at least not in Cameroon and other similar countries. It is recommended that laboratories involved in DNA PCR detection implement early HIV infection diagnosis in children born from HIV-positive mothers.

In conclusion, our study on lymphocyte subsets is the first study performed in Africa on neonates and very young children. It is also the first with the single-platform technology using Multitest reagents with Trucount tubes, while other studies are performed by using the double-platform technology. In addition, we provide here additional information on lymphocyte subsets in Cameroon with respect to subject age and gender. Our findings suggest that caution should be used when the immunologic status of HIV-infected African children is being assessed; clinicians should take into consideration the clinical status of the child along with other laboratory parameters. The findings presented here may have application to other countries with similar socioeconomic, cultural, and environmental conditions.

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