

T-Cell Subsets in Healthy Teenagers: Transition to the Adult Phenotype¹

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Little is known about the normal range and variability of T-cell subsets in older children. We analyzed peripheral blood mononuclear cell subsets in 112 healthy children, ages 12-19 years (mean \pm SD: 15.4 \pm 1.9 years), using monoclonal antibodies and flow cytometry. The study population included 28 blacks and 84 whites, with 59 boys and 53 girls. The mean \pm SD cell subset values were: CD3+ T cells, 74.0 \pm 7.8%; CD4+ helper-inducer T cells, 46.8 \pm 6.9%; CD8+ suppressor-cytotoxic T cells, 27.3 \pm 5.7%; CD4:CD8 helper:suppressor ratio, 1.81 \pm 0.57; CD16+ natural killer cells, 4.4 \pm 3.1%; CD19+ B cells, 10.0 \pm 5.3%; CD14+ monocytes, 20.0 \pm 6.5%; and HLA-DR cells, 15.4 \pm 4.8%. Overall, boys had a higher proportion of HLA-DR+ cells than girls, attributable to an increase in CD19+ B cells. Blacks tended to have a higher proportion of HLA-DR+ cells than whites, apparently due to an increase in activated T cells. Detailed analysis by age group revealed a striking transition in the pattern of CD4+ and CD8+ cell populations. The CD4:CD8 ratio, higher in boys than girls for ages 12-16, was reversed to the "adult" pattern in 17-19 year olds, with a higher CD4:CD8 ratio in girls. These data provide important baseline values for healthy children and stress the importance of establishing normative ranges for pediatric subjects separately from adults. © 1990 Academic Press, Inc.

INTRODUCTION

Technical advances in monoclonal antibody production and flow cytometry have allowed these techniques to be utilized in a wide variety of clinical and epidemiological settings (1, 2). T-cell subset determinations are frequently employed to study patients with lymphoproliferative malignancies or immunodeficiency states and to monitor rejection in organ transplant recipients (3-5). With the rapid accumulation of flow cytometry data has come the recognition that age, race, gender, and environmental exposures (e.g., cigarette smoking) may significantly influence mononuclear cell subset values, even in healthy individuals (6-16). The influence of demographic variables on T-cell subsets has been exten-

¹ Supported in part by USPHS Contract Y01-CP-30500 and NIH Grant HL07427.

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sively studied in adults, but considerably less is known about the normal range and variability of these values in children. Data on neonates and young children have begun to accumulate, but few studies have addressed the period of transition between childhood and adulthood. To study children in this critical age range, we determined peripheral blood mononuclear cell subset levels in 112 healthy teenage children.

METHODS

Population Selection

Children for this investigation were enrolled from households participating in a large population-based study of T-cell subsets. Selection of the households has been described in detail elsewhere (17). Briefly, random digit dialing was utilized to select a population-based random sample of adult subjects in the Washington, D.C., metropolitan area. Demographic, life-style, and medical information was collected through telephone and self-administered questionnaires, and approximately one-third of potential adult study subjects were excluded on the basis of life-style characteristics or medical conditions which might affect the immunologic parameters under investigation. Racial groups other than whites and blacks were excluded due to the small number of subjects predicted from census data. Participating adults with children between the ages of 12 and 19 were asked to enroll one or two children from the household in the present study. A health questionnaire was administered to each child (with parental assistance as required) and a blood sample was drawn for cell subset analysis and routine chemistry and hematology studies. From the 374 households included in the adult study, 112 children from 83 households participated in the present investigation. Also included for comparison purposes in part of the analysis were 114 nonsmoking adults aged 20-39 who participated in the adult study (13).

Sample Preparation and Flow Cytometry Analysis

Phlebotomy was performed by a nurse phlebotomist at a specially equipped mobile van. Blood samples were submitted to a commercial laboratory for routine hematology and chemistry analyses. Peripheral blood mononuclear cells (PBMC) were separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation, washed, counted, and resuspended in modified RPMI 1640 medium as previously described (18). Aliquots of 10 million mononuclear cells were then cryopreserved in DMSO using a controlled rate freezer and stored in liquid nitrogen until needed for flow cytometry analysis.

The following directly fluorescein-conjugated monoclonal antibodies, purchased from Ortho Diagnostics, Raritan, New Jersey (ORTHO), or Becton-Dickinson Monoclonal Center, Mountain View, California (BD), were utilized: OKT3 (CD3+ T cells; ORTHO); OKT4 and OKT4A (CD4+ helper-inducer T-cell subset; ORTHO); OKT8 (CD8+ suppressor-cytotoxic T-cell subset; ORTHO); anti-Leu 12 (CD19+ B cells; BD); anti-Leu M3 (CD14+ monocytes; BD); anti-Leu 11A (CD16+ natural killer cells; BD) anti-HLA-DR (nonpolymorphic HLA-DR antigen; BD); and mouse IgG1 (clone 11-63; BD) and IgG2(a+b)

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(clones 11-4.1 and MPC-11; BD) as negative control reagents (13). The surface antigen recognized by anti-HLA-DR is reported to be on the surface of B cells, monocytes, and activated T cells (19, 20). To help separate the contribution of each cell type to the pool of HLA-DR+ cells, the relative proportion of activated T cells (ACT-T) was estimated by subtracting from the HLA-DR+ cell pool the number of B cells (Leu 12+) and the number of monocytes (Leu M3+) not eliminated by right-angle scatter gating.

Prepared samples were analyzed on a FACS II cell sorter (Becton-Dickinson) interfaced to a PDP 11/24 DEC computer (Digital Equipment Corp., Landover, MD). The forward-angle light scatter window was set to exclude electronic noise, debris, and damaged or dying cells, while the right-angle light scatter window was set to exclude monocytes (21). For analysis of monocyte surface markers, the right-angle light scatter window was opened to allow for viewing of all mononuclear cells. Ten thousand gated events were collected for each monoclonal antibody tested.

In comparisons of flow cytometry analyses on fresh cells with that of frozen samples in our laboratory, freezing did not significantly alter the proportion of positively stained cells for CD3+, CD4+, CD8+, CD14+, CD16+, and CD19+ cells. A small but statistically significant increase in the proportion of positively stained cells was observed for HLA-DR+ cells after freezing (fresh cells, 15.0 ± 1.1% HLA-DR+ vs frozen cells, 16.7 ± 1.2% HLA-DR+; $P = 0.01$).

Statistical Analysis

Study subjects were divided into three age groups: 12–14 years ($n = 38$); 15–16 years ($n = 41$); and 17–19 years ($n = 32$). Gender-related effects were analyzed using analysis of variance, controlling for age group and race (22). The same approach was applied to race-related effects, controlling for age group and gender. Multiple linear regression analysis was employed to evaluate the independent effect of age, controlling for race and gender (23). All reported P values were derived from multivariate models, adjusting for the effects of all other independent variables of interest. To achieve a more normal distribution of values, all analyses were repeated utilizing log-transformed values for cell counts and arcsin-transformed values for cell proportions. When significance estimates for the transformed analyses differed from those of nontransformed analyses, P values from the transformed analyses are presented.

RESULTS

Population Characteristics

The study population included 59 boys and 53 girls. Subjects ranged in age from 12 through 19 years (mean ± SD: 15.4 ± 1.9 years), and 26% were black. Data on pubertal status or state of maturation were not available. Therefore, study subjects were categorized into three age groups to approximate developmental stages: 12–14 years (mostly prepubertal); 15–16 years (pubertal); and 17–19 years (mostly postpubertal). Hemoglobin and hematocrit values were similar in boys and girls aged 12–14 years, but were significantly lower in girls compared to boys

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for 15–16 year olds and for 17–19 year olds ($P < 0.05$), consistent with our developmental stratification scheme.

Mononuclear Cell Subsets

The distribution of PBMC subsets in boys and girls by age group is shown in Table 1. Overall, the only statistically significant gender-related differences were a higher proportion of HLA-DR+ cells among boys ($P = 0.03$), attributable to an increase in the proportion of CD19+ cells (B cells). CD3+ (T cells) were correspondingly lower in boys than in girls, but the difference did not reach statistical significance ($P = 0.11$). There was no indication of an increase in activated T cells among boys, and no age-related changes in HLA-DR+ cells were observed. There were no significant gender-related differences in the proportion of CD16+ cells (natural killer cells) or CD14+ cells (monocytes).

Although overall mean values for the major T-cell subsets were similar in boys and girls, striking boy:girl differences in the pattern of cell subset proportions were apparent over the three age groups (Fig. 1). The percentage of CD4+ cells was similar in boys and girls for 12–14 year olds and 15–16 year olds, but was substantially higher among girls in the 17–19 year age group. Conversely, CD8+ cells were higher in girls than boys for 12–14 year olds and 15–16 year olds, but equal in boys and girls aged 17–19 years. The CD4:CD8 (helper:suppressor) ratio, higher in boys than girls for ages 12–14 and 15–16 years, was reversed in the 17–19 year olds, with a higher CD4:CD8 ratio among girls.

TABLE 1
MONONUCLEAR CELL SUBSETS IN BOYS AND GIRLS BY AGE GROUP^a

Cell surface antigen		Age group (years)			All ages
		12–14	15–16	17–19	
CD3	Boys	72.2 ± 1.5	72.9 ± 1.4	73.7 ± 2.0	73.0 ± 1.0
	Girls	74.1 ± 1.6	75.6 ± 0.8	76.3 ± 2.1	75.1 ± 0.8
CD4	Boys	47.5 ± 1.6	46.7 ± 1.4	45.8 ± 1.9	46.6 ± 1.0
	Girls	47.1 ± 1.7	45.9 ± 1.2	45.9 ± 1.7	46.9 ± 0.9
CD8	Boys	25.4 ± 1.0	25.4 ± 1.2	28.2 ± 1.2	26.6 ± 0.7
	Girls	26.7 ± 1.4	29.3 ± 1.2*	28.3 ± 1.8	28.1 ± 0.8
CD4:CD8	Boys	1.93 ± 0.1	1.93 ± 0.13	1.73 ± 0.14	1.85 ± 0.08
	Girls	1.88 ± 0.1	1.64 ± 0.10	1.84 ± 0.19	1.77 ± 0.08
CD16	Boys	5.3 ± 0.9	4.6 ± 0.9	4.6 ± 0.7	4.8 ± 0.5
	Girls	3.7 ± 0.6	4.0 ± 0.7	4.1 ± 0.9	3.9 ± 0.4
CD19	Boys	11.6 ± 1.5	12.8 ± 1.1	9.5 ± 1.2	11.1 ± 0.7
	Girls	9.4 ± 1.1	8.1 ± 1.0*	8.6 ± 1.6	8.7 ± 0.7*
CD14	Boys	19.4 ± 1.0	18.2 ± 1.2	20.5 ± 1.3	19.5 ± 0.7
	Girls	21.1 ± 2.0	20.1 ± 1.5	20.1 ± 1.2	20.5 ± 1.0
HLA-DR	Boys	17.3 ± 1.5	16.0 ± 1.2	16.2 ± 0.9	16.4 ± 0.7
	Girls	14.0 ± 1.0	14.6 ± 1.1	14.6 ± 1.5	14.4 ± 0.6*
ACT-T	Boys	3.5 ± 1.6	2.1 ± 0.9	5.0 ± 1.4	3.7 ± 0.8
	Girls	2.7 ± 0.9	3.0 ± 1.4	4.1 ± 1.0	3.5 ± 0.7

^a Values expressed as percentage of positively staining lymphoid cells (means ± SE).

* $P < 0.05$ for boys vs girls by analysis, adjusting for age group and race.

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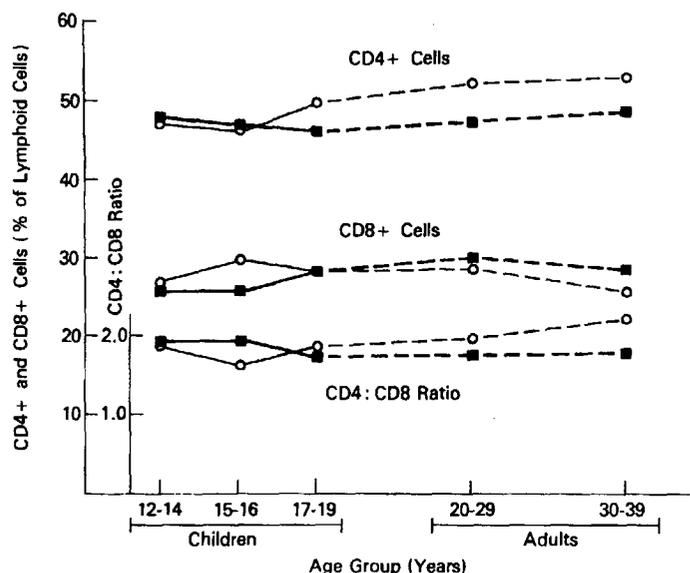


FIG. 1. Proportion of CD4+ and CD8+ cells and the CD4:CD8 ratio. Values for male (squares with solid line) and female (circles with solid line) children by age groups are compared to values among nonsmoking adult males (squares with broken line) and females (circles with broken line) aged 20-29 years ($n = 53$) and 30-39 years ($n = 61$). Data for adults from Ref. (13).

Statistically significant differences between blacks and whites were limited to the HLA-DR+ cell subset (Table 2). The higher proportion of HLA-DR+ cells among black children appeared to be due to an increase in activated T cells among blacks ($P = 0.01$), not B cells or monocytes. The proportion of CD3+ cells (T cells) was consistently higher among whites than blacks, but the difference was not statistically significant.

DISCUSSION

Information on normal values for peripheral blood T-cell and other mononuclear cell subsets in children is extremely limited, particularly for older children. To date, most reports have documented T-cell subset alterations in a variety of childhood disease states, including premature birth (25), congenital rubella (26), pertussis (27), allergic disorders (28, 29), and congenital immunodeficiency states (30). Recently, analyses of T-cell subsets in human umbilical cord blood (31), neonates (32, 33), and healthy children ages 2 months to 13 years (34) have begun to provide data on healthy subjects. The report by Yanase *et al.* (34) is noteworthy for the application of stratified and multivariate statistical approaches to dissect the independent influences of age and gender on T-cell subset proportions. Whether their observations in Japanese children can be generalized across international and racial boundaries, however, has not been determined. These reports have concentrated on young, prepubertal children, where gender differences might be expected to be minor. Information on racial differences has been mini-

TABLE 2
MONONUCLEAR CELL SUBSETS IN WHITE VS BLACK CHILDREN BY AGE GROUP^a

Cell surface antigen		Age group (years)			
		12-14	15-16	17-19	All ages
CD3	Whites	73.6 ± 1.2	74.9 ± 1.0	75.8 ± 1.6	74.7 ± 0.7
	Blacks	71.7 ± 2.6	73.3 ± 1.2	71.0 ± 4.1	72.1 ± 1.5
CD4	Whites	46.9 ± 1.2	47.1 ± 1.1	46.2 ± 1.7	46.8 ± 0.7
	Blacks	48.8 ± 3.3	44.1 ± 1.4	48.0 ± 3.4	46.5 ± 1.5
CD8	Whites	26.3 ± 1.0	27.8 ± 1.22	28.9 ± 1.22	27.6 ± 0.7
	Blacks	25.2 ± 2.0	27.1 ± 1.0	27.6 ± 1.9	26.8 ± 0.9
CD4:CD8	Whites	1.87 ± 0.10	1.81 ± 0.10	1.69 ± 0.11	1.80 ± 0.06
	Blacks	2.03 ± 0.23	1.67 ± 0.11	1.85 ± 0.31	1.82 ± 0.12
CD16	Whites	4.0 ± 0.5	4.1 ± 0.6	4.2 ± 0.7	4.1 ± 0.3
	Black	6.0 ± 1.6	4.8 ± 1.1	5.0 ± 1.1	5.2 ± 0.7
CD19	Whites	10.4 ± 1.0	10.4 ± 1.0	9.3 ± 1.2	10.1 ± 0.6
	Blacks	10.2 ± 2.6	9.8 ± 1.7	8.2 ± 0.9	9.4 ± 1.0
CD14	Whites	19.9 ± 1.4	20.2 ± 1.3	19.7 ± 1.0	19.9 ± 0.7
	Blacks	22.6 ± 2.0	17.2 ± 1.1	22.7 ± 2.5	20.3 ± 1.1
HLA-DR	Whites	14.5 ± 0.8	15.1 ± 1.0	15.0 ± 0.9	14.8 ± 0.5
	Blacks	19.6 ± 2.5*	15.5 ± 1.6	17.4 ± 1.6	17.1 ± 1.1*
ACT-T	Whites	2.1 ± 0.8	2.9 ± 1.1	3.8 ± 1.3	2.8 ± 0.6
	Blacks	7.8 ± 2.8*	3.8 ± 1.8	7.7 ± 1.5	6.0 ± 1.0*

^a Values expressed as percentage of positively staining lymphoid cells (means ± SE).

* $P < 0.05$ for whites vs blacks by analysis of variance, adjusting for age group and gender.

mal. The immunologic changes which occur during the transition between childhood and adulthood have not been characterized. To investigate this age group, we enrolled teenage children from households participating in a population-based survey of mononuclear cell subsets in healthy adults.

A number of significant age, race, and gender effects observed in this population differed from those observed among nonsmoking adults in the study from which these children were recruited (13). Particularly notable was the pattern of T-cell subset distributions in boys and girls over the three age groups. For comparison, T cell subset levels for nonsmokers aged 20-29 (29 males, 24 females) and 30-39 (31 males, 30 females) from the adult study (13) have been included in Fig. 1. For CD4+ cells, CD8+ cells, and CD4:CD8 ratio, there was a transition from the "childhood" pattern for children in the youngest age group to the "adult" pattern for children in the oldest age group. In adults, the proportion of CD4+ cells was higher in women than in men and increased with age for both sexes. Among teenagers, the proportion of CD4+ cells was similar in younger boys and girls, with a slight increase among older girls. In adults, CD8+ cells were lower in women than in men and decreased with age, while among teenagers the proportion of CD8+ cells was slightly higher in girls than in boys for the younger age groups but decreased among older girls. Viewed as a continuum, there was a clear transition from the "childhood" pattern in the youngest age group to the "adult" pattern in the 17-19 year olds. Determination of the precise relationship between hormonal and physiological development and the pattern of T-cell subset distri-

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The ratio of CD4+ to CD8+ cells (helper:suppressor ratio) has been the subject of numerous studies, particularly in patients with the acquired immunodeficiency syndrome (AIDS) and other immunodeficiency states (3, 4). A low ratio, especially when accompanied by a decrease in the absolute number of CD4+ cells, has been highly correlated with the clinical diagnosis of AIDS. A recent report of CD4:CD8 ratios in a large population of blood donors suggested that a ratio less than 0.85 should raise the suspicion of AIDS (10). The mean CD4:CD8 ratio among the 112 healthy children in the present study was 1.81 (95 confidence interval 0.87–2.29). The four values found to be less than 1.0 were 0.87, 0.90, 0.93, and 0.96.

Unlike previous reports of pediatric subjects, the children in this study were recruited from households participating in a larger, well-defined population of healthy adults. Since selection of the children was not population based, the adult and adolescent groups were not strictly comparable. However, the common geographic base, well-defined health status, and similarity in socioeconomic status and racial distribution gave credibility to comparisons between the two groups. For comparison purposes, only nonsmoking adults were included in Fig. 1, to avoid confounding by the known effects of cigarette smoking on T-cell subsets (14–16). Such cross-sectional comparisons may provide insight into the influence of adolescent developmental changes on the immune system. From these observations, testable hypotheses can be developed for future longitudinal studies. In addition to individual host factors and hormonal influences, longitudinal studies could address in more depth a number of environmental influences on the immune system, particularly such prevalent exposures as cigarette smoking (14–16, 35).

Care must be exercised when generalizing the specific values presented here to other laboratories since cell subset proportion may be influenced by the choice of whole blood vs mononuclear cell-enriched preparations, cryopreserved vs fresh cells, and fixed vs nonfixed cells. However, comparisons of fresh vs cryopreserved cells in our laboratory showed only a small increase in the proportion of HLA-DR+ cells with freezing. Other investigators have reported that T-cell subset analyses give similar results in geographically distinct laboratories and populations when equipment and protocols are comparable (9, 36). Even if the absolute values are not directly applicable to other laboratories, the associations with age, race, and gender are unlikely to vary with preparation techniques.

Studies such as this provide important baseline data and stress the need to consider age, race, and gender when analyzing immunologic parameters in children. The next challenge is to confirm and extend these observations in prospective cohort studies, correlating within-subject longitudinal changes with demographic characteristics, genetic, physiologic, and developmental effects, and environmental influences.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of Phillip Virgo and Richard Switalski for

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assistance in computer analyses, Darlene Bramble for secretarial support, and Barbara Greenberg, Sharon Fogel, Ronald Kase, Elizabeth Maloney, Karen O'Dell, and Daniel Ringer for technical assistance.

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Received December 13, 1989; accepted with revision March 6, 1990