CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, July 2001, p. 740–746 Vol. 8, No. 4
1071-412X/01/$04.00+0 DOI: 10.1128/CDLI.8.4.740–746.2001

Immune Function in Healthy Inner-City Children

JACQUELINE A. BARTLETT,* ARIELLE R. GOLDKLANG, STEVEN J. SCHLEIFER, AND STEVEN E. KELLER

Department of Psychiatry, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07107

Received 23 October 2000/Returned for modification 23 January 2001/Accepted 8 April 2001

The importance of investigating immunity in healthy children has been underscored in the last few years by studies of the immune pathology of childhood illnesses, including human immunodeficiency virus. This study reports both ennumerative and functional immune measures in healthy inner city children. A total of 152 of 207 children studied were completely heathy at the time of venipuncture and were included in this study. Laboratory immune batteries were completed (or begun) the same day as venipuncture. Relationships between age, gender, ethnicity, and immunity were then analyzed. We found that gender predicted both the absolute number and the percentage of T cells and helper cells and the percentage of natural killer cells. Total leukocyte counts and percentages of lymphocytes and granulocytes were related to ethnicity, as was the response to mitogen stimulation (concanavalin A and pokeweed mitogen) and phagocytic ability. In conclusion, age, gender, and ethnicity factors were found to contribute to differences in various immune measures in children and require further investigation.

As with all bodily functions and systems, the immune system develops and matures over time. While there is substantial data on immune measures and functioning among adult and newborn populations, there has been little research on immune parameters for young populations such as adolescents and children. Further, literature has often focused on Caucasian populations, with less research available concerning other ethnic groups, or on children with chronic illnesses rather than healthy children.

Descriptions of the immune system of children have often been derived from consideration of control groups used for studies investigating diseases in children (3, 12). Studies that do include a large sample size of normal control comparisons (8) may be limited in the normative data provided since they focus on specified immunological variables or sociodemographic influences (4). Other studies include a large population of children in the research but are focused solely on a single symptom, such as the exacerbation of asthma in children (5). Further, studies of immunity in children need to focus on influences of such factors of age, gender, and sexual maturity, in order to enhance our understanding of developmental immunity.

We present here results of an investigation of immune function in healthy children from an inner city population which was obtained as part of a longitudinal study of factors affecting immunity in children, such as mood or cognition. The data are presented in terms of the relationships to age, gender, level of sexual development, and ethnicity within this selected population of healthy inner city children. Normative data on this understudied population has become increasingly important with respect to immune-related diseases. Since the advent of diseases such as AIDS, the need for further and more descriptive data on immunity in children, as well as the need for tracking the progression of such disease states, and how that compares to normal immunologic development in children has increased dramatically.

* Corresponding author. Mailing address: Department of Psychiatry, New Jersey Medical School, UMDNJ, Medical Science Building, Rm. E565, Newark, NJ 07107. Phone: (973) 972-6528. Fax: (973) 972-7935. E-mail: bartleja@umdnj.edu.

MATERIALS AND METHODS

This investigation was conducted in the context of a longitudinal study of behavior, mood, and immunity in children from an inner city minority population recruited from public school third-and fourth-grade classes. These data are from the 152 healthy African American and Latino children and utilized fresh blood cells and a battery of immune assessments, which provide data on enumerative and functional immunological measures. These measures included total leukocyte count, counts of both granulocytes and lymphocytes, and counts of subsets of the lymphocyte populations, including those that may have implications in certain disease states such as the human immunodeficiency virus. The functional measures that were chosen involve in vitro assays only. Therefore, no exposure to antigen or other invasive procedure was necessary. This was thought to decrease subject risk and increase subject participation.

Subjects. This study was approved by the Institutional Review Board of University of Medicine and Dentistry of New Jersey–New Jersey Medical School. Informed assent from each of the subjects, along with informed consent from their parent or guardian, was obtained. A total of 207 children ranging in age from 8 to 12 years participated in the study and were recruited as part of a project assessing behavior, immunity, and health from a local elementary school. All psychosocial (e.g., ethnicity and age) data were obtained in interview format.

A medical history, assessment of bodily systems, vital signs, and a complete physical examination at the time of venipuncture were obtained for each child seen in the study. Potential subjects were excluded from data analyses if they had chronic diseases thought to have possible effects on immune function (e.g., asthma) or if they were taking any medications with known immunological effects. Subjects with any acute medical conditions, such as minor infections (upper respiratory illness), were deferred for 2 weeks and then studied.

Medical group evaluation. Each subject was evaluated by a pediatric nurse practitioner or a physician. A complete physical examination was given just prior to venipuncture. All medically relevant data was checked and reviewed by the physician (J.A.B.), who made the final judgment of the child's medical status and Tanner stage of sexual development (10). Only those who were determined to be medically healthy (n = 156) were included in these analyses. Four Caucasian children were also excluded from the analyses due to the small sample size of this ethnic group, leaving the total subjects included in these analyses at 152 children.

Immunological evaluation. Assays were carried out by lab technicians blind to the subject's medical status. Subject blood samples were collected in a heparinized syringe (preservative-free heparin), and heparinized whole blood was used in the phenotypic analysis of lymphocytes, monocytes, and ganulocytes. Results

presented were all obtained from the same venipuncture for each child, as we had done previously (1). Total leukocyte and differential counts were conducted following standard guidelines. Peripheral blood mononuclear cells (PBMC) were separated from whole blood by centrifugation on a Ficoll-Hypaque gradient and were used to assess mitogen-induced lymphocyte stimulation and natural killer (NK) cell function in the subjects. Granulocytes isolated from the remaining erythrocytes by a Percoll gradient were utilized to determine phagocytosis and killing ability.

Cell phenotype. Cell phenotypes were assessed by flow cytometry utilizing a Becton Dickinson FACScan. All reagents were supplied by BD. Appropriate filter combinations were used to simultaneously measure emissions from fluorescein isothiocyanate (FITC) and Phycoerythrin (PE). Gates are obtained using forward-and 90°-angle light scatter to select the lymphocyte cell population. A minimum of 2,500 gated lymphs were utilized for a standard analysis. Dual-color immunofluorescence (FL1 versus FL2) was used for immunophenotyping and allows precise definition of lymphocytes and lymphocyte subpopulations. Lymphocytes (bright $CD45^+$ $CD14^-$) were measured utilizing CD45FITC/CD14PE. T cells ($CD3^+$) and B cells (CD19) were measured utilizing CD3 FITC/CD19PE, T helper cells ($CD3^+$ $CD4^+$) were measured utilizing CD3FITC/CD4PE, T cytotoxic-suppressor ($CD3^+$ $CD8^+$) cells were measured utilizing CD3FITC/CD8PE, and NK ($CD3^-$, $CD16^+$ $CD56^+$) cells were measured utilizing CD3FITC/CD16+56PE.

Mitogen-induced lymphocyte stimulation. Mitogen-induced lymphocyte stimulation was performed according to the techniques modified by Keller et al. (11) with dose-response curves for concanavalin A (ConA; Calbiochem, San Diego, Calif.), phytohemagglutinin (PHA; Wellcome Reagents, Ltd., Beckenham, England), and pokeweed mitogen (PWM; GIBCO-BRL Products). The doses were as follows: for ConA, 3.75, 7.5, and 15 μg/well; for PHA, 0.05, 0.25, and 2.0 μg/well; and for PWM, 0.25, 0.5, and 5.0 μg/well. Lymphocyte stimulation data were presented as counts per minute in the stimulated cultures minus the counts per minute in the unstimulated cultures. All counts were log transformed to reduce variance. The mean of the two higher doses was utilized as a single dependent variable for regression analyses. (The lowest dose was included to establish a basal response for the dose-response curve.)

NK cell activity. NK cell activity was assessed with K562 target tumor cells according to standard methods modified by Georgescu and Keller (6, 7). Target K562 cells were maintained by passaging every 2 to 3 days. Cell viability was always >98%. On the day of the assay, target cells were collected, washed, and labeled with 500 μCi of ^{51}Cr for 2 h at 37°C. Target cells were then washed three times, and 10^4 cells were plated in microtiter plate wells. The NK cells (PBMC) were isolated from the whole blood as described above and prepared in three dilutions in RPMI with 15% normal human serum. The final concentrations of NK cells were 25×10^4, 50×10^4, and 100×10^4. This provided three effector/target ratios (25:1, 50:1, and 100:1). The mixture of target cells and NK cells was then incubated for 4 h at 37°C. The microtiter plates were then centrifuged; the supernatant from each well was assessed for ^{51}Cr activity, and the data are presented as a percentage of specific cytotoxicity. While we examined the NK cell activity results both as specific killing and the number of lytic units, we have presented the data as a percentage of killing for several reasons. First, the use of specific killing was closer to the raw data and less manipulated and allows direct inspection of the levels of specific cytotoxicity. Second, not all of our data fit the assumptions required for lytic unit transformation and so requires either the dropping of particular data points, the dropping of particular cases, or the truncation of the data. The mean for the two highest effector/target killing ratios for NK cell activity was utilized as the dependent variable for the regression analyses.

Granulocyte activity. Granulocyte activity assessment included the ability to phagocytize and kill *Staphylococcus aureus* according to the methods described by Weir (21), with the following modifications. After separation of the lymphocytes from the peripheral blood by use of a Ficoll-Hypaque gradient, the granulocytes were separated from the erythrocytes by a Percoll gradient. Heat-inactivated normal human AB serum was used to opsonize the bacteria. The granulocytes were then incubated with the opsonized *S. aureus* for 20 min. The unphagocytized *S. aureus* was then removed. Granulocytes were resuspended in RPMI, and aliquots were prepared for further processing. One aliquot was processed immediately to assess the phagocytic ability of the granulocytes, two were incubated at 37°C to assess killing ability, and two more were incubated on ice as controls. At time zero and at 1 and and 2 h postincubation with *S. aureus*, the granulocytes were lysed with 0.5% bovine serum albumin in distilled water and plated on blood agar plates. The plates were incubated at 37°C for 24 h. The number of colonies was counted; each valve represents an ingested *S. aureus* bacterium. Differences between the numbers of colonies at 37 and 0°C represent specific killing of bacteria.

TABLE 1. Demographic data

Demographic characteristic	No. of subjects		
	Female (n = 84)	Male (n = 68)	Total (n = 152)
African-American	53	50	108
Latino	31	18	49

Data analyses. Data were first examined descriptively for distribution, means, and variance. Then, independent sample t tests were run to compare males to females and to compare African American to Latino subjects. Pearson correlations were used to evaluate effects of age and Tanner stage of sexual development. We also investigated Tanner stage effects within groups (boys and girls separately) using a Pearson bivariate correlation. We also looked at the effects of menarche in girls using a t test (pre- or postmenarchal). We then ran analyses for each immune measures controlling for all of the demographic variables in a regression analysis simultaneously to investigate independent significant relationships between any of the demographic variable(s) and immune measures.

RESULTS

Demographics. As shown in Table 1, there were 68 males and 84 females in the 152 healthy children studied. This sample of 152 children consisted of 103 African Americans (50 males) and 49 Latino (18 males) children. The mean age ± the standard deviation (SD) for the entire sample was 9.34 ± 1.33 years. The mean age of the males was 9.32 ± 1.32 years, and that of the females was 9.35 ± 1.35 years. The age and gender distributions provided sufficient power to allow us to meaningfully assess their contributions to the variance of the immune measures.

(i) Gender effects. (a) Cell phenotypes. The relationships between gender and cell phenotypes for the entire sample are presented in Table 2. Gender significantly contributed both to the percentage and absolute numbers of helper cells and T cells and to the percentage of NK cells. No other enumerative measures were significantly related to gender.

(b) Mitogen-induced lymphocyte stimulation. For the proliferation assays, gender had no significant effect on the mean lymphocyte responses to the mitogens ConA, PWM, or PHA.

(c) NK cell activity. There was no significant relationship between gender and NKCA.

(d) Granulocyte activity. While there was a trend for lower phagocytic activity in males, there was no gender-related difference in bactericidal activity at 1 or 2 h of incubation.

(ii) Effects of ethnicity. (a) Cell phenotypes. The relationships between ethnicity and white blood cell (WBC) counts for the entire sample are presented in Table 3. The total WBC count was lower in African Americans than in Latinos. The African American children also had a lower percentage of granulocytes but a higher percentage of lymphocytes than the Latino children.

(b) Mitogen-induced lymphocyte stimulation. There were significant ethnicity differences among the proliferation assays. The mean proliferative responses (to the top two doses) to both ConA and PWM were greater in the African-American children. The mean response to PHA was not found to be affected by ethnicity.

(c) NK cell activity. There was no relationship observed between ethnicity and NK cell cytotoxicity.

TABLE 2. Gender and peripheral leukocyte enumerative and functional measures

Variable	Mean result ± SD		*t*	*P*
	Male subjects	Female subjects		
Enumerative measures				
WBC count (no. of cells [10^6]/ml)	5.88 ± 2.06	6.33 ± 1.98	−1.40	0.17
Absolute lymphocytes (no. of cells [10^6]/ml)	2.45 ± 0.71	2.61 ± 0.78	−1.14	0.26
Absolute cells (no. of cells [10^6]/ml)	0.90 ± 0.28	1.01 ± 0.35	−2.24	0.027
Absolute T cells (CD3) (no. of cells [10^6]/ml)	1.56 ± 0.45	1.74 ± 0.56	−2.14	0.034
Absolute B cells (no. of cells [10^6]/ml)	0.50 ± 0.19	0.48 ± 0.22	0.09	0.93
Absolute granulocytes (no. of cells [10^6]/ml)	1.22 ± 0.44	1.29 ± 0.41	−1.01	0.31
Absolute suppressor cells ($CD8^+$) (no. of cells [10^6]/ml)	0.58 ± 0.19	0.64 ± 0.24	−1.60	0.11
Absolute NK cells ($CD56^+$) (no. of cells [10^6]/ml)	0.25 ± 0.13	0.23 ± 0.16	0.44	0.66
% Lymphocytes	43.84 ± 9.79	42.27 ± 9.22	1.02	0.31
% Helper cells	36.42 ± 5.48	38.80 ± 5.79	−2.58	0.011
% T cells	63.69 ± 7.70	67.34 ± 5.39	−3.43	0.001
% Granulocytes	49.54 ± 11.05	50.16 ± 10.08	−0.36	0.72
% Monocytes	6.65 ± 3.75	6.23 ± 3.72	0.70	0.49
% Suppressor cells	23.62 ± 4.82	24.84 ± 5.59	−1.42	0.16
% NK cells	10.17 ± 4.69	8.51 ± 4.26	2.28	0.024
Functional measures				
Phagocytosis	227 ± 96.85	260.46 ± 117.65	−1.89	0.061
Log mean ConA	4.57 ± 0.33	4.57 ± 0.27	−0.06	0.96
Log mean PHA	4.73 ± 0.28	4.74 ± 0.25	−0.23	0.82
Log mean PWM	4.48 ± 0.31	4.48 ± 0.33	−0.01	0.99
% Kill (1 h)	63.54 ± 19.68	64.47 ± 19.71	−0.29	0.77
% Kill (2 h)	76.88 ± 12.59	76.62 ± 16.45	0.11	0.92
NK, 100:1	37.21 ± 14.34	33.68 ± 14.26	1.52	0.13
NK, 50:1	29.22 ± 12.76	25.67 ± 13.09	1.68	0.10
NK, 25:1	20.23 ± 10.85	17.34 ± 10.28	1.68	0.10
NK, mean	33.49 ± 12.81	29.80 ± 13.36	1.72	0.09

(d) Granulocyte activity. African-American children had significantly lower phagocytic activity than Latino children, but there were no differences in bactericidal activity at 1 or 2 h related to ethnicity.

(iii) Age effects. (a) Cell phenotypes. Age within the group as a whole was not significantly related to any enumerative immune measures.

(b) Mitogen-induced lymphocyte stimulation. There were no significant age effects for the mean lymphocyte responses to the mitogens ConA, PWM, or PHA.

(c) NK cell activity. There was no significant relationship between age and the mean NK cell cytotoxicity.

(d) Granulocyte activity. There was no relationship between age and phagocytosis of *S. aureus* or bactericidal activity at 1 or 2 h of incubation.

(iv) Analyses for separate groups defined by gender.

(a) Age effects in groups defined by gender. While there were no age effects for the sample as a whole, when each gender was examined as a group separately age effects became apparent. Age in boys (Table 4) was inversely related to the absolute number of B cells and the percentage of T cells. Further, age tended to be positively correlated with bactericidal activity at 2 h of incubation in boys.

The only findings in girls (Table 5) were that age was inversely correlated to the total WBC and tended to be correlated with absolute numbers of granulocytes.

(b) Effects of sexual development. As expected, Tanner stage and age in boys were significantly related, as were Tanner stage and age for girls (Table 6, note the descriptive statistics of Tanner stage by gender).

Significant Tanner stage and immune relationships were found for boys but not for girls using the Pearson correlation analyses (Table 7). Specifically, Tanner stage in boys was inversely related to the percentage of T cells but positively correlated to the number of B cells.

While no Tanner stage and/or immune relationships were found in girls, 8.3% ($n = 7$) of the 84 girls had already begun to menstruate, specifically all girls in Tanner stages IV and V. We therefore used independent sample *t* tests to investigate for possible effects of menarche. Girls menstruating at the time of venipuncture were significantly older ($P < 0.000$). The menstruating girls had a mean age of 12.35 ± 056 years versus those not yet menstruating, who had a mean age of 9.57 ± 1.11 years. No significant differences in menarchal status between ethnic groups was found; 57.1% of those already menstruating and 63.6% of those not yet menstruating were African American. The two groups, pre- or post menarche, showed significantly different immune measures. Specifically, the absolute numbers of lymphocytes, helper cells, NK, and T cells, as well as the percentage of NK cells, were lower in girls who were postmenarche, with a trend for them to have a lower WBC count as well. Further, girls who were postmenarche had significantly lower phagocytic ability. (Table 8).

(c) Ethnicity effects in groups defined by gender. We ran independent sample *t* tests in order to examine ethnicity differences in girls and boys as separate groups. Ethnicity was significantly related to immune measures in both groups.

In girls, the absolute numbers of lymphocytes, suppressor cells, and T cells and the percentage of lymphocytes were higher in the African Americans, while the percentage of gran-

TABLE 3. Ethnicity and peripheral leukocyte enumerative and functional measures

Variable	Mean result ± SD		*t*	*P*
	African-American subjects	Latino subjects		
Enumerative measures				
WBC count	5.87 ± 1.88	6.67 ± 2.21	−2.32	0.022
Absolute lymphocytes	2.57 ± 0.78	2.49 ± 0.69	0.67	0.51
Absolute helper cells	0.97 ± 0.34	0.94 ± 0.28	0.62	0.54
Absolute T cells (CD3)	1.68 ± 0.56	1.63 ± 0.42	0.55	0.59
Absolute B cells	0.49 ± 0.21	0.51 ± 0.20	−0.53	0.60
Absolute granulocytes	1.23 ± 0.42	1.32 ± 0.43	−1.25	0.22
Absolute suppressor cells ($CD8^+$)	0.62 ± 0.24	0.60 ± 0.17	0.30	0.77
Absolute monocytes	0.16 ± 8.93	0.15 ± 0.10	0.78	0.44
Absolute NK cells ($CD56^+$)	0.25 ± 0.14	0.22 ± 0.16	1.06	0.29
% Lymphocytes	44.88 ± 8.92	38.99 ± 9.56	3.71	0.000
% Helper cells	37.67 ± 6.17	37.87 ± 4.83	−0.20	0.84
% T cells	65.59 ± 7.28	65.45 ± 5.55	−0.30	0.76
% Granulocytes	48 ± 9.30	53.82 ± 11.82	−3.30	0.001
% Monocytes	6.61 ± 3.73	6.01 ± 3.73	0.92	0.36
% Suppress or cells	24.20 ± 5.70	24.49 ± 4.30	−0.32	0.75
% NK cells	9.60 ± 4.32	8.53 ± 4.88	1.37	0.17
Functional measures				
Phagocytosis	231.17 ± 105.03	275.61 ± 114.43	−2.37	0.019
Log mean ConA	4.61 ± 0.29	4.49 ± 0.31	2.34	0.02
Log mean PHA	4.74 ± 0.27	4.74 ± 0.26	−0.03	0.98
Log mean PWM	4.52 ± 0.29	4.40 ± 0.39	1.99	0.048
% Kill (1 h)	63.21 ± 19.56	65.81 ± 19.87	−0.76	0.45
% Kill (2 h)	77.90 ± 12.94	74.29 ± 18.07	1.41	0.16
NK, 100:1	36.31 ± 14.97	33.05 ± 12.83	1.31	0.19
NK, 50:1	28.40 ± 13.88	24.86 ± 10.73	1.57	0.12
NK, 25:1	19.60 ± 11.51	16.61 ± 8.11	1.64	0.10
NK, mean	32.65 ± 13.76	28.93 ± 11.66	1.63	0.11

ulocytes was lower (Table 9). The mean response to ConA and to PWM was also higher in the African-American girls (Table 9).

In boys, ethnic differences also existed; specifically, higher absolute numbers of B cells and higher total WBC counts were found in Latino boys (Table 10).

(v) Regression analyses. We next undertook a series of exploratory regression analyses investigating the independent effects of all the sociodemographic variables on each dependent immune variable. This was done in order to determine if there remained a significant unique contribution of the individual independent variable after partialing out the other demography-related variance. Since no causal priority can be assigned, all three demographic variables (age, ethnicity, and gender) were entered into the model simultaneously (Table 11).

Effects of gender after accounting for age and ethnicity. Consistent with the original findings with respect to gender, the absolute number of helper cells, the absolute number of T cells, the percentage of helper cells, and the percentage of T cells remained significantly related to gender while controlling age and ethnicity. The percentage of NK cells also retained a significant relationship with gender when ethnicity and age were partialed out. However, phagocytosis, which was related to gender at the 0.06 level in the initial correlation, was not significantly (0.10) related to gender after the individual contributions of age and ethnicity were controlled.

Effects of ethnicity when age and gender were taken into account. The WBC count retained a significant relationship with ethnicity after age and gender were accounted for, as did the percentage of lymphocytes and the percentage of granulocytes. With the proliferation assays, the mean responses to ConA and PWM were also significantly related to gender after age and ethnicity were accounted for. Additionally, phagocytosis remained significantly related to ethnicity.

TABLE 4. Age findings in boys (n = 68; mean age, 9.85 ± 1.32 years)

Immune measure	Mean ± SD	Pearson *R* coefficient	*P*
WBC count (no.)	5.88 ± 0.19	0.25	0.04
Absolute granulocytes (no.)	1.22 ± 0.44	0.093	0.45
Absolute B cells (no.)	0.50 ± 0.19	0.250	0.04
% T cells	63.69 ± 7.70	−0.253	0.038
% Kill (2 h)	76.88 ± 12.57	0.213	0.082

TABLE 5. Age findings in girls (n = 84; mean age, 9.80 ± 1.32 years)

Immune measure	Mean ± SD	Pearson *R* coefficient	*P*
WBC count (no.)	6.33 ± 1.98	−0.230	0.036
Absolute granulocytes (no.)	1.29 ± 0.41	−0.205	0.061
Absolute B cells (no.)	0.49 ± 0.22	−0.136	0.22
% T cells	67.34 ± 5.39	0.163	0.14
% Kill (2 h)	76.62 ± 16.50	0.098	0.37

TABLE 6. Tanner stage by gender

Group (*n*)	No. (% total) for Tanner stage:				
	I	II	III	IV	V
Girls (84)	41 (48.8)	22 (26.2)	14 (16.7)	6 (7.1)	1 (1.2)
Boys (68)	52 (76.5)	11 (16.2)	4 (5.9)	1 (1.5)	0

DISCUSSION

These data demonstrate differences in immune measures in relation to ethnicity, gender, age, and stage of sexual development in normal healthy minority children. Differences in absolute and percent helper cells, absolute and percent T cells, and percent NK cells were all significantly different in girls and boys. Differences in relationship to ethnicity were observed for the following measures: phagocytosis of bacteria, WBC counts, and percent lymphocytes and percent granulocytes. Further, mitogen stimulation responses by lymphocytes (ConA and PWM) were related to ethnicity.

While no significant contribution in terms of age or Tanner stage of development on immune function were found in these healthy children as a group, when boys and girls were examined as separate groups effects related to age and to level of sexual development were detected. Since the hormonal milieu of puberty is very different in boys and girls, these findings might be expected to be undetectable unless gender differences were controlled in some fashion.

These findings contribute significantly to the existing data on normal immunity in children. Further, these data broaden the contributions of previous research in the field by providing data concerning immunological functioning among minority populations. Chen et al. (4) previously reported ethnic differences in immune function among children with acute lymphoblastic leukemia (ALL). These authors reported that African-American children with ALL had higher WBC and T cell counts and suggested that an elevated risk for childhood leukemia may be related to, among other factors, socioeconomic factors and different levels of metabolizing processes or enzymes between ethnic or racial groups. This leads us to postulate that the possible links between childhood diseases and illnesses and environmental and genetic factors can be more closely understood through the study of immune function among a healthy population.

Having demonstrated gender, age, and ethnic differences in immunity during childhood, we may consider the possible mechanisms involved. Hormonal factors involved with growth in general or in sexual maturation may play a role in these differences. Certainly growth hormone (GH), prolactin (PR), insulin-like growth factor 1 (IGF-1), and somatostatin have been demonstrated to influence immune function in addition to their role in physical growth and development (14; W. Burgess et al., Neuro immunomodulation **6:**56–68 [abstr.], 1999; V. H. Heemskerk et al., Cytokine Growth Factor Rev. **10:**5–14 [abstr.], 1999; E. L. Hooghe-Peters and R. Hooghe, Cell Mol. Life Sci. **54:**1057–1058 [abstr.], 1988). Receptors for these hormones have been found on immunocytes, and the release of at least PR from within the immune system (e.g., synthesized and released by subpopulations of lymphocytes) has been reported (12a, 14). Both GH and PR are thought to influence immune system development as well as function (17). Rapaport et al. (R. Rapaport et al., J. Pediatr. **109:**434–439 [abstr.], 1986; R. Rapaport et al., Life Sci. **41:**2319–2324 [abstr.], 1987) studied children with GH deficiency and found effects of GH on immunity both in vitro and in vivo. In children with short stature treated with human GH, immunoglobulin M production decreased during treatment (M. Bozzola et al., Acta Pediatr. Scand. **77:**675–680 [abstr.], 1988). In another study, children with growth retardation and GH deficiency were found to have impaired phagocytic function (13). In a study of young and elderly adults, plasma levels of IGF-1 were related to NK cell numbers in peripheral blood in young but not elderly subjects (11a). This is supportive to our finding of NK counts related to age, since growth-related hormones change over the course of development. Further, in studies of aging populations, age-related immune changes can be reversed by IGF-1 (Burgess et

TABLE 7. Tanner stage and immune effects in boys ($n = 68$)

Immune measure	Mean ± SD	Pearson *R* coefficient	*P*
Absolute B cells (no.)	0.50 ± 0.19	0.296	0.014
% T cells	63.69 ± 7.70	−0.264	0.030

TABLE 8. Menses and immune effects in girls ($n = 84$)

Immune measure	Mean ± SD in girls (*n*)		*t*	*P*
	Postmenarche (7)	Premenarche (77)		
Absolute helper cells (no.)	0.86 ± 0.08	1.03 ± 0.36	3.43	0.002
Absolute lymphocytes (no.)	2.21 ± 0.22	2.64 ± 0.80	3.52	0.002
Absolute NK cells ($CD56^+$) (no.)	0.16 ± 0.05	0.24 ± 0.16	3.38	0.003
Absolute T cells (CD3) (no.)	1.49 ± 0.14	1.76 ± 0.58	3.24	0.003
% NK cells	6.46 ± 1.13	8.70 ± 4.39	3.40	0.002
Phagocytosis	198.00 ± 65.04	266.14 ± 119.96	2.42	0.035
WBC count (no.)	5.22 ± 1.42	6.44 ± 2.00	2.10	0.068

TABLE 9. Ethnicity differences in immune measures in girls ($n = 84$)

Immune measure	Mean ± SD in girls (*n*)		*t*	*P*
	African-American (53)	Latina (31)		
Absolute lymphocytes (no.)	2.74 ± 0.82	2.37 ± 0.67	2.27	0.026
Absolute suppressor cells ($CD8^+$) (no.)	0.67 ± 0.27	0.57 ± 0.17	2.09	0.040
Absolute T cells (CD3) (no.)	1.84 ± 0.60	1.58 ± 0.44	2.12	0.037
% Granulocytes	47.65 ± 0.843	54.45 ± 11.31	−3.14	0.002
% Lymphocytes	45.07 ± 8.32	37.47 ± 8.83	3.88	0.000
Log mean ConA	4.64 ± 0.25	4.46 ± 0.28	3.18	0.002
Log mean PWM	4.55 ± 0.26	4.37 ± 0.41	2.49	0.015
Absolute helper cells (no.)	1.06 ± 0.38	0.92 ± 0.28	1.82	0.073
Phagocytosis	242.02 ± 109.09	292.00 ± 126.64	−1.84	0.072

TABLE 10. Ethnicity difference in immune measures in boys ($n = 68$)

Immune measure	Mean ± SD in boys (n)		t	P
	African-American (50)	Latino (18)		
Absolute B cells (no.)	0.47 ± 0.17	0.58 ± 0.23	−2.30	0.025
WBC count	5.56 ± 1.99	6.74 ± 2.05	−2.12	0.043
Absolute granulocytes (no.)	1.16 ± 0.44	1.39 ± 0.43	−1.99	0.056
Absolute T cells (CD3) (no.)	1.51 ± 0.46	1.72 ± 0.39	−1.86	0.072

al. Neuroimmunomodulation). Future studies in healthy children are needed to investigate the potential impact and influences of physical growth and development on immune function.

The literature does contain other evidence suggestive that, even after early childhood, immune differences may be expected in conjunction with the age of subjects studied. For example, younger renal transplant patients are reported to have more episodes of acute rejection than older patients (16). We have previously reported that preadolescent children with major depressive disorder may have elevated responses to mitogen stimulation, unlike adults with major depressive disorder, who are more likely to have diminished responses (2).

However, finding age related differences in cell phenotypes in this age group (age 8 to 12 years) is different from our findings in healthy adolescents (ages 12 to 18) (1) in whom only the total WBC count was related to age. These interstudy differences may be related to the issues of sexual maturity in the two samples, differences which were only modestly addressed in this study by physical examination and the assessment of Tanner stage and pre- or postmenarchal status. Other differences between these two studies related to growth and development may also be postulated. For example, height, weight, and body mass index were not assessed, nor was growth percentile addressed in either study. No hormonal assays have been undertaken in either study, although this may occur in the future.

The ethnic differences that existed between the Latino and African-American children studied here cannot be compared to other investigations, since racial and/or ethnic differences are even less well studied than are age and gender effects. However, data suggestive of possible underlying racial or ethnic differences have been reported. Ishitani et al. reported that in children receiving living related kidney transplants, race was significantly related to graft survival (10). There are several similar studies of racial differences in outcome of organ transplantation in children (19) and adults (16). Further, Tench and Isenberg (20) reported significant ethnic differences in anti-DNA in patients with systemic lupus erythematosus. Similarly, Irwin and Miller (9) reported significantly lower NK cell activity in a group of African-American alcoholic men compared to alcoholic men of different ethnicities. In our study and many others, it is important to note that the racial and ethnic groups are often self-identified and do not constitute racial groups demonstrated to be of specific genetic origin. However, these data do suggest that ethnic or racial differences must be considered whenever immune-related diseases or functions are investigated.

TABLE 11. Independent effects of demographic variables

Variable	Overall model		Independent effects		
	ΔR^2	Significant F Δ	Gender	Ethnicity	Age
Absolute helper cells (no.)	0.038	0.12	0.023	0.39	0.75
Absolute T cells (CD3) (no.)	0.035	0.15	0.030	0.44	0.66
% Granulocytes	0.08	0.006	0.98	0.001	0.15
% Helper cells	0.043	0.09	0.012	0.94	0.86
% T cells	0.08	0.008	0.001	0.98	0.48
% NK cells	0.044	0.085	0.033	0.27	0.62
Phagocytosis	0.053	0.043	0.10	0.031	0.10
WBC count	0.062	0.023	0.26	0.024	0.088

The possible selection bias in this study must be discussed. The subjects for this study were recruited from an inner city elementary school and from subject nomination. Results from studies with children from other urban or suburban settings or students with severe academic problems requiring special education facilities may differ from the present study's findings. However, the random selection of subjects, the physical assessment by physicians, the collection of psychobehavioral data and venipuncture on the same day, and the subsequent immediate processing of the blood for each of the multiple immune assays were important factors in ensuring the quality and accuracy of these normative data.

This investigation of the effects of age, gender, and ethnicity on a variety of immune measures demonstrates the need to have normative data for the many specific populations in existence. Although our subjects were randomly sampled, there existed significant differences with regard to ethnicity, age, and gender in immune functioning. How these effects may influence immune function in disease, in health, and in preventive measures undertaken in clinical settings requires much additional investigation.

REFERENCES

1. **Bartlett, J. A., S. J. Schleifer, M. Demetrikopoulos, B. Delaney, S. Shiflett, and S. E. Keller.** 1998. Immune function in healthy adolescents. Clin. Diagn. Lab. Immunol. **5:**105–113.
2. **Bartlett, J. A., S. J. Schleifer, M. K. Demetrikopoulos, and S. E. Keller.** 1995. Immune differences with and without depression. Biol. Psychiatry **38:**771–774.
3. **Camorlinga-Ponce, M., J. Torres, G. Perez-Perez, Y. Leal-Herrera, et al.** 1998. Validation of a serologic test for the diagnosis of *Heliobacter pylori* infection and the immune response to urease and CagA in children. Am. J. Gastroenterol. **93:**1264–1270.
4. **Chen, C. L., Q. Liu, C. H. Pui, G. K. Rivera, J. T. Sandlund, R. Ribeiro, W. E. Evans, and M. V. Relling.** 1997. Higher frequency of gluthione *S*-transferase deletions in black children with acute lymphoblastic leukemia. Blood **89:** 1701–1707.
5. **Cunningham, A. F., S. L. Johnston, S. A. Julious, F. C. Lampe, and M. E. Ward.** 1998. Chronic *Chlamydia pneumoniae* infection and athsma exacerbations in children. Eur. Rep. J. **11:**345–349.
6. **Georgescu, R., and S. E. Keller.** 1987. Overnight storage at 1°C does not affect NK cytotoxicity. J. Immunol. Methods **103:**151.
7. **Georgescu, R., and S. E. Keller.** 1987. Decreasing the spontaneous release in NK cell assays, J. Immunol. Methods **103:**143.
8. **Gissler, M., M. R. Jarvelin, P. Louhiala, O. Rahkonen, and E. Hemminiki.** 1999. Can children's health be predicted by perinatal health? Int. J. Epidemiol. **28:**276–280.
9. **Irwin, M., and C. Miller.** 2000. Decreased natural killer cell responses and altered interleukin-6 and interleukin-10 production in alcoholism: an interaction between alcohol dependence and African-American ethnicity. Alcohol Clin. Exp. Res. **24:**560–569.
10. **Ishitani, M., R. Isaacs, V. Norwood, S. Nock, and P. Lobo**. 2000. Predictors

of graft survival in pediatric living-related kidney transplant recipients. Transplantation **70:**288–292.
11. **Keller, S. E., S. J. Schleifer, J. Sherman, M. Camerino, H. Smith, Jr., and M. Stein.** 1981. Comparison of a simplified whole blood and isolated lymphocyte stimulation technique. Immunol. Commun. **10:**417–431.
11a. **Krishnaraj, R. A. Zaks, and T. Unterman.** 1998. Relationship between plasma IGF-1 levels, in vitro correlates of immunity, and human senescence. Clin. Immunol. Immunopathol. **88:**264–270.
12. **Kuznetsova, A. A., Y. V. Natochin, and A. V. Papayan.** 1998. Osmoregulatory function of the kidney in enuretic children. Scand. J. Urol. Nephrol. **32:**132–137.
12a. **Ly, Y.-L.** 1997. Molecular actions of prolactin in the immune system. Proc. Soc. Exp. Biol. Med. **215:**35–52.
13. **Manfredi, R., F. Tumietto, L. Azzaroli, A. Zucchini, F. Chiodo, and G. Manfredi.** 1994. Growth hormone and the immune system: impaired phagocytic function in children with idiopathic GH deficiency is corrected by treatment with biosynthetic GH. J Pediatr. Endocrinol. **7:**245–251.
14. **Matera, L.** 1996. Endocrine, paracrine and autocrine actions of prolactin on immune cells. Life Sci. **59:**599–614.
15. **Meier-Kriesche, H. U., A. O. Ojo, D. M. Cibrik, J. A. Hanson, A. B. Leichtman, J. C. Magee, et al.** 2000. Relationship of recipient age and development of chronic allograft failure. Transplantation **70:**306–310.
16. **Meier-Kriesche, H. U., A. O. Ojo, D. M. Cibrik, J. A. Hanson, A. B. Leichtman, J. C. Magee, and B. Kaplan.** 2000. African-American renal transplant recipients experience decreased risk of death due to infection: possible implications for immunosuppressive strategies. Transplantation **70:**375–379.
17. **Murphy, W. J., S. K. Durum, M. R. Anver, and D. L. Longo.** 1992. Immunologic and hematologic effects of neuroendocrine hormones studies on DW/J dwarf mice. J. Immunol. **148:**3799–3805.
18. **Tanner, J. M.** 1975. The measurement of maturity. Trans. Eur. Orthodontic Soc. **1975:**45–60.
19. **Tejani, A. H., E. K. Sullivan, S. R. Alexander, R. N. Fine, W. E. Harmon, E. C. Kohaut.** 1999. Predictive factors for delayed graft function (DGF) and its impact on renal graft survival in children: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). Pediatr. Transplant **3:**293–3000.
20. **Tench, C. M., and D. A. Isenberg.** 2000. The variation in anti-ENA characteristics between different ethnic populations with systemic lupus erythematosus over a 10-year period. Lupus **9:**374–376.
21. **Weir, D. M. (ed.).** 1978. Handbook of experimental immunology, 3rd ed. Blackwell Scientific Publications, Ltd., Oxford, England.