

Prospective 5-year study of peripheral blood CD4⁺, CD8⁺, and CD19⁺/CD20⁺ lymphocytes and serum Igs in children born to HIV-1⁺ women

William T. Shearer, MD, PhD,^a Kirk A. Easley, MS,^b Johanna Goldfarb, MD,^b Howard M. Rosenblatt, MD,^a Hal B. Jenson, MD,^c Andrea Kovacs, MD,^d and Kenneth McIntosh, MD,^e for the P2C2 HIV Study Group *Houston and San Antonio, Tex, Cleveland, Ohio, Los Angeles, Calif, and Boston, Mass*

Background: Peripheral blood CD4⁺ and CD8⁺ T cells, CD19⁺/20⁺ B cells, and serum Igs are known to be altered by the progression of pediatric HIV-1 infection, but their evaluation as predictors of survival needs further definition. **Objective:** To determine the natural history of these immune factors and their importance in predicting survival, we studied 298 HIV-1 vertically infected (HIV-1⁺) children over a 5-year period. **Methods:** These immune factors and serum HIV-1 RNA levels were measured in two groups: (1) a birth cohort of children enrolled up to age 28 days postnatally, including 93 HIV-1⁺ and 463 HIV-1 uninfected infants (HIV-1⁻), and (2) an older cohort of 205 HIV-1⁺ children enrolled after the age of 28 days, who were classified as survivors or nonsurvivors. **Results:** In the birth cohort HIV-1⁺ children had significantly lower CD4⁺ T-cell counts, higher CD8⁺ T-cell counts, and lower CD19⁺/20⁺ B-cell counts and higher IgG, IgA, and IgM levels than HIV-1⁻ children. In the older cohort survivors had significantly higher CD4⁺ and CD8⁺ T-cell and CD19⁺/CD20⁺ B-cell counts and higher IgG, lower IgA, and lower IgM levels than did nonsurvivors. In univariable analysis factors affecting survival in the older cohort were baseline CD4⁺ and CD8⁺ T-cell and CD19⁺/20⁺ B-cell counts and IgG and HIV-1 RNA levels (all $P < .05$). In multivariable analysis high baseline CD4⁺ T-cell count and low baseline HIV-1 RNA load remained important. **Conclusion:** The longitudinal mean profiles of CD4 and CD8 T-cell and CD19/20 B-cell counts and serum IgG levels helped to describe the natural progression of HIV-1 disease in children. However, only baseline CD4 T-cell count independently predicted survival. (*J Allergy Clin Immunol* 2000;106:559-66.)

Key words: Pediatric HIV-1 infection, survival, CD4⁺ T cells, CD8⁺ T cells, CD19⁺/20⁺ B cells, serum Igs

Death in pediatric HIV-1 infection is a consequence of a failed immune system caused by dysfunction of cellular elements, principally T lymphocytes, and humoral components, mostly antibodies.¹ Of these two arms of immune resistance, the cellular-mediated immunity seems to be most profoundly affected because the CD4⁺ T cell is one of the principal targets of HIV-1 attack.²⁻⁷ By virtue of the virion's cognate recognition of the CD4 surface molecule itself and the CXCR4 chemokine receptor, it enters the CD4⁺ T cell, becomes incorporated into the cell's genome, and converts the cell into a factory for mature virions until final rupture and release of its replicated descendants.⁸ When sufficient numbers of CD4⁺ T cells have been destroyed by the virus, secondary (usually opportunistic) infections lead to marked morbidity and eventual death.⁹ In the antibody dysfunction the earliest clue of altered antibody production is the extraordinarily elevated serum concentrations of the 3 principal Igs (IgG, IgA, and IgM), particularly in children.¹⁰⁻¹² When measured by antibody responses to T cell-dependent recall antigens (eg, diphtheria and tetanus toxoid) or neoantigen (eg, bacteriophage ϕ X174), mostly weak primary antibody (IgM) responses and few secondary antibody (IgG) responses were obtained in children.¹³⁻¹⁵ This indicates failure to switch from an IgM to an IgG antibody (long-lived, high-affinity, memory antibody) is most likely caused by the lack of CD4⁺ (helper) T cells, which generate a second signal to B cells on cognate recognition of antigen.¹⁶

A preliminary report of the immune function of some of the children enrolled in the National Institutes of Health National Heart, Lung and Blood Institute P2C2 HIV-1 Study consisted of measurement of CD4⁺ T cells and CD8⁺ T cells during a follow-up of less than 2 years.¹⁷ This report of the peripheral blood immune cells and serum Igs in the completed P2C2 HIV Study cohort will add important information to the body of knowledge on the role of these immune factors in survival of HIV-1⁺ children.¹⁸⁻²⁴ In addition, this report will add new information to the surprisingly scarce reports on serum Ig concentrations and peripheral blood B-cell populations in children born to HIV-1⁺ women.

METHODS

Study population and informed consent

The P2C2 HIV Study population has been described fully elsewhere, with explanations of recruitment, examinations, laboratory

From ^aBaylor College of Medicine, Houston; ^bCleveland Clinic Foundation, Cleveland; ^cUniversity of Texas Health Science Center, San Antonio; ^dLAC and University of Southern California Medical Center, Los Angeles; and ^eBoston Children's Hospital/Harvard Medical School, Boston.

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Reprint requests: William T. Shearer, MD, PhD, Texas Children's Hospital, 6621 Fannin St (MC 1-3291), Houston, TX 77030.

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and clinical tests, quality assessment, and data analysis.²⁵ Briefly, a group of 600 study subjects born to HIV-1⁺ women were enrolled at birth or by 28 days of life beginning in 1990 and followed prospectively for up to 6 years. The birth cohort comprised 93 HIV-1⁺, 463 HIV-1⁻, and 44 HIV-1-indeterminate infants. Another group of 205 infants and children with HIV-1 infection were enrolled at greater than 28 days of life (older cohort) between 1990 and 1993 and were similarly followed for up to 6 years. Infants and children in both groups were examined at regular intervals of 3 to 6 months. Almost all study subjects (>90%) took antiretroviral medications (principally zidovudine and dideoxyinosine) at some time during the study period, 29 (9.7%) HIV-1⁺ children (9.7%) took protease inhibitors (ritonavir, nelfinavir, saquinavir, or indinavir) when they were over 2 years of age, and 38% of study subjects received intravenous IgG at some time during the study period.

The 44 infants of indeterminate HIV-1 infection status were excluded from all analyses. Demographic characteristics for these 44 infants were similar to those of the cohorts described in this study.

Informed consent from parents or guardians of the children in this study was obtained on forms approved by the National Institutes of Health and institutional review boards.

Definitions of HIV-1 disease survival in the older cohort

A survivor was defined as a child who survived for 5 years in this natural history study, regardless of age at enrollment, or a child who was alive when lost to follow-up. A nonsurvivor was defined as a child who died during the course of this study. These simple definitions of survivor and nonsurvivor did not address the phenomenon of long-term survival in HIV-1⁺ children discussed in other studies.²⁶⁻³¹

Examinations of subjects

All patients underwent periodic physical examinations and laboratory tests, including complete blood count, lymphocyte counts (CD4⁺, CD8⁺, and CD19⁺/20⁺ lymphocytes), and serum Ig measurements (IgG, IgA, and IgM). Laboratory tests and interpretation of physical measurements were quality controlled, as described in the reference article.²⁵ Serum specimens from children who had IgG replacement therapy within 90 days (≥ 4 half-lives of IgG) were excluded from Ig analysis. Lymphocyte phenotypes were determined by 2- or 3-color fluorescence-activated flow cytometry by using commercially available mAbs. Absolute numbers of lymphocyte subsets were calculated arithmetically on the basis of complete blood counts performed on the same blood sample. Serum obtained on 82% of the birth cohort and 81% of the older cohort was frozen at -70°C, stored in a central repository, thawed once, and analyzed for serum Ig concentrations by using laser nephelometry. Serum was analyzed for HIV-1 RNA concentration in the older cohort by using quantitative HIV-1 RNA PCR with the Amplicor HIV-1 Monitor Test (Roche Diagnostic Systems, Branchburg, NJ).^{20,24}

Statistical analysis

Repeated-measures analyses of lymphocyte phenotypes (cube-root transformation of counts), serum Ig (natural log), and HIV-1 RNA (\log_{10}) were performed by SAS Proc Mixed, which provided estimates of the mean and 95% confidence intervals at each age for the birth cohort by HIV-1 status and for the older cohort by survivorship. Reported *P* values are two-sided and are considered significant at a value of less than .05.

Longitudinal data were more complete early in the study for both cohorts. For the HIV-1⁻ children, loss to follow-up was high, and as dictated by the P2C2 HIV protocol, approximately half of the HIV-1⁻ cohort was randomly selected to remain in the study as a control group, and the remainder were randomized off study.²⁵ Regardless of HIV-1 status, approximately 15% of the scheduled laboratory

tests were not performed because of missed visits by the patients. Sample sizes were also smaller in the HIV-1⁺ children with increasing age as a result of mortality (approximately 7% per year).

Cumulative survival for the older HIV-1⁺ cohort was estimated with the Kaplan-Meier method. Log-rank tests were used to compare survival according to the baseline measurements of lymphocyte counts, serum Ig levels, and HIV-1 RNA viral burden, with groups defined as above or below the median value for each covariate. The Spearman rank-order correlation coefficient was used to assess the associations between baseline laboratory measurements.

To assess the simultaneous effect of baseline factors on survival time, the Cox proportional-hazards regression model was used. Forward and backward stepwise selections were used to choose variables for the multivariable model. Only factors that were significant at a *P* value of .05 or less in the univariable analyses were included in the multivariable analyses.

RESULTS

Patient study groups

Most children (87%) were of minority groups, and the distributions of races in the survival categories were roughly equal. Similarly, the sex distribution of children in the disease categories was approximately equal. Over 60% of the HIV-1⁺ birth cohort was asymptomatic at 3 months of age, but only 12.2% of the HIV-1⁺ older cohort was asymptomatic at enrollment. Median ages of survivors and nonsurvivors were 22 and 26 months, respectively. By 2 years of age, only 10.5% of the birth cohort remained asymptomatic, cumulative mortality was 16.3%, and 46.8% had died or reached Centers for Disease Control and Prevention category C.¹⁸

Lymphocyte subsets

In the birth cohort the mean CD4⁺ T-cell counts were significantly lower in HIV-1⁺ children than in HIV-1⁻ children at all times except the first week of life ($P \leq .002$; Fig 1, A). In both the HIV-1⁺ and HIV-1⁻ children, there were rises in mean CD4⁺ T-cell counts between 1 week and 1 month, but afterward, a sharper decline in mean CD4⁺ T cells occurred in the HIV-1⁺ children out to 15 months, with a subsequent decline approximating that of the CD4⁺ T cells in the HIV-1⁻ children out to 60 months of observation. In the older cohort of HIV-1⁺ children, survivors had significantly higher mean CD4⁺ T-cell counts compared with the nonsurvivors at all ages ($P < .001$; Fig 1, D).

The CD8⁺ T-cell comparison demonstrated increases in the birth cohort in both the HIV-1⁺ and HIV-1⁻ children from 1 week to 1 month of age, with falling mean CD8⁺ T-cell counts in the HIV-1⁻ children after 1 month (Fig 1, B). The HIV-1⁺ children had a sustained elevation in the number of CD8⁺ T cells for almost the entire 60-month period. At most time points, there were significant differences in mean CD8⁺ T-cell numbers between the HIV-1⁺ and HIV-1⁻ children ($P \leq .05$ between 15 and 54 months of age). These increases in absolute CD8⁺ T-cell counts in the HIV-1⁺ children occurred despite the fact that the absolute lymphocyte count was significantly lower compared with the HIV-1⁻ children (except at 1 week and 1 month, data not shown). Mean CD8⁺ T-cell numbers in the older cohort survivors were almost

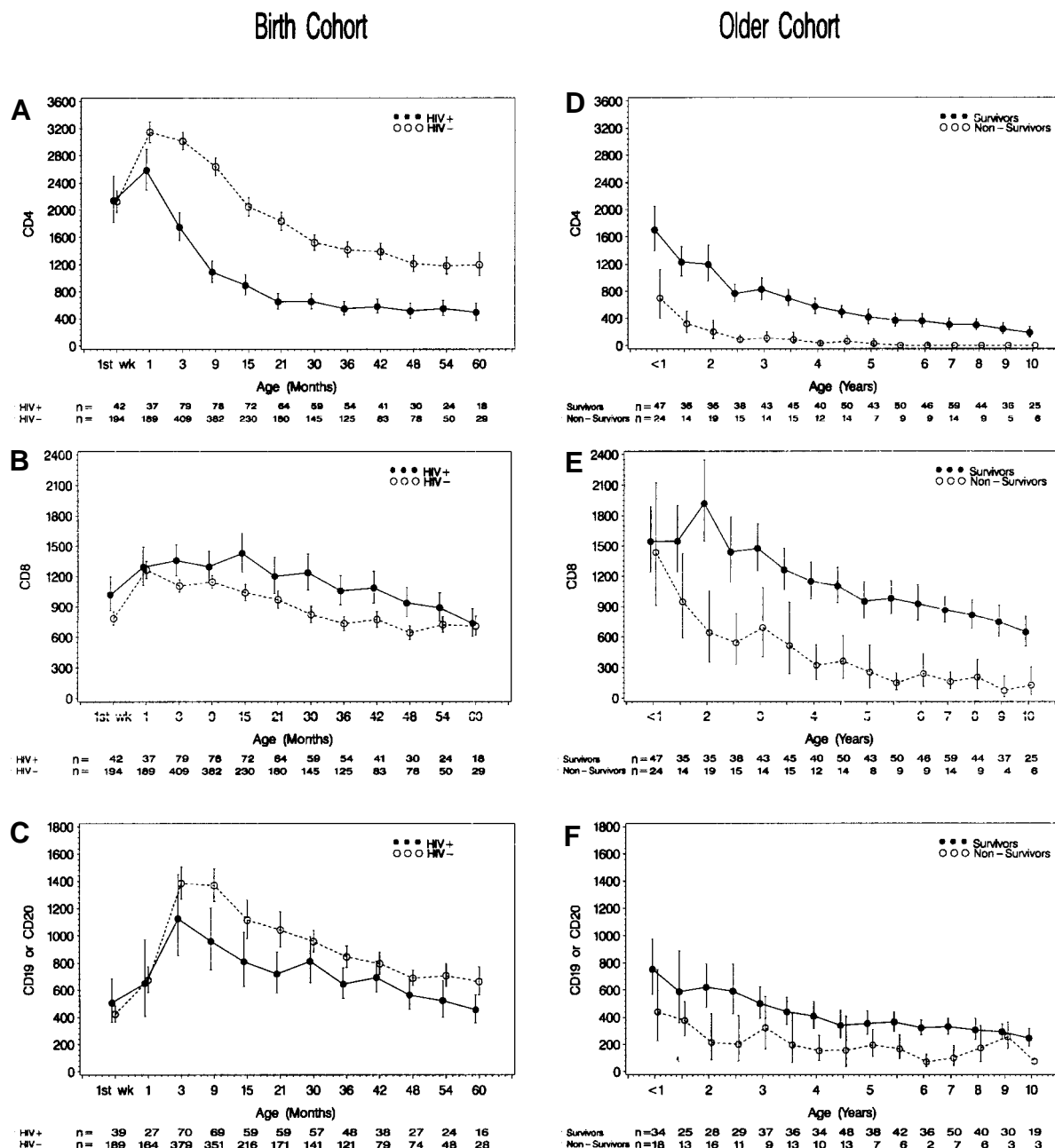


FIG 1. Longitudinal changes in mean lymphocyte counts per microliter in children born to HIV-1+ women. Lines represent the model-based means and 95% confidence intervals. **A** and **D**, CD4+ T-cell counts per microliter for the birth cohort (n = 92 HIV+ and n = 461 HIV-; **A**) and for the older cohort (n = 133 survivors and n = 65 nonsurvivors; **D**). **B** and **E**, CD8+ T-cell counts per microliter for the birth cohort (n = 92 HIV+ and n = 461 HIV-; **B**) and for the older cohort (n = 133 survivors and n = 65 nonsurvivors; **E**). **C** and **F**, CD19+ or CD20+ T-cell counts per microliter for the birth cohort (n = 91 HIV+ and n = 447 HIV-; **C**) and for the older cohort (n = 125 survivors and n = 55 non-survivors; **F**).

always higher than those of the nonsurvivors ($P \leq 0.004$ at all ages ≥ 2 years; Fig 1, *E*).

In the birth cohort the mean absolute B-cell numbers were similar in HIV-1+ infants (mean, 506 counts/ μ L) and HIV-1- infants (mean, 423 counts/ μ L) at birth ($P = .32$; Fig 1, *C*). Between 1 week and 3 months of age, the mean

CD19/20 B-cell counts increased significantly in both groups ($P < .001$), with a mean increase of 620 and 960 counts/ μ L, respectively, in the HIV-1+ and HIV-1- infants. The mean CD19/20 B-cell counts began to decline after 3 months of age in both groups and were significantly lower in the HIV-1+ children at 9, 15, 21, 36, 54, and 60 months

of life ($P = .004, .02, .002, .006, .03$, and $.006$, respectively). In the birth cohort CD19 was used in 69.4% and CD20 in 30.6% of the analysis of 2494 measurements. The pattern of change when B cells were counted by either CD19 or CD20 analysis was not different. In a subset analysis of 594 measurements, where both CD19 and CD20 were available, the B-cell counts remained lower in the HIV⁺ children, but the differences were statistically significant only after 4 years of age. In our primary analyses differences were not found between HIV-1⁺ and HIV-1⁻ children for CD19/20 cell percentages, but these percentages tended to be lower in the HIV-1⁺ children. In the older cohort there was a gradual decline with age in mean B-cell numbers. Survivors had significantly higher mean CD19/20 B-cell counts than did nonsurvivors at most time points between 2 and 7 years of age ($P \leq .05$; Fig 1, *F*). Mean differences were not identified in the older children, perhaps because of the small sample sizes among nonsurvivors. (The same limitation applies to the serum immunoglobulin data.)

Serum Ig concentrations

In the birth cohort there were significant differences between mean serum IgG concentrations of HIV-1⁺ children and HIV-1⁻ children at each time point from birth to 60 months of life ($P = .03$ at birth; $P < .001$ at other ages; Fig 2, *A*). From birth to 6 months, there was a significant decrease in IgG concentration (904 mg/dL at birth to 444 mg/dL at 6 months) for only the HIV-1⁻ children ($P < .001$). After 6 months of life, the mean serum IgG concentrations in the HIV-1⁺ children rose to a sustained plateau of approximately 1900 mg/dL by 18 months of life, and the HIV-1⁻ children's mean serum IgG concentrations began to climb to a plateau of approximately 1050 mg/dL by age 5 years. In the older cohort survivors had generally higher mean serum IgG values than nonsurvivors ($P = .007$ at <1 year, $P = .002$ at 2 years, $P = .04$ at 3 years, $P = .004$ at 4 years, and $P = .02$ at 5.5 years of age). Survivors also had higher mean serum IgG values at 3, 4, and 5.5 years of age ($P = .04, .004$, and $.02$, respectively).

Except for the birth and 60-month time points, the birth cohort of HIV-1⁺ children had higher mean serum concentrations of IgA ($P \leq .001$ through 42 months, $P = .04$ at 48 months, and $P = .001$ at 54 months; Fig 2, *B*). At age 60 months, both the HIV-1⁺ and HIV-1⁻ children had similar mean serum IgA concentrations of approximately 120 mg/dL. The survivors in the older cohort, in general, had lower mean serum IgA concentrations than the nonsurvivors through 4 years of age ($P = .009, .02, .02$, and $.03$ between 2.5 and 4 years of age; Fig 2, *E*).

In the birth cohort the HIV-1⁺ children had higher mean serum IgM concentrations from birth to 42 months of life (Fig 2, *C*; $P \leq .001$ through 42 months). By 24 months of life, the HIV-1⁺ children's values reached a sustained mean level of approximately 164 mg/dL, but the HIV-1⁻ children's values had reached only 119 mg/dL and climbed slowly to about 129 mg/dL by 60 months. Nonsurvivors in the older cohort had generally higher mean serum IgM concentrations ($P = .001, .13, .07, .16$, and $.06$ between 2.5 and 4.5 years of age; Fig 2, *F*).

HIV-1 RNA viral burden of the older cohort

Mean levels of HIV-1 RNA were consistently lower at all ages for surviving children compared with nonsurvivors. For infants less than 1 year of age, mean HIV-1 RNA levels were significantly higher in nonsurvivors (geometric mean, 168,927 copies/mL) compared with survivors (geometric mean, 35,424 copies/mL; $P = .05$). Mean HIV-1 RNA levels were also statistically higher at 2.0 years ($P < .001$), 2.5 years ($P = .03$), and at most time points between 4 and 10 years of age.

Univariable survival analysis of the older cohort

Table I summarizes cumulative survival among 205 HIV⁺ children (older cohort) according to 7 baseline laboratory measurements. Seventy-one of the 205 HIV⁺ children died during follow-up, producing a 5-year cumulative survival rate of 64.6% (95% confidence interval, 57.8%-71.5%). Univariable analyses suggested survival was associated with higher baseline CD4⁺ T-cell counts and higher baseline age-adjusted CD4⁺ T-cell count Z scores, higher baseline CD8⁺ T-cell counts, and higher baseline CD19⁺ or CD20⁺ B-cell counts. Children with higher baseline levels of IgG had better 5-year survival than children with lower baseline levels of IgG (80.9% and 55.9%, respectively), and this difference was most pronounced in children less than 2 years of age (84.5% and 54.0%, respectively). Lower baseline HIV-1 RNA levels were also associated with survival.

Multivariable modeling of survival

Multivariable analyses were affected by missing data and by the correlation among the laboratory measurements. For example, only 153 (75%) of 205 children had a CD19⁺ or CD20⁺ B-cell count measurement within 12 months of enrollment. Sample sizes for the other measurements were 204 for CD4⁺ and CD8⁺ T-cell counts and 168 for IgG (82.0%; the others were excluded for having IgG replacement therapy within 90 days). The correlation between CD4⁺ T-cell counts and CD19⁺/20⁺ B-cell counts was high (Spearman $\rho = 0.52$, $P < .001$), as was the correlation between CD4⁺ T-cell counts and CD8⁺ T-cell counts ($\rho = 0.66$, $P < .001$).

The Cox regression model was fit separately for serum IgG, CD8⁺ T cells, and CD19⁺/CD20⁺ B cells, adjusting for CD4⁺ T-cell count, encephalopathy, clinical center, and left ventricular dysfunction, factors previously determined to be independently associated with survival.³² None of the other baseline laboratory measures except HIV-1 RNA remained associated with survival after adjusting for the other factors. In a subset analysis of 154 children for whom HIV-1 RNA copy number was available, all covariates remained associated with survival, including HIV-1 RNA copy number ($P = .04$). Thus of the immune factors studied, only the baseline CD4⁺ T-cell count independently predicted 5-year survival (Fig 3).

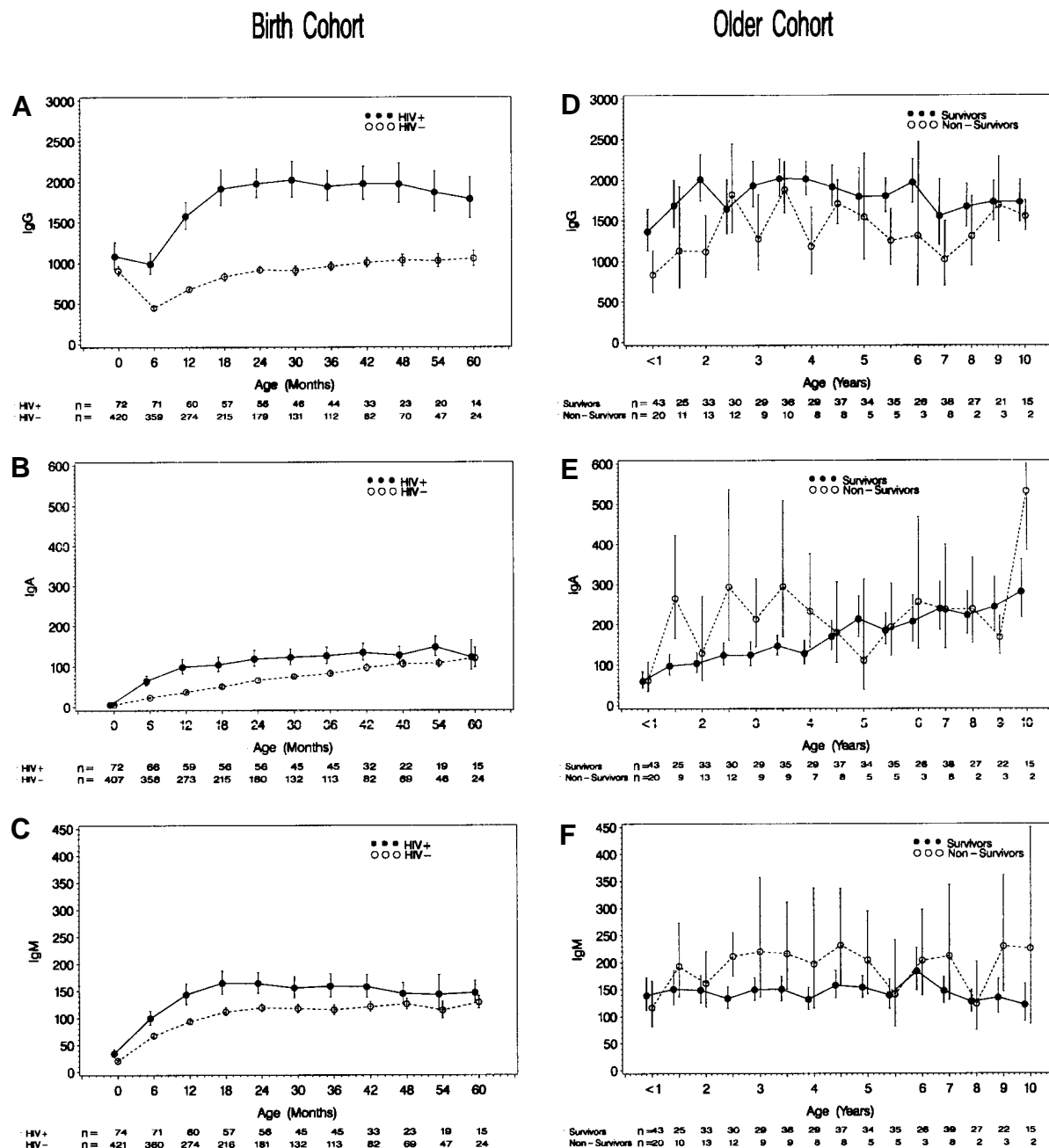


FIG 2. Longitudinal changes in mean serum Igs (in milligrams per deciliter) in children born to HIV-1+ women. *Time trend lines* are the model-based means and 95% confidence intervals. **A** and **D**, Serum IgG (in milligrams per deciliter) for the birth cohort (n = 90 HIV+ and n = 456 HIV-; **A**) and for the older cohort (n = 115 survivors and n = 49 nonsurvivors; **D**). **B** and **E**, Serum IgA (in milligrams per deciliter) for the birth cohort (n = 89 HIV+ and n = 453 HIV-; **B**) and for the older cohort (n = 115 survivors and n = 48 nonsurvivors; **E**). The upper limit of the 95% confidence interval at age 10 for nonsurvivors was truncated at 600 mg/dL (actual upper limit, 732 mg/dL). **C** and **F**, Serum IgM (in milligrams per deciliter) for the birth cohort (n = 90 HIV+ and n = 456 HIV-; **C**) and for the older cohort (n = 115 survivors and n = 48 nonsurvivors; **F**).

DISCUSSION

Higher CD4⁺ T-cell counts were found in survivor children at every age, emphasizing the central role of this T-cell subset in protection from HIV-1 disease progression. At early ages, the numbers of CD8⁺ T cells in both

survivor and nonsurvivor children were similar, illustrating the protective role these cells play in HIV-1 infection. The increased number of CD8⁺ T cells in survivor children may be caused in part by EBV and cytomegalovirus coinfections in HIV-1⁺ children.^{33,34} Lower baseline

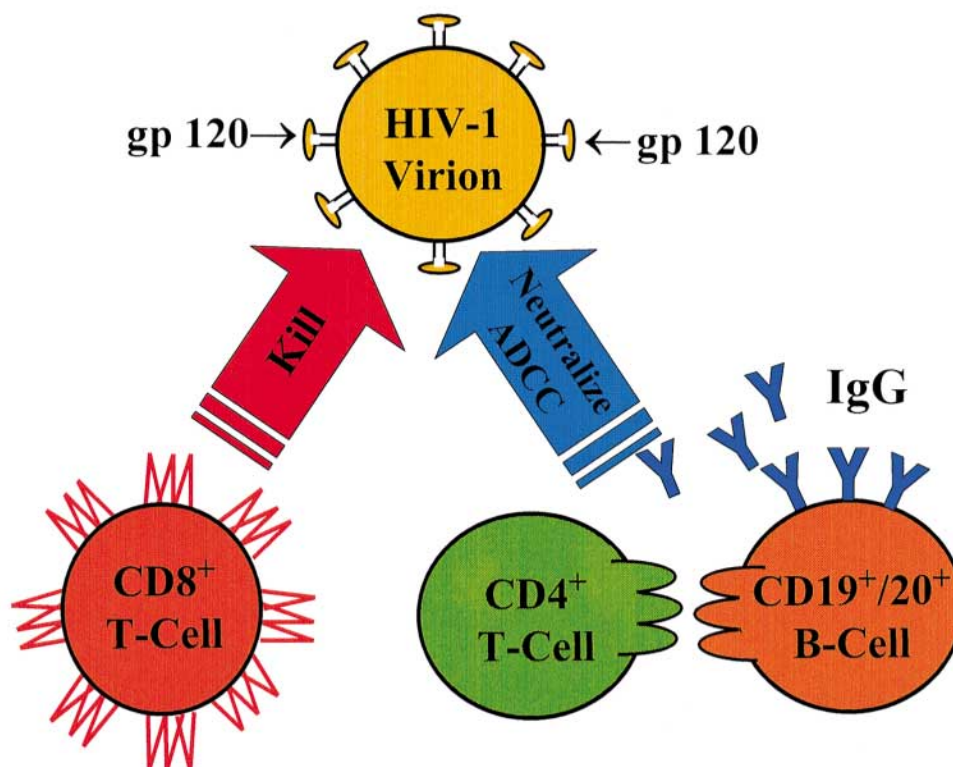


FIG 3. Possible immune survival factors in pediatric HIV-1 infection. CD4⁺ T cells are both the central power of the immune response to HIV-1 and the central target of HIV-1. CD8⁺ T cells are cytotoxic T cells that kill HIV-1. CD19⁺/20⁺ B cells are stimulated (with the help of CD4⁺ T cells) to produce antibodies specific for HIV-1 antigens, such as gp120. Antibodies participate in the neutralization of HIV-1 and destruction of HIV-1 by antibody-mediated cellular cytotoxicity (ADCC). Multivariable analysis of these immune factors, however, showed that only the number of CD4⁺ T cells predicted 5-year survival in this study.

TABLE I. Cumulative survival among 205 HIV-1⁺ children (older cohort) according to baseline laboratory measurements

Measurement	n	Deaths		5-year cumulative survival \pm SE	P value
		n	%		
CD4 cell count					
\geq Median	102	11	10.8	89.1 \pm 3.3	<.001
<Median	102	59	57.8	40.2 \pm 5.4	
CD8 cell count					
\geq Median	102	21	20.6	80.2 \pm 4.3	<.001
<Median	102	49	48.0	49.5 \pm 5.3	
CD19 or CD20 cell count					
\geq Median	77	19	24.7	75.8 \pm 5.2	.004
<Median	76	33	43.4	54.3 \pm 6.2	
IgG					
\geq Median	85	19	22.4	80.9 \pm 4.5	.003
<Median	83	33	39.8	55.9 \pm 6.0	
IgA					
\geq Median	84	30	35.7	64.2 \pm 5.5	.12
<Median	83	21	25.3	74.4 \pm 5.2	
IgM					
\geq Median	84	27	32.1	69.7 \pm 5.2	.97
<Median	83	24	28.9	68.7 \pm 5.6	
HIV-1 RNA					
\geq Median	83	32	38.6	60.5 \pm 5.6	.04
<Median	83	19	22.9	79.4 \pm 4.7	

serum HIV-1 RNA levels were associated with the survival of study subjects.

From univariable analysis of this natural history study of 205 older cohort children, the number of peripheral blood CD19⁺/CD20⁺ B cells and serum IgG concentration were both associated with predicted survival of HIV-1⁺ children. Serum IgG concentration was higher, and serum IgA and IgM concentrations of the survivors were generally lower than those of the nonsurvivors. These observations are consistent with the previous reports of lack of acquired antibody production in HIV-1⁺ children caused most likely by the profound loss of CD4⁺ T cells, which initiate class switching through signaling mechanisms to B cells.¹³⁻¹⁵ Thus patients who succumb to HIV-1 disease are likely those who can only mount IgM (primary) antibody responses caused by lack of adequate T-cell help to switch from IgM to IgG production. Our data do not support faulty IgM to IgA class switching as a basis for patient death.

These two aspects of B-cell metabolism in pediatric HIV-1 infection (peripheral blood B-cell count and serum Ig concentrations) have not been studied in great depth in the last decade, and perhaps the findings of this natural history study may prompt additional investigations of their importance. The few intervening publications have documented a decrease in the CD19⁺/CD5⁺ B-cell compartment in 7- to 12-month-old HIV-1⁺ infants,³⁵ lower CD19⁺ B-cell numbers in more symptomatic HIV-1⁺ children,³⁶ low numbers of functionally active B cells in HIV-1⁺ patients with low p24 antibody serum titers,³⁷ and high serum IgG and IgA levels in HIV-1⁺ children.³⁸ Recently, Rubinstein et al³⁹ have documented progressive immunologic attrition in 17 relatively asymptomatic HIV⁺ adults by using bacteriophage ϕ X174 immunizations. Recently, significant increases in CD19⁺ B cells have been described in pediatric HIV-1⁺ patients given the protease inhibitor zidovudine.^{40,41} The possible clinical importance of recent CD19⁺/CD20⁺ and serum IgG measurements has received confirmation by a recent publication, which showed that both of these factors were important in the prediction of bacterial infections.⁴² In addition to these clinical observations is the *in vitro* evidence that HIV-1 glycoprotein 120 acts as a superantigen in a dose-related fashion and produces stimulation of several human B-cell functions, including the increased production of Ig.⁴³

In summary, the P2C2 HIV-1 Study has examined the natural history of peripheral blood T-cell and B-cell counts, serum Ig concentrations, and predictive survival value of these measurements in a 5-year study of 298 HIV-1⁺ children. HIV-1 infection decreased the CD4⁺ T-cell count, increased the CD8⁺ T-cell count, decreased the CD19⁺/CD20⁺ B-cell count, and increased the levels of serum IgG, IgA, and IgM. Higher values of CD4⁺ T cells, CD8⁺ T cells, CD19⁺/CD20⁺ B cells, and serum IgG were significant predictors of 5-year survival in univariable analysis, but only the CD4⁺ T-cell count was shown to be a significant predictor of survival in multivariable analysis.

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APPENDIX

A complete list of study participants can be found in reference 25. Principle investigators are listed with asterisks.

National Heart, Lung and Blood Institute

Hannah Peavy, MD, (Project Officer); Anthony Kalica, PhD; Elaine Sloand, MD; George Sopko, MD, MPH; Margaret Wu, PhD

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Presbyterian Hospital in the City of New York/Columbia University, New York, NY: Robert Mellins, MD*; Anastossios Koumbourlis, MD; Jane Pitt, MD; Thomas Starc, MD; Anthony Brown; Margaret Challenger; Kim Geromanos, MS, RN

UCLA School of Medicine, Los Angeles, Calif: Samuel Kaplan, MD*; Yvonne Bryson, MD; Joseph Church, MD; Arno Hohn, MD; Andrea Kovacs, MD; Barry Marcus, MD; Arnold Platzker, MD; Helene Cohen, PNP, RN; Lynn Fukushima, MSN, RN; Audrey Gardner, BS; Sharon Golden, RDMS; Lucy Kunzman, RN, MS, CPNP; Karen Simandle, RDMS; Ah-Lin Wong, RDMS; Toni Ziolkowski, RN, MSN

Clinical coordinating center

The Cleveland Clinic Foundation, Cleveland, Ohio: Michael Kutner, PhD*; Mark Schluchter, PhD (through April, 1998); Richard Martin, MD (Case Western Reserve University); Johanna Goldfarb, MD; Douglas Moodie, MD; Cindy Chen, MS; Kirk Easley, MS; Scott Husak, BS; Victoria Konig, ART; Sunil Rao, PhD; Paul Sartori, BS; Lori Schnur, BS; Amrik Shah, ScD; Sharayu Shanbhag, BSc; Susan Sunkle, BA, CCRA

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