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VARIABILITY OF SERIAL ABSOLUTE AND PERCENT CD4+ LYMPHOCYTE COUNTS IN HEALTHY CHILDREN BORN TO HUMAN IMMUNODEFICIENCY VIRUS 1-INFECTED PARENTS

The management of infants and children born to human immunodeficiency virus (HIV-1)-infected mothers is complicated by the presence of maternal antibody during the first 18 months of life and the normal, physiologic decline of CD4+ cell counts and percentage in infants.1,2 Because early diagnosis of HIV-1 infection in infants and children born to HIV-1-infected mothers is difficult, T cell subset analysis, particularly absolute CD4+ lymphocyte number (CD4#), has been used to guide decisions regarding the institution of Pneumocystis carinii pneumonia prophylaxis and antiretroviral therapy.3,4 Although age-specific normal values for T cell phenotypes have been described recently, the clinical significance of CD4# to predict the risk for or the development of immunocompromised status is not well-defined except for the advent of P. carinii pneumonia in the first year of life.5,6,7

Clinically useful surrogate markers of disease progression are stable in a given patient over time in the absence of a pathologic process. Although several investigators have evaluated T cell subsets in healthy children born to HIV-1-infected or normal mothers, a limiting aspect of these studies is that they have either not measured or not analyzed the intrinsic biologic variability of CD4# or CD4+ percentage (CD4%) in individual patients over time.3-5 Without knowledge of the background rate of CD4+ variability, it is difficult to interpret lymphocyte subset analysis in individual HIV-1-infected or at risk patients. The purpose of this study was to evaluate the intrinsic variability in patient lymphocyte subsets over time. Because CD4% is a directly derived value, we hypothesized that for any given patient, CD4% values would approximate a steady state more closely than CD4#. We evaluated this hypothesis by comparing the variability of CD4# and CD4% values over time in healthy infants and children born to HIV-1-infected mothers.

Methods. Subjects. Patients evaluated were Department of Defense beneficiaries less than 4 years of age cared for at military medical facilities and enrolled in a pediatric HIV-1 natural history study. Informed consent was obtained from the guardians of all patients before enrollment. Uninfected patients born to HIV-1-infected mothers (30) or fathers (5) were included in the analysis if they had no known immune dysfunction or other chronic illnesses. Infants and children were defined as HIV-1-negative (uninfected) if anti-HIV-1 antibodies could no longer be detected at 18 months of age. Patients were also categorized as uninfected if 2 HIV-1 cultures and two polymerase chain reaction tests for HIV-1 proviral DNA were negative. All tests were performed outside the neonatal period and at least one test when the patient was older than 6 months of age. Analysis was confined to those children who had at least 3 CD4# and CD4% determinations performed at at least 2-month intervals during the first 4 years of life with at least 2 T cell subset determinations performed in the first 24 months of life.

Lymphocyte immunophenotyping. Venous blood specimens were anticoagulated with ethylenediaminetetraacetic acid. Complete blood cell counts were performed with Coulter Counters (Coulter Hematology, Hialeah, FL) Standard two-color immunophenotyping of lymphocytes was performed with a lysed whole blood method and conjugated murine antibodies (Becton-Dickinson Immunocytometry Systems, San Jose, CA). The percent of labeled cells was determined using conventional flow cytometry. The absolute CD4# was calculated as the product of the absolute lymphocyte count and the percent of CD3+ CD4+ labeled cells. All participating laboratories have successfully met the standard of a strict quality assurance program.8

Statistical analysis. The number of CD4+ lymphocyte values recorded differed between subjects and subjects were measured at different ages, but each subject had a corresponding CD4# for each CD4%. Using the method of the European Collaborative Study,9 age-appropriate z scores were computed for each CD4# and CD4% for each subject at each time point.

To assess the stability of CD4# and CD4% for each subject, the standard deviation of each subject’s z scores over time were calculated. Comparison of the standard deviations for CD4# and CD4% z scores were made with the Wilcoxon signed rank test. To examine consecutive changes in z scores over time, the mean of the absolute value of changes between consecutive time points was computed for each patient. Differences between CD4# and CD4% z score changes were examined using the Wilcoxon signed rank test.

Serial CD4+ cell counts and percentage values were plotted on previously described age-adjusted centile curves with 9 isobars (3rd% to 97th%).10 For each patient a curve connecting serial CD4+ cell count or percentage values was drawn and the number of isobar lines that the curve crossed was recorded. The disparity between the number of isobars crossed by serial CD4# and CD4% values was also compared using the Wilcoxon signed rank test.

All P values are two-sided.

Results. We analyzed 151 separate lymphocyte subset determinations from 35 patients (22 male, 13 female). The population was ethnically diverse with 45% Caucasian, 31% African-American, 9% Hispanic, 3% Asian and 12% mixed ethnic background. The mean age of the patients at entry into the study was 0.6 months (range, birth to 15.8 months) whereas the mean duration of follow-up was 15.0 months (range, 7.3 to 34.3 months). The mean number of lymphocyte subset determinations per patient was 4.3 (range, 3 to 8).

Although both CD4# and CD4% varied considerably within individual patients, CD4# was associated with significantly greater variance. The number of age-appropriate isobar curves crossed connecting individual patient CD4+ lymphocyte values was significantly greater for CD4# than for CD4% (P = 0.001). Likewise the mean standard deviation of z scores for CD4# was 0.66 (range, 0.11 to 2.25) whereas for CD4% it was 0.43 (range, 0.01 to 1.04) (P = 0.01). Figure 1 demonstrates the distribution of the standard deviations of z scores for CD4# and CD4%.

Serial CD4+ lymphocyte values (n = 151) in 35 individual patients were analyzed to determine the amount consecu-
tively measured CD4+ lymphocyte values varied from the previously measured value. The absolute change in consecutively determined z scores was significantly less for patients CD4% (0.51 ± 0.05 (SEM)) than CD4+ values (0.70 ± 0.06 (SEM)) (P = 0.03).

Discussion. Adults infected with HIV-1 demonstrate predictable changes in T cell lymphocyte subsets. Because these changes observed in populations of HIV-1-infected adults, CD4# has become the standard surrogate marker for monitoring HIV-1 disease progression and antiretroviral drug effects in adults. Although the data are more limited, in populations of HIV-1-infected children, T cell lymphocyte subset declines generally similar to those seen HIV-1-infected adults have been observed.

Although CD4# and CD4% in both HIV-1-infected adults and children decline over time, the analysis of pediatric CD4+ lymphocyte values is complicated by normal physiologic changes in these values during the first 4 years of life. Age-related standards for T cell lymphocyte subsets in uninfected children have been published and several demonstrate that CD4# initially rises after birth and then decrease with advancing age. One problem with applying these population-derived standards to individual patients is the potential biologic variability of CD4# and CD4% values in individual patients over time. In the European Collaborative Study most variation in T cell subsets was caused by within child variation.

The practitioner uses individual patient T cell phenotype data to guide decisions regarding the likelihood of HIV-1 infection, the need for opportunistic infection prophylaxis and when HIV-1 infection is confirmed, the institution of antiretroviral therapy. A surrogate marker of HIV-1 infection that is not subject to normal biologic fluctuation in infants and children would therefore be a great asset in the management of both HIV-1-infected and unexposed children. This study compared the two most commonly used lymphocyte subset surrogate markers of HIV-1 infection, CD4# and CD4%, to determine the degree of variability intrinsic to each in individual patients over time. The data demonstrate that in our population of HIV-1-uninfected children born to HIV-1-infected mothers or fathers, serial absolute CD4+ cell counts were highly variable and did not follow any predictable pattern with regard to previously published age-specific CD4# centile curves. Comparing CD4+ lymphocyte values from individual patients to age-specific standards demonstrated that CD4% was associated with significantly less variation than CD4#. CD4% values more closely paralleled age-specific CD4+ lymphocyte isobars. CD4% values were also significantly more predictive of subsequent CD4+ lymphocyte values than CD4#.

This difference between CD4# and CD4% variability in individual patients is both statistically and clinically significant. The Centers for Disease Control recommends institution of P. carinii pneumonia prophylaxis based on age-specific CD4+ cell counts even in the absence of documented HIV-1 infection.4 Age-specific CD4+ cell counts also soon guide decisions regarding both the initiation and response to antiretroviral therapy in HIV-1-infected infants and children.2 Dramatic fluctuations in individual CD4+ cell counts make determining the optimal timing for implementing drug therapies or clinical interventions difficult. Interventions based on CD4% may be less subject to intrapatient variation and hence more reliable.

Although further studies with larger numbers of both HIV-1-uninfected and -infected infants and children will be needed to confirm these findings, the data from this small study suggest that CD4% may be more valuable as a predictor of pathologic T cell changes than absolute CD4+ cell counts in infants and children.

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