T-Lymphocyte Subsets in HIV-Infected and High-Risk HIV-Uninfected Adolescents

Retention of Naive T Lymphocytes in HIV-Infected Adolescents

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Background: The capacity of the immune system of adolescents to generate and repopulate naive and memory cell populations under conditions of normal homeostasis and human immunodeficiency virus (HIV) infection is largely unknown.

Objective: To assess lymphocyte subsets in HIV-infected and high-risk HIV-negative adolescents.

Design: The Reaching for Excellence in Adolescent Care and Health Project of the Adolescent Medicine HIV/AIDS Research Network recruits a cohort of HIV-infected and high-risk HIV-uninfected adolescents, aged 13 to 18 years 364 days, into a study of biomedical and behavioral features of HIV infection as seen in the context of full availability of primary care and HIV-related consultative services. Lymphocyte phenotypes were determined using standard 3-color flow cytometry.

Setting: The Reaching for Excellence in Adolescent Care and Health Project is carried out at 16 clinical sites in 14 urban areas.

Participants: T-lymphocyte subsets are reported in 192 HIV-positive and 78 HIV-negative youths.

Results: For HIV-positive subjects, the total CD4+ cell count and the percentage of CD4+ cells are decreased when compared with those of the HIV-negative controls (P<.001). The reduction in total CD4+ cells reflects a loss of naive, and memory, CD4+ cells compared with HIV-negative youths. Human immunodeficiency virus–infected adolescents, many of whom have been infected recently (ie, those with CD4+ cell counts ≥0.500 × 10^9/L [500/µL]), have a significant increase in naive CD8+ cells compared with HIV-negative youths (P<.01). There also is a significant increase in memory CD8+ cells at all strata of total CD4+ cells compared with HIV-negative youths (P<.01). The increase in naive CD8+ cells in those subjects with CD4+ cell counts of 0.500 × 10^9/L or greater is a unique finding in this cohort.

Conclusions: This study demonstrates high levels of naive CD8+ cells in response to HIV infection in adolescents with CD4+ cell counts of 0.500 × 10^9/L or greater. The presence of high levels of naive CD8+ cells suggests functioning thymic tissue in some adolescents infected with HIV. Furthermore, the normal level of naive CD4+ cells in adolescents with CD4+ levels of 0.500 × 10^9/L or greater provides additional support for the concept of a more robust immune system in HIV-infected adolescents compared with HIV-infected adults. These observations suggest that the immune system of HIV-infected adolescents may be capable of better responses to neontatogens and cytotoxic T-lymphocyte responses to HIV than the immune system of infected children or adults. Human immunodeficiency virus–infected adolescents may have an immune system that is capable of reconstitution following highly active antiretroviral therapy.


Editor’s Note: This important study has great implications for the treatment of human immunodeficiency virus–infected adolescents. As in most things, they are quite different than children and adults.

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PATIENTS AND METHODS

The Reaching for Excellence in Adolescent Care and Health (REACH) Project of the Adolescent Medicine HIV/AIDS Research Network recruits HIV-infected and high-risk HIV-uninfected adolescents, aged 13 to 18 years, over 364 days, into a study of biomedical and behavioral features of HIV infection as seen in the context of full availability of primary care and HIV-related consultative services. Characteristics of the cohort, recruitment and eligibility criteria, and study design are reported elsewhere.13 The group of HIV-infected youths includes antiretroviral therapy-naive and treated youths. We have reported hematologic and immunologic data on a subset of antiretroviral therapy-naive youth.14 We have characterized the T-lymphocyte profiles for this cohort to better understand the immune system of these youths as a basis for subsequent studies of HAART and the potential for immune reconstitution.

All HIV-positive subjects had positive results on an HIV enzyme-linked immunosorbent assay, with a confirmatory Western blot analysis performed before enrollment into the REACH study. The HIV-negative subjects had negative results on an HIV enzyme-linked immunosorbent assay reported within 30 days of enrollment into the REACH study. Human immunodeficiency virus–positive subjects included only those infected either through sexual contact or from needle-sharing behavior; those HIV-infected youths infected through perinatal exposure, early childhood sexual abuse, or blood product exposure were excluded from the study. The HIV-negative youth, to qualify for enrollment into the study, had a history of either sexual intercourse or injection (intravenous and intradermal) drug use. Demographic profiles were similar for the 2 groups.13

Blood samples were obtained from 16 clinical sites. Complete blood cell counts and the basic flow cytometry panel were performed at each of the clinical sites, as described previously.14 For the expanded flow panel, the specimens were collected in EDTA tubes with a minimum of 2 mL of blood, and the specimens were processed within 30 hours from the time of collection.14 Sample viability was greater than 97%, and temperature variability during shipment was less than 2°C. Cell phenotypes were determined using standard 3-color flow cytometry.14 The data are expressed as the percentage of mononuclear cells bearing the specific marker and as the absolute number of cells per unit volume bearing the marker. These denominator data are derived from the absolute lymphocyte count in the complete blood cell count on the same samples.

Immunologic variables, cell counts and percentages, were summarized by arithmetic means, SDs, and percentiles (5% and 95%). Comparisons within the REACH sample, between HIV-negative and HIV-positive male and female subjects, in a given stratum of total CD4+ cell count, were done by 2-sided Wilcoxon rank sum tests, with P < 0.05 defined as significant. Comparisons of the REACH sample with an external, published standard15 were done by reducing all data to counts of subjects with markers in quartiles (or median split from 2 markers). Using computer software,16 the REACH and Roederer et al15 cohorts were compared by Cochran-Mantel-Haenszel χ2 test for trend, using 2 × 4 tables for uninfected subjects, and within each CD4+ stratum (cell counts of 0-0.199, 0.200-0.499, and ≥0.500 × 109/L). For infected subjects, a pooled Cochran-Mantel-Haenszel test combined results across the 3 CD4+ strata. All other calculations were done with statistical software (SAS, version 6.12; SAS Institute Inc, Cary, NC).

that recovery of circulating naive CD4+ and CD8+ T lymphocytes occurs after 4 to 6 months, and usually only in children and young adults. Highly active antiretroviral therapy (HAART) leads to increases in peripheral CD4+ T-lymphocyte counts. Whether HAART will lead to different degrees of reconstitution in individuals at different ages has not been investigated. In a recent study,10 the presence of thymic tissue as measured by a chest computed tomographic scan correlated with CD4+/CD45RA+/CD62L+ T lymphocyte number, suggesting that the thymus may be functional in some, but not all, adults with HIV.10

In adults with HIV, most patients who died of acquired immunodeficiency syndrome complications did not have functional thymic tissue, and when present, thymopoiesis did not prevent lymphopenia.11 In HIV-positive infants, those with T-lymphocyte lymphopenia within the first 6 months of life were at greater risk for rapid progression to acquired immunodeficiency syndrome than nonlymphopenic infants.12 Data of this type are not available for adolescents. The capacity of the immune system of adolescents to generate and replenish naive and memory cell populations in normal homeostasis and HIV infection is largely unknown. This study demonstrates high levels of naive CD8+ cells in HIV-infected adolescents with CD4+ cell counts of 0.500 × 109/L (500/µL) or greater and suggests the presence of functioning thymic tissue in some youths infected with HIV. Adolescents may have the greatest opportunity for immunologic response to HAART. This observation has several important clinical implications. First, it is clear that we must do a better job of getting at-risk youth into HIV counseling and testing that is culturally and developmentally appropriate for adolescents. Second, linking infected youth into comprehensive care programs becomes an even greater charge for the practitioner. And finally, helping youth to understand HIV infection and helping them with adherence to complicated antiretroviral regimens becomes a major focus of care.

RESULTS

T-lymphocyte subset data were analyzed for 192 HIV-infected youths (mean age: females, 16.8 years; males, 17.1 years) and 78 HIV-uninfected youths (mean age: females, 16.3 years; males, 16.8 years). The results reported are from the first visit for which the expanded immunologic panel data were available, which for most subjects was their baseline enrollment visit.

In the Table, the mean percentage and absolute counts for CD4+ and CD8+ cell subpopulations by HIV
status and sex are shown. For HIV-positive males and females, the total CD4+ cell counts and percentage of CD4+ cells decreased when compared with HIV-negative controls. The reduction in total CD4+ cell count reflects loss of naive and memory CD4+ cells compared with HIV-negative youths. Figure 1, top, shows a significant decrease in naive CD4+ cells, as measured by CD4+/CD45RO−/CD45RA+ cells in those subjects with total CD4+ cell counts of 0.500 × 10^9/L; however, for those subjects with total CD4+ cell counts of 0.500 × 10^9/L or greater, naive CD4+ cell counts were similar to those of control subjects. Figure 1, bottom, shows the loss of memory CD4+ cells, which is significant at all levels of total CD4+ cell count in HIV-positive youths compared with high-risk HIV-negative youths.

As shown in the Table, total CD8+ cell counts are significantly higher in HIV-infected females and males compared with the HIV-negative youths. The Table also shows that the increase in total CD8+ cells is the consequence of increases in naive, and memory, CD8+ cells. As noted in the Table, absolute naive CD8+ cells are significantly higher in the HIV-infected females and males compared with the HIV-negative youths. Figure 2, top, shows the absolute naive CD8+ cells categorized across various total CD4+ strata. Those HIV-infected adolescents who are presumably in an early stage of infection (those with CD4+ cell counts = 0.500 × 10^9/L) have a significant increase in naive CD8+ cells compared with HIV-negative youths. As shown in Figure 2, bottom, there is a significant increase in memory CD8+ cells at all strata of total CD4+ cells compared with HIV-negative youths. The increase in naive CD8+ cells in those subjects with CD4+ cell counts of 0.50 × 10^9/L or greater is a unique finding in this cohort. Studies in HIV-infected adults have demonstrated that the increase in total CD8+ cell count occurs only in the CD8+ memory cells, without significant increases in the CD8+ naive cells. 

We compared our findings with the data on adults (n = 109) reported by Roederer et al. The data for the REACH subjects are counted in quartiles (or median split from 2 markers), as defined by Roederer et al, and then compared by the Cochran-Mantel-Haenszel test. So arranged, the data have 1 stratum for each CD4+ level among HIV-positive subjects and a single stratum for HIV-negative subjects. There are differences between the subjects in the REACH and those in the Roederer et al studies in CD4+ naive and memory cells across all 3 CD4+ strata. Of major interest in the context of the observations reported is a Cochran-Armitage trend test, which pools results across all CD4+ and HIV categories. This test indicates that the populations in the REACH and Roederer et al studies differ when analyzed for CD8+ naive cell counts, CD8+ naive cell percentages, and CD4+ naive cell percentages. CD4+ naive cell counts did not differ between the adult populations studied by Roederer et al and the REACH population. Furthermore, whereas the total CD8+ naive cell counts were comparable between the group of adults studied by Roederer et al and the REACH subjects, the number of CD8+ naive cells in HIV-positive subjects in the REACH cohort were higher at each of the 3 CD4+ strata studied.

Activated cytotoxic T-lymphocyte levels (as measured by 3-color flow cytometry as CD8+/CD38+/HLA-DR+ cells) in HIV-infected adolescents are shown in the
Table. Levels are significantly elevated in HIV-infected adolescent females and males compared with HIV-negative youths. Activated cytotoxic T lymphocytes are increased in HIV-infected persons, most likely in response to viral replication. Since these are predominately baseline data and many of the HIV-infected subjects were not yet undergoing antiretroviral combination therapy (52 [35%] females and 14 [33%] males), this finding most likely reflects active HIV replication in the HIV-infected adolescent females and males.

**COMMENT**

The decline in total CD4+ cell counts observed for HIV-infected adolescent females and males reflects loss of CD4+ cells related to HIV infection; however, the mean ± SD cell count for these subjects at baseline is 0.542 ± 0.253 × 10^9/L for females and 0.432 ± 0.211 × 10^9/L for males, indicating that this population is quite immunologically robust. For those infected adolescents with total CD4+ cell counts of 0.500 × 10^9/L or greater, there was no significant reduction in naive CD4+ cells compared with HIV-negative controls. Among those with total CD4+ cell counts below 0.500 × 10^9/L, the absolute number of naive CD4+ was significantly reduced. Memory CD4+ cell counts are lower than for HIV-negative subjects across all CD4+ cell count strata. The mechanism for selective loss of cell subpopulations is not yet determined. The results of in vitro experiments of viral replication in naive vs memory CD4+ cells showed preferential HIV replication in memory CD4+ cells.17,18 This selective replication does not appear to be related to either cell surface receptors or the infectability of the different cell subsets by HIV. The mechanism of T-lymphocyte destruction for naive compared with memory CD4+ cells may, therefore, differ. Early loss of memory CD4+ cells may reflect direct viral replication, resulting in cell
cytes (CD8+/CD38+/DR+ cells) reflect active HIV viral replication. For those infected adolescents with total CD4+ cell counts of 0.500 × 10^9/L or greater, there is a significant increase in naive CD8+ cells above the levels found in HIV-negative youths. It is not until the total CD4+ cell count fell below 0.20 × 10^9/L that there was a significant decrease in absolute numbers of naive CD8+ cells. This finding is unique to the adolescent population.

The thymus is the primary source of generation of naive CD4+ and CD8+ cells. T-lymphocyte progenitors migrate from the bone marrow to the thymus, where they undergo maturation to either naive CD4+ or CD8+ cells. These cells have an expanded T-lymphocyte receptor repertoire that allows them to respond to a wide variety of antigenic stimuli. The memory cells, which express CD45+/RO+ antigen on the cell membrane, are produced primarily in the peripheral blood. These cells have a far more restricted T-lymphocyte receptor repertoire and thus have limited capacity for responding to antigenic stimuli than naive cells.

In studies by Roederer et al. and more recently by McCune et al., CD8+, CD45RA+, and CD62L+ were enumerated by flow cytometry, and the CD45+/CD45RO+ subset was calculated by subtraction. In our study, CD4+ and CD8+ subsets were measured directly; we did not, however, determine CD62L+ expression. Our analysis, however, does distinguish the phenotype of naive from memory subsets by the flow cytometry gating technique used.

A recent study by McCune and colleagues demonstrated that some HIV-infected adults have a detectable thymic mass. The detection by computed tomography correlated directly with higher levels of naive CD4+ cells. In our study, there was a marked increase in naive CD8+ cells in those adolescents earliest in their HIV infection (those with total CD4+ cell counts ≥0.500 × 10^9/L).

Thymic size is not measured in our study, but the marked elevation in naive CD8+ cells suggests that many HIV-infected adolescents have persistent functioning thymic tissue. The higher levels of naive CD8+ cells may be a direct immunologic response to recent HIV infection. In those youth with moderate immunodeficiency as evidenced by CD4+ cell counts less than 0.500 × 10^9/L but greater than 0.20 × 10^9/L, the level of naive CD8+ cells was similar to that in control subjects. Thus, even with moderate immunodeficiency and a probable longer duration of HIV infection, adolescents may retain the capacity for production of naive T lymphocytes. Adolescents may, therefore, be excellent candidates for aggressive HAART and potential immune reconstitution. With control of HIV replication in CD4+ cells, there may be greater potential for repletion of thymic-derived naive CD4+ cells and naive CD8+ cells even in advanced HIV disease.

The high levels of activated cytotoxic T lymphocytes (CD8+/CD38+/DR+ cells) reflect active HIV viral replication. Many of the HIV-positive subjects were not yet undergoing antiretroviral therapy when enrolled in the REACH study. Since high levels of activated cytotoxic T lymphocytes are observed in proximity to the time of seroconversion, this may also reflect recent HIV infection in some subjects.

This study demonstrates high levels of naive CD8+ cells in response to HIV infection in adolescents with CD4+ cell counts of 0.500 × 10^9/L or greater. The presence of high levels of naive CD8+ cells suggests functioning thymic tissue in some adolescents infected with HIV. Furthermore, the normal naive CD4+ cell count in adolescents with total CD4+ cell counts of 0.500 × 10^9/L or greater provides additional support for the concept of a more robust immune system in HIV-infected adolescents compared with HIV-infected adults. These observations suggest that the immune system of HIV-infected adolescents may be capable of better responses to neoantigens and cytotoxic T-lymphocyte responses to HIV than the immune system of infected children or adults. Thus, adolescents may be the population of HIV-infected patients who have the greatest opportunity for an immunologic response to HAART, and these are ongoing investigations.
Accepted for publication August 16, 1999.

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This study was supported by the Adolescent Medicine HIV/AIDS Research Network (AMHARN), which is funded by the National Institute of Child Health and Human Development, with supplemental funding from the National Institute on Drug Abuse, National Institute of Allergy and Infectious Diseases; and the National Institute of Mental Health (grants U01-HD32842 and N01-HD33162), Bethesda, Md.

We thank the members of the Community Advisory Board for their insight and counsel and particularly for making this study happen; and Rick Mitchell, Zangwei Xu, Darlene Ghavimi-Alagha, Nancy Tustin, and Ann Reath, for their special contributions to this article.

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REFERENCES


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**Correction**

Errors in Table. In the original article by Douglas et al titled “T-Lymphocyte Subsets in HIV-Infected and High-Risk HIV-Uninfected Adolescents: Retention of Naive T Lymphocytes in HIV-Infected Adolescents,” published in the April issue of the ARCHIVES (2000;154:375-380), 2 errors occurred in the Table on page 377. The total CD4+ cell count, $\times 10^9/L$, for human immunodeficiency virus (HIV)–negative females should have read 0.879 (0.281) [0.487-1.393]; for HIV-positive males, it should have read 0.432 (0.211) [0.090-0.837]. These data are given as the mean (±SD) [5th and 95th percentiles].