Lymphocyte Subset Changes in Persons Infected with Human Immunodeficiency Virus*

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ABSTRACT

The severe complications of the acquired immunodeficiency syndrome represent the final phase of a prolonged course of immune system destruction during the infection by human immunodeficiency virus (HIV). Many of these complications can be predicted by measuring the depletion of CD4 positive lymphocytes. The CD4 positive lymphocyte counts are now widely accepted as a surrogate marker to assess the stage of disease and to determine immune response in major clinical trials. Other lymphocyte subsets are candidate surrogate markers for antiretroviral therapy.

Our laboratory has utilized flow cytometry to perform lymphocyte subset testing, including CD4, CD8, CD4/CD8 ratio, and others for more than three years on persons with suspected immune deficiency. Results from our laboratory are presented to illustrate the use of these procedures in an urban, predominantly inner city population. The role of flow cytometry in monitoring patients with HIV infection is discussed.

Introduction

The laboratory measurement of CD4 levels is now established as a reliable indicator of progression to AIDS in HIV positive individuals and serves as a surrogate marker for major clinical trials. Few individuals with CD4 counts above 500 per mm$^3$ will progress to AIDS within three years. In contrast, the majority of patients with initial counts below 200 per mm$^3$ will show progression. Since 1988, our laboratory has performed lymphocyte phenotype analysis on known or suspected HIV positive patients by flow cytometry, utilizing a two-color method on the FACS-SCAN analyzer. Using CD4 levels to divide the patients into three groups, the changes in total T cells, in T suppressor cells, in activated T cells, and in B cells and natural killer cells were assessed. Only the first measurement on each patient was employed for the comparison.

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Materials and Methods

Over three years, lymphocyte subset analyses were performed on 791 patients. The differentiation antigens measured are listed in table I. Three hundred and four patients were identified as being HIV positive by a positive confirmatory test, such as the Western Blot assay. After excluding data from patients less than seven years of age, data were evaluated for 295 patients.

Lymphocyte subset analyses were performed on the FACS-SCAN† analyzer utilizing a whole blood lysis procedure. Reagents from Becton-Dickinson were utilized for all procedures, with the exception of CD56, for which Coulter‡ NKH-1-RD1 was employed. For all cases, an isotope control consisting of IgG1/IgG2 was performed. The lymphocyte population was identified, and monocytes were excluded by simultaneous measurement of CD45/CD14.§

Both CD4/CD8 and CD3/HLA-DR were determined. When ordered, CD20 and CD56 were also performed. There were 194 determinations of CD20, and 101 determinations of CD56. Since CD4 levels are closely associated with the stage of disease, the HIV positive patients identified were separated into three groups: CD4 >500 (n = 82), 200 to 500 (n = 90), and <200 (n = 123). The comparison of values in table III were done using independent one-way analysis of variance and the Tukey-Kramer comparison of all pairs.

Data were also compiled for 84 control samples of laboratory volunteers for the same three year period (1989, 1990, and 1991). The comparison of values with control was done using the t test.

### Results

Results for the control group are shown in table II. The male:female ratio for the patient population was 70:30. The mean age was 33 with the vast majority of patients being in the 20 to 45 year age range. The mean values for the total white count (WBC), percentage and total of lymphocytes, CD4/CD8 ratio, as well as the total and percentage of lymphocytes gated for the markers tested is shown in table III. The values shown for HLA-DR represent those cells which are both HLA-DR and CD3 positive.

In table III, all groups with CD4 <500 were compared with each other and with the group with CD4 >500. Means desig-

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† Coulter Electronics, Hialeah, FL 33010.
‡ Leuko Gate™, Becton-Dickinson Immunocytometry Systems, Rutherford, NJ 07070.
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The CD4/CD8 ratio demonstrated a predictable and continuous decline with the lowest values found in those with CD4 values <200 per mm³. The total lymphocyte population, as measured by CD3, demonstrated a gradual decline from highest to lowest CD4 levels. The percentage of CD3 positive cells remained relatively constant, with a small but significant decline in the T helper cell <200 group.

The mean CD8 percentage was elevated in all groups. Total CD8 levels were elevated in the first two groups, dropping to low levels in the group with CD4 <200 per mm³. The percentage of HLA-DR, CD3 positive cells was elevated in all groups. The number of HLA-DR, CD3 positive cells was elevated in the first two groups, declining in the group with lowest CD4 values, while remaining above mean control values.

The B cells, as measured by CD20, did not vary significantly in percentage but showed a significant decline in absolute numbers in both groups with CD4 <500. Natural killer cells, determined by CD56, remained slightly below control values in terms of percentage in all groups except for CD4 <200, in which the percentage was elevated. Absolute values were below control values in all groups and showed a small but significant decline in the group with CD4 <200.

Discussion

Human immunodeficiency virus infection results in profound changes in the immune system. The HIV envelope glycoprotein GP 120 binds to the CD4 receptors, resulting in infection of T helper lymphocytes. Through mechanisms thought to be both direct and indirect, HIV infection leads to depletion and destruction of T helper lymphocytes. The HIV virus may infect monocytes and macrophages, as well as other cells. Functional abnormalities of T4, other T cell subsets and B cells ensue. Following infection by HIV, peripheral blood CD4+ cell levels fall from approximately 1,000 per mm³ to 600 per mm³ within 12 to 18 months. Following a period of relative stability usually continuing for several years, CD4 levels fall again, leading to the development of frank AIDS, after which CD4 levels fall even further. Opportunistic infection may alter CD4 as well as other marker values.
The changes in the CD4/CD8 ratio, in the CD8 and the HLA-DR, CD3 positive compartment (an important indicator of T cell activation) are consistent with those previously reported in the literature.\textsuperscript{2,5,6}

However, CD8 lymphocytosis was not sufficient to prevent a decline in the CD3 positive compartment which was observed with CD4 values less than 500 per mm\textsuperscript{3}. The B cells and natural killer cells, which are not as frequently reported in the literature, demonstrated a decline in absolute values in the advanced stage of the disease.

Coexpression of markers may have influenced some of the results. There is coexpression of a natural killer cell subset with CD8 and of dim T cells with CD20 and CD56. To exclude these subsets, it may be advisable to measure these latter markers simultaneously with CD3.

The flow cytometric measurements of CD4 levels provide reliable information as to the stage of the disease and may be used to guide therapeutic decisions as well as potentially demonstrate response to therapy. Measure of CD8, CD3, and HLA-DR may provide further relevant information. Although CD20 and CD56 levels showed significant change in the patient population, the clinical utility of these latter determinations is unclear. More detailed lymphocyte subset analysis\textsuperscript{7} is currently a valuable research tool and may provide clinically relevant information in the future.

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\textbf{References}