Hematological Reference Ranges among Healthy Ugandans

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Reference values are essential for the interpretation of hematologic data in clinical practice and research studies. Symptom-free human immunodeficiency virus antibody-negative Ugandan adults (183 subjects, aged 15 to 74 years, 37.7% women and 62.3% men) were studied to establish hematological reference ranges. The central 95% areas under the distribution curves were 1,453 to 4,448 cells per µl for the absolute lymphocyte count, 559 to 2,333 cells per µl for the absolute CD4 count, 253 to 1,396 cells per µl for the absolute CD8 count, and 0.68 to 4.4 for the CD4/CD8 ratio. Women had significantly higher mean absolute lymphocyte counts (2,826 versus 2,568/µl), absolute CD4 counts (1,425 versus 1,154/µl), and absolute CD4/CD8 ratios (2.58 versus 1.88) than did men. These reference ranges differ from those reported for populations outside Africa.

An estimated 8 million individuals living in sub-Saharan Africa (including Uganda) are infected with the human immunodeficiency virus (HIV) (2). Infection with HIV results in progressive generalized immune suppression due predominantly to cytopathic effects of HIV type 1 (HIV-1) on $CD4^+$ T-helper–T-inducer lymphocytes (6). HIV also suppresses normal hematopoiesis and is associated with a broad spectrum of hematologic abnormalities. Measurements of the peripheral blood absolute CD4 cell count (ACD4), CD4 percentage (%CD4), and CD4/CD8 ratio have been found to be useful surrogates for determining the risk of progression of HIV infection and are extensively used in observational studies and AIDS clinical trials (7).

Many AIDS-related research studies are being conducted in Uganda and other African nations with a high prevalence of HIV infection. Because of the lack of normal reference ranges for hematological parameters in healthy subjects, many investigators interpret their data using normal values derived from populations in Europe and the United States. There is a need to establish appropriate normal reference values for hematologic parameters in African populations. In this report, we describe hematologic reference ranges obtained by studying 183 symptom-free HIV-seronegative Ugandans.

(Results of this investigation were presented at the Eighth International Conference on AIDS in Africa, Marrakesh, Morocco, December 1993.)

MATERIALS AND METHODS

Subjects. Blood samples were obtained from volunteers who visited the AIDS Information Center, Kampala, Uganda, for counselling and confidential HIV testing between 7 October 1991 and 18 May 1992. All clients at the AIDS Information Center were informed of the need to establish hematological reference values and gave verbal informed consent to have an additional 5 ml of blood drawn for this purpose. All subjects received routine pre-HIV-test counselling. This report includes data for those adults who were found to be HIV-1 and HIV-2 seronegative and who reported no medical complaints during the

initial screening. Subjects were excluded prior to HIV antibody testing if their spouses had died or had suffered serious illness recently.

Sample collection. Two blood samples (drawn by using one 5-ml SST Gel and Clot Activator Vacutainer and one 5-ml EDTA-containing Vacutainer; Becton-Dickinson, Rutherford, N.J.) were drawn from each subject by antecubital venipuncture. The former sample was used for HIV enzyme immunoassay (EIA) testing, and the latter was used for determination of absolute lymphocyte count (ALC), ACD4, absolute CD8 count (ACD8), and CD4/CD8 ratio (ABSR). All blood samples were obtained between 8:30 a.m. and 1:00 p.m. and were processed within 4 h after venipuncture.

HIV antibody determination. After separation of serum from whole blood, sera were screened for the presence of anti-HIV-1 and anti-HIV-2 antibodies at the Uganda Blood Transfusion Service, Kampala, Uganda, by using a commercially available EIA kit (Welcozyme HIV-1 and -2 kit, Murex Diagnostics Limited, Temple Hill, Dartford, England). This assay kit utilizes highly purified immunodominant antigens of the core and envelope proteins of HIV-1 and an immunodominant epitope of the HIV-2 envelope, prepared by peptide synthesis techniques to ensure specificity (4). Confirmatory testing was performed at the Joint Clinical Research Centre (JCRC), Kampala, Uganda, by using the Recombigen (*env & gag*) HIV-1 assay kit (Cambridge Biotech Corporation). This assay is based on recombinant antigens containing immunodominant regions of the envelope and *gag* gene products, manufactured from genetically engineered gp41, and $gp120^{env}$ and $p24^{gag}$ gene products of HIV-1, expressed in *Escherichia coli*. All detectable contaminating bacterial proteins are removed, and the recombinant antigens are applied to polystyrene microwells. This assay is an indirect EIA (1).

ALC, ACD4, ACD8, and ABSR determinations. Hematologic testing was performed at the JCRC. Five-milliliter samples of blood collected in EDTA-containing Vacutainer tubes (Becton-Dickinson) were used for ALC, %CD4, and %CD8 determinations. ALC was measured as the total leukocyte count multiplied by the percentage of lymphocytes by using an automated method (Coulter T-540 counter; Coulter Corporation, Miami Lakes, Fla.). Immunological parameters (%CD4 and %CD8) were measured by using a FACScan (Becton Dickinson) flow cytometer after staining the leukocytes with specific fluorescent monoclonal antibodies (FMAb). FMAb used were two-color cytostat Mo2-RD/KC56-FITC (monocytes/leukocytes), MsIgG-RD/FITC (nonspecific staining), and T4-RD/T8-FITC (CD4/CD8 lymphocyte subpopulations) (Coulter Immunology, Hialeah, Fla.). One hundred microliters of blood was mixed with 10 µl of each FMAb pair. After incubation at room temperature for 15 min, the erythrocytes were lysed either manually (whole-blood lysis) with lytic reagents or by using Q-prep (Coulter Immunology). The samples were centrifuged at $400 \times g$ for 5 min, and the supernatants were decanted. The remaining cell pellet was resuspended in 1,000 µl of phosphate-buffered saline (PBS) and centrifuged for 5 min. The supernatant was removed, and the pellet was resuspended in 500 µl of PBS. The fluorescent-antibody-labelled samples were then analyzed by flow cytometry. The FACScan flow cytometer was calibrated by using Becton Dickinson caliBRITE flow cytometer beads (Becton Dickinson, San Jose, Calif.) with BDIS FACScan AutoCOMP software (version 2.0.2, 1990). The number of stained cells was determined, and %CD4 and %CD8 were calculated by using SimulSET software (version 2.3.2). ACD4 and ACD8 were calculated from the ALC,

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TABLE 1. Hematological reference ranges for entire study population[a]

Parameter	Mean value	95% Range
ALC	2,666 μl^{-1}	1,453–4,448 μl^{-1}
ACD4	1,256 μl^{-1}	559–2,333 μl^{-1}
ACD8	668 μl^{-1}	253–1,396 μl^{-1}
ABSR	2.16	0.68–4.4

[a] n = 183.

%CD4, and %CD8 by the following formula: ACD4 or ACD8 = %CD4 or %CD8 × ALC. ABSR was calculated as the ratio of %CD4 cells to %CD8 cells.

Quality control to assure the accuracy of lymphocyte subpopulation determinations during the course of the study was performed biweekly by comparison of values obtained from analysis of divided clinical samples and 4C (abnormal high, normal, and abnormal low) blood controls (Coulter Diagnostics, Hialeah, Fla.) analyzed at both the JCRC and the Makerere University-Case Western Reserve University-University of California, San Francisco, Core Laboratory at Old Mulago Hospital, Kampala, Uganda. The latter laboratory uses a Coulter T-540 counter (Coulter Diagnostics) and an EPICS II flow cytometer (Coulter Corporation).

Statistical analysis of data. All data were analyzed by using SPSSPC+ and EpiInfo software. The Kolmorogov-Smirnov test was used to test for normal distribution of parameters. The central 95% area under the distribution curve of the parameters measured was considered the reference range. Mean laboratory values were compared by using Student's t test and the Mann-Whitney U test as noted below. Significance was determined at the 95% confidence level.

RESULTS

A total of 183 subjects, 69 (37.7%) women and 114 (62.3%) men, with an age range of 15 to 74 years and a median age of 26 years were enrolled in the study.

For quality control, ALC results for divided samples were measured on instruments in different laboratories and were comparable ($P > 0.05$; Mann-Whitney U test), as were values for ACD4 and ACD8 obtained by using Cytotrol cells (Coulter).

Reference ranges for ALC, ACD4, ACD8, and ABSR for the entire study population are shown in Table 1. Reference ranges for men and women are shown in Table 2. Women had significantly higher mean ALCs (2,826 versus 2,568 μl^{-1}; $P < 0.05$, Student's t test), ACD4s (1,425 versus 1,154 μl^{-1}; $P < 0.001$, Student's t test), and ABSRs (2.58 versus 1.88; $P < 0.001$, Student's t test) than did men.

DISCUSSION

Uganda is one of the countries of central East Africa with the highest prevalence of HIV-1 infection. The peripheral

TABLE 2. Hematological reference values stratified by sex[a]

Parameter	Sex	Mean value	95% Range
ALC	Male	2,568 μl^{-1}	1,383–4,153 μl^{-1}
	Female	2,826 μl^{-1b}	1,645–4,527 μl^{-1}
ACD4	Male	1,154 μl^{-1}	466–2,112 μl^{-1}
	Female	1,425 μl^{-1c}	641–2,370 μl^{-1}
ACD8	Male	686 μl^{-1}	245–1,368 μl^{-1}
	Female	640 μl^{-1}	238–1,460 μl^{-1}
ABSR	Male	1.88	0.64–3.92
	Female	2.58[c]	0.80–4.85

[a] For men, n = 114; for women, n = 69.
[b] $P < 0.05$, Student's t test.
[c] $P < 0.001$, Student's t test.

blood ACD4, %CD4, and CD4/CD8 ratio are among the best surrogate markers for the assessment of the risk for progression to AIDS in HIV-infected individuals and are clinically useful in assessing the risk of developing certain AIDS-related opportunistic infections and timing the initiation of antiretroviral and prophylactic antimicrobial therapies (7). Current guidelines for the initiation of zidovudine therapy and preventive therapy against *Pneumocystis carinii* and *Mycobacterium avium* complex are based on serial assessments of the CD4 lymphocyte count. As a surrogate marker of the stage of HIV infection and the overall degree of immunosuppression, quantification of CD4 lymphocyte counts is widely used in stratification and follow-up of HIV-infected individuals in clinical trials (8).

The primary objective of this study was to establish hematological reference ranges among healthy Ugandans. In the absence of published hematologic reference data derived from African subjects, many researchers use reference ranges derived from North American or European populations. These values may not be representative of those for African people. This study presents preliminary data needed to establish normal hematological reference ranges facilitating interpretation of AIDS-related research studies in Africa.

Our study has several limitations. The HIV EIA kit used to exclude subjects with HIV infection has high degrees of sensitivity and specificity in detecting HIV infection in African populations (4, 8). All subjects were bled only once; therefore, it is possible that some subjects were in the "window" period of HIV infection, having been recently infected with HIV but not yet having developed anti-HIV antibodies. We did not perform medical or other laboratory examinations to evaluate the subjects' general state of health but relied on self-reported symptom histories which may be inaccurate in excluding disease in individuals presenting for voluntary HIV screening. CD4 counts have been found to have significant diurnal and day-to-day variation in the same subjects and to vary with storage time and temperature (3). While all blood samples were obtained during the late morning, stored at room temperature, and processed within a maximum of 4 h after phlebotomy to minimize variability due to time of day and storage time and temperature among different subjects, we performed only single determinations of ALC, ACD4, and ACD8 for each subject and cannot, therefore, comment on variability due to day-to-day variation among individuals.

The reference ranges we observed for ALC are lower than those published by reference laboratories from North America (3). Because ACD4 and ACD8 are calculated from ALC and %CD4 or %CD8, ACD4 and ACD8 values may appear to be significantly lower for African subjects. The use of %CD4, which is also less subject to fluctuation, may therefore be desirable in comparing CD4 lymphocyte values as surrogates for determining the stage of HIV infection obtained from studies done in other areas of the world and those obtained from studies performed in Africa.

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