NOTES

Immunohematological Reference Values for Healthy Adults in Burkina Faso

N. Klose,1 B. Coulibaly,3 D. M. Tebit,1 † F. Nauwelaers,4 H. P. Spengler,4 G. Kynast-Wolf,2 B. Kouyaté,3 ‡ H.-G. Kräusslich,1 and T. Boehler1*

Department of Virology3 and Department of Tropical Medicine and Public Health,2 Institute of Hygiene, University of Heidelberg, Heidelberg, Germany; Nouna Health Research Center, Nouna, Burkina Faso4; and BD Biosciences, Erembodegem, Belgium4

Received 21 January 2007/Returned for modification 20 March 2007/Accepted 10 April 2007

Reference ranges for peripheral blood lymphocyte subsets were generated for 186 healthy adults in Burkina Faso using single-platform flow cytometry. CD4+ T-cell counts ranged from 631 to 1,696 cells μl−1; they were lower in males (n = 97) than in females (n = 89), whereas natural killer cell counts were higher.

Immunohematological parameters may be influenced by genetic and environmental factors, differ between sexes (2), and change with age (11). Recent studies reveal important differences in lymphocyte normal ranges between different populations in Asia (3), Europe (5), and Africa (6, 7, 9, 12, 13). We therefore undertook a population-based study in Nouna, Burkina Faso, in order to generate site- and gender-specific reference values for immunohematological parameters in healthy adults.

The study was part of an ongoing longitudinal prevention of mother-to-child transmission trial which is embedded in the National Programme for AIDS Prevention in Burkina Faso. It was approved by the Institutional Ethics Committees of the University of Heidelberg, Germany, and of the Nouna Health Research Centre, Burkina Faso.

Donors were recruited from female clients of the mother and infant clinic at the local hospital and during repeated blood donation campaigns in the community of Nouna town. Donors were classified as “clinically healthy” if they did not show or report signs and symptoms of illness (>5 kg weight loss in the preceding month, fever during the last 2 weeks, asthma, diabetes, and cardiovascular and renal diseases). The presence of antibodies against the human immune deficiency virus (HIV) or syphilis or the presence of the surface antigen of the hepatitis B virus in serum led to subsequent exclusion of the individual from data analysis.

Donors were excluded from analysis irrespective of their health status if they had used drugs or traditional treatments within the preceding month, had undergone any surgical treatment, had received any blood transfusions in the past, or could not document their age or if they reported ear piercing, tattooing, and acupuncture during the last 3 months or unprotected sex with multiple partners. Female donors were excluded if they were pregnant or had delivered within the past 6 months. “Nonpregnant” status was defined as having had a menstrual bleed within the past 30 days.

Venipuncture was performed in the morning between 7 and 11 a.m., and samples were accessed for hematology analysis and single-platform flow cytometry (FCM) staining within 6 h following venipuncture. Samples were prepared at the climatized room temperature (range, 20 to 30°C) according to the instructions of the manufacturers.

Total leukocyte counts and absolute numbers of lymphocytes were determined by using an automated device (Sysmex KX21N; Sysmex Corporation, Kobe, Japan). Flow cytometric analysis was performed on a BD Biosciences three-color instrument (BD FACScan). All reagents, hard- and software, and consumables were generously provided by BD. Instrument setup was accomplished by using analysis of BD CaliBRITE beads and BD FACSComp software (initially in daily intervals, later twice a week, and then weekly). Analyses were interpreted according to the Centers for Disease Control and Prevention guidelines (8). Instrument settings were manually controlled by visual inspection of typical staining patterns of peripheral blood lymphocytes.

Eligible donors were retrospectively selected from 364 individuals originally recruited. Eighty-one study participants were excluded because the hematology analyser indicated sample agglutination during measurement. Of the remaining 283 donors, 232 were seronegative for HIV, hepatitis B virus, and syphilis. FCM (MultiSET and TruCount) was performed on 223 samples, 186 of which were ultimately included in the calculation of reference values for healthy adults (20 samples had to be excluded because of incomplete FCM measurements due to temporary lack of reagents, and 17 samples did not pass the MultiSET internal quality control and visual inspection by the operator did not allow manual analysis).

The donors’ ages ranged from 18 to 78 years: 28 donors were younger than 20 years (15%), 99 were between 20 and 29 years...
significantly lower CD4/H9262 eases. Whether clinically inapparent parasitic infestation influenced lymphocyte subset distribution for the total study group, males (n = 97) and females (n = 89). Male blood donors had significantly lower CD4 T-cell counts than females (mean difference, −140 cells μl⁻¹; 95% confidence interval, −43 to −238 cells μl⁻¹; P < 0.005), whereas their NK cell counts were significantly higher (mean difference, 163 cells μl⁻¹; 95% confidence interval, 83 to 242 cells μl⁻¹; P < 0.0001). Similar gender-specific differences in lymphocyte subpopulations have been reported from Singapore and Tanzania (2, 3, 13).

The absolute CD4⁺ T-cell counts of healthy adults living in Nouna, Burkina Faso, are among the highest ever reported in published normal-range studies from African and Asian populations (Table 2). This observation supports the hypothesis that a specific genetic factor influences peripheral blood CD4⁺ T-cell counts (1, 4). Our results, however, may also be influenced by differences in study design and laboratory performance between the different studies. The higher CD4⁺ T-cell counts may reflect the specific selection process of blood donors in our study, since we tried not to include individuals suffering from infectious or other clinically recognizable diseases. Whether clinically inapparent parasitic infestation influences CD4⁺ T-cell counts in Nouna, Burkina Faso, is not known and remains to be studied.

Reference values of lymphocyte subpopulations generated with a single-platform lysis-no-wash procedure should not be used for the interpretation of individual measurements with dual-platform flow cytometry. As already described by Nicholson et al. (10) and confirmed in our study, CD45-based lymphocytes and absolute cell μl⁻¹ counts of lymphocyte subpopulations, with lower and upper limits of normal given in parentheses (2.5th and 97.5th percentiles, respectively, or 5th and 95th percentiles if indicated by an asterisk). MultiSET reference ranges were provided by BD Biosciences. All data were generated using the MultiSET flow cytometric methodology in combination with TruCount tubes and a specia.
phocyte counting with TruCount tubes yields absolute CD4+ T-cell counts that are about 15% higher than those obtained with dual-platform flow cytometry using lymphocyte numbers obtained with the Sysmex hematology analyzer.

Elevated normal ranges of absolute CD4+ T-cell counts in healthy adults may have an impact on the immunological staging of HIV disease and AIDS. Samples from several other areas in West Africa should therefore be studied to confirm our data. At present, we recommend that treatment decisions be based on repeated measurements of CD4+ T-cell percentages and absolute counts and not on absolute counts alone.

This study was supported by Deutsche Forschungsgemeinschaft (SFB 544, project A6).

We are indebted to all blood donors for their willingness to participate and to the clinical and laboratory staff in Nouna.

REFERENCES
1. Ahmadi, K. R., M. A. Hall, P. Norman, R. W. Vaughan, H. Snieder, T. D. Spector, and J. S. Lanchbury. 2001. Genetic determinism in the relationship between human CD4+ and CD8+ T lymphocyte populations? Genes Immun. 2:381–387.
2. Bisset, L. R., T. L. Lung, M. Kaelin, E. Ludwig, and R. W. Dubs. 2004. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. Eur. J. Haematol. 72:203–212.
3. Chung, W. J., G. B. Tan, and P. Kuperan. 2004. Establishment of adult peripheral blood lymphocyte subset reference range for an Asian population by single-platform flow cytometry: influence of age, sex, and race and comparison with other published studies. Clin. Diagn. Lab. Immunol. 11:168–173.
4. Hall, M. A., P. J. Norman, B. Thiel, H. Tiwari, A. Peiffer, R. W. Vaughan, S. Prescott, M. Leppert, N. J. Schork, and J. S. Lanchbury. 2002. Quantitative trait loci on chromosomes 1, 2, 3, 4, 8, 9, 11, 12, and 18 control variation in levels of T and B lymphocyte subpopulations. Am. J. Hum. Genet. 70:1172–1182.
5. Jentsch-Ullrich, K., M. Koenigsmann, M. Mohren, and A. Franke. 2005. Lymphocyte subsets’ reference ranges in an age- and gender-balanced population of 100 healthy adults—a monocentric German study. Clin. Immunol. 116:192–197.
6. Kassu, A., A. Tsegaye, B. Petros, D. Wolday, E. Hailu, T. Tilahun, B. Hailu, M. T. Roos, A. L. Fontanet, D. Hamann, and T. F. De Wit. 2001. Distribution of lymphocyte subsets in healthy human immunodeficiency virus-negative adult Ethiopians from two geographic locales. Clin. Diagn. Lab. Immunol. 8:1171–1176.
7. Lugada, E. S., J. Mermin, F. Kaharuza, E. Ulvestad, W. Were, N. Langeland, B. Asjo, S. Malamba, and R. Downing. 2004. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clin. Diagn. Lab. Immunol. 11:29–34.
8. Mandy, F. F., J. K. Nicholson, and J. S. McDougal. 2003. Guidelines for performing single-platform absolute CD4+ T-cell determinations with CD45 gating for persons infected with human immunodeficiency virus. Morb. Mortal. Wkly. Rep. Recomm. Rep. 52:1–13.
9. Menard, D., M. J. Mandeng, M. B. Tothy, E. K. Kelembho, G. Gresenguet, and A. Talarmin. 2003. Immunohematological reference ranges for adults from the Central African Republic. Clin. Diagn. Lab. Immunol. 10:443–445.
10. Nicholson, J. K., D. Stein, T. Mui, R. Mack, M. Hubbard, and T. Denny. 1997. Evaluation of a method for counting absolute numbers of cells with a flow cytometer. Clin. Diagn. Lab. Immunol. 4:309–313.
11. Saule, P., J. Trauet, V. Dutricez, V. Lekeux, J. P. Desaint, and M. Labalette. 2006. Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4(+) versus effector memory and terminally differentiated memory cells in CD8(+) compartment. Mech. Ageing Dev. 127:274–281.
12. Tsegaye, A., T. Messele, T. Tilahun, E. Hailu, T. Sahlu, R. Doorly, A. L. Fontanet, and T. F. Rinke de Wit. 1999. Immunohematological reference ranges for adult Ethiopians. Clin. Diagn. Lab. Immunol. 6:410–414.
13. Urassa, W. K., E. M. Mbenza, A. B. Swai, H. Gaines, F. S. Mhalu, and G. Biberfeld. 2003. Lymphocyte subset enumeration in HIV seronegative and HIV-1 seropositive adults in Dar es Salaam, Tanzania: determination of reference values in males and females and comparison of two flow cytometric methods. J. Immunol. Methods 277:65–74.