

Normal Values for CD4 and CD8 Lymphocyte Subsets in Healthy Chinese Adults from Shanghai

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Received 19 December 2003/Returned for modification 26 January 2004/Accepted 20 April 2004

The aim of this study was to establish reference ranges for lymphocyte subsets in Chinese adults. Venous blood specimens were obtained from 614 healthy, human immunodeficiency virus (HIV)-seronegative adults in Shanghai. Flow cytometry was used to determine percentages and absolute numbers of CD4 and CD8 T lymphocytes. Mean values for CD4 and CD8 lymphocytes were 727 and 540 cells/ μ l, respectively, yielding a CD4/CD8 ratio of 1.49. While CD8 lymphocyte values varied with age and gender, no significant differences in CD4 lymphocyte values were observed. Shanghai adults had approximately 100 fewer CD4 lymphocytes/ μ l on average than Caucasians, suggesting that lower CD4 lymphocyte cutoffs for classifying and monitoring HIV infection may be needed in China.

Measurements of CD4⁺ lymphocytes are essential for assessing human immunodeficiency virus (HIV) disease course, clinical staging, epidemiological studies, and decisions regarding prophylactic therapies against opportunistic infections (2, 3, 8, 9, 21). Only in industrialized countries has it been feasible to routinely monitor CD4 lymphocyte subsets during routine HIV clinical care. In China, HIV has spread to all 31 provinces, regions, and municipalities and is currently moving into new segments of the populace (22). It is estimated that more than 1 million people are infected in China, and this number may reach 10 million by 2010 (11). Information is generally lacking on the normal range of lymphocyte subpopulations, including CD4 and CD8 lymphocytes, in China.

To provide normal ranges for CD4 and CD8 lymphocyte subsets, and for CD4/CD8 ratios in normal Chinese adults, blood specimens were collected from healthy native adult residents of Shanghai who received routine annual health evaluations at Huashan Hospital between August 2000 and February 2001. Subjects were excluded if they were diagnosed with HIV type 1 (HIV-1) infection or other recent viral or bacterial infections or chronic organ diseases, were immunocompromised, or were recently exposed to toxic chemicals. Whole-blood samples were collected using sterile EDTA Vacutainer tubes. The Science and Research Bureau of Fudan University approved the study.

Flow cytometry of lymphocyte subsets was carried out using a lamp-based flow cytometer (Bryte-HS, Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions. Briefly,

white blood cell counting and differentiation were performed using a Symex-SF3000 Coulter counter (Coulter Electronic, Luton, London). Blood samples were then stained using OptiClone CD4/CD8, immunoglobulin G1–fluorescein isothiocyanate, and immunoglobulin G1–phycoerythrin monoclonal antibodies (Coulter-Immunotech, Miami, Florida). The monoclonal antibodies, 13B8.2 and B9.11, were used to bind specifically to CD4 and CD8 subsets of peripheral blood T lymphocytes, respectively (7, 17). The determination of positive and negative cells for any combination of reagents was set with directly conjugated antibodies of irrelevant specificity as negative controls. Positive and negative controls were included in each run according to guidelines issued previously (4, 5). Statistical analysis was performed using Epiinfo software (version 6; Centers for Disease Control and Prevention, Atlanta, Ga.). Means, standard deviations, standard errors, medians, and confidence intervals were calculated. Single variance analysis was used to compare mean values for lymphocyte subsets among different age and gender groups. A two-tailed *P* value of ≤ 0.05 was considered significant.

During the study period, a total of 614 healthy native Chinese adults who met inclusion criteria were studied. Of these, mean age was 33.4 years (range, 16 to 50 years), and 377 (61.4%) were male. Assay reproducibility was confirmed by having 49 blood specimens from individual subjects analyzed by different technicians on different days. There were no significant differences in CD4 and CD8 lymphocyte subset values between the two runs (*P* > 0.05), indicating acceptable intra-laboratory variation. Peripheral white blood cell counts, lymphocyte percentages, CD4 and CD8 lymphocyte subset values, and CD4/CD8 ratios from 614 subjects are shown in Table 1; age and gender distribution of CD4 and CD8 subset values and CD4/CD8 ratios are listed in Table 2. CD8 lymphocyte counts,

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TABLE 1. Peripheral white blood cell count, lymphocyte percentage, CD4 and CD8 subset values, and CD4/CD8 ratios for healthy adults in Shanghai, China

Category ^a	Mean value	SD	SE	Median value	Minimum value	Maximum value	95% Range	99% Range
Age	33.4	9.5	0.4	32	16	50	NA ^b	NA
Total no. of white blood cells/ μ l	6,503	1,678	67	6,200	3,180	10,500	NA	NA
Lymphocytes (%)	31.6	7.2	0.3	31.6	12.7	64.0	NA	NA
CD4 value	727	255	10.3	679	252	2,082	415–1,189	390–1,634
CD8 value	539	134	5.3	503	193	1,071	336–780	283–881
CD4/CD8 ratio	1.49	0.57	0.02	1.42	0.47	4.62	0.72–2.56	0.60–3.29

^a CD4 and CD8 subset values are expressed as no. of cells per microliter.

^b NA, not applicable.

which averaged 539.6 ± 134.1 cells/ μ l, were significantly higher in men than in women ($P < 0.001$), resulting in a significantly lower CD4/CD8 ratio in men (1.42 versus 1.52; $P < 0.001$). We observed a mean CD4 level of 727 cells/ μ l for the healthy, HIV antibody-negative Chinese adult residents of Shanghai, with no significant differences according to age or gender ($P > 0.05$).

The hypothesis that lymphocyte subset values in healthy HIV-seronegative individuals may be affected by race, geographical location, gender, circadian changes, and physical exercise has been demonstrated previously (15, 16, 19). Studies from other countries support the presence of variation in both number and percentage of lymphocytes and their subsets (1, 13, 19, 20). Few studies have examined normal values for lymphocyte subsets in the Chinese population (10, 14), especially in persons residing on the mainland of China. Based on reported differences in normal values for lymphocytes in different populations, there are questions whether CD4 lymphocyte count thresholds used to guide therapy in Europe and in North America are appropriate for China.

Our study showed that the normal CD4 subset value among healthy, HIV-seronegative Chinese adults in Shanghai was 727 ± 255 cells/ μ l. This was significantly lower than what has been reported for Caucasians (844 ± 247 cells/ μ l) (12) and is consistent with previous findings from Saudi Arabia and Singapore

(13, 18). This finding is important because CD4 lymphocyte counts are used for clinical classification, to determine prognosis, and to decide whether to prescribe prophylaxis for opportunistic infections. Based on our study, we believe it will be very important to determine whether somewhat different guidelines and CD4 lymphocyte cutoffs will be needed for persons living with HIV infection in China.

Our data also indicated slightly lower CD8 lymphocyte subset values for the native Chinese population than has been reported for Caucasians (539 ± 134 versus 593 ± 214 cells/ μ l) (12). Previous studies indicated that variable CD8 levels were detected during the clinical course of HIV-1 infection. Numbers of CD8 cells are elevated in early HIV infection and may decline somewhat over time. At the time of AIDS diagnosis, they have often already returned to normal levels (6). The present study demonstrated variations of CD8 subset values for healthy native Chinese adults, with significantly higher values for men and different values depending on age.

In conclusion, Chinese adults residing in Shanghai have significantly lower CD4 lymphocyte subset values than has been reported for Caucasian populations. A modified and lower CD4 value for classifying and monitoring HIV-1 infection may be needed for Chinese adults. Since there were no significant differences in CD4 values according to age or gender, a single

TABLE 2. Age and gender distribution of CD4 and CD8 subset values and CD4/CD8 ratios in Shanghai, China^a

Age group	Result by gender								Result, total			
	Males				Females							
	No. tested	CD4 value	CD8 value	CD4/CD8 ratio	No. tested	CD4 value	CD8 value	CD4/CD8 ratio	No. tested	CD4 value	CD8 value	CD4/CD8 ratio
16–20	26	637 \pm 195	553 \pm 147	1.27 \pm 0.41	24	789 \pm 292	561 \pm 175	1.58 \pm 0.59	50	710 \pm 256	499 \pm 147	1.42 \pm 0.53
21–25	82	649 \pm 218	581 \pm 106	1.21 \pm 0.37	37	796 \pm 386	543 \pm 150	1.53 \pm 0.48	119	694 \pm 288	531 \pm 106	1.31 \pm 0.43
26–30	63	726 \pm 229	525 \pm 148	1.56 \pm 0.60	52	690 \pm 205	522 \pm 157	1.42 \pm 0.48	115	710 \pm 218	474 \pm 127	1.50 \pm 0.55
31–35	48	788 \pm 311	593 \pm 148	1.51 \pm 0.55	25	773 \pm 275	455 \pm 134	1.87 \pm 0.53	73	783 \pm 297	486 \pm 127	1.63 \pm 0.57
36–40	44	717 \pm 234	584 \pm 126	1.34 \pm 0.46	26	718 \pm 181	452 \pm 145	1.74 \pm 0.60	70	717 \pm 214	442 \pm 126	1.49 \pm 0.55
41–45	66	781 \pm 267	599 \pm 106	1.37 \pm 0.49	41	677 \pm 205	469 \pm 169	1.66 \pm 0.72	107	741 \pm 246	501 \pm 126	1.48 \pm 0.60
46–50	48	811 \pm 281	585 \pm 126	1.70 \pm 0.74	32	658 \pm 620	436 \pm 161	1.69 \pm 0.63	80	750 \pm 254	442 \pm 106	1.70 \pm 0.69
Total	377	730 \pm 256	569 \pm 142	1.42 \pm 0.55	237	722 \pm 255	495 \pm 118	1.62 \pm 0.59	614	727 \pm 255^b	540 \pm 134	1.49 \pm 0.57

^a Mean \pm standard deviation. CD4 and CD8 subset values are expressed as no. of cells per microliter.

^b Bold type identifies the CD4 values which are significantly lower than those in Caucasians (844 ± 247 cells/ μ l) (12).

reference range be suitable for healthy HIV-seronegative Chinese adults.

We thank Xiaohong Chen, Wei Zhang, Anqi Chen, Fuqin Gao, Guozhen Zhu, Yongxing Zhang, and Yongmei Shou for their excellent assistance and David Haas for his critical review of the manuscript.

REFERENCES

1. **Al Qouzi, A., A. Al Salamah, R. Al Rasheed, A. Al Musalam, K. Al Khairy, O. Kheir, S. Al Ajaji, and A. H. Hajeer.** 2002. Immunophenotyping of peripheral blood lymphocytes in Saudi men. *Clin. Diagn. Lab. Immunol.* **9**:279–281.
2. **Anonymous.** 1992. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Morb. Mortal. Wkly. Rep.* **41**:RR-17.
3. **Anonymous.** 1990. Acquired immunodeficiency syndrome (AIDS). Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. *Wkly. Epidemiol. Rec.* **65**:281–283.
4. **Anonymous.** 1997. Guidelines for the enumeration of CD4+ T lymphocytes in immunosuppressed individuals. *Clin. Lab. Haematol.* **19**:231–241.
5. **Anonymous.** 1997. Revised guidelines for performing CD4 T-cell determination in persons infected with human immunodeficiency virus (HIV). *Morb. Mortal. Wkly. Rep.* **46**:RR-2.
6. **Cooper, D. A., B. Tindall, E. J. Wilson, A. A. Imrie, and R. Penny.** 1988. Characterization of T lymphocyte responses during primary infection with human immunodeficiency virus. *J. Infect. Dis.* **157**:889–896.
7. **Corbeau, P., M. Benkirane, R. Weil, C. David, S. Emiliani, D. Olive, C. Mawas, A. Serre, and C. Devaux.** 1993. Ig CDR3-like region of the CD4 molecule is involved in HIV-induced syncytia formation but not in viral entry. *J. Immunol.* **150**:290–301.
8. **Fahey, J. L., J. M. Taylor, R. Detels, B. Hofmann, R. Melmed, P. Nishanian, and J. V. Giorgi.** 1990. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N. Engl. J. Med.* **322**:166–172.
9. **Freedberg, K. A., J. A. Scharfstein, G. R. Seage III, E. Losina, M. C. Weinstein, D. E. Craven, and A. D. Paltiel.** 1998. The cost-effectiveness of preventing AIDS-related opportunistic infections. *JAMA* **279**:130–136.
10. **Kam, K. M., W. L. Leung, M. Y. Kwok, M. Y. Hung, S. S. Lee, and W. P. Mak.** 1996. Lymphocyte subpopulation reference ranges for monitoring human immunodeficiency virus-infected Chinese adults. *Clin. Diagn. Lab. Immunol.* **3**:326–330.
11. **Kaufman, J., and J. Jing.** 2002. China and AIDS—the time to act is now. *Science* **296**:2339–2340.
12. **Lebranchu, Y., G. Thibault, D. Degenne, and P. Bardos.** 1991. Abnormalities in CD4+ T lymphocyte subsets in patients with common variable immunodeficiency. *Clin. Immunol. Immunopathol.* **61**:83–92.
13. **Lee, B. W., H. K. Yap, F. T. Chew, T. C. Quah, K. Prabhakaran, G. S. Chan, S. C. Wong, and C. C. Seah.** 1996. Age- and sex-related changes in lymphocyte subpopulations of healthy Asian subjects: from birth to adulthood. *Cytometry* **26**:8–15.
14. **Lin, S. C., C. C. Chou, M. J. Tsai, K. H. Wu, M. T. Huang, L. H. Wang, and B. L. Chiang.** 1998. Age-related changes in blood lymphocyte subsets of Chinese children. *Pediatr. Allergy Immunol.* **9**:215–220.
15. **Maini, M. K., R. J. Gilson, N. Chavda, S. Gill, A. Fakoya, E. J. Ross, A. N. Phillips, and I. V. Weller.** 1996. Reference ranges and sources of variability of CD4 counts in HIV-seronegative women and men. *Genitourin. Med.* **72**:27–31.
16. **Miyawaki, T., K. Taga, T. Nagaoki, H. Seki, Y. Suzuki, and N. Taniguchi.** 1984. Circadian changes of T lymphocyte subsets in human peripheral blood. *Clin. Exp. Immunol.* **55**:618–622.
17. **Moebius, U., G. Kober, A. L. Griselli, T. Hercend, and S. C. Meuer.** 1991. Expression of different CD8 isoforms on distinct human lymphocyte subpopulations. *Eur. J. Immunol.* **21**:1793–1800.
18. **Shahabuddin, S., I. Al-Ayed, M. O. Gad El-Rab, and M. I. Qureshi.** 1998. Age-related changes in blood lymphocyte subsets of Saudi Arabian healthy children. *Clin. Diagn. Lab. Immunol.* **5**:632–635.
19. **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.
20. **Uppal, S. S., S. H. Verma, and P. S. Dhot.** 2003. Normal values of CD4 and CD8 lymphocyte subsets in healthy Indian adults and the effects of sex, age, ethnicity, and smoking. *Cytometry* **52B**:32–36.
21. **Yarchoan, R., D. J. Venzon, J. M. Pluda, J. Lietzau, K. M. Wyvill, A. A. Tsiatis, S. M. Steinberg, and S. Broder.** 1991. CD4 count and the risk for death in patients infected with HIV receiving antiretroviral therapy. *Ann. Intern. Med.* **115**:184–189.
22. **Zhong, P., L. Kang, Q. Pan, F. Konings, S. Burda, L. Ma, Y. Xue, X. Zheng, Z. Jin, and P. Nyambi.** 2003. Identification and distribution of HIV type 1 genetic diversity and protease inhibitor resistance-associated mutations in Shanghai, P. R. China. *J. Acquir. Immune Defic. Syndr.* **34**:91–101.