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The distribution of immunoregulatory cells in the peripheral blood of normal Malaysian adults

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Abstract

The distribution of imrnunoregulatory cells in the peripheral blood of an individual has now been established as an important tool in helping the management of several diseases. It is necessary to set the normal ranges of these cells for the laboratory. We have undertaken in this study to establish the reference ranges for normal Malaysian adults. We found that the mean percentages of T cells, B cells, T Helper cells (CD 4), T suppressor cells (CD 8), NK cells and the ratio of CD4/CD8 were 70.91%, 11.38%, 38.15%, 37.76%, 17.45% and 1.00 respectively. There was no significant difference between the sexes. In certain parameters, there was significant differences between Malay, Chinese and Indians. The Chinese and Indians were significantly different in the distribution of B cells and in the CD4/CD8 ratio. In the case of CD4 and NK cells, the Indians were different from the other two groups.

Key words: Immunoregulatory cells, FACScan, normal range

INTRODUCTION

Determination of the distribution of immunoregulatory cells in the peripheral blood is important in helping the management of diseases that involve alterations in lymphocyte subpopulations. Notable examples include the depletion of T helper (CD4+) cells during the course of HIV infection[1-2] as well as in idiopathic CD4+ T-lymphocytopenia,[3-7] the increase in T helper/T suppressor ratios in autoimmune liver diseases[8-9] and relative decreases in both proportion and number of identifiable T cells in addition to periodic increases in certain B-cell populations during active systemic lupus erythematosus (SLE).[10-11]

The objective of this study is to determine the distribution of immunoregulatory cells in the peripheral blood of normal individuals so as to establish a reference range for normal adults for this laboratory.

MATERIALS AND METHODS

Selection of subjects and collection of samples

Blood was collected from 74 normal volunteers, 39 males and 35 females, ranging in age from 20 to 79 years. The volunteers, from three different ethnic groups, were 22 Malays, 30 Chinese and 22 Indians. Peripheral blood was obtained by venipuncture and collected in EDTA containers.

Staining and flow cytometric analysis

The methodology of the test performed was the lysed whole blood technique of Becton Dickinson.[12] An appropriate amount of murine monoclonal antibodies against the different cell surface markers (Table 1) was mixed and incubated in separate tubes with one hundred microlitres of blood at room temperature. The erythrocytes were lysed when the above mixture was incubated with FACS Lysing solution. The stained leucocytes were washed with phosphate buffered saline (PBS) and fixed in 1% paraformaldehyde. The cells were subsequently analysed using the Simulset Analysis software in the FACScan within 24 hours of preparation.

Statistical analysis

All results were expressed as means ± 1SD (standard deviation). Data obtained conformed to the normal distribution plot. Therefore, the data were analysed in its original form. Analysis of variance (ANOVA) was performed to see if factors such as sex and race played a role in determining the immune status of an individual. The Duncan Multiple Range test was performed to analyse the effect of race on every parameter

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TABLE 1: Panel of reagents employed in this study

Antigen (Cellular distribution)	Antibodies/fluorochrome (Simultest reagents)
CD3 (All T cells)	Anti-CD3/FITC (Simultest T & B cells test)
CD19 (B cells)	Anti-CD19/PE (Simultest T & B cells test)
CD4 (T helper/inducer cells)	Anti-CD4/FITC (Simultest CD4 & CD8 cells test)
CD8 (T suppressor/cytotoxic cells)	Anti-CD8/PE (Simultest CD4 & CD8 cells test)
CD16+56 (Natural killer cells)	Anti-CD16+56/PE (Simultest CD3 & CD16+56 cells test)
CD45 (Leucocytes)	Anti-CD45/FITC (Simultest Leucogate)
CD14 (Monocytes)	Anti-CD14/PE (Simultest Leucogate)

Note: All the above Simultest reagents used in this study were obtained from Becton Dickinson, USA.

studied. Statistical analysis was performed using the STATGRAPHICS program (Statistical Graphics System by the Statistical Graphics Corporation).

RESULTS

Results of each immune status parameter (T cells/CD3, B cells/CD19, T helper cells/CD4, T suppressor cells/CD8, T Helper (CD4)/T Suppressor (CD8) ratio and natural killer (NK) cells) determined in this study are shown in Table 2. The lymphocyte subpopulations in the Caucasian population, obtained by Becton Dickinson researchers,[13] are listed in Table 2 for comparison. No significant difference ($p>0.05$) between the males and females was seen in the distribution of the regulatory mononuclear cells in the peripheral blood (Table 3). Significant differences occured in certain parameters among the different ethnic groups (Table 4).

TABLE 2: Comparison between the distribution of immunoregulatory cells in the peripheral blood of Caucasians and Malaysians

Parameter	Malaysian				Caucasian*			
	N	Mean	SD	Mean±2SD	N	Mean	SD	Mean±2SD
T cells	74	71.0	8.0	55-87	304	73	6.5	60-86
B cells	74	11.4	4.1	3.2-19.6	319	14	4.2	5.6-22.4
CD8 cells	74	37.7	8.4	20.9-54.5	304	33	7.4	18.2-47.8
CD4 cells	74	38.3	8.1	22.1-54.5	304	43	7.6	27.8-58.2
CD4/CD8	74	1.1	0.3	0.5-1.7	304	1.4	0.6	0.2-2.6
NK cells	74	17.2	7.2	2.8-31.6	319	14	6.0	2-26

Note: CD4 cells = T helperlinducer cells; CD8 cells = T suppressor/cytotoxic cells; NK cells = Natural killer cells; SD = standard deviation; Mean ± 2SD = reference range.

All results except for CD4/CD8 ratio are reported in percentages of the lymphoid cells gated in the flow cytometric analyses.

* Caucasian data was obtained from: Forrest J, Lowder J N, Reichart T A. Normal values: Definition of a reference range for lymphocyte subsets of healthy adults. In: Bach, B A, ed. Clinical Monograph No. I. San Jose: Becton Dickinson Immunocytometry Systems, 1988.

TABLE 3: Distribution of immunoregulatory cells in the peripheral blood of male and female subjects

Parameters	Male	Female
CD3 cells	69.75 ± 8.18	72.20 ± 7.47
CD19 cells	11.05 ± 4.49	11.74 ± 3.58
CD4 cells	37.53 ± 8.22	38.83 ± 7.94
CD8 cells	37.69 ± 9.81	37.83 ± 6.45
CD4/CD8 ratio	1.08 ± 0.38	1.07 ± 0.27
CD16+56 cells	18.87 ± 8.12	15.90 ± 6.03

Note: Results are presented in the form of mean ± 1SD. No significant difference was observed between males and females in all the parameters measured ($p>0.05$).

DISCUSSION

It is common practice for every clinical laboratory to set its own reference range for any parameter being studied in both the normal and pathological population. There is bound to be variation in results produced by different laboratories due to differences in methodology, operator variability and inherent differences of the subjects studied.

The reference range for normal adults used in this laboratory before this study was based on that prepared by Becton Dickinson researchers in the United States of America.[13] It was not suitable for the local population since the subjects were Caucasians. When we compared the reference range (mean ± 2SD) we obtained with that of Becton Dickinson,[13] we found that the ranges were different. The difference in the distribution of peripheral blood immunoregulatory cells between the two population may be attributed to genetic and environmental factors such as diet, climate and exposure to different antigens. Interlaboratory variation may also have contributed to this difference.

Statistically, it was shown that sex was not a contributing factor in determining the distribution of immunoregulatory cells in the peripheral blood. Our observation was not in concordance with the results obtained by Becton Dickinson.[13] They found that the percentages of T cells and T helper cells were significantly higher in females than in males. Caucasian males were also found to have a higher percentage of natural killer cells.

We found that for certain parameters, significant differences between the three ethnic groups were observed. Prince et *al* (1985)[14] have reported that variations in the distribution of cellu-

TABLE 4: Distribution of immunoregulatory cells in peripheral blood according to ethnic groups and Multiple **Duncan** Test results on the effect of each ethnic group on parameters studied

Parameter	Ethnic group		
	Malay (M)	Chinese (C)	Indian (I)
T cells	C I (69.14 ± 10.30)	M I (70.77 ± 7.19)	M C (72.86 ± 5.68)
B cells	C I (11.14 ± 3.62)	M (10.27 ± 3.91)	M (13.14 ± 4.29)
CD8 cells	C I (36.23 ± 9.96)	M I (39.37 ± 8.48)	M C (27.09 ± 6.03)
CD4 cells	C (34.14 ± 8.41)	M (37.33 ± 6.40)	– ✳ (43.27 ± 7.30)
CD4/CD8 cells	C I (1.07 ± 0.43)	M (1.00 ± 0.22)	M (1.20 ± 0.32)
NK cells	C (19.14 ± 9.60)	M (18.67 ± 6.74)	– ✳ (14.09 ± 4.58)

Note: The symbols in each column show that the ethnic group which the symbol represents is homogenous with the ethnic group of the column. Values in brackets represent the mean ± 1SD for each the parameters studied.

– ✳ which is noted in the Indian column for both CD4 and NK cells means that for these two parameters the Indian differs significantly ($p < 0.05$) from the Malay and Chinese.

lar immune status due to different races do occur. We also noted that the Chinese have a higher percentage of NK cells when compared to the other ethnic groups. This observation is in concordance with the results of a preliminary study by Becton Dickinson which showed that Orientals have a significantly higher percentage of NK cells.[13]

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REFERENCES

1. Kidd PG, Vogt R F J. Report of the workshop on the evaluation of T cell subsets during HIV infection and AIDS. Clin Immunol Immunopathol 1989; 52:3-9.
2. Taylor J, Fahey J, Detels R, Giorgi J. CD4 percentage, CD4 number and CD4:CD8 ratio in HIV infection: which to choose and how to use. J AIDS 1989; 2:114-24.
3. Daar ES, Moudgil T, Ho DD. Persistently low T-helper (CD4+) lymphocyte counts in HN-negative asymptomatic men. Clin Res 1990; 38:114A (abstract).
4. Ho DD, Cao Y, Zhu T, et al. Idiopathic CD4+ T-lymphocytopenia - immunodeficiency without evidence of HIV infection. N Engl J Med 1993; 328:380-5.
5. Weiss SH, Klein CW, Mayur RK, Besra J, Denny TN. Idiopathic CD4+ T-lymphocytopenia. Lancet 1992; 340: 608-9.
6. Unexplained CD4+ T-lymphocyte depletion in persons without evident HIV infection - United States. MMWR Morb Mortal Wkly Rep 1992; 41:541-5.
7. Update: CD4+ T-lymphocyte depletion in persons without evident HIV infection - United States. MMWR Morb Mortal Wkly Rep 1992; 41:578-9.
8. Thomas HC, Brown D, LaBrooy J, et al. T cell subsets in autoimmune and HBV-induced chronic liver disease, HBs antigen carriers with normal histology and primary biliary cirrhosis:a review of the abnormalities and the effects of treatment. J Clin Immunol 1982; 2:57S-60S.
9. Bhan AK, Dienstag JL, Wands R, er al. Alterations in T cell subsets in primary biliary cirrhosis. Clin Exp Immunol 1982; 47:351-8.
10. Scheinberg MA, Cathcart ES. B and T cell lymphopenia in systemic lupus erythmatosus. Cell Immunol 1974; 12:309-15.
11. Gilliam N, Hurd ER. Comparison ofcirculating T and B lymphocytes in discoid versus systemic lupus erythmatosus. Clin Immunol lmmunopathol 1976; 6:149-55.
12. Anonymous. Methods in Clinical Flow Cytometry. San Jose: Becton Dickinson Immunocytometry Systems, 1988.
13. Forrest J, Lowder JN, Reichert TA. Normal values: Definition of a reference range for lymphocyte subsets of healthy adults. In: Bach, BA, ed. Clinical Monograph No.l San Jose:Becton Dickinson lmmunocytometry Systems, 1988.
14. Prince H, Hirji K, Waldbeser S, Plaeger-Marshall S, Kleinman S, Lanier L. Influence of racial background on the distribution of T cell subsets and Leu 11 positive lymphocytes in healthy blood donors. Diag Immunol 1985; 3:33-9.