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Immunophenotyping of Peripheral Blood Lymphocytes in Saudi Men

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Flow cytometry is an important tool for the diagnosis and follow-up of immunodeficiency patients, as well as for pateints with leukemia and lymphoma. Lymphocytes and their subsets show variations with race. The aim of this study was to establish reference ranges for lymphocytes and their subsets in an Saudi adult population by using flow cytometry. Blood samples obtained from 209 healthy Saudi men were used for this study. All blood donors were between 18 and 44 years old. Lymphocytes and their subsets were analyzed by flow cytometry, and the absolute and percentage values were calculated. We investigated the expression of T-cell markers (CD3, CD4, and CD8), B cells (CD19), and natural killer cells (CD16 and CD56). The absolute and percent values of each cell subset were compared with published data from different populations by using the Student *t* test. Reference ranges, each expressed as the mean ± the standard deviation, were as follows: leukocytes (6,335 ± 1759), total lymphocytes (2,224 ± 717), CD3 cells (1,618 ± 547), CD4 cells (869 ± 310), CD8 cells (615 ± 278), CD19 cells (230 ± 130), and CD3-CD16^{+}/CD56+ cells (262 ± 178). The CD4/CD8 ratio was 1.6 ± 0.7. Our results for B cells, CD4 cells, and CD8 cells and for the CD4/CD8 ratio fell in between the reported results for Ethiopian and Dutch subjects. Our results were also different from previously reported findings in an Saudi adult population that showed no increase in CD8 T cells. We thus establish here the reference ranges for lymphocytes and their subsets in a large cohort of Saudi men. The CD8 cell count was not abnormally high, as previously reported, and fell in between previous results obtained for African and European populations.

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Flow cytometry is a powerful tool in the diagnosis and management of congenital and acquired immunodeficiency syndromes. It is also used for the diagnosis of leukemia and lymphomas. Results of studies from different countries support the presence of variation in absolute and percent quantities of lymphocytes and their subsets (2, 3, 6, 8, 12). Several factors were found to affect these results, including age, gender, race, and environmental factors (2, 9, 11). For example, low CD4^{+}-T-cell counts were reported in Asians (1) and Chinese (7). In order to establish a reference range for the different lymphocyte populations, Shahabuddin (8) investigated a cohort of 150 male Saudi blood donors. He showed that there was a marked increase in the CD8 cell count, a decreased CD4/CD8 ratio, and a low natural killer (NK) cell count compared to 32 Caucasian males.

In this report, we carried out the immunophenotyping of lymphocytes and their subsets in order to establish our own reference range in an adult Saudi population.

MATERIALS AND METHODS

Subjects. A total of 209 Saudi men were recruited for this study. These were random blood donors that were used as an internal control each time we conducted an immunophenotyping procedure.

Blood collection. EDTA peripheral blood was collected and analyzed initially on Advia 120 (Bayer) hematological analyzer for total and differential blood counts. A blood smear was then prepared for a manual differential count.

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Flow cytometric analysis. Lymphocyte subsets were analyzed on FACScalibur (Becton Dickinson) with the following monoclonal antibody combinations: immunoglobulin G1-immunoglobulin G1 control (with different fluorescent dyes), CD14 (fluorescein isothiocyanate [FITC])-CD45 (PerCP), CD2 (phycoerythrin [PE])-CD19 (PerCP), CD3 (PerCP)-CD4 (FITC), CD3 (PerCP)-CD8 (PE), CD4 (FITC)-CD8 (PE), and CD3 (PerCP)-CD16/CD56 (PE). In brief, 100 μl of whole blood was mixed and incubated, in the dark, with 20 μl of each monoclonal antibody in separate tubes, at room temperature. Red blood cells were then lysed by adding 2 ml of lysing solution (Becton Dickinson), and the tubes were vortexed and incubated in the dark at room temperature for 10 min and finally centrifuged at 2,500 rpm for 5 min. The pellet was then washed once with 2 ml of phosphate-buffered saline (PBS), resuspended in 500 μl of PBS, and finally analyzed with CellQuest software (Becton Dickinson). The FACScalibur was calibrated with Calibrite beads (Becton Dickinson) and AutoComp weekly.

Statistical analysis. Data were entered and analyzed by using STATA v6.0 statistical software. The mean and standard deviation (SD) were calculated for each marker. The Student's *t* test was used to compare the means and SD of our results to those from other published sources.

RESULTS

A total of 209 Saudi men aged between 18 to 44 years (mean ± SD = 26 ± 6.6 years) were recruited for this study. All were blood donors at King Fahad Hospital, Riyadh, Saudi Arabia. The percentages of the different lymphocyte subsets were as follows (mean ± SD): CD3 cells (72.9 ± 7.7), CD4 cells (39.4 ± 7.9), CD8 cells (27.6 ± 7.5), NK cells (11.7 ± 5.9), and B cells (10.4 ± 4.7). An average CD4/CD8 ratio of 1.6 was obtained, with the lowest ratio of 0.6 and a maximum value of 5.0. A mean total white blood cell (WBC) count of 6,334 was obtained with a range of 3,200 to 11,600.

Table 1 compares our results in the Saudi adult male population with mixed groups (males and females) from Ethiopian and Dutch populations. Compared to the Ethiopian studies, our results were significantly different except for total WBC, T-lymphocyte, and NK cell counts. For all parameters, our

TABLE 1. Comparison of absolute lymphocyte subset counts from the present study to those obtained in an Ethiopian study and a Dutch study

Cell type	Mean absolute count (cells/μl) ± SD[a] in:		
	Present study (n = 209)	Ethiopian study (n = 142)[c]	Dutch study (n = 1,365)[d]
Lymphocytes	2,224 ± 717	1,857 ± 576[1]	2,054 ± 573[1]
T lymphocytes	1,618 ± 548	1,555 ± 463[NS]	1,525 ± 458[3]
CD4 T cells	869 ± 310	775 ± 225[2]	993 ± 319[1]
CD8 T cells[b]	615 ± 278 (1.6 ± 0.7)	747 ± 333[1] (1.2 ± 0.5)[1]	506 ± 220[1] (2.2 ± 1.0)[1]
B cells	230 ± 130	191 ± 94[2]	313 ± 147[1]
NK cells	262 ± 178	250 ± 137[NS]	NA

[a] Mean and SD values were compared by using the Student t test. Significance is indicated by superscripts as follows: 1, $P \leq 0.0001$; 2, $P \leq 0.005$; 3, $P \leq 0.01$; and NS, not significant. NA, not available.
[b] The CD4/CD8 ratio is indicated in parentheses.
[c] Tsegaye et al. (12).
[d] Tsegaye et al. (12).

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Saudi population presented with higher values than did the Ethiopians except for the CD8$^+$ cell counts. Compared to the Dutch population, our results were also significantly different for all lymphocyte cell types. However, the Dutch population had higher CD4 and lower CD8 counts, which was reflected in a higher CD4/CD8 ratio than in our results.

In Table 2, we compare our results with those from two previous studies, one on Saudi males and the other on Ethiopian males. The Ethiopian male population gave results similar to those seen in the mixed (male and female) population presented in Table 1. All results were significantly different from ours except for the total lymphocyte and NK cell counts. Our population had higher absolute counts for total lymphocytes and lymphocyte subsets except for CD8 and NK cells. Compared to the previously published results from a male Saudi population, our cell counts were lower than the published data for T cells, CD4 cells, CD8 cells, and B cells. However, the CD4/CD8 ratio was significantly higher in our population. This was reflected by the lower CD8 counts in the population in our study. In addition, NK cell counts were significantly higher among subjects in the present study.

DISCUSSION

The main aim of the present study was to establish reference values for lymphocytes and their subsets in healthy Saudi adults. We investigated a large number of Saudi men who donated blood at King Fahad Hospital.

In 1995 Shahabuddin (8) reported on the immunophenotypes of lymphocytes and their subsets in male Saudi blood donors of ages similar to those of the subjects in the present study. Those results were significantly different from ours except for the CD4 cell counts. One noticeable finding was the high CD8 cell count, which rendered a low mean CD4/CD8 ratio (1.1). Those findings were similar to a recent report on Ethiopians (12) (Table 2). The mean CD4/CD8 ratio in our population was 1.6, with a wide interval (0.6 to 5.0); this reflected the wide ranges obtained for both CD4 and CD8 cells. It is interesting that in the CD4 cell count, the lower limit was below 300 cells/μl. This should not be confused with human immunodeficiency virus (HIV) infection profile. HIV infection is event in Saudi Arabia, and none of the 209 subjects investigated had HIV (data not shown). The number of lymphocytes and their subsets (including CD4) is influenced by both genetic and environmental factors (3, 11).

Our results were significantly different compared to the previously published results for the Ethiopian and Dutch populations. The total lymphocyte counts in our population were significantly higher than either the Ethiopian or Dutch study results. However, for B cells, CD4 cells, CD8 cells, and CD4/CD8 ratio our results fell in between the results from the two populations (Table 1). Both populations were HIV negative and of mixed gender. It is interesting that the results from the Ethiopian male and female populations were not different (12).

In 1998, Shahabuddin et al. (7) reported on the age-related

TABLE 2. Comparison of adult male population results from the present study to those from other studies

Cell type	Mean absolute count (cells/μl) ± SD[a] in:		
	Present study (n = 209)	Saudi study (n = 150)[c]	Ethiopian study (n = 92)[d]
Lymphocytes	2,224 ± 717	NA	1,857 ± 606
T lymphocytes	1,618 ± 548	1,750 ± 520[3]	1,564 ± 485[NS]
CD4 T cells	869 ± 310	880 ± 270[NS]	753 ± 227[3]
CD8 T cells[b]	615 ± 278 (1.6 ± 0.7)	890 ± 290[1] (1.1 ± 0.3)[1]	777 ± 362[1] (1.1 ± 0.4)[1]
B cells	230 ± 130	290 ± 90[1]	184 ± 96[2]
NK cells	262 ± 178	190 ± 80[1]	277 ± 143[NS]

[a] Mean and SD values were compared by using the Student t test. Significance is indicated by superscripts as follows: 1, $P \leq 0.0001$; 2, $P \leq 0.005$; 3, $P \leq 0.01$; and NS, not significant. NA, not available.
[b] The CD4/CD8 ratio is indicated in parentheses.
[c] Shahabuddin (8).
[d] Tsegaye et al. (12).

changes in peripheral blood lymphocytes from Saudi children. They found that CD8 cell population was increasing with age, whereas the CD4 cell count decreased, resulting in a decreasing CD4/CD8 ratio with age, and this result was also confirmed in adults (8). This finding was not confirmed in our study, however. In addition, published work from different laboratories showed that both $CD4^+$ and $CD8^+$ cell counts increase with age with an unchanged CD4/CD8 ratio (13, 14).

The difference between our results and those reported by Shahabuddin (8) is significant. Other reports in the literature suggested that many variables could contribute to differences seen in the same population; these variables includeg the kind of instrument, the monoclonal antibodies, and the washing procedures (5). The only difference we could see was in the machine used; in our study we used FACSCalibur (Becton Dickinson), whereas Shahabuddin (8) used a FACScan (Becton Dickinson), an older machine model. This could be the origin of the variation between our results and the previously published data. However, other variables could also have contributed to these variations. These variables include the method used for absolute lymphocyte counts and whether an automated or a manual differential was utilized. There are three levels of variations in the manual differential: the WBC count and the lymphocyte percentage obtained from the hematology analyzer, as well as the percentage of the different lymphocyte subpopulations obtained through the flow cytometer. In an effort to compare the accuracy of single-platform technology versus conventional flow cytometry, Reimann et al. (4) found single-platform methodology to be much more accurate than the conventional method, with improved precision in the absolute lymphocyte counts (both within and between instruments and laboratories) compared to the conventional multiplatform technology (4).

Lymphocyte subsets can differ significantly between healthy males and females (9, 10), although this was not the case in the Ethiopians (12). In the present study we report on healthy adult men from Saudi Arabia; all were blood donors. No females were included since very few women donate blood. We are currently trying to recruit healthy females for additional lymphocyte subset immunophenotyping studies.

In this study we report the reference ranges and means of lymphocyte cells and their subsets in adult Saudi males. Our study investigated a larger number of subjects than did an earlier report (8) and does not support the finding that CD8 cell counts are high in Saudi adults.

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