B and T Lymphocytes: Methodology and Normal Ranges

ARMAND B. GLASSMAN, M.D. and CAROL E. BENNETT

Department of Laboratory Medicine, Medical University of South Carolina, Charleston, SC 29403

ABSTRACT

B and T lymphocytes separation by use of various cell markers has been previously described. These differential lymphocyte subpopulation counts have been helpful in elucidating immunodeficiency, lymphoproliferative and other disease states. Expanding clinical utility has moved these assays into the general laboratory for routine determination. Specific methodology for differentiation of B and T cell subpopulations is described. The mean T cell population is 68.1 percent ± 4.2 and the mean B cell population is 8.4 percent ± 2.1 for 30 ambulatory normals. A bi-modal distribution of T and B lymphocyte cell lines is noted in a family with a high incidence of carcinoma. Preliminary data on patients in the diabetic clinic of the Medical University of South Carolina shows a statistically significant difference in the T cell population of diabetics versus normal.

Introduction

Lymphocyte subpopulations have been reported in varying percentages for humans in the literature. This variability probably reflects differences in methodology used to determine T and B cell lines. The purpose of our study was to investigate reproducible methods for determining T and B cell percentages and to define a normal range for T and B lymphocytes in an ambulatory population. The applicability of T and B lymphocyte cell subpopulations related to specific disease entities was investigated.

Thirty ambulatory laboratory personnel were screened by history, hematocrit, white blood cell and differential count. These volunteers then had blood drawn for T and B population percentages to be determined. Two of the subjects were drawn on multiple occasions to determine individual variability of lymphocyte counts from day to day and a coefficient of variation of the procedure both intra and inter assay. B and T cell differentiation was performed by fluorescent staining of surface immunoglobulins using an isothiocyanate fluorescein conjugated anti-human immunoglobulins (with anti IgG, IgM, and IgA specificity) for B cells, E-rosetting for T cells, EAC rosetting for B cells, and inert latex particle ingestion for macrophage identification.
Materials and Methods

Ten (10) ml of heparinized peripheral blood are required for the performance of the assay. A 7 ml EDTA tube is drawn for determinations of the complete blood count and differential. Blood is processed for separation within one hour. The blood is diluted 1:3 with Hank's balanced salt solution, without calcium and magnesium, and layered over 10 ml of sterile ficole-isopaque. This is centrifuged at 150 x gravity for 45 min at room temperature. Extreme caution must be taken to accelerate the centrifuge slowly in order to prevent mixing at the interface. After centrifugation, the plasma layer is removed to within 6 mm of the white blood cell layer using suction. The white blood cell layer is removed with a Pasteur pipet and washed three times in Hank's balanced salt solution.

The expected number of recovered cells can be estimated from the white blood cell count and differential. The maximum number of cells for latex incubation should not exceed 5 x 10^7 cells per ml. Exactly 1.5 ml of RPMI* 1640 and 0.5 ml of fetal calf serum are added to washed, packed cells and the suspension is mixed thoroughly. One tenth ml of 1 percent latex suspension is added, mixed, and incubated for 45 min at 37° C. After incubation, the cells are layered over 1 ml of 100 percent fetal calf serum in a 12 x 75 mm plastic tube to remove uningested latex particles. The tubes are centrifuged at 200 x gravity for five min at room temperature and the supernatent is removed. The packed cells are resuspended in 2 ml of Hank's balanced salt solution and the lymphocytes are counted on a hemocytometer. The suspension is relayered over 100 percent fetal calf serum and centrifuged again in order to remove any residual particles. The lymphocytes should be brought to a final concentration of 5 x 10^7 cells per ml in RPMI 1640.10

One tenth ml of titered fluorescin conjugated polyclonal anti-human immunoglobulin antisera is added to glass tubes containing pellets of mononuclear cells (3 to 5 x 10^6 total). The tubes are mixed by vortexing to resuspend the cells and are incubated at 4° C for 30 to 60 min. The cells are washed twice in phosphate buffered saline (PBS) containing 5 percent fetal calf serum and 0.2 percent sodium azide at 4° C. One tenth ml of PBS with 5 percent fetal calf serum and 0.2 percent sodium azide are added to each cell pellet. The cells are resuspended by vortexing and 10 µl are placed on a clean slide and a cover slip is placed over the cells. Using the oil immersion objective and a fluorescent microscope, all the cells in the field are counted using direct light. Latex positive cells or clumped cells are not counted. The transmitted light is blocked while using the UV light. The fluorescent cells which do not contain latex are counted as a proportion of 200 cells.

From a cell suspension of 1 x 10^7 cells per ml in RMPI 1640, 0.1 ml is withdrawn and placed in a plastic tube. Two tenth ml of absorbed fetal calf serum and 0.1 ml of 2 percent sheep red blood cells are added, gently mixed and incubated for five min at 37° C. The tubes are centrifuged for three min at 200 x gravity and incubated in a refrigerator at 4° C overnight. The pellets are resuspended gently and one drop is placed on a clean slide and a cover slip placed over it. Two hundred lymphocytes are counted using the 40 x objective of a light microscope; the number of rosetted and nonrosetted lymphocytes is recorded for each sample. A rosette forming cell is defined as one which has three or more sheep red blood cells adhering to the lymphocyte surface. The number of rosetted lymphocytes divided by the number of rosetted plus nonrosetted lymphocytes, times 100, yields the percent E-rosettes.10

---

* Rosewell Park Memorial Institute (RPMI)
Results

NORMALS

B and T cell population of 30 ambulatory normal volunteers were as follows: B cell population was 8.4 percent ± 2.1 (one standard deviation) and T cell population was 68.1 percent ± 4.2 (one standard deviation). The remainder of the cells were nonclassifiable by these methodologies and are considered "null cells." Repeated assays on the same individual revealed that there was close agreement of T and B cell populations for that individual (table I). In 10 assays performed on a 25 year old white female over a nine month span, T cells were 65.8 percent ± 1.5 with a coefficient of variation of 2.3 percent; B cells were 7.0 percent ± 1.3 with a coefficient of variation of 18.6 percent. Intra-assay coefficient of variation derived from data of four observers was 2.5 percent for T cells and 13.95 percent for B cells. The correlation coefficient of EAC-rosetting and fluorescent staining of surface immunoglobulins exceeded 0.93; because of this close correlation, B cell determinations were subsequently performed by surface immunofluorescence alone.

PATIENT DATA

Determinations on nine members of the "Smith Family" revealed unusual data (table II). There is a bi-modal distribution of the B and T cell populations within this family. Of interest in this family is that Patient 1 and Patient 5 in the elevated B lymphocyte group are known to have neoplasms. Patient M. A. has carcinoma of the thyroid; Patient J. S. has renal cell carcinoma.

Studies on 42 patients attending the Diabetic Clinic at the Medical University of South Carolina revealed the following: T cell line = 72.7 percent ± 7.8, B cell line = 8.0 percent ± 2.6. The majority (38/42) of these patients were black. In a group of 20 black controls, the T cell line is 66.2 percent ± 1.7 and the B cell line is 7.7 percent ± 1.1. The difference in T cell populations for 35 white and 20 black controls is significant with a p value of less than 0.05. The increase in T cells found in the black diabetics is statistically significant (p < 0.001) (table III). No statistical correlation could be determined related to whether the patient was on insulin or oral hypoglycemic therapy.

Discussion

Interest in B and T cell counting has been spurred by the clinical applicability to immunodeficiency diseases, lymphoproliferative disorders and in an expand-
TABLE III
Results of Assays for T and B Lymphocytes in Patients with Diabetes Mellitus

<table>
<thead>
<tr>
<th></th>
<th>T</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black controls</td>
<td>20 66.2 ± 1.7</td>
<td>7.7 ± 1.1</td>
</tr>
<tr>
<td>White controls</td>
<td>35 68.1 ± 3.5</td>
<td>7.9 ± 1.5</td>
</tr>
<tr>
<td>Diabetics</td>
<td>42 72.7 ± 7.8</td>
<td>8.0 ± 2.6</td>
</tr>
</tbody>
</table>

*p values are the result of paired t-tests applied to the T and B cells of black versus white controls and black controls versus diabetics (since 38/42 diabetics were black). No statistically significant difference was seen between the B cell populations of any groups.

The findings of a bi-modal distribution of T and B lymphocyte subpopulation in the “Smith Family” and the high incidence of carcinoma is striking. Whether or not use of classification of lymphocyte populations will be useful in predicting subsequent development of carcinoma in members of a family remains to be proven.

The information related to differential B and T subclasses in the diabetic population is of interest. It is known that diabetics have a greater occurrence of a variety of infections. Why this occurs is multifaceted and includes, at least in part, alterations in the neutrophilic macrophage function which is noted in this disease. Whether or not there are additional correlations or relationships with the alteration in T cell population that are noted will require further investigation.

Summary and Conclusion

1. Specific methodology for differentiation of B and T cell subpopulations is described.
2. These methodologies are reproducible in a clinical laboratory.
3. The normal T cell population is 68.1 percent ± 4.2. The normal B cell population is 8.4 percent ± 2.1 for 30 ambulatory normals.
4. A bi-modal distribution of T and B lymphocyte cell lines is noted in a family with a high incidence of carcinoma.
5. Preliminary data on patients seen in a diabetic clinic of the Medical University of South Carolina indicates that there are statistically significant differences in the T cell populations of diabetics versus normals.

Acknowledgment

Sincere thanks are extended to Dr. Nick M. Burdash for aid in developing and performing methodology and to Diane Johnson, MT(ASCP), Joyce Christopher, CLA(ASCP), Tricia Buchner, B.S., M.S., and Valerie Papadopalous, B.S., MT(ASCP) for their technical and professional assistance.

References


