HUMAN T- AND B-LYMPHOCYTE POPULATIONS IN BLOOD: LOCAL POPULATION STUDIES

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INTRODUCTION

THE RAPID increase in immunological knowledge in the past 10 — 15 years has been accompanied by an increasing demand for laboratory services in clinical immunology. Such laboratories, especially in the developing countries, restrict themselves to tests concerned mainly with the humoral aspects of immunity i.e. detection of antibodies in a variety of infections (serology) or the measurement of serum protein components (e.g. complement, immunoglobulins). However, and as pointed out recently (see reference 7), there is also a need to have available tests on *cellular* immunity. This communication reports the introduction of one of these tests, the enumeration of T and B lymphocytes in the University Hospital. Local population values, of which little is known, are reported together with the methods used and the application of the test in clinical conditions.

MATERIALS AND METHODS

Donors and patients

Healthy adult donors were selected from the Blood Bank, University Hospital, Kuala Lumpur or from students of the Faculty of Medicine, University of Malaya. ALL (acute lymphoblastic leukaemia) patients were selected according to the criteria of Brouet *et al.* (1976) and the diagnosis confirmed by standard cytological procedures.

Specimens

4 — 5 ml blood collected into glass containers containing EDTA or heparin.

Lymphocyte isolation

Lymphocytes were isolated using Ficoll-Paque density gradients by a technique modified from Boyum (1968). Diluted blood (3.5 mls blood and 3.5 mls balanced salt solution, BSS) was layered on 3 mls of Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) in siliconized glass tubes. Tubes were then centrifuged at 400g for 30 mins, the lymphocyte layer recovered and the cells washed 3 times with BSS. Lymphocytes were then counted in a haemocytometer and viability determined by trypan blue dye exclusion.

T cell enumeration

T cells were enumerated by formation of rosettes with sheep erythrocytes (erythrocyte-rosette-forming cells, E-RFC) using a technique extensively modified from Winchester & Ross (1976). Sheep red blood cells (SRBC) were first washed 3 times with phosphate-buffered saline (PBS). 100 µl of packed SRBC were then treated with 400 µl AET (2-aminoethyl isothiouranium bromide, Sigma Chem. Co., St. Louis, MO., U.S.A.; 0.402 gm AET made up with 10 mls distilled water, adjust to pH 9.0 with 4N NaOH) for 15 mins at 37° C. AET treatment of SRBC has been shown to improve the stability and speed of binding of rosettes (Pellegrino *et al.*, 1975). SRBC were then washed 5 times with PBS and 50 ul packed SRBC added to 10 mls BSS to give a final concentration of 0.5% SRBC. Rosette formation was carried out by mixing 0.5 ml of 0.5% SRBC suspension with 0.5 ml lymphocytes (conc. = 2 x 10^6 cells/ml), incubating for 15 mins at 37° C and then centrifuging for 10 mins at lowest speed on the bench centrifuge (MSE bench top centrifuge). The tubes were then allowed to stand at 0 — 4° C (on ice) for 15 mins and the cell pellets resuspended by *gentle* shaking in a small volume of BSS. The proportion of rosetting cells was then counted under the light microscope. A lymphocyte is counted as rosette-forming if three or more erythrocytes adhere (Winchester & Ross, 1976).

B cell enumeration

B cells were enumerated by direct immunofluorescence to detect the presence of surface

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membrane immunoglobulin (SmIg). Lymphocytes were suspended in PBS at a concentration of 2 x 10^7 cells/ml. 20 µl of this suspension was then mixed with 20 µl of fluorescein-conjugated, heterologous anti-human immunoglobulin (antiserum to human IgG + IgA + IgM, fluorescein-conjugated, Behringwerke, W. Germany). Final dilution of antiserum was 1 in 2 or 1 in 4. The mixture was incubated for 30 mins at room temperature and then washed 3 times with PBS-albumin (2% bovine albumin in PBS). Pellets were finally resuspended in 0.1 ml PBS plus 1 drop PBS-glycerol (10% PBS in glycerol) and examined under a fluorescent microscope (Leitz ortholux II).

RESULTS

The method used for isolation of lymphocytes (Ficoll-Paque density gradients) regularly gave > 35% recovery of input lymphocytes (mean recovery = 45%) with a viability of > 95% for the isolated lymphocyte population.

As shown in Table I, a value of 70% T lymphocytes (E-RFC) and 9% B lymphocytes (SmIg-positive cells) in peripheral blood was obtained in a study of the local population. The range of values was 55 — 86% for T cells and 3 — 20% for B cells (Table I). Further analysis of the data showed no significant differences between the various racial groups tested i.e. Chinese, Malays and Indians (Table II). Similarly, the values obtained did not seem to be influenced by the sex of the donors (Table II). In addition, the test was also used to analyze peripheral blood lymphocytes from 3 cases of ALL (Table III). According to the classification of Brouet *et al.* (1976) two patients had a T-derived ALL and one had a non-T non-B (null) ALL (Table III).

Table I

Percentage of T and B cells in normal peripheral blood

Donors*	% T cells (E-RFC)	% B cells (SmIg)
Normal	70.5 ± 8 (Range: 55 — 86)	9.4 ± 4 (Range: 3 — 20)

* Total no. of donors = 33

Table II

Percentage of T and B cells in normal peripheral blood: Effect of race and sex of donors.

Parameter*	% T cells (E-RFC)	% B Cells (SmIg)
Race		
Chinese (13)	71.5 ± 8	7.8 ± 4
Malay (10)	71.9 ± 7	11.5 ± 5
Indian (10)	67.6 ± 8	10.1 ± 5
Sex		
Male (21)	71.0 ± 8	10.5 ± 5
Female (12)	67.9 ± 8	6.6 ± 2

* No. in brackets indicate number of donors in each group

Table III

Percentage of T and B cells in peripheral blood of ALL patients

Patient	% T cells (E-RFC)	% B Cells (SmIg)	Classification
P	82	8	T-derived ALL
Z.Y.	44	22	T-derived ALL
T.S.P.	7	1	Non-T non-B ALL

DISCUSSION

The detection of E-RFC and SmIg-positive cells are the most commonly used techniques for the identification of human T and B lymphocytes respectively (Strober & Bobrove, 1975; Winchester & Ross, 1976; Hayward & Greaves, 1977). Conclusive evidence has also been obtained that E-RFC's are in fact T cells and that SmIg-positive cells are B cells (Hayward & Greaves, 1977; Chess & Schlossmann, 1977). In the present report, these two methods were used to obtain values for T and B lymphocytes in peripheral blood in a local, Asian population. These values are of obvious importance if the test is to be used in a local context. The values obtained in the present study appear to be in agreement with those obtained for Caucasians where the following values have been reported for T and B lymphocytes respectively: 85% and 9% (Winchester & Ross, 1976), 77% and 21% (Strober & Bobrove, 1975), 75% and 10% (Hayward & Greaves, 1977), 70 — 80% and 5 —

15% (Waller & MacLennan, 1977). The results also indicated that approximately 10 — 20% of peripheral blood lymphocytes appeared to possess neither T- nor B-lymphocyte characteristics. These cells have been referred to as 'unclassified" cells (Hayward & Greaves, 1977) and probably include K cells and null cells (Winchester & Ross, 1976; Hayward & Greaves, 1977; Chess & Schlossmann, 1977).

It is important to note, however, that several factors may influence the tests. Firstly, monocytes are found together with lymphocytes isolated by the Ficoll-Paque technique (Winchester & Ross, 1976). The monocyte shares several cell surface markers with the B lymphocyte so that in disease states where monocytes are significantly increased (e.g. Hodgkins disease) they could be erroneously counted as B lymphocytes (Strober & Bobrove, 1975; Winchester & Ross, 1976). In the present study, the degree of monocyte contamination was about 10%. Secondly, antilymphocyte antibodies which adhere to the lymphocyte surface can confer positive surface staining to an otherwise SmIg-negative cell. These antibodies are often found in diseases such as rheumatoid arthritis and systemic lupus erythematosus (Strober & Bobrove, 1975; Winchester & Ross, 1976). Other factors which may influence the tests include physiological variations (Hayward & Greaves, 1977), antiserum specificity and technical factors (e.g. mechanical or thermal disruption of erythrocyte rosettes) (Winchester & Ross, 1976).

There are several areas in which the above tests could be used as clinical diagnostic aids. Firstly, the diagnosis of immunodeficiency disorders during infancy and early childhood can be supported by the above tests (Strober & Bobrove, 1975; Hayward & Greaves, 1977; Cooper & Seligmann, 1977). For example, infants with DiGeorge syndrome (thymic hypoplasia) have significantly decreased T cells (Strober & Bobrove, 1975) and, at the other extreme, the absence of peripheral blood B lymphocytes is characteristic of X-linked hypogammaglobulinaemia (Cooper & Seligmann, 1977). Secondly, these tests are also of use in the immunological categorization of leukaemic cells and lymphomas (Brouet *et al.*, 1976; Hayward & Greaves, 1977; Barrett *et al.*, 1977). These studies indicate that ALL patients, for example, could be classified into three groups: (i) non-T non-B ALL (ii) T-derived ALL (iii) B-derived ALL (Brouet *et al.*, 1976). Of the three patients studied in the present report, two seemed to have T-derived ALL and one a non-T non-B (null) ALL. A more extensive study involving more ALL cases is under way. In contrast, CLL (chronic lymphocytic leukaemia) appears to have predominantly B cell features (Cooper & Seligmann, 1977). Whether or not immunological typing of a patient's cells possesses any prognostic significance remains to be seen (Hayward & Greaves, 1977). Other areas in which these tests may be of use include sarcoidosis, hepatitis, various infections and certain non-lymphoid malignancies (Hayward & Greaves, 1977).

SUMMARY

The relative percentages of T and B lymphocytes in peripheral blood in a local, Asian population was determined. The values obtained were 70.5% T cells (by sheep erythrocyte-rosetting test) and 9.4% B cells (by immunofluorescence to detect surface membrane immunoglobulin). There appeared to be no significant differences between males and females and between the various racial groups tested (Chinese, Malays, Indians). The test was also used to type leukaemic cells from 3 ALL patients.

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