ABERRANCIES OF HUMAN T — AND B — LYMPOCYTE POPULATIONS IN PERIPHERIAL BLOOD

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INTRODUCTION

About 50% of all current immunological papers published in journals have some aspects of T and B cell function being studied. Warner and Szenberg (1961) were the first to define the two cell types: B stands for bursa of fabricius and T stands for the thymus. Since then there is this general concept that B-cells are responsible for antibody production and T-cells fro graft versus host type of reaction. Claman *et al.* (166) first gave evidence for the need for cooperation between T and B cells, if antibody is to be produced normally. It was in mid-1974 that the controversy on the nature of the immune receptors on T-cells arose that remains unresolved till today. Until we are able to recognize, prepare and manipulate monoclonal populations of T-cells, any suggested interpretations is wholly tentative. The enumeration of T and B lymphocytes were started in 1978 at the Institute for Medical Research, Kuala Lumpur, and some of the clinical cases reported (Gan *et al.*, 1979a and 1979b). Pang et al., (1979) published some results on the T and B lymphocyte percentages of our local populations. In agreeing with him that there is no known values of our local populations prior to our independent studies, this paper intends to supplement the previous publication on the same.

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MATERIALS AND METHODS

Blood samples

5 mls. of venous blood were collected in glass containers containing preservative free heparin. The control group were healthy blood donors and volunteers. All clinical cases presented were confirmed clinically.

Lymphocyte seperation

Lymphocytes were recovered by ficoll-isopaque centrifugation (Boyum, 1964 & 1968). In instances of poor lymphocyte seperation due to red cells adhering to the lymphocytes, the contaminated erythrocytes were removed by agglutinating it with the appropriate red cell anti-serum before recentrification in ficoll-isopaque (modifications of Ting and Morris, 1971).

T-cell enumeration

T-cells were enumerated by the formation of rosettes with sheep erythrocytes following the recommendations of the WHO/IAR sponsored workshop on human T and B cells in London (1974) special technical report. 0.4 ml. of $2X10^6$ cells/ml. of lymphocyte suspension in RPMI 1640 was added to 0.4 ml. of 0.5% sRBC and 0.2 ml. of absorbed heat-inactivated foetal calf serum. Centrifuge at 50g for 5 mins. and incubate at 4°C overnight. Immediately before taking the reading add one drop of 0.5% trypan blue and gently tapping at the bottom of the tube. Using a haemocytometer score all the lymphocytes with three or more adherent erythrocytes as rosettes and express it as a percentage of about 400 peripheral blood lymphocytes. sRBS treated with 2-aminoethylthiouronium bromide (AET) gives better rosette formation though not significantly different in the total number of rosettes formed.

B-cell enumeration

B-cells were enumerated by direct membrane

immunofluorescence to detect the presence of surface membrane immunoglobulin (SmIg). 0.1 ml. of 2 X 10^7 cells/ml. of lymphocyte suspension was added to 0.1 ml. of diluted fluorescein-conjugated antiserum to human immunoglobulins (polyvalent Igs: 1gG, IgM, IgA) and incubated for 30 mins. at 4° C. The lymphocytes were washed three times in 0.145 M Phosphate Buffered Saline (PBS) pH 7.2, supplemented with 2% bovine serum albumin and 0.2% sodium azaide. Thin smears were made on clean glass slides with the washed cells suspended in a drop of foetal calf serum. The smears were fixed in absolute ethanol for five mins., gently washed with 0.145 M PBS (pH 7.4) and air dried. The fixed smears were stained with peroxidase (Graham & Karnovsky, 1966) for seven mins., then washed with distilled water and counter stained with 0.01% methyl green (Scheuk & Churukian, 1974) for six mins. The slides were washed in distilled water and mounted in glycerol-PBS (one part of PBS pH 8.6 to 9 parts glycerol) and read under fluorescent microscope.

Lymphocytes were identified as peroxidase negative mononuclear cells. Methyl green conter-staining aids in the exclusion of monocytes and such cells are not included in the counting procedure. B-cells were expressed as the percentage of fluorescein positive cells out of two hundred lymphocytes counted.

Table I

Effect of age on the percentage of T and B cells in normal peropheral blood

Age Group in years	Total Number	T-Cell Percentage	B-Cell Percentage
Neonates	34	80 ± 5	15 ± 6
1 – 4	118	75 ± 5	14 ± 3
12 – 25	87	72 ± 6	12 ± 3
30 – 45	30	67 ± 5	12 ± 4
55 above	25	55 ± 4	9 ± 2

DISCUSSION

As shown in Table I, age seems to have an effect on the percentage of T and B cells in the peripheral blood. There is a significant drop in percentage especially of the T cells with aging. Prof . F.M. Burnett (1976) said, "All men die, and those who escape the hazards of youth and maturity begin at about 60 years of age to lose physical power and to grow more vulnerable to accidents of every description." He advocates that aging is wholly the result of progressive inefficiency of the immune system. Similarly an inefficient immune system is observed in individuals with infections, malignancies or even malnutrition. Thus T and B cell percentages reflect the state of efficiency of an individual's immune surveillence mechanism.

Our observations agree with those of Phang *et al.* (1979) in that there were no significant differences between the T and B cell percentages of the various racial groups studied nor was there any significant differences between the sexes. Here strict precautions were taken to ensure that monocytes were not included in the total T and B cell counts.

Table II (Gan *et al.,* 1979a) shows the effect of the state of malnutrition on the T and B cell percentages. Work (1973) found that rosetting thymus-dependent T-cells were depressed before nutritional recovery, with the reduction in T-cells paralling the degree of weight loss. The proportion of B-cells in the peripheral blood was not significantly different from that in the controls. The immunology of Protein Calorie Malnutrition (PCM) was first discussed by Gan *et al.* (1979a). The existence of a defective cell mediated immunity in undernourished Malaysian children was established. The drop in T cell percentage is in relation to the state of malnutrition, more severe in kwashiorkor than in marasmus.

Table III (Gan *et al.,* 1979b) shows the aberrancies of T and B cell percentages in carcinomas. Parrish (1972) first demonstrated that there is a reciprocal relationship between the cell mediated immunity (the T-cell system) and antibody production (the B-cell system). When an antigenic determinant contacts the T and B cell receptors with adequate affinity and the cells are not adjacent, the T-cell is stimulated to proliferate and produce functional delayed hypersensitivity and/or "helper" cells, and the B-cell is rendered unresponsive. On the other hand, when reactive T and B cells are adjacent in the presence of an antigen with which both can react, the B-cells are stimulated to proliferate and produce antibody and T-cells are rendered unresponsive. Antigenic determinants of different affinity are being produced by the different types of carcinomas. In instances where treatment causes a drop in T and B cells, the drug used is probably cytotoxic or having an immunosuppressive effect.

Table II

T-Cells and B-Cells expressed as a percentage of peripheral blood lymphocytes in normal and malnourished Malaysian children

Clinical Status	Total Number	Race						T-Cell Percentage	B-Cell Percentage
		Malay	Chinese	Indian	Others	Male	Female		
Kwashiorkor	8	–	–	8	–	3	5	35 ± 3	15 ± 4
Marasmus	25	4	3	14	4	11	14	43 ± 8	11 ± 3
Marasmic Kwashiorkor	19	2	–	17	–	7	12	30 ± 6	17 ± 4
Marginally Undernourished	12	5	–	5	2	5	7	55 ± 3	10 ± 2
Significantly Undernourished	8	4	–	4	–	2	6	42 ± 7	13 ± 2
Normal (with persistent infections: 1 – 4 years old)	25	3	8	12	2	11	14	29 ± 6	12 ± 4
Normal (1 – 4 years old)	20	2	11	7	–	10	10	67 ± 5	12 ± 3
Neonates	24	8	9	7	–	10	14	80 ± 5	15 ± 6

Table III

T-Cell and B-Cell percentages of total peripheral blood lymphocytes in carcinoma patients

Type of Carcinoma		Number of cases	Average age	Sex		Race			T-Cell %	B-Cell %
				Male	Female	Chinese	Malays	Indians		
Hepatoma:	Non-treated	14	45	12	2	11	1	2	69 ± 4	10 ± 2
	Treated	11	46	11	–	9	2	–	45 ± 5	22 ± 4
Organs and tissues capable of forming antibodies:	Non-treated	18	57	14	4	10	6	2	49 ± 6	10 ± 2
	Treated	11	54	9	2	6	3	2	25 ± 4	7 ± 3
Hormone secreating tumors:	Non-treated	6	60	4	2	6	–	–	41 ± 2	11 ± 2
	Treated	7	62	6	1	5	2	–	69 ± 6	10 ± 2
Squamous-cell Carcinoma:	Non-treated	11	47	9	2	7	2	2	58 ± 3	7 ± 2
	Treated	9	46	7	2	6	1	2	32 ± 5	7 ± 3

We have enumerated T and B cell percentages of over 600 cases in the last 18 months, covering a myraid of diseases and their corresponding age-match controls. In this paper only results on malnutrition and certain malignancies were presented. Thus besides all the clinical significance in typing a patient's cells as outlined by Pang *et al.* (1979) we strongly assert its prognostic significance as well.

SUMMARY

Strict precautions were taken in our metodology to exclude any monocytes from being included in the total T and B cell estimation. There is a progressive drop in the percentage of T and B cells with age, but no significant differences between the races nor between the sexes of the same age group. Aberrancies of T and B cell percentages were noted in most infections, malignancies and even malnutrition.

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