Clin. exp. Immunol. (1972) 32, 346–351.

Age-dependent changes of human blood lymphocyte subpopulations

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(*Received* 9 *December* 1977)

SUMMARY

T- and B-lymphocyte populations were enumerated at four stages of life: at the newly born, infant, adult and aged stages. The proportion of T cells detected by E rosettes and an anti-human T-lymphocyte antigen (HTLA) serum increased from new-born children to adults, then decreased with ageing. The antiserum detected less mature T cells in aged people. The percentages of cell forming 'active' E rosettes increased with ageing. Lower numbers of B cells bearing surface immunoglobulins were found in adults. Complement receptor-bearing lymphocytes (percentages and absolute numbers) decreased from new-born children to aged humans. Finally, the number of monocytes were significantly greater in the young than in adult and aged people. Such results bring new data concerning the age-dependent changes of lymphocyte subpopulations and concerning the significance of various techniques used together to detect mononuclear cell populations in the human peripheral blood.

INTRODUCTION

In man, as in many animal species, cell-mediated and humoral immune responses are quite effective at birth, but tend to become less adequate with ageing (Walford, 1969). The increased incidence of infections (Rowley, Buchanan & Mackay, 1968), neoplasia, monoclonal gammapathies and autoimmune diseases (Burnet, 1974; Walford, 1969) in ageing people may reflect some degree of inadequacy of the immune response to self or non-self antigens. A first probe of the age-associated immunological impairment was obtained by the study of proliferative responses of circulating lymphocytes to plant mitogens: already maximal with foetal or cord blood lymphocytes (Brochier *et al.*, 1970; Kay, Doe & Hockley, 1970), they tend to decrease with ageing (Girard *et al.*, 1977; Pisciotta *et al.*, 1967).

The purpose of this study was to estimate lymphocyte subpopulations at three stages of life: newly born, adult and aged. Lymphocytes were enumerated using six different markers in order to discriminate between varying T- and B-cell subpopulations. It was shown that the T lymphocytes and the complement receptor-bearing cells were mainly affected during ageing.

MATERIALS AND METHODS

Subjects. Peripheral blood lymphocytes were obtained from: (a) twenty-six newly born babies (thirteen males and thirteen females) without infection or immunological disease. They were 3–21 days old (gestational age range : 32 weeks to full term); (b) twelve infants (three males and nine females) 3 months old; (c) forty healthy adult volunteers (20–50 years old); and (d) forty aged subjects, including twenty females (76–97 years) and twenty males (75–90 years) without generalized infection, chronic uraemia, neoplasia, chronic alcoholic disease or tuberculosis, and not on corticosteroid therapy.

Lymphocyte suspensions. Lymphocytes were obtained from heparinized blood centrifuged (400 **g**, 30 min, 20°C) on Ficoll–Hypaque gradient (density : 1·077 kg/m³). The cell suspensions contained 85–95% of small lymphocytes and 5–15% of

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monocytes, as judged by peroxidase staining (Preud'homme & Flandrin, 1974). The total numbers of lymphocytes were determined by total white blood cell counts and differential counts on May–Gründwald–Giemsa–stained smears. The total numbers of lymphocytes were calculated in the whole blood before separation and in purified cell suspensions (Holm *et al.*, 1975). The lymphocyte yield was generally greater than 70%; in nine cases where it was lower than 70% the cells were not studied and another sample was drawn up.

Lymphocyte markers. T cells were enumerated by the formation of rosettes with sheep erythrocytes (E rosettes) (WHO/IARC Report, 1974) and with a horse anti-human T-lymphocyte antigen (HTLA) serum (Brochier *et al.*, 1976).

The anti-HTLA serum was prepared by extensive absorptions of horse anti-human thymocyte globulins on lymphoblastoid cell lines bearing B-cell markers, and on fractionated non-T tonsillar lymphocytes. The criteria of specificity are reported elsewhere (Brochier *et al.*, 1976). Briefly, it delineated to a plateau, either by cytotoxicity or indirect immunofluorescence, a subpopulation of lymphocytes which averaged 75% in the blood, which was found to be different from the cells bearing surface immunoglobulins, which formed E but not complement-coated erythrocyte (EAC) rosettes, and which did not phagocytose latex particles. HTLA-positive cells were identified by the microcytotoxicity technique (Brochier *et al.*, 1976) and their percentage was expressed by the cytotoxic index calculated according to the formula:

$$\text{cytotoxic index} = \frac{\text{percentage dead cells with anti-HTLA serum} - \text{percentage dead cells with C alone}}{100 - \text{percentage dead cells with C alone}}$$

In no case was the number of dead cells in the controls with C alone higher than 3%.

A subpopulation of T cells was detected according to the method of 'early' E rosettes (Yu, 1975) or 'active' E rosettes (Wybran & Fudenberg, 1973).

B cells were identified by the presence of surface immunoglobulins (SmIg), using a fluoresceinated anti-polyvalent Ig serum (Meloy Laboratories). C3 receptor-bearing lymphocytes were detected according to Bianco and colleagues (Bianco, Patrick & Nussenzweig, 1970; Clot ; Charmasson, 1976) and Fc gamma receptor- bearing cells by the EA-rosette technique (Brochier, Samarut & Revillard, 1975). Monocyte contamination was determined by peroxidase staining (Preud'homme & Flandrin, 1974).

In each case, the results were expressed as mean percentages and mean absolute numbers of cells per $mm^3 \pm$ one standard deviation (s.d.). Comparisons of these values were performed by the classical Student's *t*-test.

RESULTS

The results obtained with the different markers are summarized in Table 1. No statistical difference was found between mean percentages from the new-born children and infants, so that these groups were taken together for comparison with the other groups.

The proportion of T cells determined by the E-rosette technique and the anti-HTLA serum increased from new-born children to adults, then decreased with ageing (Fig. 1). However, the anti-HTLA serum detected more T cells in new-born children and adults and less in aged people than the E rosettes did (Table 2). The absolute numbers of T lymphocytes measured by both techniques decreased with ageing.

In contrast, the percentages of cells forming 'early' E rosettes increased with ageing.

The lowest numbers (percentages and absolute counts) of B cells bearing SmIg were found in adults. Conversely, C3 receptor-bearing lymphocytes (percentages and absolute counts) decreased from new-born children to aged humans. The ratio of EAC rosettes : SmIg thus decreased from 1·38 in the young and adults to 0·68 in elderly subjects. The absolute counts of Fc receptor-bearing cells decreased with ageing, but their percentages remained unchanged (Fig. 2).

The percentages of monocytes were significantly greater in the young than in adults and in aged people. Null cells, defined by the absence of SmIg and E receptor or HTLA, were found to be higher in young and aged people than in adults (Table 3).

Finally, no statistical difference in the lymphocyte markers was observed between males and females from the young and adult groups. In contrast, a sex-related difference appeared among aged people for EAC- and 'early' E-rosette percentages (Table 4).

DISCUSSION

Few data are yet available on the changes of lymphocyte subpopulations during life. Our results confirm previous partial reports, concerned mainly about T cells detected by the E-rosette technique and

TABLE 1. Percentages and absolute counts (ct/mm³) of cells forming E, 'early 'E, EAC or EA rosettes, or bearing HTLA or SmIg in the four groups of subjects studied

Patients			E rosettes	HTLA	'early' E rosettes	SmIg	EAC rosettes	EA rosettes	Monocytes*
New-born children	Percentage	Mean	55·3	63·4	12·9	14·8	20·4	8·3	13·5
		s.d.	8·5	16·0	5·3	4·5	6·3	2·9	8·7
($n = 26$) (a)†	ct/mm³	Mean	2875·7	3297·0	670·8	796·6	1060·9	341·6	
		s.d.	147·8	278·1	35·6	78·2	109·5	50·4	
Infants	Percentage	Mean	53·3	64·3	13·7	13·5	18·4	10·9	
($n = 12$) (b)†		s.d.	10·5	14·1	8·3	4·2	2·8	3·2	
	ct/mm³	Mean	2379·2	2870·2	611·5	602·6	821·3	486·6	
		s.d.	163·0	218·9	125·3	65·1	43·5	49·6	
Adults	Percentage	Mean	70·9	75·9	21·9	11·5	15·8	12·3	7·05
($n = 40$) (c)†		s.d.	6·4	3·1	10·0	3·1	3·3	3·5	4·2
	ct/mm³	Mean	1253·3	1341·5	387·6	203·3	279·3	217·4	
		s.d.	112·7	16·3	52·7	16·8	17·5	18·4	
Aged	Percentage	Mean	66·0	62·9	29·7	14·9	10·1	10·7	7·4
(n 40) (d)†		s.d.	12·2	13·1	10·4	3·5	3·1	5·2	3·1
	ct/mm³	Mean	1021·3	950·3	450·2	225·1	168·6	161·7	
		s.d.	28·0	39·2	31·1	10·5	9·3	15·6	
Student's t-test	a *vs* b		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	
on mean	(a + b) *vs* (c)		< 0·0001	< 0·001	< 0·001	< 0·01	< 0·001	< 0·01	< 0·001
percentage	(c) *vs* (d)		< 0·05	< 0·001	< 0·01	< 0·001	< 0·0001	n.s.	n.s.
Student's t-test	(a) *vs* (b)		< 0·0001	< 0·001	< 0·05	< 0·0001	< 0·0001	< 0·01	
on mean ct/mm³	(a + b) *vs* (c)		< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	
	(c) *vs* (d)		< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	< 0·0001	

* Monocyte determination by the peroxidase staining was carried out only in twenty-eight new-born children and infants, forty adults and twenty-five aged subjects.

† Total lymphocyte count in whole blood from: (a) new-born children : 5200·3 ± 1738·3; (b) infants : 4463·8 ± 1552·6; (c) adults : 1767·5 ± 526·9; (d) aged : 1542·2 ± 300·5.

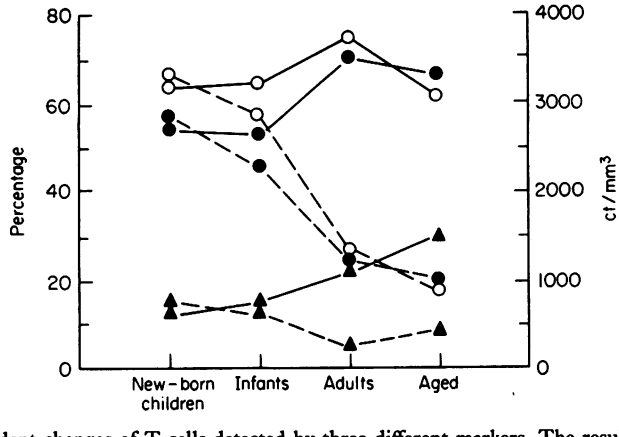

FIG. 1. Age-dependent changes of T cells detected by three different markers. The results are expressed as mean percentages and absolute counts per mm³. Continuous lines and broken lines only join together the results obtained at four stages of life and do not represent a curve. (●) E rosettes, (○) HTLA, (▲) Early E rosettes, (———) percentages, (– – –) ct/mm³.

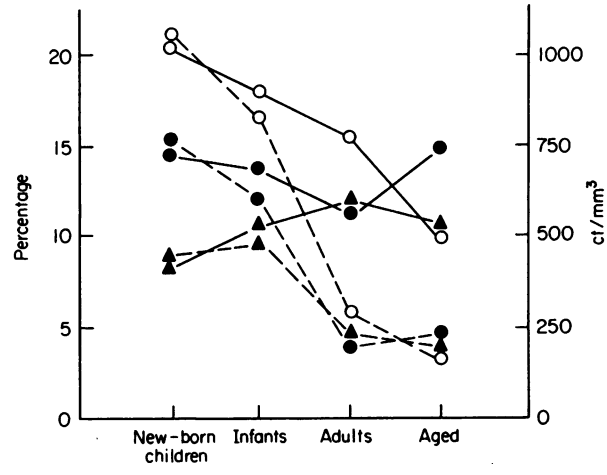

FIG. 2. Age-dependent changes of B cells bearing SmIg and C3 receptors and of cells bearing Fc receptors. The results are expressed as mean percentages and absolute counts per mm^3. Continuous lines and broken lines only join together the results obtained at four stages of life and do not represent a curve. (●) SmIg, (○) EAC rosettes, (▲) EA rosettes, (———) percentages, (– – –) ct/mm^3.

TABLE 2. Comparison of the percentage of T cells detected by the anti-HTLA serum and the E rosette technique

Patients	(HTLA − E rosettes)*	*P* value†
New-born children	9·9 ± 1·8	< 0·001
Adults	4·1 ± 0·9	< 0·01
Aged	−3·5 ± 1·4	< 0·01

* (HTLA − E-rosettes) = mean percentage ± SD of the difference between the numbers of cells detected by anti-HTLA serum and the E-rosette technique for each individual.

† *P* obtained by the Student's *t*-test on paired values.

TABLE 3. Null cells without T or B markers in the peripheral blood

Markers added	New-born children and Infants	Adults	Aged
E + SmIg + Monocytes	82·4	89·5	88·3
HTLA + SmIg + Monocytes	91·5	94·5	85·2
Undetected cells	8·5–17·6	5·5–10·5	11·7–14·8

Results are expressed as percentages.

B cells assessed by SmIg or EAC rosettes (Augener *et al.*, 1974; Ben-Zwi *et al.*, 1977; Campbell *et al.*, 1974; Carossella, Mochanko & Braun, 1974; Diaz-Jouanen, Strickland & Williams, 1975; Fernandez, MacSween & Langley, 1976; Fleisher *et al.*, 1975; Froland & Natvig, 1972; Gajl-Peczalska, Hallgren & Kersey, 1974; Girard *et al.*, 1977; Rowe *et al.*, 1973; Smith, Evans & Steel, 1974). Most of these studies, in agreement with our data, reported age-dependent changes of E rosette-forming cells (Augener *et al.*, 1974; Campbell *et al.*, 1974; Carossella *et al.*, 1974; Diaz-Jouanen *et al.*, 1975; Fleisher *et al.*, 1975; Girard *et al.*, 1977; Smith *et al.*, 1974), sometimes associated with an increase of B cells with ageing

TABLE 4. Comparison of lymphocyte markers between aged males and females

Patients		E rosettes	HTLA	'early' E rosettes	SmIg	EAC rosettes	EA rosettes
Aged males ($n = 20$)	Mean	69·1	63·5	33·4	14·4	12·0	12·3
	s.d.	11·0	14·4	10·4	3·3	2·7	6·3
Aged females ($n = 20$)	Mean	63·0	62·3	25·6	15·5	8·3	9·3
	s.d.	12·9	12·0	9·0	3·6	2·2	3·6
Student's t-test	(P)	n.s.	n.s.	$< 0{\cdot}05$	n.s.	$< 0{\cdot}001$	n.s.

Results are expressed as mean percentages ± s.d.

(Augener *et al.*, 1974; Diaz-Jouanen *et al.*, 1975; Gajl-Peczalska, Hallgren & Kersey, 1974; Girard *et al.*, 1977) or without any change (Ben-Zwi, *et al.*, 1977; Fernandez *et al.*, 1976).

T cells were enumerated in the present study by the E rosette technique and anti-HTLA serum: both techniques showed a maximal proportion in adults, whereas the absolute counts constantly decreased from new-born to aged. Comparisons of the two markers elicited a few percentages of non-rosetting cells (9·9% in new born children and 4·1% in adults) detected by the anti-HTLA serum, whereas 3·5% of E rosettes were not assessed by our antiserum in aged people. This suggests a detection of immature T cells by this anti-HTLA serum and not by the E-rosette test, these cells being present in the circulating pool mainly in new-born children and disappearing with ageing. In agreement, it has been demonstrated that during *in vitro* T-cell maturation HTLA appears before the ability to bind sheep erythrocytes (J.L. Touraine, personal communication).

In contrast, 'early' or 'active' E-rosette proportions increased with ageing. If this technique detects T cells involved in delayed hypersensitivity reactions (Felsburg 1976), the increase of these rosettes might represent a more mature stage of the E rosette-forming cells. This agrees also with the decrease of immature T-cells detected by the anti-HTLA serum, and may reflect the persistence of normal secondary humoral and cell-mediated responses in ageing people (Rowley *et al.*, 1968). This evolution seems to be correlated with a cell-mediated impairment in ageing (Fernandez *et al.*, 1976; Foad *et al.*, 1974; Hallgren *et al.*, 1973; Walford, Willkens & Decker, 1968).

Proportions of B cells bearing SmIg were found to be minimal in adults, but the total counts remained unchanged in adult and aged people. Conversely, the age-dependent change of C3 receptor-bearing lymphocytes is different, since the EAC-rosette percentages and absolute numbers were higher than SmIg-bearing cells in the young and adults, and lower in the elderly subjects. Such data disagree with those recently reported by Ben-Zwi *et al.* (1977), who used ox red cells and human complement instead of the sheep erythrocytes and mouse complement employed in the present study. It has been reported that C3 receptors are not only present on B cells with SmIg, but also on other mononuclear cells (Ross *et al.*, 1973). The phenomenon observed here could be due to the presence of precursor cells among the C3 receptor-bearing lymphocytes (Gatien, Schneeberger & Merler, 1975) lacking in the aged population. An alternative explanation could be the occurrence of circulating immune complexes related to the autoimmune process in ageing (Burnet, 1974); such complexes could interfere with the EAC-rosette formation (Ezer & Hayward, 1974). Moreover, it must be noted that EAC-rosette percentages are significantly lower in aged females than in aged males. This is yet unexplained, but might be due to the differences of mean age (females 85·7 years *vs* males 80·5 years).

Finally, it appears that undetected mononuclear cells (null cells) are mainly present in young and aged subjects. This might be related to the presence of immature cells in young children and to the immunocompetence decrease in aged people. However, it must be noted that our data represent the changes in the circulating pool of lymphocytes and could be different in lymphoid organs.

The subjects of this study were kindly provided to us by Dr J. Astruc and by Dr E. Marcais, A. Mandin and J. Dauverchain. We gratefully acknowledge Dr J. P. Revillard and Professor J. F. Soothill for their valuable suggestions and criticisms.

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