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Age-related changes in human thymus

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SUMMARY

Thymic biopsies obtained during open heart surgery were examined for age-related involution of the parenchyma and changes in cell surface characteristics. In parallel with the parenchymal atrophy, E-rosette-forming cells continuously decreased with age and were progressively replaced by an increasing proportion of 'null' lymphoid cells. These changes appeared to be independent of each other. The significance of these findings in the context of the host's immune mechanism is discussed.

INTRODUCTION

Human thymus undergoes progressive involution during adult life. This is characterized by a decrease in thymic weight and is accompanied by regression of the lymphoid parenchyma with a concomitant replacement of cortex and medulla by mature adipose tissue (Hammar, 1926; Young & Turnbull, 1931; Mackay, 1966). Histometric techniques have recently been used to reveal different patterns of age-related thymic involution. Thus in males, cortical atrophy is uniformly progressive whilst in females it is biphasic, due to a retarded rate of atrophy just before the menopause (Simpson, Gray & Beck, 1975).

Cells obtained from foetal and post-natal thymuses behave predominantly as lymphocytes; they respond to mitogenic stimulation, form rosettes with sheep erythrocytes and show specific antigen binding sites on the membrane surface (Dwyer & Mackay, 1970; Papiernik, 1972; Whittingham & Mackay, 1973; Roberts, Whittingham & Mackay, 1975). The subsequent fate of these cell surface characteristics does not seem to have been examined in relation to other criteria of age-related thymic involution.

The present study was therefore undertaken to examine the surface markers of thymic lymphocyte populations in relation to the histometric criteria of the ageing human thymus.

MATERIALS AND METHODS

Thymic biopsies were obtained from fifty-two subjects (forty-one males and eleven females) at the time of open heart surgery. Their ages ranged from 5 to 75 years. None of these patients had received corticosteroids or immunosuppressive drugs prior to this study.

The bulk of the biopsied material was used to obtain a suspension of thymic cells, all steps being carried out at room temperature (18–22°C). Freshly obtained tissue was finely minced in ice-cold TC199 and coarse debris was removed by sedimentation for 5–10 min. The resulting supernatant was aspirated and cells were obtained by centrifugation (250 ***g*** 10 min). The contaminating erythrocytes were removed by lysis with 0·83% ammonium chloride and the remaining cells, after three further washes, were finally resuspended in TC199 containing 10% (v/v) foetal calf serum. Before use the foetal calf serum had been heat-inactivated and repeatedly adsorbed with sheep erythrocytes. Cell viability, as judged by exclusion of Trypan blue dye, always exceeded 95%.

Smears were prepared from the above cell suspension for the evaluation of cell morphology. Aliquots (0·3–0·5 ml) of cell suspension (1–2 $\times$ 10^6/ml) were placed in cuvettes of a 'Cytospin' centrifuge and the cells were transferred to glass slides by

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centrifugation at 90 *g* for 10 min. These were dried in air, fixed in 95% methanol and stained with Giemsa for further examination. A differential count of 500 cells was performed in each instance.

A portion of the original biopsy was fixed in Bouin's solution and embedded in paraffin wax. Sections (6 μm thickness) were stained with haematoxylin and eosin for histological examination and histometric measurements.

The age involution of the thymus parenchyma was assessed by the histometric method described by Simpson *et al.* (1975). The relative areas occupied by cortex, medulla and non-parenchymal tissue (fat, connective tissue and blood vessels) were measured using a × 4 objective and a × 10 eye-piece which contained a graticule (1 cm^2) subdivided into 100 small squares. The volume percentages of the different components of the gland were subsequently derived from these data according to the principle that the area of the graticule overlying any particular component in the section was directly proportional to the volume of that component in the tissue sample (Hennig & Mayer-Arendt, 1963). Depending on their size, up to six sections of an individual biopsy were examined.

Spontaneous rosette (E-rosette) formation between sheep erythrocytes and thymic lymphocytes was demonstrated by the method of Wybran, Chantler & Fudenberg (1973), modified so that incubation was carried out at 4°C for 20 hr.

Membrane-bound immunoglobulins (SmIg) were examined by a direct immunofluorescent method in which a polyvalent antibody having activities against all known classes of human immunoglobulins was used (Papamichail, Brown & Holborow, 1971; Preud'homme & Seligmann, 1976). Complement binding (C3 receptor) sites were examined by EAC-rosette formation using IgM antibody and complement-coated ox red cells (Papamichail & Holborow, 1977).

RESULTS

Cell population

Cells obtained from the biopsied material were mainly lymphocytes (Table 1). A majority of these (68·9±7·1%) were well differentiated small lymphocytes with scanty cytoplasm and dense nuclear chromatin. Others (14·9±4·5%) were medium sized lymphocytes with a moderate amount of pale blue cytoplasm and rather clumped nuclear chromatin. The remaining lymphoid cells (8·9±4·6%) were large lymphocytes which had intensely basophilic cytoplasm and relatively immature nuclei characterized by finely distributed nuclear chromatin and distinct nucleoli. The non-lymphoid cell population (5·6±1·5%) comprised epithelial cells, macrophages and granulocytes, as well as a small proportion of cells which could not be definitely identified.

TABLE 1. Differential count of cells obtained from thymus (all values are expressed in percentages)

	Range	Mean±s.d.
Large lymphocytes	4·5–19·0	8·9±4·6
Medium lymphocytes	8·5–26·0	14·9±4·5
Small lymphocytes	54·5–79·2	68·9±6·1
Total lymphocytes	90·0–96·0	92·8±1·54
Non-lymphoid cells (epithelial cells, granulocytes, macrocytes, etc.)	3·3–8·3	5·6±1·5
Others (unidentified cells)	0·7–2·9	1·7±0·6

E-rosette-forming cells

E-rosettes in all fifty-two subjects had a range of 45–96% (mean 74·5±12·0). Although a differential count of cells forming these rosettes was not performed, all three type- of lymphocytes formed E-rosettes. In those instances where E-rosette-forming cells were relatively few, all three different types of lymphocytes were observed to be incapable of forming these rosettes. The findings in the forty-one males (73·9±11·2%) and eleven females (76·9±15·1%) were similar ($P > 0·05$).

E-rosette formation, when examined in relation to the age of the individual, showed a progressive decline with increasing age ($r = -0·81$; $P < 0·001$). These changes, shown in Fig. 1, were equally evident in males ($r = -0·76$; $P < 0·001$) and females ($r = -0·91$; $P < 0·001$).

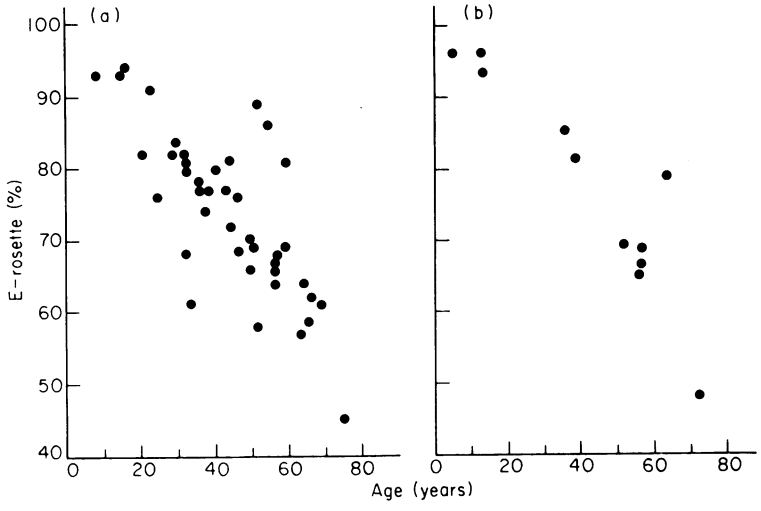

FIG. 1. Relationship between E-rosettes and age shown separately for (a) males and (b) females.

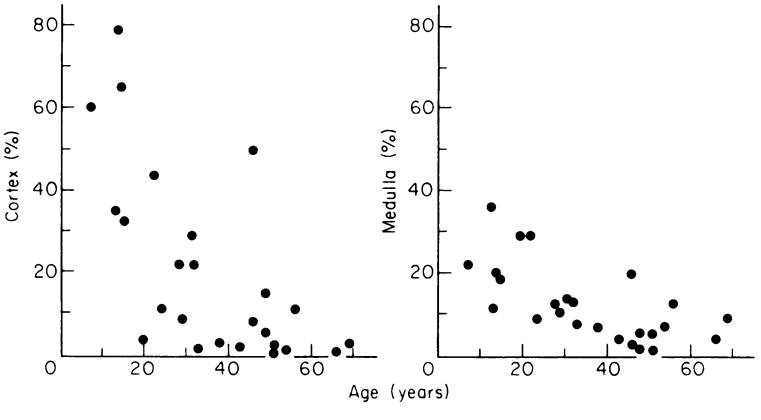

FIG. 2. Relationship between residual parenchyma and age shown separately for cortex and medulla. The equation $y = a + bx$ was as follows: cortex = 52·28+(−0·881) years, medulla = 25·20+(−0·350) years.

In ten instances where E-rosette-forming cells amounted to less than 60%, the cells carrying SmIg and C3 receptors, surface markers of B lymphocytes, never exceeded 1%.

These findings show that the thymic lymphocytes undergo two distinct but inter-related changes with increasing age. As the population of E-rosette-forming cells progressively declines, its place is taken up by 'null' cells which are devoid of the characteristic surface markers of either T or B lymphocytes. It is possible that these 'null' cells are variants of T lymphocytes which have not yet acquired the characteristic membrane markers.

Changes in parenchyma

Biopsies from twenty-six subjects, all males, were available for histological and histometric assessment. The distinction between cortex and medulla was clear in twenty-five of these. In the remaining single instance the parenchyma comprised foci of lymphoid cells scattered amongst fat and connective tissue. The total parenchyma in all twenty-six subjects had a range of 1·5–90·2%. The cortex comprised 0·4–78·8% (20·6±22·9) whilst the medulla varied from 1·7–35·9% (12·6±9·2) of the tissue. As shown in Fig. 2, both cortex ($r = -0{\cdot}67$; $P < 0{\cdot}001$) and medulla ($r = -0{\cdot}67$; $P < 0{\cdot}001$) involuted progressively with ageing. The involution of cortex appeared to proceed at a rate approximately two and a half times faster than that of the medulla ($b_{cortex} = -0{\cdot}881$, $b_{medulla} = -0{\cdot}350$), (Fig. 2).

Inter-relationship between E-rosettes and parenchyma

In view of the fact that both changes are age-related, the relationship between E-rosettes and parenchymal involution was examined by calculating the partial correlation between these variables independently of age. The findings summarized in Table 2 show that the correlation coefficients between E-rosettes on the one hand and the residual parenchyma and its components on the other were not significant.

Therefore, it appears that the various age-related changes observed in this study, i.e. the involution of parenchyma and changing characteristics of lymphoid cells, occurred simultaneously but independently of each other.

TABLE 2. Partial correlation between E-rosettes and residual thymus parenchyma and its cortex and medulla determined independently of age. Figures in parenthesis indicate the number of pairs included

		r	*P*
E-rosettes *vs* total parenchyma	(26)	0·16	>0·30
E-rosettes *vs* cortex	(25)	0·09	>0·40
E-rosettes *vs* medulla	(25)	0·16	>0·30

DISCUSSION

The human thymus, after an initial burst of rapid growth in childhood and early adolescence, undergoes progressive atrophy. The findings in the present study lend further support to this well established view. It is nevertheless of considerable interest that the organization of the parenchyma into cortex and medulla was preserved in spite of marked atrophy and the fact that the cellular elements remained predominantly lymphoid. Inside this continuously involuting parenchyma the lymphoid cells underwent profound changes. E-rosette-forming cells progressively decreased and were replaced by an increasing proportion of cells devoid of the three principal surface markers. As there was no correlation between the regression of parenchyma and the changing lymphoid cell characteristics, these two concurernt and age-related changes are likely to be independent of each other.

Thymectomy in adult animals often leads to a diminution of circulating T lymphocytes and a progressive impairment of thymus dependent immune mechanisms (Good *et al.*, 1962; Kappler *et al.*, 1974). It is likely that the thymus, instead of being a vestigial organ, remains an important reservoir of immunocompetent T lymphocytes, and that this reservoir, by virtue of progressive parenchymal atrophy, is continuously diminishing in size. Within this framework a preferential loss of E-rosette-forming cells indicates a progressive impairment of the thymus-dependent immune competence of the host, which is further accentuated by an increasing population of 'null' cells.

It is possible that the 'null' cells are undifferentiated T lymphocytes which have not yet acquired the distinctive surface markers. Diminishing production of thymic hormones in relation to age would seem relevant in this context, for *in vitro* studies have shown that 'null' lymphoid cells may differentiate into E-rosette-forming cells under the stimulus of thymic extracts (Bach, Fournier & Bach, 1975; Incefy, L'Esperance & Good, 1975; Lewis *et al.*, 1978). *In vitro* studies to determine whether 'null' cells in the aged thymus will become E-rosette-forming cells in the presence of thymosin should help to clarify this issue.

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