Age-Specific Dynamics of Human Thymus Immune Cell Differentiation

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The expression of markers of undifferentiated $CD5^+$ cells, B cells, T-killer/suppressors, and T-helpers was verified by immunohistochemical methods in the thymuses of elderly, senile, and long-lived humans. The counts of $CD5^+$ cells in the thymus progressively decreased with age. Positive correlations between the counts of $CD5^+$ cells and differentiated T cells were detected. The capacity of $CD5^+$ thymocytes to differentiate into T-killer/suppressors decreased with age.

Key Words: *undifferentiated $CD5^+$ cells; T cells; B cells; thymus; aging*

The lifespan of a human being and the tempo of age-specific disease development are largely determined by the immune system status, to be more exact, by its central organ, the thymus [2]. By the age of 60 years, the counts of immature lymphoid cells of the thymus (thymocytes) and of their differentiated forms decrease significantly [4]. The causes of this process are (1) degeneration of the thymic epithelial cells maintaining differentiation, proliferation, and survival of thymocytes, and (2) reduced secretion of bioactive thymic factors (IL-7 and thymic serum factor) regulating differentiation of T and B cells [5]. However, it remains unclear to what measure the involution of the thymus in humans aged over 60 years involves the processes of thymocyte and immature B cell proliferation and subsequent differentiation.

We studied age-specific dynamics of thymocyte counts and evaluated the relationships between the proportions of various populations of mature T and B cells and their undifferentiated forms.

MATERIALS AND METHODS

The thymuses (autopsy material from humans of different age) were distributed into groups in accordance with WHO classification: 1) from elderly subjects (60-74 years); 2) from senile subjects (75-89 years); and 3) from long-lived subjects (90 years and older).

Fragments of the thymus were fixed in formalin (pH 7.0), dehydrated, and embedded in paraffin by the standard method. Sections of the thymus (3-5 μ) were sliced on a Leica 540 M microtome and mounted on slides coated with poly-L-lysine. The preparations were stained with hematoxylin and eosin in order to detect various populations of immune cells.

Immunohistochemical detection of the expression of undifferentiated immune cells, mature T-helpers (Th), T-killer/suppressors (TKS), and B cells was carried out using the respective monoclonal antibodies CD5 (1:30, Novocastra), CD4 and CD8 (ready to use; Novocastra), and CD20 (1:30, Novocastra). A universal kit of biotinilated antimouse and antirabbit immunoglobulins served as the second antibodies. Staining was visualized using avidin complex with horseradish peroxidase (ABC-kit) with subsequent development of horseradish peroxidase with diaminobenzidine (all reagents from Novocastra).

CD5 marker was selected for thymocyte verification because the corresponding cell receptor is involved in stimulation of immature immune cells of the thymus [6]. In addition, the CD5 molecule is present on mature B cells, Th, and TKS [3], which enables

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evaluation of the differentiation of immature lymphoid cells into various mature populations.

Morphometry was carried out using a system of computer-aided analysis of microscopic images consisting of a Nikon Eclipse E400 microscope, Nikon DXM1200 digital camera, PC, and VideotestMorphology 5.0 software. Ten visual fields at magnification 400 were analyzed in each case. The area of the above listed markers expression was calculated as the proportion of the area occupied by immunopositive cells to total cell area in the visual field and expressed in percent. Optical density of expression was measured in arbitrary units.

The data were statistically processed using two-way Student's test. Linear relationships between the areas of expression of the studied immunohistochemical markers were evaluated by Pearson analysis of correlations. The significance of the linear regression equation was evaluated by coefficient of determination, calculated as the square value of Pearson coefficient of correlation.

RESULTS

The area of CD5 expression in group 1 was 7.07± 1.15%, which was 1.4 times higher than in group 2 (5.07±0.62%; $p<0.05$). In group 2, the area of CD5 expression was 1.6 times larger than in group 3 (3.26± 0.72%, $p<0.05$). Optical density of CD5 expression was virtually the same in all 3 groups. The data indicate a progressive decrease in thymocyte and immature B cell counts in the thymus after 60 years of age, the maximum reduction of the counts of undifferentiated $CD5^+$ immune cells being found in long-lived subjects. The levels of receptors to this marker per cell did not change with age. We hypothesized that age-specific reduction of the counts of thymocytes and undifferentiated B cells led to a decrease in the levels of mature T- and B cells in the thymus.

The relationship between the areas of expression of CD20 (mature B cell marker) and CD5 was moderate, which was shown by a low Pearson coefficient of correlations (Table 1). On the other hand, the approximating equation (Fig. 1) indicated that the level of

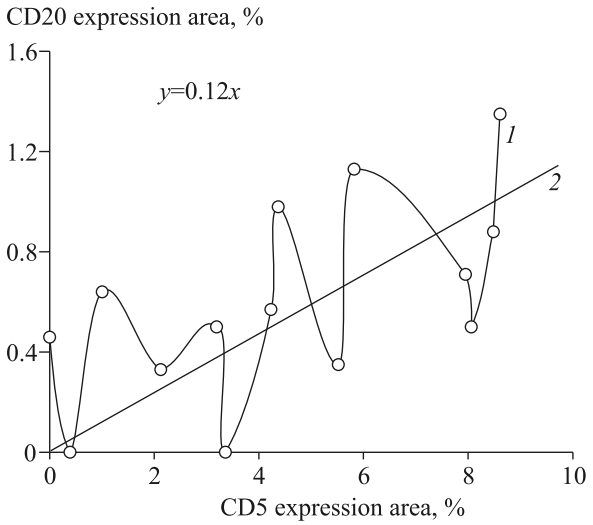

Fig. 1. Relationship between the areas of expression of CD20 (B cell marker) and CD5 (marker of undifferentiated T and B cells) in the thymuses of humans of groups 1-3. Here and in Figs. 2, 3: *1*) experimental data; *2*) approximating straight line.

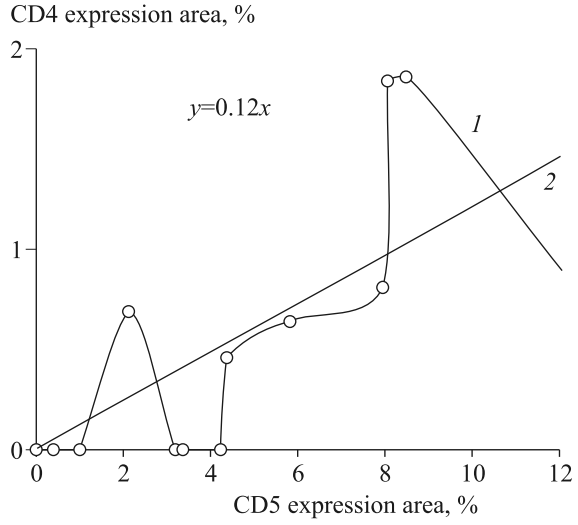

Fig. 2. Relationship between the areas of expression of CD4 (Th marker) and CD5 (marker of undifferentiated T and B cells) in the thymuses of humans of groups 1-3.

$CD20^+$ B cells in the population of $CD5^+$ cells reached 12%. Coefficient of determination indicated that the level of mature B cells was by only 34% determined

TABLE 1. Relationships between Expression of Various Immune Cell Markers in Human Thymus

Age groups	Comparison of expression areas of		Coefficient of correlations	Intensity of relationship between parameters	Coefficient of determination
1, 2, 3	CD20	CD5	0.59	Moderate	0.34
1, 2, 3	CD4	CD5	0.78	Strong	0.60
1	CD8	CD5	0.92	Strong	0.85
2, 3	CD8	CD5	0.80	Strong	0.64

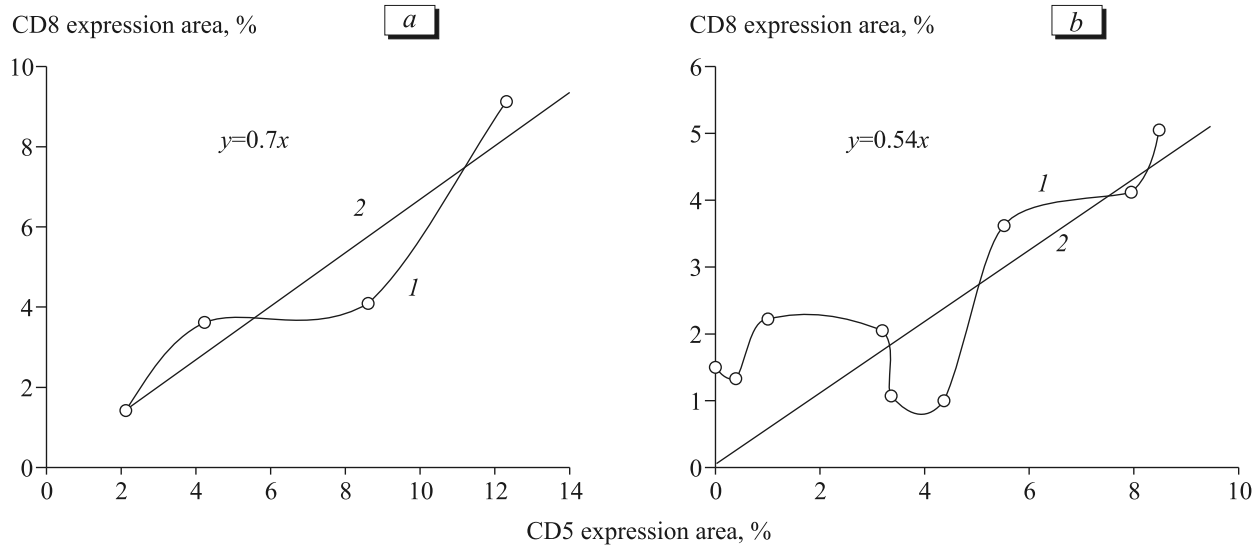

Fig. 3. Relationship between the areas of expression of CD8 (TKS marker) and CD5 (marker of undifferentiated T and B cells) in the thymus at different age. *a*) group 1; *b*) groups 2 and 3.

by the level of immature $CD5^+$ cells in the thymus and largely depended on other factors.

The expressions of Th marker CD4 and thymocyte marker CD5 were in a strong correlation (Table 1), and the significance of the data approximating linear equation, according to which 12% thymocytes differentiated into Th (Fig. 2), was confirmed by high coefficient of determination.

Study of correlations between the areas of CD8 (TKS marker) and CD5 (thymocyte marker) expression showed that it depended on the age group. Therefore, analysis of correlations was carried out separately for group 1 and pooled groups 2 and 3.

In group 1, coefficient of correlations for CD8 and CD5 expression areas exhibited a trend to 1 (Table 1), indicating that the relationship between the experimental data were close to the linear one. The approximating relationship indicated that 70% thymocytes differentiated into TKS (Fig. 3, *a*), and the coefficient of determination confirmed that this conclusion was well-based (Table 1). In groups 2 and 3, the areas of CD8 and CD5 expression also formed a strong correlation, but the linear equation describing this relationship indicated that only 54% thymocytes were capable of differentiation into TKS (Fig. 3, *b*).

The data indicated that the levels of undifferentiated $CD5^+$ cells in the thymus decreased after the age of 60 years and this decrease progresses with age. Twelve percents of total count of $CD5^+$ cells were $CD20^+$ B cells, the level of B cells in the thymus not depending on the level of $CD5^+$ cells. Another 12% of the total count of $CD5^+$ cells were Th, their count depending on the count of $CD5^+$ thymocytes. The process of TKS differentiation from $CD5^+$ thymocytes differed in elderly people and subjects over 74 years. In elderly age 70% $CD5^+$ cells differentiated into TKS, while after 74 years thymocyte capacity to differentiate into cytotoxic T cells decreased to 54%.

The count of undifferentiated $CD5^+$ cells in the thymus decreased with age, which led to reduction of the count of mature T cells (Th and TKS), but was inessential for the level of mature B cells. The capacity of $CD5^+$ thymocytes to differentiate into TKS was low in senile and in long-lived subjects, which was an additional cause of reduction of this population of immune cells.

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