Role of Dendritic Cells in the Formation of Subpopulation of Cytotoxic T-Lymphocytes in the Thymus during Its Aging

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The counts of dendritic cells and cytotoxic T-lymphocytes in the thymus decrease during its aging. The counts of dendritic cells decrease in senile age, while low counts of cytotoxic T-cells are observed only in long-living individuals. Presumably, reduction of the counts of thymic dendritic cells causes disorders in the differentiation of T-cells, particularly of cytotoxic ones, which can represent a mechanism of thymus involution during its aging.

Key Words: *dendritic cells; cytotoxic T-lymphocytes; thymus; aging*

The counts of T-cells, particularly of their mature forms, in the thymus decrease during its age-related involution [2,3,5]. Dendritic cells (DC) are involved in T-lymphocyte differentiation in the medullary layer of the thymus [6]. They release IL-1 and prostaglandins E2 promoting T-lymphocyte maturation and selection [3,4]. Microscopic studies showed the formation of T-lymphocyte rosettes around DC, the majority of these T-cells with cytotoxic function [1]. Despite the data on DC involvement in T-cell differentiation, this problem, particularly as regards the age-associated involution of the thymus, remains little studied.

We studied the relationship between the counts of DC and cytotoxic T-lymphocytes (CTL) in the thymus and human age.

MATERIALS AND METHODS

Thymus specimens (autopsy material) were distributed into groups in accordance with the WHO classification of age: group 1) elderly people (60-74 years; *N*=10); group 2) senile people (75-89 years; *N*=10); and group 3) long-living individuals (90 years and older; *N*=10).

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Fragments of the thymus were plunged in formalin (pH 7.0), dehydrated, and embedded in paraffin by the standard method. Sections of 3-5 μ were sliced on a Leica 540 M microtome and mounted onto poly-L-lysine-coated slides. Markers of DC (CD35) and CTL (CD8) were detected by immunohistochemical method using mouse monoclonal antibodies to CD35 (1:60, Novocastra) and CD8 (ready to use, Novocastra) as primary and biotinylated antimouse immunoglobulins (Novocastra) as second antibodies. The reaction was visualized with avidin complex with horseradish peroxidase (ABC-kit) and diaminobenzidine.

Morphometry was carried out by computer analysis of microscopic images. The system included a Nikon Eclipse E400 microscope, a Nikon DXM 1200 digital camera, a computer, and VideotestMorphology 5.0 software. Ten visual fields at ×400 were analyzed in each case. The area of the expression of markers was calculated as the proportion of the area occupied by immunopositive cells to total area of cells in the visual field. Optical density of expression was measured in arbitrary units.

The data were statistically processed using Student's bilateral test. Nonlinear relationship between the studied markers and age was evaluated by Spearman's rank test. Linear relationship was evaluated by Pearson's test and determination coefficient. The sig-

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nificance of the resultant linear regression equation was evaluated by determination coefficient.

RESULTS

Optical density of $CD35^+$ cells in the thymus did not differ much in all three groups, though the long-living individuals exhibited a trend to an increase in their number (Table 1). Optical density characterized the number of signal molecules (of CD35 in this case) on the surface of one cell. Hence, functional activity of DC did not change with aging.

The area of CD35 expression in thymic tissue in groups 2 and 3 was 2-fold lower than in group 1 (Table 1; Fig. 1, *a*, *b*). Correlation analysis was carried out for characterization of the age-specific time course of DC count reduction. Spearman's coefficient of correlations between the area of $CD35^+$ cells and age was -0.8 for a sample of 3 pooled groups, this indicating a strong negative correlation between these parameters.

The area of expression characterized the number of cells carrying the antigen corresponding to the immunohistochemical marker. A significant reduction of the number of $CD35^+$ cells in the thymus with aging indicated its functional involution. Reduction of DC count with aging could be significantly (R=0.77) approximated by a quadratic equation $y=0.003x^2-0.562x+25.848$, where x was age and y area of CD35 expression.

Optical density and area of CTL marker expression in the thymus showed a universal age-dependent dynamics. Optical density of $CD8^+$ cells was the same in groups 1 and 2, while in group 3 this parameter was 1.5 times lower than in group 2 (Table 1). Reduction of CD8 marker optical density in thymic tissue of individuals aged over 89 years indicated a reduction of CTL functional activity in long-living individuals. The area of $CD8^+$ cells in the thymus was also unchanged in groups 1 and 2 and was 2-fold lower in group 3 compared to group 2 (Table 1; Fig. 1, *c*, *d*). Hence,

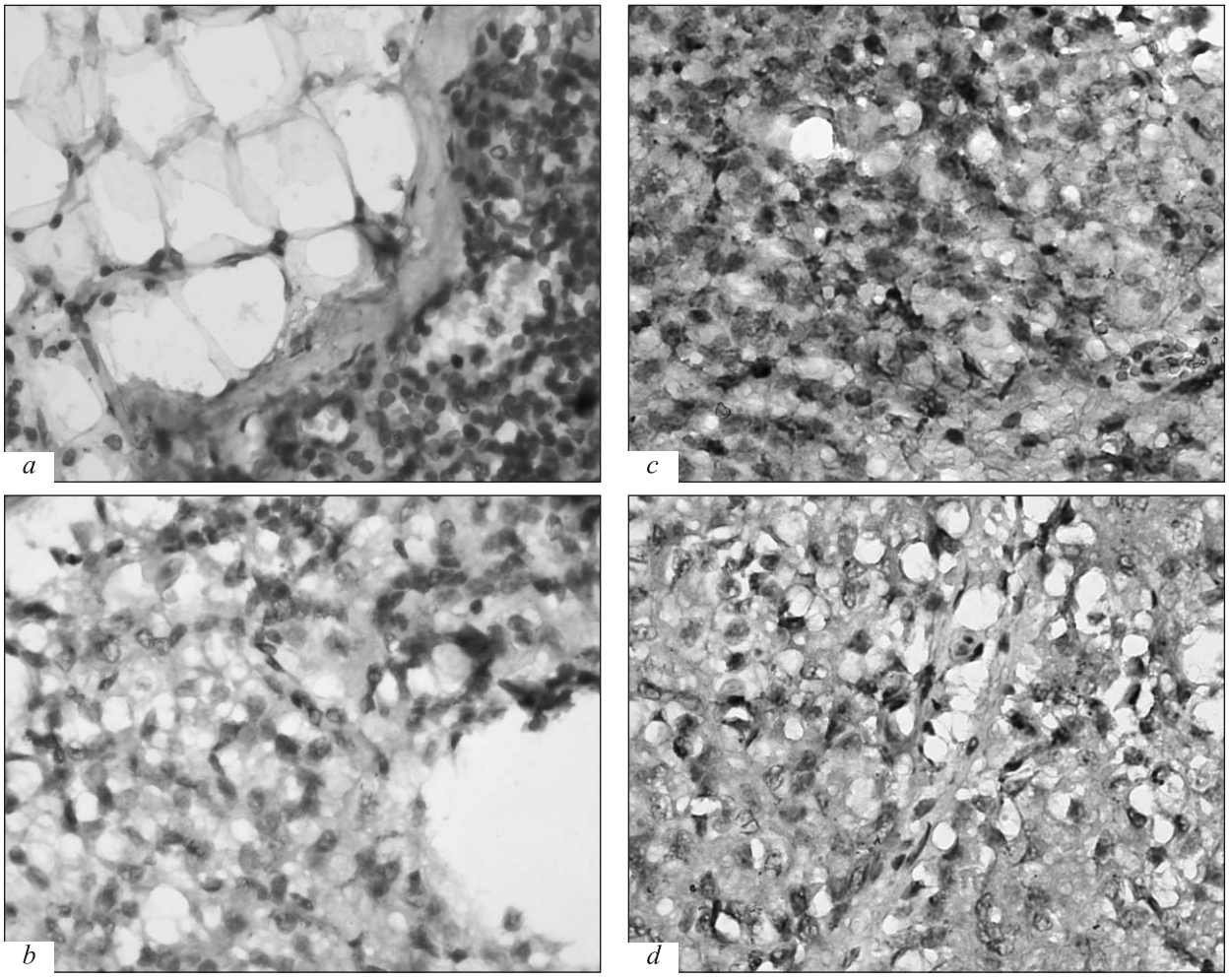

Fig. 1. Expression of DC marker (CD35) and CTL marker (CD8) in thymuses of individuals aged over 60, ×400. *a*) DC, group 1; *b*) DC, group 2; *c*) CTL, group 1; *d*) CTL, group 3.

TABLE 1. Area of Expression and Optical Density of Immune Cell Markers in the Thymus in Different Age Groups

Group	CD35		CD8	
	area of expression, %	optical density, arb. units	area of expression, %	optical density, arb. units
1	2.26±0.42	0.29±0.08	3.88±0.97	0.55±0.09
2	0.98±0.31*	0.23±0.06	3.91±0.86	0.56±0.10
3	1.02±0.23*	0.39±0.12	2.03±0.41**	0.39±0.05**

Note. $p<0.05$ compared to: *group 1, **group 2.

the counts of CTL do not change in elderly and senile age, but drop in long-living individuals, which was confirmed by the findings of analysis of correlations.

Pearson's coefficient of correlations between patient's age and area of $CD8^+$ cells in the thymus for a united sample of groups 2 and 3 was -0.99, this characterizing the relationship between the studied parameters as a linear one. Coefficient of determination for approximating equation $y=-0.2x+18$, where x was age and y area of CD8 expression, was 0.98. Determination coefficient showed that the reduction of CTL count in the thymus was by 98% determined by age and by only 2% could be determined by other causes.

The findings indicate that the counts of DC and CTL decrease 2-fold with aging, this indirectly indicating a relationship between these processes. Since reduction of DC count is observed in senile age and of CTL only in long-living individuals, it seems that reduction of DC population is one of the causes of reduction of T-cell count in the thymus. Presumably, the reduction of the counts of DC, releasing bioactive substances stimulating T-lymphocyte differentiation and presenting foreign antigens to T-cells, inhibits the maturing of CTL and hence, leads to immunity reduction, this becoming particularly obvious in long-living individuals.

The reduction of DC counts in subjects aged over 74 years can be one of the causes of CTL differentiation disorders, particularly manifest in long-living individuals, and can be one of the causes of functional insufficiency of the thymus during its aging.

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