Interrelation of Cell Apoptosis and Proliferation in the Thymus during Its Involution

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In people over 60 years, thymocytes contain proliferation protein Ki67 and proapoptotic protein P53. During aging, apoptosis of thymus cell prevails over proliferation, though thymocytes retain proliferative capacity even in long-livers.

Key Words: *thymus; aging; proliferation; apoptosis*

Age-related involution of the thymus leads to impairment of immune reactivity and is one of principal causes of age-related pathologies [1,4,5]. The ratio of apoptosis and proliferation processes in the thymus, like in any other organ, is an important marker of thymus aging [3]. Evaluation of apoptosis and proliferation of thymocytes can be useful to determine functional activity of the thymus during aging.

Here we studied the ratio between the expression of proapoptotic protein P53 and proliferative protein Ki67 in the thymus in elderly and senile individuals and in long-livers.

MATERIALS AND METHODS

Thymus samples obtained during autopsy from individual at the age of 60 years and more were divided into 3 groups according to patient's age. Group 1 (n=6) included elderly people (60-74), group 2 (n=6) comprised senile patients (75-90 years), and group 3 (n=6) consisted of long-livers (>90 years). Immunohistochemical reaction was carried out using antibodies to proapoptotic protein P53 (1:30 Novocastra) and proliferation protein Ki67 (1:30 Novocastra) according to standard one-step protocol with high-temperature antigen demasking in citrate buffer (pH 6.0). Universal kit with biotinylated anti-mouse immunoglobulin was used as secondary antibodies. The reaction was visualized using avidin complex with biotinylated horseradish peroxidase and diaminobenzidine (ABC-kit Dako).

Morphometry was carried out using computer system for microimage analysis consisting of Nikon Eclipse E400 microscope, Nikon DXM1200 digital camera, computer, and VideoTest Morphology 5.0 software. In each case, 10 fields of view were analyzed at ×400. The area of expression (AE) of the markers was determined as the percent of immunopositive cell area to the total area of cells in the field of view. Optical density (OD) of the expression was measured in conventional units. Apoptosis index (AI) was calculated as the ratio of P53 AE to Ki67 AE (both parameters were measured on the same thymus sample).

The data were processed using two-way Student's test. Nonlinear relationship between the studied markers and age was assessed using Spearman's rank correlation test. Linear relationship between AI and patient's age was calculated using Pearson's test. Determination coefficient was used to assess significance of obtained linear regression model.

RESULTS

OD of immunostained thymocytes containing proapoptotic protein P53 was about 0.5 arb. units and did not change with age.

AE P53 in thymus cells was significantly higher in elderly people than in senile individuals and in

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long-livers (Fig. 1, *a*). Increased P53 AE in elderly in comparison with senile people, was apparently associated with increased apoptosis and reflected age-related involution of the thymus. Lack of differences between senile individuals and long-livers can be explained by genetically determined preservation of the thymus structure and function in people over 90 years. The main cause of death in elderly and senile age is known to be impairment of thymus and immune system functions, which results in the development of chronic diseases at the age of 60-74 years, and to their progression and lethal outcome after 74 years [2,4]. It can be hypothesized that low level of thymocyte apoptosis and preservation of thymus function correlate with lifespan.

OD of Ki67-immunopositive cells was about 0.5-0.6 arb. units and was similar in all groups. Ki67 AE in thymocytes significantly decreased with age (Fig. 1, *b*). In elderly, this value was 3-fold lower than in senile people. In long-livers, Ki 67 AE was 8-fold lower than in 60-74- and 74-90 year-old people by 2.7 times, respectively. These findings demonstrate age-related decrease in the number of thymocytes synthesizing Ki67 protein, which attests to a decrease in thymocyte proliferation capacity and almost complete loss of this function in individuals over 90. Spearman's correlation coefficient for the relationship between Ki67 AE and age was -0.56. Minus sign indicates a decrease in KI67 AE with age, whereas numerical value reflects close relationship between the mentioned parameters. The relationship between Ki67 AE in thymus cells and age can be described by a parabolic equation $y=0.1x^2+8x+216$, where y is Ki67 AE and x is age (Fig. 2, *a*).

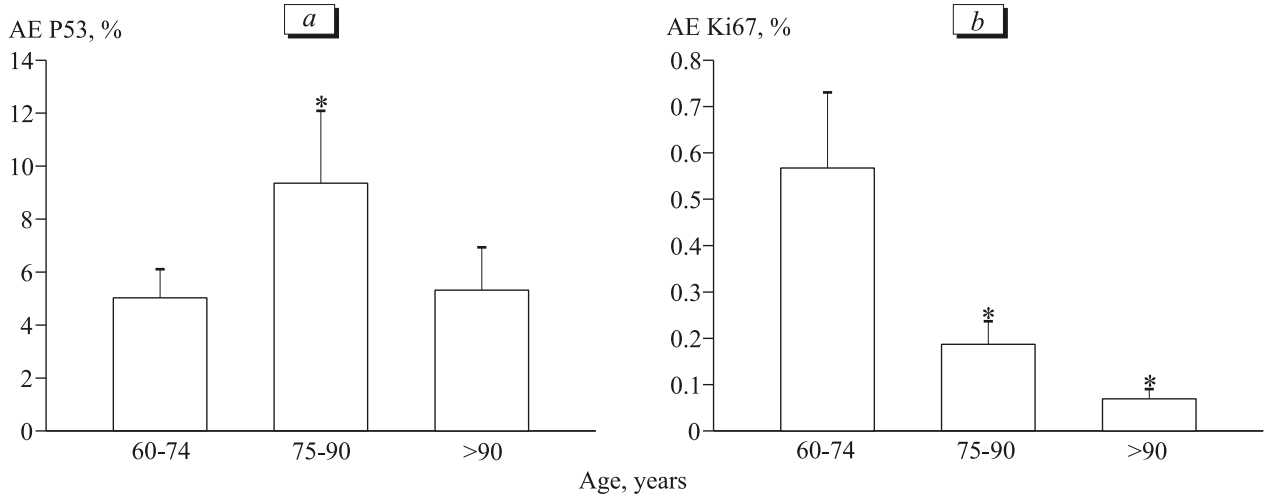

Fig. 1. Expression of apoptosis (P53) and proliferation (Ki67) markers in people of different age groups. *a*) AE for apoptosis protein P53; *b*) AE for proliferation protein Ki67. *$p<0.05$ in comparison with group of elderly people (60-74 years).

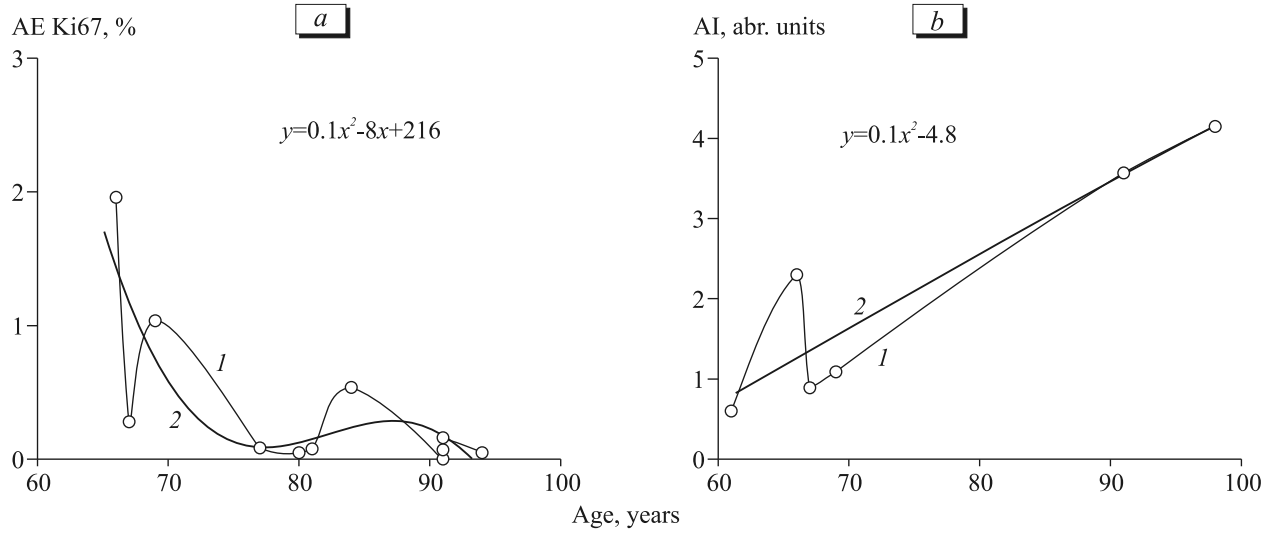

Fig. 2. Relationship between AE Ki67 (*a*), AI (*b*) in the thymus and the age. *1*) experimental data, *2*) approximating model.

AI changed linearly with age, and therefore it was assessed using Pearson's correlation test and determination coefficient. Pearson's coefficient between AI sample and age sample was 0.93, which attested to the presence of a positive near-linear correlation between the studied parameters. Relationship between AI and age can be approximated by the equation: y=0.1x+4.8, where y is AI and x is age (Fig. 2, *b*). Determination coefficient for the approximating relationship was 0.86, which indicated adequacy of the mentioned equation for the described data. Assessment of the coefficient of determination showed that differences between AI values are explained by 86% by differences in the age and only by 14% by other factors.

At the age of 60-65 years, AI was close to 1 (Fig. 2, *b*), *i.e.* corresponded to equilibrium between the number of dying and proliferating cells. Starting from the age of 65 years, programmed cell death prevails over cell division, and after the age of 100 years apoptosis was 4-times more active than proliferation.

The intensity of P53 synthesis did not change with age, whereas the number of P53 synthetizing cells in senile individuals was more than in elderly. In long-livers, P53 synthesis was maintained at the level of elderly; hence, genetically determined low ability of thymus cell to apoptosis can be regarded as a factor contributing to lifespan prolongation.

The number of Ki67-synthetizing cells dramatically decreased with age; proliferation capacity of thymus cell in long-livers was 8-fold lower than in elderly. Moreover, the intensity of Ki67 synthesis in the thymus did not depend on age. Our findings showed that despite age-related thymus involution, proliferation potential of thymocytes was preserved not only in elderly people, but even in senile individuals and partially in long-livers.

The ratio of apoptosis and proliferation processes in thymus cells changed with age. At the age of 60-65 years, apoptosis and proliferation were equilibrated, while in individuals over 65 years, cell death prevailed over proliferation.

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