BIOGERONTOLOGY

Peptidergic Regulation of Thymocyte Differentiation, Proliferation, and Apoptosis during Aging of the Thymus

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The effects of T-31, AB-17, and AB-9 peptides on old (passage 8) thymocyte culture were studied. Only AB-9 peptide exhibited a complex geroprotective effect on thymocytes during their aging. Peptide AB-9 stimulated proliferative activity and differentiation of thymocytes and inhibited their apoptosis.

Key Words: *geroprotective peptides; proliferation; apoptosis; differentiation; thymocytes*

The thymus is the central object of studies in gerontology, because its involution starts earlier in comparison with other organs and is the most pronounced [1,2]. The main factor reflecting the involution processes in the thymus during its aging is reduction of the counts of thymocytes and mature T-lymphocytes [2,4,5]. The count of thymocytes during sexual maturation is 8-fold lower than at the age of 3 years. By the age of 60 years, just solitary islets of lymphoid cells are left in the thymus. Involution of the thymus is also characterized by reduction of blood levels of thymic hormones, which are essential for the development and activities of T and B cells and for various types of immune reactions [6-8]. Hence, the search for peptide preparations stimulating the immune function of the thymus during its aging is an important problem of gerontology [3,4].

We studied the effects of synthetic peptides normophthal (AB-17; (H-Lys(H-Glu-OH)-OH), AB-9 (Nα-(γ-L-Glu)-L-Lys), and cartalax (T-31; (H-Ala-Glu-Asp-OH) on the T-cell component and thymocytes in an old culture.

MATERIALS AND METHODS

Synthetic peptides normophthal (AB-17; (H-Lys(H-Glu-OH)-OH), AB-9 (Nα-(γ-L-Glu)-L-Lys), and cartalax (T-31; (H-Ala-Glu-Asp-OH) were used in the study. The cells were incubated with the peptides in a concentration of 200 ng/ml for 1 h at 37°C.

Peptide effects on the expression of membrane markers on thymocyte surface were evaluated in six experiments. Fetal thymocytes (14-20 week gestation) were used in three experiments. In other experiments, thymocytes of children aged under 1.5 years were used (the cells were isolated from operation material). The cells were cultured during 8 passages in 24-well plates (Costar) at 37°C and 5% CO_2 in RPMI-1640 (Flow) with 10% fetal calf serum (Sigma), 300 μg/ml L-glutamine (Flow), 0.02 HEPES buffer (Serva), and 100 μg/ml gentamicin (Farmakhim). The initial cell concentration was 10^6/ml.

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The expression of molecules on cell surface was evaluated by two-color flow cytofluorometry on a FACSCalibur (Becton Dickinson). Monoclonal antibodies CD3, CD4, CD8, labeled with FITC and phycoerythrin (Sorben-Service, Becton Dickinson) were used. In order to evaluate antigen expression, the cells were incubated with antibodies (30 min, 37°C). Cells not treated with the antibodies served as control 1, cultures treated with antibodies without peptide treatment served as control 2. A total of 10,000 cells per sample were analyzed in argon laser rays (15 mW, 488 nm) at cell flow velocity of 6000 cells/sec using CellQuest 3.1 software.

Mitosis inductor phytohemagglutinin (PHA) was added to control cultures of thymocytes before cytofluorometry, into experimental cultures before addition of peptides, after which cytofluorometric analysis was also carried out.

Apoptosis of immature T-lymphocytes was evaluated by accumulation of hypodiploid thymocytes. The cells were fixed in 70% ethanol for 1 h and after washing stained with 0.15% propidium iodide for 15 min. The cells were subjected to flow cytofluorometry. Their count was estimated in the peak located left from the peak corresponding to diploid cells. Counts of hypodiploid cells were evaluated over 24 h after co-culturing.

RESULTS

Peptide effects on differentiation of old (after 8 passages) cultured thymocytes were evaluated in experimental series I. The percentage of $CD3^+$ cells among $CD4^+$ and $CD8^+$ cells changed under the effects of peptide preparations. In the control, the overwhelming majority of $CD4^+$ (more than 90%) and $CD8^+$ (70-80%) co-expressed CD3. The percentage of $CD3^+$ cells among $CD4^+$ thymocytes decreased to 65-70% and among $CD8^+$ thymocytes increased to 90-95% under the effect of AB-9 peptide (Fig. 1). Cartalax exhibited similar effect on $CD8^+$ cells.

Hence, AB-9 and cartalax demonstrated a geroprotective effect towards thymocytes in old cultures, stimulating their differentiation into mature $CD4^+$ and

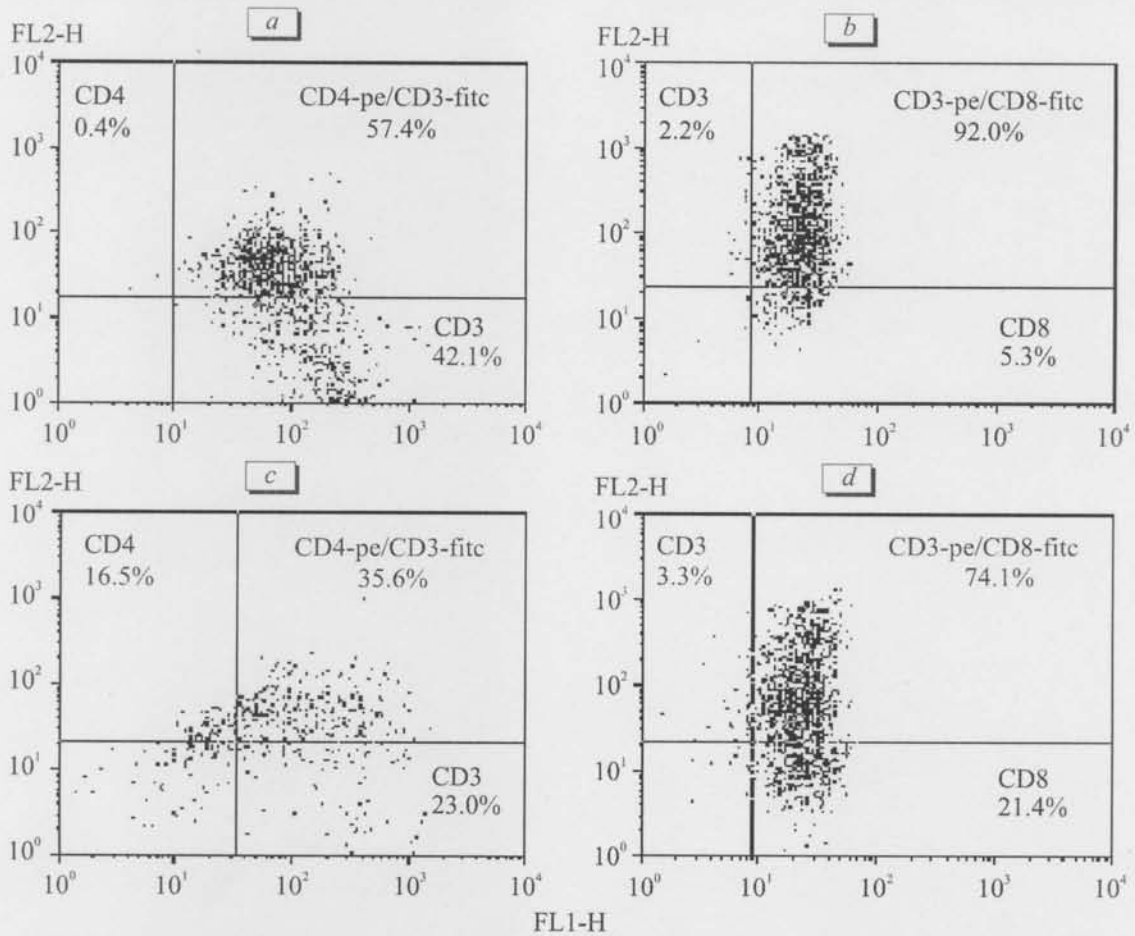

Fig. 1. Redistribution of $CD4^+$ and $CD8^+$ co-receptors on $CD3^+$ thymocytes in the control and under the effect of AB-9 peptide. *a*) control (CD3/CD4 markers); *b*) control (CD3/CD8 markers); *c*) AB-9 peptide (CD3/CD4 markers); *d*) AB-9 peptide (CD3/CD8 markers).

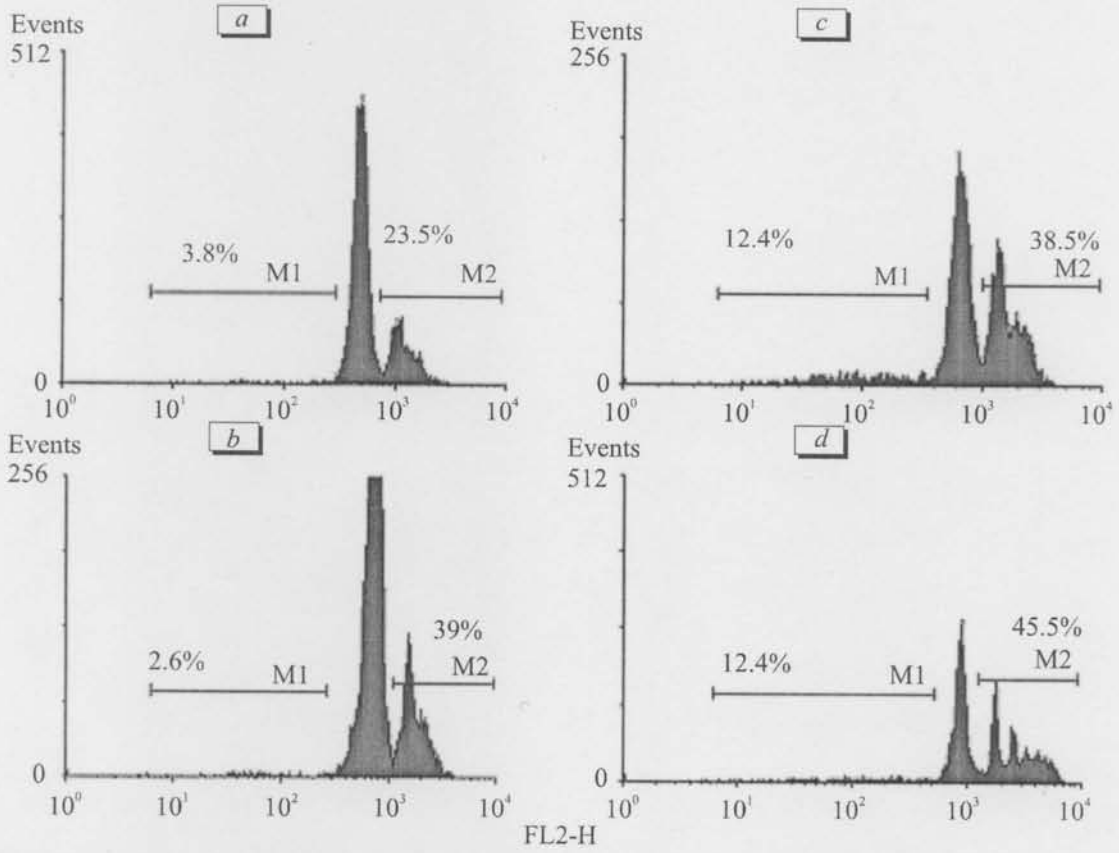

Fig. 2. Peptide effects on spontaneous (*a*, *b*) and PHA-induced (*c*, *d*) proliferation of thymocytes. *a*) spontaneous proliferation; *b*) proliferation induced by normophthal; *c*) proliferation induced by PHA; *d*) proliferation induced by PHA and AB-9 (counts of thymocytes in the cell cycle in histograms reflect the peaks located to the right from the main diploid peak). M1: non-dividing cells; M2: cells after 1 division.

$CD8^+$ lymphocytes. Although the peptide effects varied from one experiment to another, the decrease in the counts of immature $CD4^+CD8^+$ cells predominated and was paralleled by an increase in the counts of mature $CD4^+$ and $CD8^+$ cells.

Peptide effects on thymocyte proliferation and apoptosis were evaluated in experimental series II. Thymocytes in the control culture were characterized by a high proliferative activity (up to 20-25%). Incubation with AB-9 peptide caused no appreciable changes in this parameter. Normophthal increased the percentage of proliferating thymocytes from 24 to 39% (Fig. 2).

Phytohemagglutinin treatment increased the percentage of thymocytes capable to proliferation to 39%. The relative count of thymocytes in the growth phase increased to 45-49% in the presence of AB-9 and normophthal. This increase was most pronounced in the presence of AB-9 peptide (52%). These data indicated that the peptides, particularly AB-9, promoted transition of thymocytes to the stage of functionally mature cells capable of developing a proliferative response to stimulation.

Cartalax and AB-9 peptides virtually did not change the level of apoptosis in thymocyte cultures. Apoptosis increased in the presence of PHA, while after addition of AB-9 to the culture apoptotic death of thymocytes seemed to stop. Hence, the tested peptides demonstrated different effects on thymocyte proliferation/apoptosis balance.

The effects of cartalax (T-31) and normophthal (AB-17) on old thymocyte culture were not uniform, without complex effect on all the studied parameters.

Of the studied peptide preparations, AB-9 demonstrated the most pronounced geroprotective effect on old thymocyte culture. This peptide inhibited thymocyte apoptosis and increased their proliferative activity and differentiation into mature $CD4^+$ and $CD8^+$ lymphocytes. Since the decrease in the counts of mature T cells and T-lymphocyte precursors are the main signs of thymus aging reflecting functional involution of the gland, the effects of AB-9 peptide suggest that it can be considered as a drug restoring the immune function of the thymus.

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