BIOGERONTOLOGY

In Vitro Effect of Short Peptides on Expression of Interleukin-2 Gene in Splenocytes

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Synthetic peptides Vilon (Lys-Glu), Epithalon (Ala-Glu-Asp-Gly), and Cortagen (Ala-Glu-Asp-Pro) *in vitro* activated interleukin-2 mRNA synthesis in splenocytes from CBA mice in the absence of specific inductors. The intensity of interleukin-2 mRNA synthesis in splenocytes depended on the type, concentration, and duration of treatment with the peptides. Vilon and Epithalon were most potent, while Cortagen produced a less pronounced effect on interleukin-2 mRNA synthesis.

Key Words: *peptides; Vilon; Epithalon; Cortagen; interleukin-2 gene; splenocytes*

Aging is associated with suppression of defense systems and increased susceptibility to various diseases [6]. T cell response to mitogenic stimuli and intensity of interleukin-2 (IL-2) synthesis decrease in aged people [10]. Expression of immediate early genes in T cells, including *c-myc*, *c-jun*, *c-fos*, and IL-2 genes, decreases during aging [13]. Normal growth and functions of T cells require cooperative activity of various genes, including IL-2 gene [13]. Dysregulation of gene expression is responsible for decreased functional activity of lymphocytes during aging.

Therapy with peptide bioregulators holds much promise for the correction of disturbances in gene expression [4]. Previous studies showed that peptide preparations from the thymus (Thymalin) and pineal gland (Epithalamin) correct functional disturbances in the immune system [5,11]. Analysis of expression of immediate early genes in lymphoid cells in the presence of various substances allows us to study the mechanisms of their effects, reveal changes induced by biologically active compounds (*e.g.*, peptides) in the early stage of genome activation, and evaluate the possibility of using these agents for the correction of immune dysfunction [2,4,5].

Here we compared *in vitro* effects of Vilon, Epithalon, and Cortagen on IL-2 gene expression in splenocytes.

MATERIALS AND METHODS

Peptides were synthesized at the St. Petersburg Institute of Bioregulation and Gerontology on the basis of amino acid composition of complex preparations Thymalin (Vilon, Lys-Glu), Epithalamin (Epithalon, Ala-Glu-Asp-Gly), and Cortexin (Cortagen, Ala-Glu-Asp-Pro) from the thymus, pineal gland, and brain cortex, respectively.

IL-2 mRNA was analyzed in splenocytes from CBA mice weighing 14-16 g. Each series was performed on 8 mice. Unstimulated splenocytes and splenocytes stimulated with concanavalin A (Con A) ser-

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ved as the control. The cells were isolated from mouse spleen in a Ficoll gradient [5] and incubated in the presence of Con A or peptides in concentrations of 0.05, 5, 50, and 100 ng/10^6 cells at 37°C for 5 or 20 h. Total mRNA was isolated, and its content was measured using dot-blot hybridization [3].

Production of plasmid DNA (pAA 1213 plasmid containing full-length cDNA for human IL-2 gene; kindly provided by Prof. E. Gren, Institute of Organic Chemistry, Riga), isolation, restriction, electrophoresis, and non-radioactive labeling of IL-2 cDNA with digoxigenin were performed routinely [3,12].

The content of IL-2 mRNA was evaluated by densitometry (LKB Ultrascan XL Law densitometer). The peaks were approximated by trapeziums and their areas were calculated.

The results were analyzed by Student's *t* test.

RESULTS

All peptides *in vitro* stimulated IL-2 gene expression in mouse splenocytes in the absence of mitogen Con A and, therefore, possessed mitogenic activity (Fig. 1). Studies of the dose-response relationships and the dynamics of cell response to incubation with peptides showed that they produced different effects. All test peptides in concentrations of 0.05 ng/10^6 cells were active. The concentrations producing the maximum stimulatory effects after 5-h incubation differed by more than 20 times (5 ng/10^6 cells for Epithalon and Vilon and 100 ng/10^6 cells for Cortagen). Activation of IL-2 mRNA synthesis in cultured cells in the presence of Cortagen was not more than 60% of the effects of Vilon and Epithalon (Fig. 1).

In contrast to Con A, synthetic peptides stimulated IL-2 mRNA synthesis even after 20-h incubation, Vilon being most active. These results are consistent with published data that biologically active peptides produce long-lasting effects [1]. Studies of IL-2 mRNA synthesis in cells after 5- and 20-h incubation with the peptides revealed differences in the duration of their stimulatory effects. The amount of IL-2 mRNA synthesized in splenocytes after 5-h incubation with Epithalon, Vilon, and Cortagen (50 ng/10^6

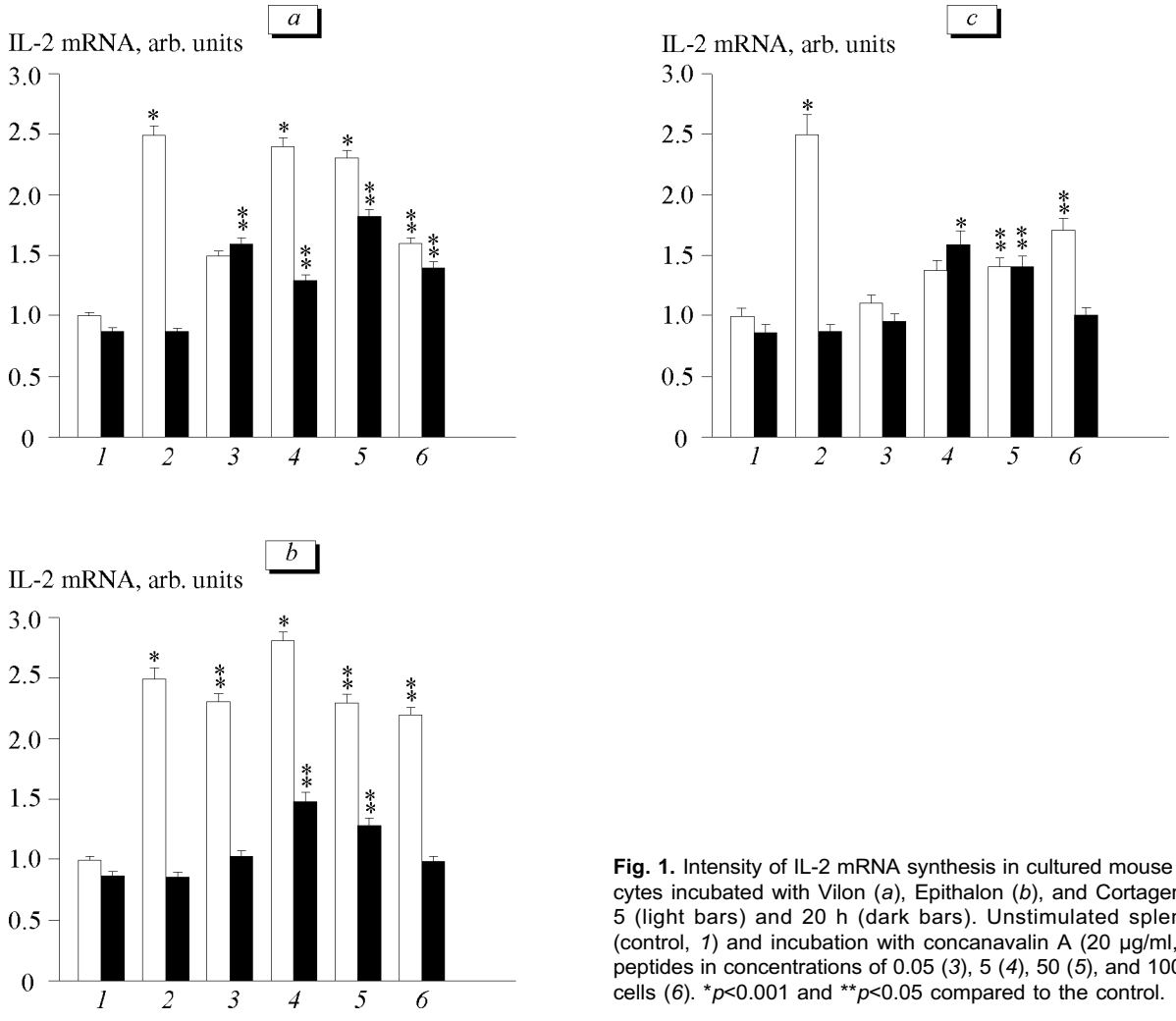

Fig. 1. Intensity of IL-2 mRNA synthesis in cultured mouse splenocytes incubated with Vilon (*a*), Epithalon (*b*), and Cortagen (*c*) for 5 (light bars) and 20 h (dark bars). Unstimulated splenocytes (control, *1*) and incubation with concanavalin A (20 μg/ml, *2*) and peptides in concentrations of 0.05 (*3*), 5 (*4*), 50 (*5*), and 100 ng/10^6 cells (*6*). *p<0.001 and **p<0.05 compared to the control.

cells) was 2.7 ($p<0.002$), 2.9 ($p<0.005$), and 1.4 arb. units ($p<0.003$), respectively.

The intensity of IL-2 mRNA synthesis sharply decreased after 20-h incubation with Epithalon in concentrations of 50 and 100 ng/10^6 cells, which attested to its shorter effect compared to Vilon and Cortagen.

Our results indicate that Vilon and Epithalon are most potent in stimulating IL-2 synthesis. These peptides intensify IL-2 gene expression in splenocytes. The thymus and pineal gland are the major organs of the immune and endocrine systems. Their functional activity decreases during aging [7]. Vilon and Epithalon hold much promise for the correction of immune deficiency associated with impaired expression of IL-2 gene, in particular, during aging.

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REFERENCES

1. I. P. Ashmarin and E. P. Karazeeva, *Biochemistry of the Brain* [in Russian], Eds. I. P. Ashmarin *et al*., St. Petersburg (1999), pp. 232-266.
2. T. B. Kazakova, S. V. Burov, O. I. Golovko, *et al*., *Byull. Eksp. Biol. Med*., **122**, No. 9, 334-337 (1996).
3. T. Maniatis, E. Frich, and J. Sembruk, *Methods of Genetic Engineering. Molecular Cloning* [in Russian], Moscow (1984).
4. V. G. Morozov and V. Kh. Khavinson, *Peptide Bioregulators (Experience of 25-Year Experimental and Clinical Studies)* [in Russian], St. Petersburg (1996).
5. V. Kh. Khavinson, V. G. Morozov, V. V. Malinin, *et al*., *Byull. Eksp. Biol. Med*., **130**, No. 9, 330-332 (2000).
6. A. Ben-Yehuda, and M. E. Weksler, *Cancer Invest*., **10**, 525-530 (1997).
7. R. G. Goya, O. A. Brown, and F. Bolognani, *Neuroimmunomodulation*, **6**, 137-142 (1997).
8. J. M. Guerrero and R. J. Reiter, *Endocrinol. Res*., **18**, 91-113 (1992).
9. J. M. Guerrero. D. Pozo, S. Garcia-Maurino, *et al*., *Ann. N. Y. Acad. Sci*., **917**, 397-403 (2000).
10. D. R. Jackola, J. K. Ruger, and R. A. Miller, *Aging Clin. Exp. Res*., **4**, 25-29 (1994).
11. V. G. Morozov and V. Kh. Khavinson, *Int. J. Immunopharmacol*., **19**, No. 9/10, 501-505 (1997).
12. *Nonradioactive in Situ Hybridization. Application Manual*, Boehringer Mannheim (1992).
13. R. L. Whisler, M. Chen, L. Beiging, and K. W. Carle, *Cell. Immunol*., **175**, 41-50 (1997).