BIOGERONTOLOGY

Effect of Peptide Lys-Glu on Interleukin-2 Gene Expression in Lymphocytes

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Lys-Glu *in vitro* stimulated interleukin-2 gene expression in mouse spleen lymphocytes. This effect depended on peptide concentration and duration of treatment. It is hypothesized that this peptide is the shortest regulatory fragment promoting the transport of trans-acting factors into the nucleus. It can not be excluded that Lys-Glu is a structural component of trans-acting factor active centers, which are necessary for the activation of interleukin-2 gene transcription in lymphocytes.

Key Words: *peptides; gene expression; interleukin-2; lymphocytes*

The effects of low-molecular-weight immunomodulators on the expression of cytokine genes are poorly understood.

Functional activity of immunocompetent cells decreases under the effect of extreme factors and during aging [4]. It was shown that interleukin-2 (IL-2) gene expression in lymphocytes, which yields the protein playing a key role in immune functions and T lymphocyte response to mitogens, decreases during aging [11]. Studies of IL-2 gene expression allow us to reveal metabolic disturbances at the early stages of cell activation and help to understand the mechanisms of immunomodulating effects of peptide bioregulators. Various peptides from the thymus and pineal gland, myelopeptides, and opiate peptides affect the immune response, phagocytic and antigen-presenting functions of macrophages, and production of IL-1, IL-2, IL-6, and tumor necrosis factor-α [1,6-8]. N-Acetylmuramyl-L-Ala-D-isoGln (muramyl dipeptide) and its derivatives stimulate gene expression and synthesis of IL-1α, IL-1β, IL-6, IL-8, and tumor necrosis factor in blood mononuclear cells [9,13]. Trans-acting factors of IL-2 gene (NF-AT, NF-κB, AP-1, AP-2, AP-3, and Oct-1) consisting of high-molecular-weight components were extensively studied [10]. It was shown that analogues of hypothalamic peptide hormone luliberin, Ns-Pro-Ser-Tyr-D-Asp-Leu-Arg-Pro-NHEt and H-Pro-D-Phe-Pro-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2, stimulate IL-2 mRNA synthesis and differentiation of mouse spleen T cells [3]. Low-molecular-weight peptides with the same N-terminal sequence Pro-Glu-Pro-Ala-Lys-Ser-Ala-Pro-Ala-Pro isolated from the nuclei of spleen and brain cells of immunized animals have similar properties [2].

Here we studied the effects of peptide Lys-Glu on IL-2 gene expression in mouse spleen lymphocytes *in vitro*.

MATERIALS AND METHODS

pAA1213 plasmid containing a full-length cDNA copy of human IL-2 gene (550 nucleotide sequences) was gifted by Prof. E. Gren (Institute of Bioorganic Chemistry, Riga). DNA isolation and restriction, isolation of IL-2 cDNA fragment, and nonradioactive labeling

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with digoxigenin (Boehringer Mannheim) were performed as described elsewhere [5,13]. IL-2 mRNA was assayed in spleen lymphocytes from 10-12-week-old male CBA mice weighing 14-16 g. Lymphocytes were isolated and incubated at 37°C for 5 or 20 h. Total RNA was isolated, and the content of IL-2 mRNA was assayed by hybridization [5]. After hybridization, filters with labeled IL-2 cDNA were washed and stained for immunoprecipitation [13]. The intensity of staining reflecting the amount of IL-2 mRNA was measured on an Ultrascan XL Law Densitometer (LKB).

Peptide Lys-Glu was synthesized on the basis of amino acid analysis of the complex thymus preparation thymaline and immunoreactive properties of amino acids. The peptide was synthesized in St. Petersburg Institute of Biological Regulation and Gerontology.

The effects of 50 pg/ml, 5, 50, and 100 ng/ml Lys-Glu were studied. Unstimulated lymphocytes and cells incubated with concanavalin A (Con A, 5 mg/ml) and rIL-2 (30 U/ml) served as the control.

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

RESULTS

After 5-h incubation, peptide Lys-Glu (especially, in a concentration of 5 ng/ml) stimulated IL-2 mRNA synthesis in lymphoid cells. Prolonged (20-h) incubation produced less pronounced changes in IL-2 gene expression, but this parameter remained above the control level (Fig. 1).

Thus, peptide Lys-Glu stimulates *in vitro* synthesis of IL-2 mRNA in mouse spleen lymphocytes. This effect depends on peptide concentration and duration of incubation. In addition, Lys-Glu activates IL-2 gene expression in cultured lymphocytes without additional stimulation with Con A and rIL-2. It should be emphasized that IL-2 gene expression in cells increased after 20-h incubation with the peptide, when addition of mitogens into the culture medium had no effect (Fig. 1). Probably, the presence of Lys-Glu in the incubation medium prolonged the effects of nuclear transacting factors regulating IL-2 gene expression.

It is known that the regulation of transcription in eukaryotes is a complex process including many stages of positive and negative regulation of gene expression. These processes include translocation of a signal from cell surface to the cytoplasm and its transduction to nuclear genetic structures. The stimulatory effect of peptide Lys-Glu on IL-2 mRNA synthesis in mouse splenocytes confirms not only the possibility of transporting dipeptides across the cell membrane, but also indicates their involvement in the regulation of gene expression. The data suggest that peptide Lys-Glu is the shortest regulatory fragment formed in cells during organic

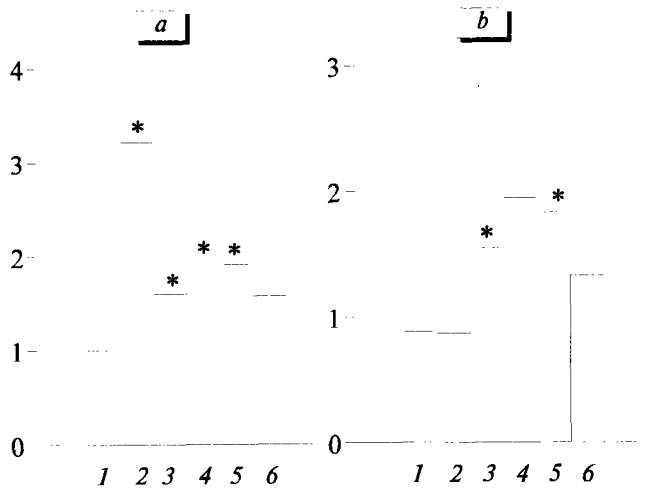

Fig. 1. Effects of peptide Lys-Glu on IL-2 mRNA synthesis (optical density units) in mouse spleen lymphocytes after incubation for 5 (*a*) and 20 h (*b*): control (*1*), concanavalin A+rIL-2 (*2*), and Lys-Glu in concentrations of 50 pg/ml (*3*), 5 (*4*), 50 (*5*), and 100 ng/ml (*6*) per 10^6 cells. *$p<0.05$ compared to the control.

proteolysis and performing specific regulatory functions. Lys-Glu is involved in the transport of transacting factors into the nucleus or enters the composition of their active centers necessary for the activation of IL-2 gene transcription in lymphocytes.

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