Biological Activity of Analogs of the Peptide Hormone Luliberin in the Regulation of the Immune Response of T Cells

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The ability of the antitumor analogs of luliberin (LH-RH), a hypothalamic peptide hormone, to stimulate the immune function of T cells is examined in experiments with the gene coding for interleukin-2 (IL-2). Recombinant MIL2C or 4xPu DNA containing the marker gene of chloramphenicol acetyltransferase (CAT) under the control of a 2.2 kb promoter of murine IL-2 gene or four copies of purine-rich element (from -292 to -246 base pairs), respectively, is injected in *Xenopus laevis* oocytes. The promoter activity is blocked by inoculation of the protein fraction of nuclear extracts from resting mouse splenic T cells. The IL-2 gene promoter is dereppressed after injection of the short LH-RH analog L1 (7 amino acid residues) into the oocyte nucleus or cytoplasm. The addition of L1 or L2 (an LH-RH analog consisting of 10 amino acid residues) to the incubation medium activates mouse splenic T cells and stimulates the synthesis of IL-2 mRNA 2- to 3-fold more intensely than ConA+rIL-2, judging from dot-blot and *in situ* hybridization data. Cytological analysis of cell culture shows that the presence of L1 and L2 peptides in the culture medium promotes differentiation of T cells. It is hypothesized that the antitumor activity of these peptides is associated with the stimulation of IL-2 synthesis.

Key Words: *luliberin; interleukin-2 gene; mRNA synthesis*

It was hypothesized that protein molecules have biologically active sites responsible for binding to cell receptors, protein-protein recognition, and formation of complexes activating gene expression [4]. The gene coding for interleukin-2 (IL-2) is a convenient tool for the investigation of the role of peptides in the regulation of the expression of eukaryotic genes. Protein product of IL-2 gene plays the key role in the immune response of T cells.

The aim of this study was to evaluate the ability of two analogs of the peptide hormone luliberin (LH-RH) to regulate the expression of IL-2 gene. These analogs are peptides consisting of seven (L1) and ten (L2) amino acid residues. The choice of luliberin is based on the fact that it is synthesized not only in hypothalamus, but also in T cells [3]. Tests for antitumor activity showed that L1 and L2 peptides inhibit tumor growth by 40 and 80%, respectively.

Previously, we showed that L1 specifically interacts *in vitro* with the promoter of bovine IL-2 gene and stimulates the production of IL-2-like growth factor in a culture of surviving mouse splenic T cells (lymphoblastic transformation data) [2]. The ability of L2 to interact with the promoter of human IL-2 gene was demonstrated by binding to the promoter of digoxin-labeled IL-2 DNA on a nitrocellulose membrane [1].

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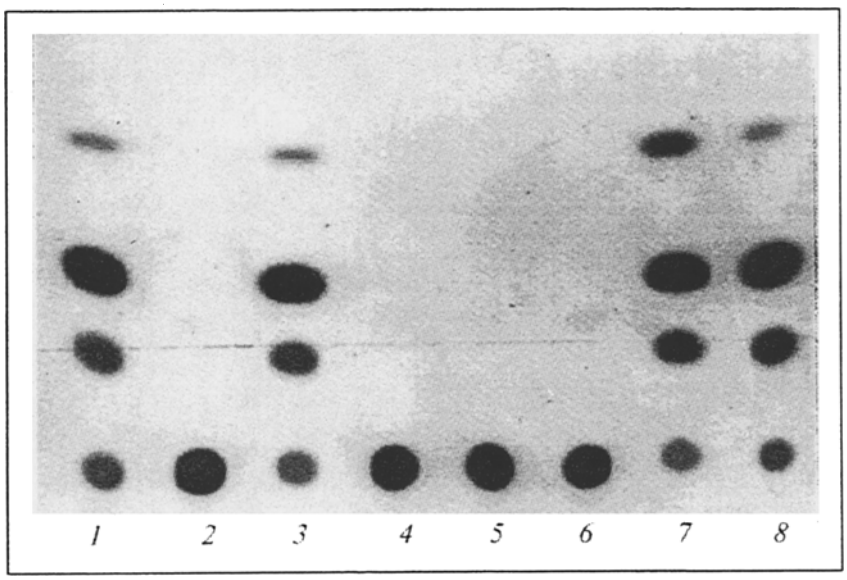

Fig. 1. Effect of L1 peptide (analysis of CAT synthesis) on the activity of the promoter of the IL-2 gene in *X. laevis* oocytes. *1*) baseline promoter activity; injection of: *2*) nuclear proteins from intact mouse splenic T cells (5 ng/oocyte); *3*) nuclear proteins from mouse splenic T cells stimulated for 8 h; *4-6*) nuclear proteins from rat splenocytes with molecular weight of 110, 60, or 30 kD, which do not stimulate the promoter of the IL-2 gene [7]; *7*, *8*) L1 peptide (20 ng/oocyte).

We hypothesized that the antitumor activity of L1 and L2 peptides is associated not only with their effect on the level of sex steroid hormones [9], but also with stimulation of the immune response of T cells and, consequently, with activation of the expression of the IL-2 gene.

MATERIALS AND METHODS

The analogs of the hypothalamic peptide hormone luliberin (LH-RH) were synthesized at the Institute of High Molecular Compounds (Russian Academy of Sciences): L1 by classic peptide synthesis in solution and L2 by solid-phase synthesis in a Neosystems Lab. NAS 4000 semiautomatic peptide synthesizer on a methylbenzhydrylamine polymer matrix. Peptide L1 consists of 7 amino acid residues: NS-Pro-Ser-Tyr-D-Asp-Leu-Arg-Pro-NHEt (Ns-beta-naphthanylsulfonyl). Peptide L2 consists of 10 residues: H-Pro-D-Phe-Pro-Ser-Tyr-D-Lys-Leu-Arg-Pro-Gly-NH_2 (the peptide is patented).

Recombinant DNA MIL2C or 4xPu (2 ng) containing the marker CAT gene controlled by the 2.2 base pairs (b.p.) promoter of mouse IL-2 gene or 4 copies of purine-rich element (from -292 to -246 b.p.) was injected into the oocyte nucleus as described elsewhere [8]. Basal DNA activity was blocked by injection of KCl (300 mM) precipitate of nuclear proteins from resting mouse splenic T cells (20 ng/μl/oocyte) into the oocyte cytoplasm 2-

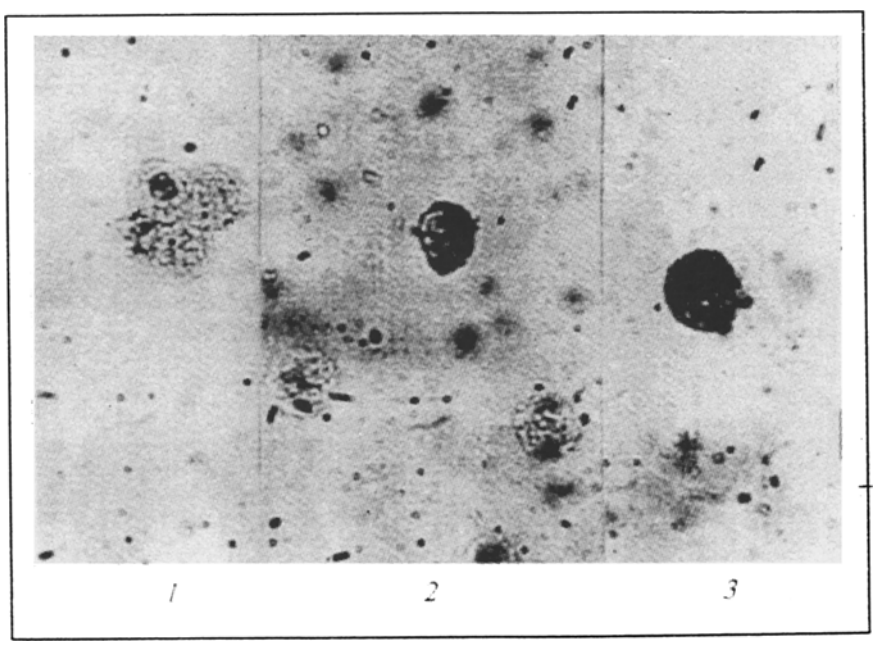

Fig. 2. Effect of L1 and L2 peptides on the differentiation of T cells. Detection of the IL-2 mRNA-IL-2 cDNA complex by *in situ* hybridization after incubation for 2 h (*1*), 4 h (*2*), and 6 h (*3*).

3 h prior to the injection of recombinant DNA. Peptide L1 (20 ng) was injected into the nucleus or cytoplasm an hour later, and the oocytes were incubated for 20-24 h at 20°C. The activity of CAT was assayed in lysates using labeled C-acetyl coenzyme A [6].

T cells were isolated from the spleen of male CBA mice (15-18 g, 10-12 weeks). Spleens of 2-3 mice were homogenized in RPMI-1640 medium, the homogenate was filtered through Becton Dickinson nylon cell strainer (pore size 70 μ), and T cells were isolated on a Ficoll-Verografin gradient (d=1.076, Pharmacia) by centrifugation (40 min, 4000*g*). Incubation with and without the peptides was performed as described previously for the nuclear trans-factor of the IL-2 gene [7].

The nucleotide sequences of IL-2 cDNA were isolated from pIL2LUC plasmid in which IL-2 cDNA was inserted by *BamH* I and *SalG* I sites. Preparative DNA electrophoresis was carried out in 1% low-melting agarose (SiKem). The IL-2 cDNA was labeled with the use of a Boehringer Mannheim Digoxigenin-labeling and detection kit.

The synthesis of IL-2 mRNA was assessed by dot-blot and by *in situ* RNA-DNA hybridization. For dot-blot hybridization the total RNA was isolated from lymphocytes as described [5] after a 6-h incubation. RNA-DNA hybridization was performed according to the recommendation of Manual Research Boehringer. The amount of IL-2 mRNA was determined in a UltraScan XL densitometer (LKB) by measuring the intensity of staining of the precipitate with antidigoxigenin antibodies.

RESULTS

The ability of L1 peptide to stimulate the IL-2 gene promoter was evaluated in *Xenopus laevis* oocytes. MIL2C or 4xPu DNA was injected in the nucleus in which basal DNA activity was blocked by "negative" protein trans-factors isolated from resting splenic T cells. The peptide was injected an hour later, which led to derepression of the IL-2 gene promoter both in MIL2C and 4xPu DNA (Fig. 1). Consequently, the purine-rich region of the IL-2 gene from -264 to -292 b.p. is the main target of L1 peptide. After being injected into the nucleus, the peptide produced a higher derepression effect than after injection in the cytoplasm. This indicates that the peptide acts on nucleotide pairs of the gene or on nuclear proteins transmitting the signal to the gene.

Peptide analogs of LH-RH activated lymphocytes after the addition to the culture medium. Both peptides were tested for the ability to stimulate the

TABLE 1. Effects of L1 and L2 Peptides on the Expression of the IL-2 mRNA in Mouse Splenic T Cells *In Vitro* (*M±m*)

Incubation period, h	Percent of Dig-labeled RNA-DNA hybrids											
	control			peptide concentration, ng/10^6 cells								
				L1					L2			
	without additives	ConA+rIL-2	NSP 110	2	5	10	20	2	5	10	20
2	0	0	0	0.5±0.9	6±4.0	5±2.5	12±3.0	25±8.0	1.5±1.5	0.8±0.5	3±0.8
4	0	12±2.0	0	1.5±1.5	4±2.8	7±4.0	17±5.2	25±11.0	4±2.0	2±1.5	4±1.8
6	0	18±5.1	0	10±2.2	9±3.1	6±1.8	9±2.0	21±7.8	5±3.1	0	0.6±0.5
20	0	0	0	0	0	0	0	3±1.8	6±1.5	2±2.0	0

Note. NSP 110: protein (Mr 100 kD) isolated from nuclear extract of splenocytes obtained from rats immunized with sheep erythrocytes. This fraction does not stimulate the synthesis of IL-2 mRNA. 500-1000 cells were used for each peptide concentration. All values are statistically significant.

synthesis of IL-2 mRNA in mouse splenic T cells *in vitro*. The expression of mRNA was analyzed by dot-blot and *in situ* hybridization. In both cases the peptides stimulated the synthesis in the concentration range 2-20 ng/10^6 cells. Stimulation of lymphocytes with ConA+rIL-2 or nuclear proteins (Mr 14-19 and 110 kD) isolated from splenocytes of immune rats. Previously, we showed that 14-19 kD protein but not 110 kD protein specifically binds to the IL-2 gene promoter *in vitro* and stimulates the expression of the gene [7].

In situ hybridization showed that the peptides stimulate the synthesis of IL-2 mRNA after 2 h of incubation, the maximum effect being observed after a 4-h incubation. It should be noted that L2 peptide produced a 50-fold higher effect in a lower concentration and after a shorter incubation (2 ng, 2 h) compared with L1 peptide (Table 1). These peptides also stimulated the differentiation of T cells: after 2 h of incubation, the synthesis of the IL-2 mRNA was detected in the nucleus, after 4 h both in the nucleus and cytoplasm with predominance of small T cells, and after a 6-h incubation medium T cells appeared (Fig. 2).

Thus, our results indicate that biologically active synthetic analogs of the peptide hormone luliberin (LH-RH) stimulate the expression of IL-2 gene and differentiation of cultured T cells. Experiments with *Xenopus laevis* oocytes show that this effect is not confined only to the membrane structures of T cells providing signal transduction: both peptides stimulated the IL-2 promoter after being injected in the oocyte nucleus. From analysis of the production of IL-2 mRNA in a culture of surviving T lymphocytes it can be suggested that the more potent antitumor effect of L2 peptide is associated with its capacity to stimulate the synthesis of IL-2, thus increasing the organism's immunity.

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