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Immunostimulating Activity of Synthetic Bursopeptides

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Biologically active peptides bursopeptide-1 (Tyr-Glu-Glu) and bursopeptide-2 (Trp-Thr-Ala-Glu-Glu-Lys-Gln-Leu) from the bursa of Fabricius were isolated and synthesized. Bursopeptide-1 stimulated expression of B cell differentiation antigens in lymphocyte culture from patients with burns. Bursopeptide-2 produced a variety of effects and stimulated expression of differentiation antigens on T and B cells and natural killer cells. Bursopeptides can modulate the mechanisms of interleukin-2-mediated lymphocyte activation. Bursopeptides stimulated the immune response in mice with cyclophosphamide-induced immunodeficiency, which manifested in increased hemagglutinin and hemolysin titers and increased count of antibody-producing cells in the spleen.

Key Words: *peptides; immunodeficiency; lymphocytes; differentiation antigens*

Synthesis of immunomodulators on the basis endogenous bioactive peptides selectively correcting impaired element of the immune system is an urgent problem of experimental and clinical medicine [1]. Much attention was given to immunoactive peptides from the central organs of immunogenesis thymus [5] and bone marrow [4]. Little is known about peptides from the bursa of Fabricius, the central organ of humoral immunity in birds. This organ is absent in humans. Chemical structure and mechanisms of action of individual peptides from the bursa of Fabricius still remained unknown [7].

Here we compared immunostimulating activity of synthetic peptide analogues from the bursa of Fabricius (bursopeptides).

MATERIALS AND METHODS

Peptides were isolated from the bursa of Fabricius in chicks by alkaline and acetic acid extraction followed by gel filtration and reversed-phase high-performance liquid chromatography. The primary structure of peptides was evaluated on a gas-phase sequenator (Model 477A, Applied Biosystems). Solid-phase peptide synthesis was performed using Boc strategy. The structure of synthesized peptides was estimated by mass spectrometry.

Lymphocytes from 32 patients with burns (IIIb-IV) were isolated on a Ficoll-Verografin density gradient (ρ=1.077 g/cm^3) and used in a concentration of 2.5 million cells/ml. The test peptides in a concentration of 5 pmol/ml were added to cultured lymphocytes and incubated at 37°C for 2 h. Control experiments were performed with physiological saline.

In series II lymphocytes from 16 patients with burns were incubated with interleukin-2 (IL-2) in a concentration of 2000 pg/ml for 60 min. Bursopeptides (5 pmol/ml) and physiological saline were added to experimental and control samples, respectively, and incubated for 2 h. Expression of lymphocyte differentiation antigens was studied by indirect membrane immunofluorescence with monoclonal antibodies (Sorbent) [2]. Fluorescence was recorded using an ES Lyumam-RPO11 luminescence microscope.

Immunodeficiency was modeled on 96 male outbred mice weighing 18-20 g. The animals received single intraperitoneal injection of cyclophosphamide in a maximum permissible dose (200 mg/kg) [9]. Group

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1 and 2 mice were intraperitoneally immunized with sheep erythrocytes in a single dose of 7×10^9 cells/kg on days 1 and 10 after cyclophosphamide administration, respectively. Bursopeptides in a dose of 5 nmol/kg were administered 1, 2, 3, and 4 days after immunization. Control animals received physiological saline. The number of antibody-producing cells (APC) in the spleen was estimated by local hemolysis in gel on day 5 of the immune response [2]. Hemolysin and hemagglutinin titers were measured [3] and blood leukocyte count was determined.

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

RESULTS

Consecutive separation of immunoactive fractions from the bursa of Fabricius allowed us to isolate 2 individual peptides and determine their primary structure. Chemical synthesis of these peptides was performed. Peptide Tyr-Glu-Gly received the name bursopeptide-1 (BP-1). Peptide Trp-Thr-Ala-Glu-Glu-Lys-Gln-Leu was designated as bursopeptide-2 (BP-2). These peptides were not described in scientific literature and patents. Therefore, they belong to a new group of immunoactive peptides.

The lymphocyte membrane carries CD markers acting as receptor, signal, or adhesive molecules. We studied the effects of bursopeptides on the expression of surface lymphocyte markers in patients with burns accompanied by severe combined immunodeficiency [8]. Table 1 shows that the count of lymphocytes carrying surface differentiation clusters (particularly antigens CD3 and CD4) decreases during burns. Incubation of lymphocytes with bursopeptides was accompanied by an increase in the number of cells with membrane CD antigens. It should be emphasized that bursopeptides produce various effects on the expression of differentiation antigens. BP-1 affectes prima-

TABLE 1. Effects of Bursopeptides on Spontaneous Expression of Differentiation Antigens on Lymphocytes from Patients with Burns (%, *M*±*m*)

Differentiation cluster	Lymphocytes from healthy donors (control 1)	Lymphocytes from patients with burns		
		physiological saline (control 2)	BP-1	BP-2
CD2	82.40±1.19	59.73±1.24*	62.13±1.25*	81.66±1.21$^+$
CD3	70.40±1.73	45.79±1.37*	47.48±1.07*	68.36±1.58$^+$
CD4	39.60±0.85	12.46±0.84*	13.96±0.97*	31.37±1.21*$^+$
CD8	25.10±0.74	18.69±0.67*	17.88±0.52*	19.54±0.85*
CD16	13.80±0.71	10.21±1.01*	10.96±0.96	13.39±0.98$^+$
CD21	8.90±0.87	4.63±0.43*	7.77±0.70$^+$	8.46±0.62$^+$
CD22	9.90±0.74	5.21±0.67*	8.81±0.74$^+$	7.41±0.69$^+$
CD38	21.30±0.98	8.86±0.63*	19.43±0.62$^+$	10.16±0.53*

Note. Here and in Table 2: $p<0.05$: *compared to control 1; $^+$compared to control 2.

TABLE 2. Effects of Bursopeptides on Expression of Differentiation Clusters on IL-2-Treated Lymphocytes from Patients with Burns (%, *M*±*m*)

Differentiation cluster	Lymphocytes from healthy donors (control 1)	Lymphocytes from patients with burns incubated with IL-2		
		physiological saline (control 2)	BP-1	BP-2
CD2	59.73±1.24	76.73±1.85*	79.18±1.97*	86.41±1.56*$^+$
CD3	45.79±1.37	57.43±2.56*	53.64±1.71	74.63±1.73*$^+$
CD4	12.46±0.84	19.30±1.01*	20.36±0.76*	29.58±1.37*$^+$
CD8	18.69±0.67	30.88±1.01*	29.13±0.84*	24.71±1.21$^+$
CD16	10.21±1.01	15.28±0.82*	14.01±0.73	15.66±0.87*
CD21	4.63±0.43	11.55±0.82*	11.86±0.86*	12.56±0.84*
CD22	5.21±0.67	11.53±0.69*	11.73±0.85*	11.66±0.82*
CD38	8.86±0.63	19.82±0.95*	25.51±0.84*$^+$	18.70±0.87*

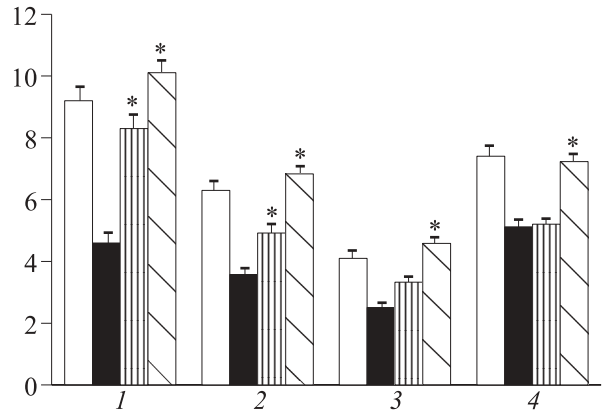

Fig. 1. Effects of bursopeptides on the immune response in mice immunized on day 10 after administration of cyclophosphamide: number of antibody-producing cells (per 10^3 cells, *1*); titers of hemagglutinins (log_2, *2*) and hemolysins (log_2, *3*); and count of peripheral blood leukocytes (10^9/liter). Light bars: intact animals. Dark bars: control (cyclophosphamide and physiological saline). Vertical shading: experiment 1 (cyclophosphamide and bursopeptide-1). Slant shading: experiment 2 (cyclophosphamide and bursopeptide-2). *$p<0.05$ compared to the control.

rily B cells and stimulates expression of CD21, CD22, and CD38. BP-2 produces a variety of effects and stimulates expression of membrane molecules on T and B cells and natural killer cells (CD2, CD3, CD4, CD16, CD21, and CD22). Both preparations have no effect on $CD8^+$ cells (Table 1). Differences in activity of bursopeptides are probably related to their influence on various subpopulations of lymphocytes.

Regulatory peptides are components of a complex system responsible for biological regulation. They act in close cooperation with other endogenous factors, including IL-2 [1]. In series II we evaluated the effects of bursopeptides on expression of differentiation antigens on IL-2-stimulated lymphocytes from patients with burns. IL-2 belongs to the family of glycoprotein immunomodulators responsible for signal induction in cascade cell-cell interactions, maturation, differentiation, and functioning of immunocompetent cells [6]. IL-2 stimulated expression of differentiation antigens on lymphocytes from patients with severe burns. Incubation of lymphocytes with IL-2 and bursopeptides changes expression of these markers. BP-1 potentiates the stimulatory effect of IL-2 on CD38 expression. BP-2 promotes IL-2-induced expression of antigens in CD2, CD3, and CD4, but abolishes this effect in CD8 cells (Table 2). These results indicate that bursopeptides can modulate the mechanisms of IL-2-mediated lymphocyte activation.

In series III we determined *in vivo* activity of bursopeptides in mice with cyclophosphamide-induced immunodeficiency. The immune response to xenogeneic erythrocytes was suppressed in mice immunized on day 1 after cyclophosphamide administration. Bursopeptides had no effect on the immune response in these animals. Injection of bursopeptides on day 10 of cytostatic-induced immunodeficiency markedly stimulated the immune response to sheep erythrocytes, which sometimes reached the control level. BP-2 was most potent and increased the number of APC in the spleen and titers of hemolysin and hemagglutinin by 2.1 times and 83.2 and 90.7%, respectively, compared to the control (Fig. 1). These data indicate that bursopeptides were most effective during recovery of the immune response in animals with cytostatic-induced immunodeficiency. This period is characterized by repopulation of lymphoid organs with lymphocytes at various stages of differentiation (target cells for bursopeptides).

Structural study, synthesis, and evaluation of mechanisms underling the effect of individual peptides from the bursa of Fabricius open new scope for the development of new immunostimulators.

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