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IMMUNOSTIMULATING PROPERTY OF ASPARTIC ACID

G. A. Belokrylov

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Not only thymus peptides, but also brain peptides and some of their synthetic analogs have immunostimulating properties [3, 12]. An important role is ascribed to them in the regulation of higher nervous activity [5, 12], in which not only peptides but also individual amino acids, such as aspartic acid (AA) participate [4]. The question arises whether individual amino acids and, in particular, AA can act on processes of immunogenesis. The aim of this investigation was to compare the effect of AA and functionally different oligopeptides containing it (thymopentin [11, 13], pentagastrin [6, 7]) on differentiation of bone marrow precursor T cells into T cells and on the immune response in mice.

EXPERIMENTAL METHOD

Experiments were carried out on 406 male CBA mice weighing 14-16 g. The substance tested included AA (from Sigma, USA), the pharmacopoieal preparation panangin, which is a mixture of potassium and magnesium aspartates, and pentapeptides, including AA in their composition: thymopentin (HArg-Lys-Asp-Val-Tyr-OH), synthesized in the Department of Natural Compounds, Scientific-Research Institute of Chemistry, A. A. Zhdanov Leningrad University, and pentagastrin (Boc-HAla-Trp-Met-Asp-Phe-NH_2 [6, 7], from Sanitas, Kaunas).

Bone marrow precursor T cells were differentiated as described previously [2], by a modified method [8]. after treatment of the bone marrow cells *in vitro* with the test preparations at 37°C for 1.5 h. The number of T lymphocytes in the bone marrow cell population was determined with the aid of rabbit antibrain serum in the complement-dependent cytotoxicity test [1]. The antiserum was used in a dilution of 1:50, in which, in the presence of fresh guinea pig complement (1:3), and after absorption by liver homogenate and mouse and sheep red blood cells [1], it caused death of 85 ± 2.5% of thymocytes and did not interact with bone marrow cells of CBA mice. In each test at least 200 cells, whose viability was estimated with a 0.2% aqueous solution of trypan blue, were counted. The experiment was repeated no fewer than 4-5 times.

The preparations were injected subcutaneously into the animals, dissolved in pyrogen-free physiological saline, daily for 10 days. The AA and panangin, like the other amino acids composing the test peptides, were injected in a dose of 1 μg, and thymopentin and pentagastrin in a dose of 5 μg daily. The control animals received pyrogen-free physiological saline by a

TABLE 1. Stimulation of Differentiation of T Precursor Cells *in Vitro* by AA, Panangin, Thymopentin, and Pentagastrin (M ± m)

Dose of preparation, μg/ml	Cytotoxicity index of antibrain serum (in %) after treatment of bone marrow cells with				
	AA	panagin	thymopentin	pentagastrin	Hanks' solution
1	20,7±2,8	6,9±1,8	0	17,5±2,7	
0,1	19,5±2,7	9,4±2	0	16,1±2,1	
0,01	19,7±2,8	9,7±2	0	16,8±1,9	0
0,001	13,5±2	3,0±0,8	0	2,8±1,2	
0,0001	1,3±0,8	0	6,9±1,8	0	
0,00001	0	0	6,5±1,7	0	

Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.
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TABLE 2. Effect of Thymopentin, Pentagastrin, and Amino Acids Composing Them on Immune Response to SRBC (M ± m)

Preparation	Dose, μg/day	Number of IgM-AFC per 10^6 splenic karyocytes	Agglutinins (reciprocal titers)
Thymopentin (HArg-Lys-Asp-Val-Tyr-OH)	5	18,5±1,8* (12)	51,6±5,5* (12)
Pentagastrin (Boc-HAla-Trp-Met-Asp-Phe-NH_2)	5	12, ±0,9* (10)	42,7±5,2* (10)
Panangin	1	14±2,9* (21)	57,5±11* (16)
AA	1	18,2±2,1* (48)	40±2,1* (48)
Alanine	1	16±1,4* (21)	33,3±5,5 (21)
Tryptophan	1	13±1,1* (12)	25±1,8 (12)
Valine	1	10,4±0,9* (12)	28,1±3,2 (12)
Methionine	1	7±1,5 (19)	19,2±2,8 (19)
Arginine	1	6,1±0,7 (22)	24,1±6,5 (22)
Tyrosine	1	6±1 (19)	18,3±2,8 (19)
Phenylalanine	1	5,8±0,9 (12)	22,5±2,8 (12)
Lysine	1	5±0,9 (10)	25,8±2,7 (20)
Pyrogen-free physiological saline (control)	—	5,8±1.1 (130)	24,5±1,9 (130)

*P < 0.01 compared with control.

Legend. Here and in Table 3, number of animals given in parentheses.

TABLE 3. Effect of AA and Panangin on Immune Response to Vi-Antigen (M ± m)

Preparation	Dose, μg/day	Number of IgM-AFC per 10^6 splenic karyocytes	Agglutinins (reciprocal titers)
AA	1	84±4,9 (14)	847±66,8 (17)
Panangin	1	78,6±2 (12)	853,3±59,2 (12)
Pyrogen-free physiological saline (control)	—	82±4 (19)	800±84,9 (19)

similar scheme. The animals were then immunized intravenously with sheep's red blood cells (SRBC, $2 \cdot 10^6$) or with Vi-antigen (0.1 μg per mouse). On the 4th day after immunization the number of IgM-antibody-forming cells (AFC) in the spleen of each mouse was determined by the method in [10] and the hemagglutinin titer was determined in the serum. To detect antibodies against Vi-antigen, the antigen was loaded on SRBC [2]. The final concentration of Vi-antigen in the solution of 20 μg/ml. To remove all unbound Vi-antigen the erythrocytes were washed with physiological saline at least eight times. The number of AFC was calculated per 10^6 splenic karyocytes.

EXPERIMENTAL RESULTS

Exposure of bone marrow cells to different doses of AA, penangin, or pentagastrin led to the appearance of T cells sensitive to the action of antibrain serum (Table 1). The number of these cells did not exceed one-fifth of the total cell mass, it was the same over a considerable dose range, and decreased only when low concentrations of the preparations were used. Thymopentin, unlike AA, panangin, and pentagastrin, potentiated differentiation of T precursors into T cells only in very low concentrations: 0.0001 and 0.00001 μg/ml. Testing other amino acids which were components of thymopentin and pentagastrin showed that tryptophan, valine, and alanine were active. However, this activity was much weaker than that of AA. Treatment with the above-mentioned amino acids in a dose of 1 μg/ml of bone marrow cells increased the number of T lymphocytes from 1.2 ± 0.5% in the control to 11.3 ± 2.6, 5.8 ± 1.7, and 5.7 ± 1.6% in the experiment. All preparations which accelerated differentiation of T precursors into T cells stimulated the immune response to SRBC. AA was the most active (Table 2). The immune response to thymus-independent Vi-antigen was unchanged by AA or panangin (Table 3).

The results show that AA, like the peptides containing it, can stimulate the immune response to SRBC. The immune response to thymus-independent Vi-antigen was unchanged under these circumstances, evidence that the effect of AA is connected with the function of T, but not of B cells.

Since the biological effect of immunologically active amino acids (alanine, tryptophan, and valine) which are components of thymopentin and pentagastrin was weaker than that of AA, and the half-life of the above-mentioned peptides in blood plasma is only 30 sec and not more than 5 min respectively [7, 13], this suggests the action of the peptide preparations is attributable mainly to the function of AA set free in the body as a result of enzymic degradation of the peptides.

The immunostimulating activity of panangin, which has been successfully used in clinical practice to normalize intracellular metabolism of potassium and magnesium ions, is an interesting fact. Activation of immunogenetic processes by this substance, connected with T cell function, suggests that panangin may be not only an active regulator of intracellular ion metabolism, but also an effective activator of the T system of the body.

The immunologic activity of the oligopeptides with different functions confirms the hypothesis [8] that common functional blocks exist in different systems, and that the immunologic activity of individual amino acids, most of which enter the body after enzymic degradation of proteins in the gastrointestinal trace, is evidence of the important role of the digestive system in processes of immunogenesis.

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SUPPRESSOR CELL HYPERACTIVITY RELATIVE TO ALLOGENEIC LYMPHOCYTE PROLIFERATION AS A MANIFESTATION OF DEFECTIVE T–T-CELL INTERACTIONS IN SYSTEMIC LUPUS ERYTHEMATOSUS

M. A. Stenina, A. A. Potapova, A. V. Biryukov, A. Yu. Skripnik, and A. N. Cheredeev

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In the contemporary literature there is much factual evidence of the varied manifestations of disturbances of the immune response in patients with systemic lupus erythematosus (SLE). The development of these disturbances is in good agreement with the view that in SLE there is a state of B-cell hyperactivity, caused by an uncertain etiologic factor and arising against the background of major disorders of immunoregulation [4], which are due to a quantitative and functional defect among immunoreulatory lymphocyte subpopulations, and also, perhaps, to an internal defect of the immunoregularoty cells (IRC) themselves and (or) of the cells controlled by them. This last factor, which has received less study, suggests an important role of disturbance of intercellular interactions in the pathogenesis of the immune changes in SLE.

The aim of this investigation was to study the state of immunoregulatory processes in SLE at the T–T-cell interaction level, and to test the possibility of their pharmacologic modulation.

Department of Immunology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, R. V. Petrov.)

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