ABILITY OF SOME AMINO ACIDS INCORPORATED INTO PROTEIN TO STIMULATE THE THYMUS-DEPENDENT IMMUNE RESPONSE

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The immunomodulating action of certain natural peptide preparations and their synthetic analogs has recently been extensively studied. The immunologic activity of the latter is linked with the definite primary structure of the peptide. The role of amino acids composing immunoactive peptides remained unknown until recently. In the case of the known preparation tuftsin — an immunoglobulin fragment (HThrLysProArgOH) possessing stimulating activity toward phagocytosis of bacteria by neutrophils, the ability of two of the four amino acids composing it, namely proline and arginine, to stimulate phagocytosis in the same way as the original preparation, was demonstrated [9].

The question arises whether individual amino acids are able to affect the specific parameters of immunity.

The aim of this investigation was to study the direct immunomodulating effect of amino acids composing a protein. For this purpose two criteria were used: the effect of the amino acids *in vitro* on differentiation of T precursor cells of bone marrow and T lymphocytes and their ability to stimulate the thymus-dependent immune response in an animal.

EXPERIMENTAL METHOD

Experiments were carried out on 674 male CBA mice weighing 14-16 g. Amino acids were obtained from Sigma (USA), Fluka (West Germany), Reanal (Czechoslovakia), and the Olaine Factory (USSR). The amino acids for testing were injected subcutaneously into the animals daily for 10 days, in a dose of 1 μg per injection, in pyrogen-free physiological saline. The animals were then immunized intravenously with sheep's red blood cells (SRBC) in a dose of $2 \cdot 10^6$. On the 4th day after immunization the number of IgM-antibody-forming cells (AFC) was determined in the spleen of each mouse by the method in [7], and the hemagglutinin titer was determined in the serum. The number of AFC was calculated per 10^6 splenic karocytes.

Differentiation of T precursor cells of bone marrow and T lymphocytes under the influence of amino acids was estimated *in vitro* by a modified method [5]. For this purpose the bone marrow cell pool from the femur, tibia, and sternum was freed from red cells by treatment with 0.65% ammonium chloride solution. After the cells had been washed five times at 1200 rpm for 7 min with cold Hanks' solution they were mixed with the test amino acids so that 1 ml of medium contained $3 \cdot 10^7$ nucleated cells and 1 μg of amino acid. The mixture of amino acids with cells was continuously shaken and kept at 37°C for 1.5 h, after which the cells were again washed five times with Hanks' solution under the same conditions and their sensitivity to antibrain serum was determined in the cytotoxicity test [1]. Antiserum was obtained by repeated immunization of rabbits with cerebral cortical tissue of CBA mice without Freund's adjuvant [1], it was absorbed with a homogenate of mouse liver and mouse and sheep red blood cells [1], and used in a dilution of 1:50. In this dilution, in the presence of complement (fresh guinea pig serum — 1:3), the antiserum led to death of 91.8 ± 1.6% of thymocytes and 1.2 ± 0.3% of bone marrow cells of CBA mice. In each sample no fewer than 200 cells were counted whose viability was estimated by the use of a 0.2% aqueous solution of trypan blue. The experiment was repeated at least 4-5 times. Altogether 115 animals were used for the experiments *in vitro*.

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TABLE 1. Differentiation of Precursor T Cells and Parameters of Immune Response under the Influence of Amino Acids (M ±

Amino acid	Number of T lymphocytes in suspension of bone marrow cells after treatment with amino acids (cytotoxicity index of antibrain serum, %)	Number of IgM-AFC per 10^6 splenic karyocytes
Asparagine (Sigma, Olaine factory)	21,5±2,9*	19,2±0,5* (36,
Glutamic acid (Sigma, Reanal)	16,6±3,1*	9,9±1,3* (13)
Cysteina (Sigma, Reanal)	16,1±1,7*	11,2±1,3* (12)
Serine (Reanal)	11,3±2,4*	9,3±1,2* (12)
Tryptophan (Reanal)	11,3±2,6*	13,0±1,1* (12)
Threonine (Sigma, Reanal)	11,1±2,3*	13,9±1,7* (45)
Asparagine (Reanal)	8,9±2,3*	14,5±1,9* (25)
Valine (Reanal)	5,8±1,7**	10,4±0,9* (12)
Alanine (Reanal)	5,7±1,6**	16,0±1,4* (21)
Glutamine (Sigma, Reanal)	3,8±1,6	6,8±0,7 (31)
Tyrosine (Sigma, Reanal)	3,3±1,7	6,0±1,0 (19)
Glycine (Olaine factory)	1,9±1,0	7,3±1,1 (22)
Lysine (Sigma, Reanal)	1,8±0,9	5,0±0,9 (20)
Methionine (Sigma, Fluka)	1,6±1,1	7,0±1,5 (19)
Isoleucine (Reanal)	1,6±1,1	7,5±0,8 (11)
Histidine (Reanal)	1,5±0,8	7,3±1,2 (22)
Leucine (Olaine factory)	1,3±0,9	5,0±0,7 (21)
Phenylalanine (Reanal)	1,3±0,9	5,8±0,9 (12)
Proline (Sigma)	1,3±0,9	5,5±0,7 (13)
Arginine (Sigma)	0,7±0,7	6,1±0,7 (22)
Control	1,2±0,3	5,9±0,5 (87)
	Bone marrow cells in Hanks' solution	Injection of pyrogen-free physiological saline

Legend. Each number is the result of four or five experiments (counting at least 800-1000 cells).

TABLE 2. Effect of Thymopentine, Fragments of α_1-Thymosine, and Their Component Immunoactive Amino Acids on the Immune Response to SRBC (M ± m)

Preparation	Dose, μg per animal per day	Number of IgM-AFC per 10^6 splenic karyocytes	Agglutinins (reciprocals of titers)
Pyrogen-free physiological saline (control)	—	6,0±1,2 (24)	24,0±2,1 (24)
Thymopentine	5,0	18,5±1,8* (12)	51,6±5,5* (10)
Fragment 20-24 of α_1-thymosine	5,0	11,3±1,4** (12)	26,5±2,8 (12)
Aspartic acid	1,0	17,2±3,8* (12)	40,0±6,5** (12)
Glutamic acid	1,0	10,6±1,3** (12)	25,0±1,9 (12)
Valine	1,0	10,5±0,9* (12)	26,6±6,5 (12)

EXPERIMENTAL RESULTS

Of the 20 amino acids studied nine accelerated differentiat-on of precursor T cells into T lymphocytes: aspartic and glutamic acids, asparagine, cysteine, serine, threonine, tryptophan, alanine, and valine (Table 1). When injected into mice, these amino acids correspondingly intensified the immune response to SRBC. Aspartic acid, which has marked ability to accelerate differentiation of bone marrow precursor T cells into T lymphocytes *in vitro*, also had the strongest stimulating effect on the level of the immune response: compared with the control, it increased AFC production by 3.2 times and antibody production by 1.6 times. All the other immunoactive amino acids stimulated AFC production only and did not affect the level of hemagglutinins: their titers varied from 20.0 ± 2.7 to 27.6 ± 3.0 compared with 24.5 ± 1.9 in the control.

In the nest (control) series of experiments we estimated the immunostimulating activity of pentopeptides synthesized by ourselves: the fragment 20-24 of α_1-thymosine (HLys Glu Val GluOH), thymopentine (HArg Lys Asp Val TyrOH) [8], and the individual immunologically active amino acids composing them. Injection of 1 μg of each amino acid into the animals daily for 10 days gave an immunostimulating effect similar to the action of the peptides, when injected by the same scheme in a dose of 5 μg. The immunostimulating action of the peptide did not exceed the effect of its most active amino acid component (Table 2).

The results are evidence that not only immunoactive peptides may possess immunologic activity, but also the individual amino acids composing them. Analysis of the primary structures of 158 known biologically active peptides showed that the immunologically active amino acids which we found are present mainly in immunoactive thymus peptides. They have a particularly high content of aspartic and glutamic acids — up to 20-28% — and α-prothymosine has up to 65% [6]. The content of the above-mentioned amino acids is high in the brain, where they have marked functional activity [2]. It can be tentatively suggested that the intersystemic connections of the body are maintained not only by identical regulatory peptides, such as vasopressin [11] and Thy-1-antigen [3, 10], for example, but also by different peptides with common biologically active amino acids. This conclusion is in full agreement with the hypothesis that different systems contain identical functional blocks [4], and this is an important and promising concept in connection with our understanding of the peptide regulation of homeostasis as a whole.

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