DIVERSITY OF ACTION OF PEPTIDES AND THEIR COMPONENT AMINO ACIDS ON ANTIBODY FORMATION AND PHAGOCYTIC ACTIVITY OF NEUTROPHILS IN MICE

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Our previous investigations [3, 4] showed that nine of the 20 amino acids found in the composition of proteins (alanine, asparagine, aspartic acid, valine, glutamic acid, serine, tryptophan, threonine, and cysteine) can stimulate antibody formation in mice to thymus-dependent antigen, while having no effect on the level of the thymus-independent immune response. Arginine, on the other hand, was found to suppress the immune response: thymus-dependent in a dose of $5 \cdot 10^{-11}$ mole/mouse, thymus-independent in a dose of $5 \cdot 10^{-8}$ mole per mouse [4]. Amino acids acting on antibody formation we described as immunoactive.

Information on the effects of peptides and their component amino acids on phagocytic activity of neutrophils is limited to data on the ability of arginine and proline, in the composition of tuftsin, to stimulate phagocytosis of staphylococci in the same way as the action of the original peptide [7].

To assess the role of individual amino acids in the peptide regulation of antibody formation and the phagocytic process, we undertook a comparative study of the action of fragments of natural peptides and their component amino acids on the thymus-dependent immune response to sheep's red blood cells (SRBC) in vivo and on phagocytosis of staphylococci in vitro by mouse peritoneal neutrophils.

EXPERIMENTAL METHOD

The experiments in vivo were carried out on 499 male CBA mice weighing 14-16 g. The following substances were tested: the immunoglobulin fragment tuftsin — Thr Lys Pro Arg (TKPR), containing both immunoactive and inactive amino acids, fragments of the biologically active Thy-1-antigen [1], consisting either only of immunoreactive amino acids: Leu Gly Ile Pro (LGIP) Pro Tyr Ile Lys (PYIk), or immunoactive and inactive acids: Thr Thr Lys Asp (TTKD); Leu Gly Ile Pro Glu (LGIPE); Pro Tyr Ile Lys Val (PYIKV). The peptides listed above were obtained by the method of classical synthesis in solution.

The preparations were injected subcutaneously in the course of 5 days in pyrogen-free physiological saline and over a wide dose range (10^{-9}-10^{-17} mole/mouse). The animals were then immunized intravenously with SRBC ($2 \cdot 10^6$), and 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 of Jerne and Nordin [6], and calculated per 10^6 karyocytes.

The phagocytic activity of neutrophils of CBA mice under the influence of peptides and amino acids was determined in experiments in vitro. For this purpose peritoneal exudate cells were used in a final concentration of $12.5 \cdot 10^6$ ml. The exudate was obtained 2.5 h after injection of a 10% solution of peptone. The object of phagocytosis was a 24-h culture of *Staphylococcus aureus,* strain 9198, in a final concentration of $250 \cdot 10^6$ cells/ml. Peptides were tested over a wide range (10^{-9}-10^{-17} mole/ml). In each experiment 900-1000 neutrophils were counted. The experiment was repeated at least twice. LPS-prodigiosan (0.005%) was used as the reference preparation. The phagocytic index and phagocytic number were determined [5].

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TABLE 1. Effects of Peptides and Amino Acids Composing them on Immune Response to SRBC in vivo and on Phagocytosis of Staphylococci by Mouse Peritoneal Neutrophils in vitro (M ± m)

Parameter	Peptide and amino acids composing them																
	TKRR	TTKD	TGIP	TGIPE	PYIK	PYIKV	F	K	P	R	D	E	V	Y	L	I	G
Number of IgM-AFC per 10^6 karyocytes after injection of preparations (M ± m)	24,0 ± 1,8* (12)	35,6 ± 3,2* (12)	9,2 ± 1,1 (20)	17,4 ± 1,6* (10)	11,3 ± 1,8 (30)	11,2 ± 3,0 (10)	27,8 ± 3,4* (30)	10,0 ± 1,8 (20)	11,0 ± 1,4 (12)	6,2 ± 0,8* (10)	38,4 ± 1,0* (36)	18,5 ± 1,4* (10)	23,0 ± 2,6* (10)	12,0 ± 2,0 (19)	10,0 ± 1,4 (21)	15,0 ± 1,6 (10)	14,6 ± 2,2 (22)
Number of IgM-AFC per 10^6 karyocytes after injection of pyrogen-free physiological saline (M ± m), control	10,8 ± 0,6 (24)	9,7 ± 0,5 (24)	9,7 ± 0,7 (18)	11,4 ± 0,8 (18)	10,8 ± 0,6 (10)	11,8 ± 1,0 (20)	11,1 ± 1,0 (10)	11,2 ± 1,1 (12)	10,5 ± 0,6 (21)	12,0 ± 1,0 (20)	12,0 ± 1,0 (20)	10,8 ± 0,6 (10)	10,8 ± 0,6 (10)	11,8 ± 1,0 (12)	11,8 ± 1,0 (12)	11,8 ± 1,0 (12)	11,8 ± 1,0 (12)
Phagocytic index, per cent, after treatment of cells with preparations (M ± m)	45,8 ± 2,3*	46,0 ± 3,6*	28,1 ± 0,6	43,2 ± 2,8*	18,8 ± 2,0	38,3 ± 0,5*	44,3 ± 1,9*	44,7 ± 2,9*	37,7 ± 1,7*	40,5 ± 2,2*	27,2 ± 1,6*	45,8 ± 3,6*	41,0 ± 2,3*	35,1 ± 1,5*	32,5 ± 2,0*	17,7 ± 1,4	21,3 ± 1,1
Phagocytic index, per cent, after treatment of cells with Hanks' solution (M ± m), control	20,8 ± 1,5	20,8 ± 1,5	28,5 ± 2,1	20,9 ± 0,5	18,7 ± 0,7	18,7 ± 0,7	19,1 ± 0,8	19,1 ± 0,8	18,7 ± 0,7	18,2 ± 1,2	18,2 ± 1,2	18,1 ± 2,3	18,7 ± 0,7	18,7 ± 0,7	23,5 ± 1,5	18,7 ± 0,7	23,5 ± 1,5
Phagocytic index after treatment of cells with LPS (M ± m)	48,0 ± 3,4	48,0 ± 3,4	42,5 ± 3,2	40,1 ± 2,6	34,1 ± 0,8	34,1 ± 0,8	43,7 ± 2,0	43,7 ± 2,0	34,1 ± 0,8	34,3 ± 3,5	34,3 ± 3,5	32,1 ± 0,7	34,1 ± 0,8	34,1 ± 1,5	42,5 ± 4,2	34,1 ± 1,5	42,5 ± 4,2

Legend. Cross in table indicates data obtained by testing peptides in vivo in a dose of 10^{-9} mol/mouse and ire vitro in a concen- tration of 10^{-9} mol/ml. Amino acids used in equimolar proportions to peptides. Asterisks indicate significant differences compared with control at $p < 0.01$.

EXPERIMENTAL RESULTS

As Table 1 shows, the peptide TTKD, with amino acids capable of stimulating antibody formation and phagocytosis at both N- and C-ends, potentiates both processes. Peptides LGIP and PYIK, consisting of immunoinactive amino acids, affected neither the immune response nor the phagocytic activity of the neutrophils. Lengthening the peptide LGIP from the C-end by an immunoactive glutamic acid residue (E) leads to the creation of a peptide LGIPE, which stimulates both the immune response and phagocytosis, whereas lengthening of the immunoinactive peptide PYIK from the C-end by an active valine (V) residue confers on the peptide PYIKV the ability to potentiate phagocytosis but not the immune response. Meanwhile the peptide TKPR (tuftsin), which has threonine (T) at the N-end, potentiating the immune response, and arginine (R) at the C-end, inhibiting the response, stimulates both antibody formation and phagocytosis.

The action of amino acids present in the above-mentioned peptides on the immune response and phagocytosis also differs. Glutamic and aspartic acids, threonine, and valine, stimulating the immune response, can also potentiate the phagocytic process. Glycine and isoleucine, which cannot stimulate the immune response, likewise do not affect the phagocytic activity of neutrophils. However, lysine, proline, tyrosine, and leucine, with no effect on antibody formation, potentiate phagocytosis of staphylococci by neutrophils. Arginine, an inhibitor of antibody formation, possesses similar properties (Table 1).

The effective dose range of the peptides and the amino acids composing them during stimulation of antibody formation and phagocytosis is commensurate, whereas the effective dose of glutamic acid is much greater than the corresponding parameter of the peptide. For instance, the peptide LGIPE stimulates the immune response and phagocytic activity of neutrophils within the dose range of 10^{-9}-10^{-13} mole/mouse and 10^{-9}-10^{-15} mole/ml respectively, whereas glutamic acid (E), which is a component of it, does so in doses of 10^{-9}-10^{-15} mole/mouse and 10^{-9}-10^{-16} mole/ml.

The action of peptides and the amino acids composing them on phagocytic activity of neutrophils is manifested as an increase in the phagocytic index of the positive control (LPS) under their influence. The phagocytic number was unchanged under the influence of different concentrations of peptides and amino acids and varied within limits of 1.7 ± 0.06-1.9 ± 0.08 compared with 1.7 ± 0.05-2.0 ± 0.08 in the control (not shown in Table 1).

These results confirm and supplement the earlier results [3, 4] indicating high immunomodulating activity of particular amino acids. Some amino acids, namely glutamic (E) and aspartic (D) acids, threonine (T), and valine (V), can stimulate not only antibody production but also phagocytosis. Others, namely lysine (K), proline (P), tyrosine (Y), and leucine (L), do not change the level of the immune response but stimulate phagocytosis, whereas arginine (R) inhibits antibody production but stimulates the phagocytic activity of the neutrophils.

The presence of individual amino acids in a peptide is important in connection with the manifestation of its immunostimulating and phagocytosis stimulating activity. Peptides LGIP and PYIK, for instance, which do not contain any immunoactive amino acids, do not affect the immune response. Lengthening of the peptide by a glutamic acid residue (E), capable of stimulating both antibody production and phagocytosis, endows this peptide with the ability to stimulate both processes. However, the ability of the peptide to stimulate antibody production and/or phagorytosis not only depends on its containing an active acid, but it is also largely determined by its structural characteristics. Peptides LGIP and PYIK, not including in their composition any amino acids stimulating the immune response, but containing amino acids stimulating phagocytosis, affect neither antibody production nor phagocytosis. If the peptide PYIK is lengthened by a valine residue (V), stimulating antibody formation and phagocytosis, this leads to the appearance of ability to stimulate phagocytosis but not the immune response by the peptide. Tuftsin (TKPR), containing amino acids stimulating phagocytosis, namely proline (P), and lysine (K) — or both phagocytosis and antibody production simultaneously — threonine (T), and also arginine, an inhibitor of the immune response and stimulator of phagocytosis, stimulates both antibody formation and phagocytosis.

Activity of peptides and their properties are thus determined by the presence of certain sequences of immunoactive amino acids in them. The amino acids themselves are evidently an independent link in the chain of regulation of immunogenesis. Several factors support this conclusion: 1) peptides and their component amino acids act variously on antibody production and the phagocytic activity of the neutrophils: PYIKV does not affect the immune response, but valine (V), which is a component of this peptide, stimulates it; thymopentin (RKDVY) [2] and tuftsin (TKPR) stimulate the immune response, but arginine (R), which is a component of the structure of these peptides, inhibits it; LGIP has no effect on the phagocytic activity of neutrophils, but its components leucine (L) and proline (P) stimulate it. 2) The effective dose range of the peptides and their component amino acids differs: in the case of glutamic acid (E), when it stimulates antibody production and phagocytosis it is much wider than when it is a component of the peptide LGIPE.

The facts described above suggest the existence of two systems of regulation of immunogenesis: an older system, represented by amino acids, and a system of peptide regulation, reflecting the later stages of evolution.

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IMMUNOMODULATING PROPERTIES OF NONSTEROID ANTI-INFLAMMATORY AGENTS

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The writers previously showed that certain iodine derivatives of pyrazolone stimulate the humeral immune response to erythrocytic and viral antigens [1]. The aim of this investigation was to study the immunostimulating and immunosuppressive properties of some representative nonsteroid antiinflammatory agents (NSAIA).

EXPERIMENTAL METHOD

The test compounds butadione, antipyrin, acetylsalicylic acid, and sodium salicylate were obtained from the Novosibirsk Pharmaceutical Chemical Factory, and 4-iodoantipyrin, 4-bromoantipyrin, and stampyrine were synthesized by E. V. Shmidt et al. (Tomsk Polytechnical Institute). In some experiments tslorone, obtained from the Research Institute of Therapeutic Substances (Stariya Kupavna, Moscow Region) was used as the control.

Experiments were carried out on 350 noninbred male mice weighing 18-20 g, obtained from the animal house of the "Vector" Research-Production Combine (Koltsevo village, Novosibirsk Region) and 80 male (CBA × C57BL)F_1 mice obtained from the animal house of the Institute of Clinical Immunology, Siberian Branch, Academy of Medical Sciences of the USSR (Novosibirsk).

To evaluate the immune response we used a heterologous antigen (sheep's red blood cells — SRBC) and also Coxsackie A13 virus (Flores strain), adapted to reproduction in J-41 cell culture in the presence of Eagle's MEM medium, liter of virus 5.0 log TCD_{50}/0.1 ml, The number of antibody-forming cells (AFC) in the spleen of mice immunized with the heterologous antigen was determined [7] against a background of intraperitoneal injection of the NSAIA, once a day for 4 days, in half the maximal tolerated dose (for 4-iodoantipyrin the dose was established experimentally previously [1]). Immunization of the mice with Coxsackie A13 virus and injection of the NSAIA were carried out by the program developed by the writers previously [1]. T-suppressor cell function was evaluated by the method in [3]. The significance of the results was assessed by Student's test.

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