FIBRONECTIN TRIPEPTIDE FRAGMENT INCREASES INGESTIVE CAPACITY OF PHAGOCYTES

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Fibronectin, which exists in the body as plasma and tissue forms, is responsible for adhesion, aggregation, and migration of cells, phagocytosis, and cellular differentiation. It is suggested that it has a twofold action on bacteria and on secondary products of tissue damage: as a nonspecific opsonin and as a stimulator of phagocytosis [4, 11]. The tripeptide (T) Arg-Gly-Asp (RGD), the reactive component for interaction of fibronectin with cell surface receptors [9], has been identified and synthesized. Besides in fibronectin, T has also been found in molecules of vitronectin, collagens, fibrinogen, and Von Willebrand's factor. Adhesive RGD-containing proteins and the corresponding cells receptors (integrins) constitute a recognizing system, which largely determines the spatial arrangement, functional state, differentiation, and growth of cells [10].

The aim of this investigation was to study the effect of the synthetic T Arg-Gly-Asp on phagocytic activity of polymorphonuclear leukocytes (PNL) and monocytes (M) of the blood in vitro.

EXPERIMENTAL METHOD

The tripeptide Arg-Gly-Asp was synthesized by classical methods of peptide chemistry in the Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR.

There were seven series of experiments with blood from Wistar rats. Each series involved one to three samples for each test dilution of T. Altogether, for each concentration of T there were 12-15 samples. For the phagocytic test, the parameters of known variants of reproduction of the reaction [1, 7] were utilized. Heparinized blood was allowed to stand and was centrifuged for 5 min at 1000 rpm. A suspension of leukocytes was obtained and its concentration adjusted to 5×10^6 cells/ml. The leukocyte suspension was transferred in volumes of 0.1 ml into wells of immunologic panels. To the leukocyte suspension, 0.1 ml of the test T, diluted in physiological saline in concentrations of 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} mM, was added to each well, adding 0.1 ml of physiological saline alone to the control wells. Incubation continued for 30 min at 37°C. Next, 0.1 ml of a suspension of latex particles (5×10^8/ml) 0.8 μ in diameter was added to each sample. The reaction mixture was incubated

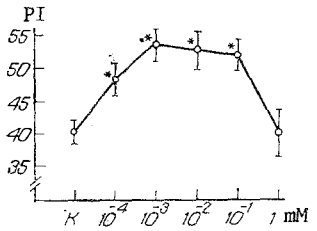

Fig. 1. Changes in PI depending on concentration of T. Here and in Fig. 2: abscissa, concentration of T (in mM). K) Control. Asterisk indicates significant difference from control ($p < 0.02$-0.001).

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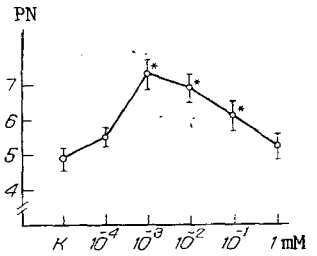

Fig. 2. Effect of concentration of T on PN.

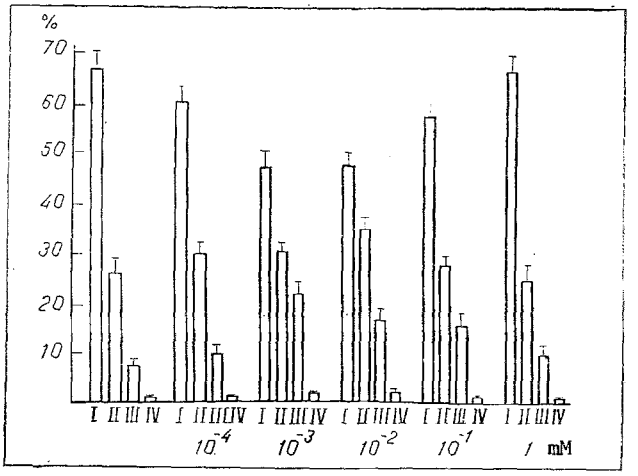

Fig. 3. Degree of phagocytosis when different concentrations of T are used. Ordinate, percentage of phagocytic PNL and M. I) Phagocytes ingesting 1-5 latex particles, II) 6-10, III) 11-20, and IV) over 20 particles. Remainder of legend as to Figs. 1 and 2. .

in a humid chamber for 30 min at 37°C with constant shaking. Films were prepared and stained by the Romanovsky-Giemsa method. At least 100 PNL and M with and without latex was counted in each preparation. The phagocytic index (PI), determined as the percentage of the total number of leukocytes counted engaging in phagocytosis, and the phagocytic number (PN), the mean number of latex particles ingested by one phagocytic cell, were determined. Altogether four degrees of phagocytosis were distinguished, depending on the number of ingested latex particles: I) 1-5, II) 6-10, III) 11-20, and IV) over 20 particles.

The numerical results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Preliminary incubation of rat blood PNL and M with T in initial concentrations of 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} mM caused a significant increase in PI (Fig. 1). The maximal level of ingestive capacity of the phagocytes was reached with T in concentrations of 10^{-3} and 10^{-2} mM, when it was 1.3 times higher than in the control. If the concentration of T in the reaction mixture was relatively high (in mM), no stimulation of phagocytosis was observed (40.3 ± 3.6 and 40.2 ± 1.7 in the control). PN differed only a little from the control level when T was used in concentrations of 10^{-4} mM and 10^{-1} mM, low and high concentrations under these experimental conditions, although a tendency was observed for it to rise (Fig. 2). The highest values of PN were obtained on incubation of leukocytes with T in concentrations of 10^{-3} mM (1.5 times higher than the control) and 10^{-2} mM (1.4 times higher).

The percentage of phagocytic cells which had ingested more than five latex particles (degrees II, III, and IV of phagocytosis) also was higher when T was used in doses of 10^{-3} and 10^{-2} mM (Fig. 3). This tendency was strongest when T was used in a concentration of

10^{-3} mM: the number of phagocytes with 11-20 or over 20 ingested latex particles was 3 times greater than in the control.

These data are evidence that the tripeptide Arg-Gly-Asp can induce dose-dependent stimulation of the ingested capacity of the blood phagocytes in vitro.

This fact is of theoretical and practical importance on its own account, for possibility of stimulating phagocytosis with RGD-tripeptide was not previously known. The results also add to existing information on stimulation of degranulation, chemotaxis, and adhesion [8, 12] during activation of phagocytic cells by fibronectin fragments and they help to make more precise our ideas on the mechanisms of stimulation of phagocytosis by fibronectin.

Among the known stimulators of phagocytosis, those distinguished by their efficacy include peptides of natural origin and their synthetic analogs, especially tuftsin [6], human IgG decapeptide fragment [5], and rigin [2]. In similar concentrations, T exhibited comparable activity, and it can therefore be regarded as a promising preparation for medical practice.

A matter of special importance, in our view, is the dependence of the intensity of the phagocytosis-stimulating effect of T on dose. Extrapolation of these data to processes in vivo does not contradict the view that fibronectin is very closely linked functionally with phagocytes, during wound healing [3], for example, when the content of fibronectin and its fragments determines the character of the course of reparative regeneration.

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