EFFECT OF TUFTSIN ON ACTIVITY OF SOME PERITONEAL MACROPHAGAL ENZYMES OF ENERGY METABOLISM

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Tuftsin* has been shown to affect the functions of various cells of the immune system [9]. The action of tuftsin on macrophages and lymphocytes, which play an important role in reactions of specific and nonspecific immunity, is particularly interesting. Tuftsin increases the bactericidal activity of macrophages *in vivo* and *in vitro* [8], stimulates phagocytic activity of peritoneal macrophages [4], and activates movement of polymorphonuclear leukocytes *in vitro* [10]. The antitumor action of tuftsin has been demonstrated: it prevents inhibition of macrophage migration by melanoma factors [6] and increases the cytotoxicity of macrophages against tumor cells both *in vivo* [5] and *in vitro* [10].

The action of tuftsin was studied in the present investigation on activity of enzymes of energy metabolism in peritoneal macrophages of rats at rest and during phagocytosis. Activity of enzymes of glycolysis, the tricarboxylic acid, and the pentose phosphate shunt — lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), and glucose-6-phosphate dehydrogenase (G6PDH) — was estimated.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200 g were used. Macrophages were isolated 1 week after intraperitoneal injection of 10 ml of a 4% solution of peptone in 0.9% NaCl solution intraperitoneally. The cells were flushed out of the peritoneal cavity with phosphate buffer (10 ml, pH 7.4), containing D(+)-glucose (2 mg/ml), bovine serum albumin (2 mg/ml), benzylpenicillin, and streptomycin (200 μg/ml of each). All subsequent manipulations were conducted in this same medium. The number of cells was counted in a Goryaev's chamber and was about $2.5 \cdot 10^6$ cells/ml medium. The proportion of viable cells was determined by staining with trypan blue, for which purpose the cells were incubated in a solution of the dye (1% solution in 0.9% NaCl solution) at 37°C for 30 min. The fraction of living cells in the suspension was 85%. The cell suspension (0.5 ml) was applied to a slide and incubated at 37°C for 2 h to obtain a cell monolayer. The slides were washed twice, 0.1 ml of tuftsin solution (Serva, West Germany) was applied, and they were incubated at 37°C for 15 min. Phagocytosis was induced on the slide by application of 1 ml of a suspension of latex (d = 1.26, concentration $2 \cdot 10^6$ particles/ml) and the cells were incubated at 37°C (30 min), after which the slides were washed. Activity of SDH, LDH, and G6PDH was determined by the method in [2]. Activity of the three dehydrogenases was estimated in accordance with Kaplow's principle [7]. The phagocytosis-stimulating activity of tuftsin was characterized, first, by the ratio between the fraction of cells containing latex particles in the presence of tuftsin and the fraction of those cells in the absence of tuftsin, and second, by the ratio of the mean number of latex particles ingested by one call in the presence of tuftsin to the number of particles ingested in the absence of tuftsin. The significance of differences between the experimental and control data, when 250 cells were counted, was evaluated by the Mann—Whitney or Wilcoxon tests for independent sets [1].

*The tetrapeptide Thr-Lys-Pro-Arg.

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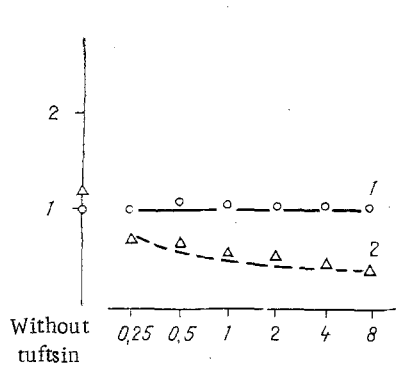

Fig. 1

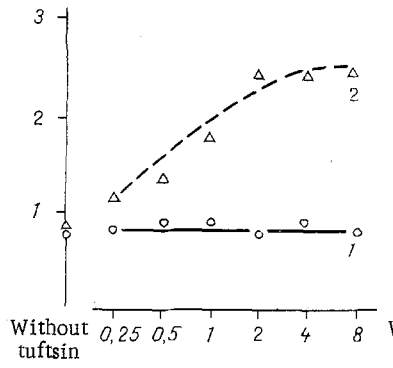

Fig. 2

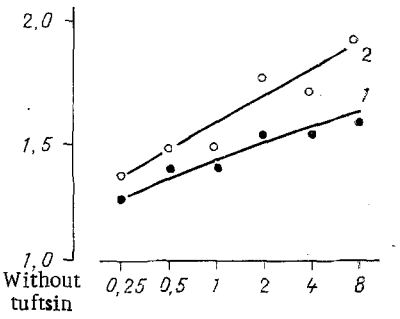

Fig. 3

Fig. 1. Effect of tuftsin on LDH activity in macrophages at rest (1) and during phagocytosis (2). Abscissa, tuftsin concentration (in μg/ml, logarithmic scale); ordinate, mean number of formazan granules per cell. Differences significant at $P < 0.01$.

Fig. 2. Effect of tuftsin on G6PDH activity in macrophages at rest (1) and during phagocytosis (2). Abscissa, tuftsin concentration (in μg/ml, logarithmic scale); ordinate, mean number of formazan granules per cell. Differences significant at $P < 0.01$.

Fig. 3. Effect of tuftsin on phagocytic activity of macrophages. Abscissa, tuftsin concentration (in μg/ml, logarithmic scale); ordinate, ratio characterizing phagocytic activity. 1) Ratio of fraction of cells containing latex particles in presence of tuftsin to fraction of those cells in control; 2) ratio of mean number of latex particles ingested by one cell in presence of tuftsin to corresponding number in control.

EXPERIMENTAL RESULTS

In the absence of tuftsin SDH and G6PDH activity in phagocytic and resting macrophages was identical. LDH activity was higher in phagocytic than in nonphagocytic macrophages (Fig. 1). Cells isolated after intraperitoneal injection of peptone solution, incidentally, were used in the experiments under review. These cells are called "stimulated" or "nonspecifically activated" by contrast with "activated" cells whose activation was the result of specific immunologic processes. This nonspecific activation of macrophages actually during isolation is probably responsible for the absence of any considerable changes in activity of the three enzymes studied during phagocytosis, observed above.

Under the influence of tuftsin in various concentrations on resting macrophages, activity of the test enzymes was unchanged (Figs. 1 and 2).

However, under the influence of tuftsin on phagocytic macrophages LDH activity fell even with tuftsin in the minimal concentration (0.25 μg/ml), and when the peptide was used in a concentration of 8 μg/ml LDH activity was only 38% of its control level (Fig. 1). In phagocytosis a special role is ascribed to glycolysis: It supplies the cell with energy to ingest particles to be phagocytosed [11]. The phagocytosis-stimulating capacity of tuftsin, described in the literature [4], may perhaps be realized at this stage. However, the fact that LDH is depressed and not activated by tuftsin is unexpected in the light of these views.

On the contrary, tuftsin had a stimulating action on G6PDH activity of phagocytic cells (Fig. 2). The difference in G6PDH activity of phagocytic and resting macrophages increased with an increase in concentration of the peptide, and at a concentration of 8 μg/ml G6PDH activity in phagocytic macrophages was 278% of the control level. Activation of G6PDH by tuftsin is in good agreement with the views that stimulation of the pentose phosphate shunt is connected with the presence of detoxication of the phagocytosed material with the aid of active compounds of oxygen.

Tuftsin did not affect SDH activity of macrophages during phagocytosis.

Evidence of the biological activity of the tuftsin used in these experiments was given by data confirming its phagocytosis-stimulating ability. The fraction of cells taking part in phagocytosis was increased by 1.5 times under the influence of tuftsin, and the number of phagocytosed latex particles was doubled (Fig. 3).

The results of an investigation of the effect of tuftsin on SDH, LDH, and G6PDH activity of peripheral blood lymphocytes of albino rats after intraperitoneal injection of the peptide in a dose of 300 μg/kg body weight were published by the writers previously [3]: After intraperitoneal injection of tuftsin a decrease in SDH activity and an increase in G6PDH activity of the lymphocytes were observed. The effect was maximal 6 h after injection of the peptide, and enzyme activity returned to its initial level after 24 h.

Tuftsin thus changes the level of activity of some enzymes of energy metabolism of phagocytic macrophages and peripheral blood lymphocytes. The minimal effective concentration of tuftsin in experiments with macrophages (0.25 μg/ml), incidentally, was close to its usual blood concentration. So far as the tuftsin concentration in the blood after intraperitoneal injection into rats in a dose of 300 μg/kg body weight is concerned, in this case its increase could not exceed tenths of a microgram per milliliter. The effects of tuftsin observed can therefore be interpreted as close to those existing under ordinary conditions of activity of the animal.

Differences in the effect of tuftsin on the macrophagal and lymphocytic enzymes studied are interesting: marked inhibition of LDH activity in macrophages but no change in lymphocytes, inhibition of SDH activity in lymphocytes but no effect in macrophages. Only the effect on G6PDH activity was identical in direction. The interpretation of these results is difficult at present. It is not clear why LDH activity is inhibited in macrophages, if glycolysis is one of the main sources of energy for phagocytosis by these cells. There is likewise no explanation of inhibition of SDH activity in lymphocytes. Only activation of G6PDH is in harmony with existing views on the role of the pentose phosphate shunt in the formation of detoxicating factors. The high degree of activation of this last enzyme in macrophages also is in harmony with this view.

LITERATURE CITED

1. I. P. Ashmarin, N. N. Vasil'ev, and V. A. Ambrosov, Rapid Methods of Statistical Analysis in Experimental Planning [in Russian], Leningrad (1975).
2. R. P. Nartsissov, Arkh. Anat., No. 5, 85 (1969).
3. M. B. Shishova, A. A. Kamenskii, V. V. Meretskov, et al., Byull. Éksp. Biol. Med., No. 5, 55 (1983).
4. A. Constantopoulos and V. A. Najjar, Cytobios, 6, 97 (1972).
5. G. Florentin, M. Bruley-Rosset, N. Kiger, et al., Cancer Immunol. Immunother., 5, 211 (1978).
6. A. Horsmanheimo, M. Horsmanheimo, and H. H. Fudenberg, Clin. Immunol. Immunopath., 11, 251 (1978).
7. L. S. Kaplow, Blood, 10, 1023 (1955).
8. I. Martinez, F. Winternitz, and I. Vindel, Europ. J. Med. Chem. Clin. Ther., 12, 511 (1977).
9. V. A. Najjar, D. Konopinska, M. K. Chaudhure, et al., Molec. Cell. Biochem., 41, 3 (1981).
10. K. Nishioka, Brit. J. Cancer, 39, 342 (1979).
11. A. I. Sbarra and M. L. Karnovsky, J. Biol. Chem., 234, 1355 (1959).
12. Z. Spirer, V. Zakuth, A. Golander, et al., J. Clin. Invest., 55, 198 (1975).
13. E. Tzehoval, S. Segal, Y. Stabinsky, et al., Proc. Nat. Acad. Sci. USA, 75, 3400 (1978).