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POTENTIATING ACTION OF METABOLIC PRODUCTS OF BLOOD LYMPHOCYTES ON POLYMORPH VULNERABILITY

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The supernatant obtained after culture of sensitized blood lymphocytes with an appropriate allergen (tuberculin) potentiates injury to polymorphs (V. A. Fradkin's index of blood neutrophil injury).
KEY WORDS: lymphocytes; polymorphs, allergy.

Until recently comparatively little attention had been paid to the study of the role of polymorphonuclear leukocytes in the development of immunity and of specific sensitization.

It was shown in the 1950s that the blood neutrophils can be modified and destroyed by the action of a specific antigen–antibody complex [4, 7]. On this basis, granulocytic diagnostic tests began to be used in allergology, including the NII (blood neutrophil injury index in vitro) test suggested by Fradkin in 1962. It was later shown [1, 2] that the mechanism of the NII test falls into the category of tests with target cells to an antigen–antibody complex.

The attention of immunologists is nowadays drawn not only to the migration of polymorphs from the bloodstream, the phagocytosis of immune complexes, and liberation of mediators, but also to humoral complement-dependent factors of neutrophil chemotaxis [5]. Papers were published in which the effect of neutrophils was examined on the level of specific responses of the blood lymphocytes such as the blast-formation phenomenon [3].

The object of this investigation was to study the effect of metabolic products of lymphocytes, transferred into liquid medium during culture of mononuclear cells in vitro, on the ameboid reaction of the blood neutrophils. The starting point was the known fact [6] that during culture of cells of lymph nodes of a sensitized guinea pig in the presence of antigen a factor with a cytotoxic action on the other elements passes into the supernatant.

EXPERIMENTAL METHOD AND RESULTS

The action of the supernatant on the intensity of ameboid activity of the blood neutrophils was studied by the NII test on 53 patients aged from 17 to 45 years with various forms of pulmonary tuberculosis, predominantly in the phase of activation of the disease. The response of the blood neutrophils was assessed in full accordance with the conditions of the NII test as described by Fradkin.

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TABLE 1. Effect of Supernatant Obtained by Culturing Lymphocytes and Various Concentrations of Tuberculin on NII for Patients with Pulmonary Tuberculosis

Variant	Conditions of NII test	Values of NII	
		in phase of active process	in phase of subsiding relapse
1	With tuberculin	0.24	0.09
2	With supernatant, containing		
	Minimal quantity of tuberculin	0.11	0.05
	Maximal quantity of tuberculin	0.48	0.39
3	With medium No. 199 containing		
	Minimal quantity of tuberculin	-	0
	Maximal quantity of tuberculin	-	0.05

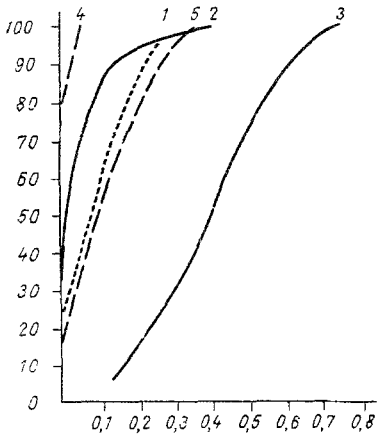

Fig. 1. Empirical distribution functions of indices of NII tests on patients with pulmonary tuberculosis in stage of subsiding relapse. Ordinate, cumulated frequencies (in %); abscissa, value of NII. 1) NII with tuberculin; 2) NII with supernatant containing minimal quantity of tuberculin; 3) NII with supernatant containing maximal quantity of tuberculin; 4) NII with medium No. 199 containing minimal amount of tuberculin; 5) NII with medium No. 199 containing maximal quantity of tuberculin.

To obtain the suspension of lymphocytes, blood from patients with active forms of pulmonary tuberculosis and with blood group I (0), for which a high NII value had previously been established, was used. Heparinized venous blood in a volume of 8-10 ml was allowed to stand at 37°C for 30-40 min. The upper layer of plasma formed after standing was drawn off. To isolate the lymphocyte the suspension of leukocytes was poured into a glass column packed with sterile cotton, so that the layer of the cotton filter was completely soaked with the plasma thus obtained. The column was incubated in the horizontal position for 30 min at 37°C. After incubation the cotton filter was rinsed with medium No. 199 in a volume equal to the volume of leukocyte suspension added, and the number of lymphocytes in 1 mm^3 of the filtrate was counted. The resulting suspension of lymphocytes was centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the cell residue resuspended in culture medium containing 30% autologous plasma and 70% medium No. 199 with an assigned number (800,000-1,200,000) of lymphocytes to 1 ml. The culture of lymphocytes was then poured in a volume of 1 ml into each of a series of penicillin flasks and tuberculin was added. To introduce the tuberculin into

the lymphocyte culture, the contents of an ampul of dry tuberculin were dissolved in 1 ml medium No. 199 and added at the rate of 0.1 ml tuberculin solution to 1 ml of the cell culture. The cells were grown for 96 h in medium containing an increased concentration of CO_2. At the end of the incubation time the lymphocyte culture was discarded by centrifugation and the supernatant was used to assess injury to the neutrophils.

The main experiments were carried out in three variants

1. The NII test with tuberculin. The contents of an ampul of dry tuberculin were diluted in 1 ml of 5% sodium citrate solution. Next, 0.08 ml of the patient's blood was added to 0.02 ml of a mixture of allergen and anticoagulant.

2. The NII test with supernatant obtained by culture of lymphocytes with tuberculin. To 0.8 ml of supernatant obtained by culturing lymphocytes with tuberculin 0.2 ml of 25% sodium citrate solution was added. For the test, 0.08 ml of blood was added to 0.02 ml of the mixture.

3. The NII test with supernatant obtained by culture of lymphocytes with tuberculin in a concentration approximately the same as the content of allergen in the first variant. However, the preparation was dissolved in 1 ml of supernatant and not in sodium citrate. Just as in the first variant, it was first mixed with 25% sodium citrate solution and the contents of an ampul of dry tuberculin were dissolved in 1 ml of the mixture, after which the NII test was set up.

The NII test with supernatant obtained by culturing lymphocytes without tuberculin served as the control: To 0.8 ml of supernatant 0.2 ml of 25% sodium citrate solution was added, after which 0.08 ml blood was added to the tube containing 0.02 ml of the mixture.

The effect of the supernatant obtained by culturing lymphocytes with tuberculin (variant 2) on the neutrophils was studied initially with blood cells from 33 patients with pulmonary tuberculosis in the phase of relapse (Table 1). The mean value of NII when the reaction was carried out with tuberculin alone was 0.24. In the case of the action of supernatant obtained by cultivation of lymphocytes with tuberculin on the cells, the mean value of NII was 0.11, but when the same supernatant, but with tuberculin dissolved in it, was used, the mean index rose to 0.48. In the control the supernatant obtained by culturing cells without the allergen caused virtually no injury to the neutrophils.

It is thus perfectly evident that the medium taken after culture of lymphocytes stimulated by tuberculin causes injury to neutrophils obtained from a specifically sensitized animal. This conclusion does not, however, rule out the probability that injury to the cells was the result of the presence of a certain quantity of allergen in the supernatant. The increased vulnerability of the neutrophils to supernatant with tuberculin additionally dissolved in it (variant 3) and also compared with the action of tuberculin alone (variant 1) could be attributed to a simple increase in the dose of the preparation.

To shed light on this problem a special investigation was made of 20 patients with pulmonary tuberculosis in the stage of subsiding relapse. Besides the three variants of the experiment described above, a further two additional NII tests were carried out with the minimal (4) and maximal (5) amounts of tuberculin, i.e., with concentrations of allergen equivalent to the concentrations of the second and third variants.

The method of the additional test was as follows.

4. Dry tuberculin in an ampul was dissolved in 1 ml of medium No. 199 and this liquid preparation was diluted 1:10 with medium No. 199. The resulting dilution of allergen corresponded to the minimal quantity which was constantly used for cell culture. This solution (0.8 ml) was mixed with a 25% solution of sodium citrate (0.2 ml) and the NII test was set up.

5. To obtain the maximal concentration of tuberculin, 1 ml of medium No. 199 containing the minimal quantity of allergen (variant 4) was treated with contents of another ampul of tuberculin.

The results of the additional tests showed (Fig. 1) that in the presence of the minimal concentration of tuberculin (when the allergen was dissolved in medium No. 199) the blood neutrophils were virtually not injured at all. On the other hand, in the presence of the maximal concentration of the preparation, the cells were injured much less than when the allergen was present in its concentration of the supernatant obtained after the end of culture of the lymphocytes (differences statistically significant: criterion λ in the first variant 1.48, in the second 1.89).

When discussing potentiation of immunological injury to the blood neutrophils by metabolic products of lymphocytes isolated from sensitized donors it must be remembered that the specific responses of lymphocytes to antigen are potentiated in the presence of polymorphonuclear leukocytes [3].

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