ACTION OF ANTILYMPHOCYTIC SERUM ON HYPERSENSITIVITY OF DELAYED TYPE IN TUBERCULOSIS

M. M. Averbakh, V. I. Litvinov, and A. M. Moroz

UDC 616-002.5-056.3-085.365.018.53

The action of antilymphocytic serum (ALS) on hypersensitivity of delayed type (HDT) was studied in experiments on 255 guinea pigs infected with tuberculosis and vaccinated with BCG. After administration of ALS the lymphocytes disappeared from the paracortical zones of the lymph glands and periarterial zones of the white pulp of the spleen. A decrease in the activity of certain enzymes (acid and alkaline phosphatases) and in the RNA content and incorporation of thymidine-H^3 were observed. Administration of ALS inhibited the development of HDT induced in vivo (tuberculin tests) and in vitro (blast transformation of lymphocytes test and inhibition of migration of peritoneal exudate cells from the capillaries).

Hypersensitivity of delayed type (HDT) plays an important role in the resistance of the organism to tuberculosis [1, 3, 5, 10]. Injury or inactivation of the HDT must influence the state of resistance to the infecting agent. Antilymphocytic serum (ALS) acts on HDT in a manner similar to antigens of mycobacteria [8, 15]. In many infections – typhus fever [4], leprosy [6], smallpox [9], etc. – it adversely affects treatment of the disease. When ALS was injected into animals infected with a virulent strain of Mycobacterium tuberculosis, it led to an increase in miliary dissemination and to earlier death of the animals [1].

The object of the present investigation was to study the action of ALS on HDT during infection with tuberculosis and vaccination.

EXPERIMENTAL METHOD

Experiments were carried out on 255 guinea pigs. Some of the animals were infected with the virulent strain M. tuberculosis Bovinus-8 in a dose of 0.0001 mg. Half of the animals of this group were infected immediately after injection of 1 ml ALS (a further 1 ml was injected after 24 h). Another group of animals was vaccinated with BCG in a dose of 1 mg. Half of the animals of this group were given two injections of ALS at an interval of 24 h 2.5 months after vaccination. All the animals of this group were then infected with the virulent strain Bovinus-8 in a dose of 0.0001 mg. The second half of both groups of animals received normal rabbit serum (NRS) by the same scheme. The ALS was injected by the method of Heise and Weiser [8]. The methods of carrying out the migration inhibition and blast-transformation tests were described previously [3]. The tuberculin tests were carried out with PPD, which was injected intradermally in a dose of 0.1 ml and a dilution of 1:10. The sections of the lymph glands and spleen were stained with hematoxylin-eosin, for acid and alkaline phosphatase and for DNA and RNA; autoradiographic tests with thymidine-H^3 and electron microscopy also were used.

EXPERIMENTAL RESULTS

Histological investigation of the lymph glands and spleen of the infected animals showed that 5-7 days after infection marked proliferation of small lymphocytes and blast cells occurred in the paracortical zones

Laboratory of Experimental Pathology, Central Research Institute of Tuberculosis, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Shmelev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 3, pp. 79-82, March, 1973. Original article submitted May 16, 1972.

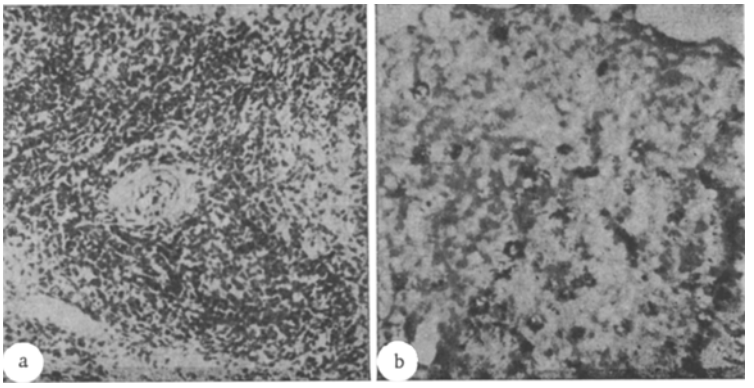

Fig. 1. Proliferative reactions in thymus-dependent zones of the spleen and lymph glands of guinea pigs vaccinated with BCG: a) white pulp of spleen 2.5 months after vaccination. Marked proliferation of lymphocytes in periarterial zone, 150 ×; b) cortex of regional lymph gland 2.5 months after vaccination. Active incorporation of thymidine-H^3, 150 ×.

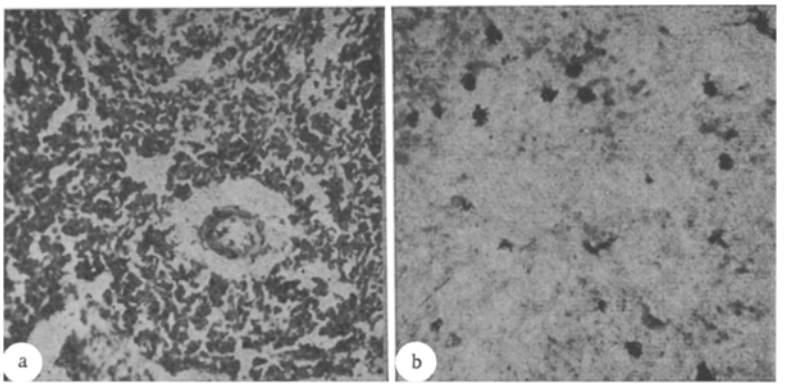

Fig. 2. Action of ALS on thymus-dependent zones of the spleen and lymph glands of guinea pigs vaccinated with BCG: a) white pulp of spleen 2.5 months after vaccination; depopulation of periarterial zones 24 h after injection of ALS, 150 ×; b) cortex of regional lymph gland 2.5 months after vaccination; 24 h after injection of ALS activity of thymidine-H^3 incorporation is reduced, 150×.

of the lymph glands and periarterial zones of the white pulp of the spleen (Fig. 1). These cells actively incorporate thymidine-H^3, stain positively for RNA, contain acid phosphatase granules, and in the electron microscope are found to contain polysomes and lysosomes. The same picture was visible in these zones 1-2.5 months after vaccination. The lymphocytes began to disappear from these zones 24 h after the injection of ALS and the process reached a maximum one week after injection of the serum (Fig. 2), when these zones were mainly filled with histiocytes and reticular cells. Histochemical investigation of these zones revealed a sharp decrease in activity of acid and alkaline phosphatases, DNA, and RNA and their thymidine-H^3 incorporation was much less marked than in animals not receiving ALS.

The blast-transformation reaction in the infected animals receiving ALS was almost completely suppressed throughout their period of survival, whereas in the infected animals not receiving ALS the number of blast cells 21-30 days after infection had almost reached the level characteristic of the vaccinated animals (Fig. 3a). In the vaccinated animals 2.5 months after vaccination the mean percentage of blast cells was 5.2. As a result of subsequent infection the number of blast cells increased very slightly after 1 week, but it then fell during the spread of the tuberculous infection. In the vaccinated animals receiving ALS a

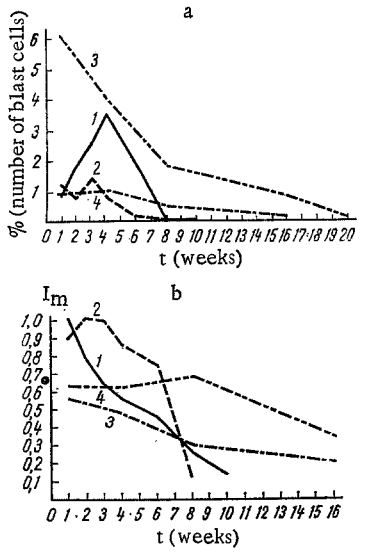

Fig. 3. Action of ALS on tests for HDT in vitro: a) blast-transformation reaction in infected and vaccinated guinea pigs, treated and not treated with ALS, respectively; b) migration-inhibition test in infected and vaccinated guinea pigs, treated and not treated with ALS, respectively. 1) Bov. 8; 2) Bov. 8 + ALS; 3) BCG + Bov. 8; 4) BCG + ALS + Bov. 8.

sharp and statistically significant decrease in the number of blast cells was observed 24 h after the injection. When these animals were subsequently infected, throughout the period of their survival the number of blast cells showed no tendency to rise.

The reaction of inhibition of migration of cells of the peritoneal exudate from capillaries was not detectable in the infected animals until the 4th week of infection, after which migration began to be inhibited very slightly, and 2 months after infection it was sharply inhibited (Fig. 3b). From 1.5 to 2 months after infection the inhibition of migration was more marked in animals treated with ALS than in animals treated with NRS (difference not statistically significant). In the animals which were vaccinated and then infected there was a gradual increase in the intensity of the reaction, which was especially marked 2 months and more after infection. In the animals of this group treated with ALS no sharp inhibition of migration was observed until 4 months after infection (the difference from the group of animals treated with NRS was statistically significant 1 and 2 months after infection), but even 4 months after infection the inhibition of migration was not so marked as in guinea pigs not receiving ALS.

The tuberculin tests on the infected animals gave the largest reactions 4-5 weeks after infection and they decreased in size shortly before death. In animals treated with ALS the tuberculin tests gave weak results throughout the period of the experiment. The intensity of the tuberculin tests was regarded as average (mean size 15 × 15 mm) 2 months after vaccination, and during subsequent infection after a slight initial insignificant increase they diminished gradually; in the vaccinated animals treated with ALS the tuberculin tests were weak throughout the period of the experiments.

ALS greatly lowers the resistance of guinea pigs to tuberculosis and also the immunity of the animals after vaccination [1, 15]. This is due chiefly to injury to the mechanisms of hypersensitivity of delayed type (ALS inhibits blast transformation, the sensitivity of the skin to tuberculin, and the ability of lymphocytes to inhibit migration of macrophages from capillaries). The marked inhibition of migration of macrophages in animals treated with ALS 2 months after infection was evidently attributable to the high vulnerability of the cells in the terminal stage of the disease. Morphological investigations of the lymph glands and spleen also show that the main action of ALS is directed against the thymus-dependent zones of these organs – the paracortical zones of the lymph glands and the periarterial areas of the white pulp of the spleen, i.e., on the population of long-living, recirculating, thymus-dependent lymphocytes responsible for the development and manifestation of hypersensitivity of delayed type [2, 12, 13, 14, etc.]. Meanwhile ALS has a negligible influence on the level of the serum antituberculosis antibodies [1].

In tuberculosis the mycobacteria are destroyed chiefly (if not entirely) intracellularly in the macrophages. From this point of view it is interesting to note that ALS, while sharply reducing resistance, has hardly any effect on phagocytosis in tuberculosis, while the antimacrophagal serum, while reducing the number of macrophages in the body, has a negligible effect on resistance to tuberculosis. It can therefore be concluded that phagocytosis of myocbacteria is ineffective without the participation of certain mechanisms of HDT.

Evidence in support of this hypothesis is already available; it has been shown, for example, that macrophages taken from vaccinated animals treated with ALS are still able to ingest, but not to destroy, mycobacteria. It has also been shown that sensitized lymphocytes and cytokinins (mediators of HDT) stimulate phagocytosis of mycobacteria in vitro [1, 11].

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