EFFECT OF SPLENIN ON THE DEVELOPMENT OF TUBERCULIN SHOCK AND BORDET'S PHENOMENON IN GUINEA PIGS

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The search for ways of making the treatment of tuberculosis more effective is proceeding in the direction not only of synthesis of new antituberculosis agents, but also the search for ways of influencing immunologic reactivity. According to preliminary data, splenin may be one such agent.

This paper describes the study of the mechanism of action of splenin on models of experimental tuberculosis.

EXPERIMENTAL METHOD

Experiments were carried out on 130 guinea pigs weighing 500 ± 30 g. The initial immunologic parameters were tested in 10 animals (group 1), and the remaining 120 guinea pigs were infected with *Mycobacterium tuberculosis* $H_{37}Rv$, injected in a dose of 0.01 mg in 0.5 ml physiological saline subcutaneously into the right inguinal region. After infection the animals were divided into six groups (20 guinea pigs in each group): group 2 was the control for infection with tuberculosis, group 3 consisted of infected animals treated with splenin (Darnitskii Pharmaceutical Chemical Factory, bath 190777) in a dose of 0.1 ml/kg body weight subcutaneously on alternate days for 1 month, group 4 consisted of animals infected with *M. tuberculosis* in which tuberculin shock was induced on the 37th day by intraperitoneal injection of old tuberculin (1:10, 1 ml), group 5 consisted of animals in which tuberculin shock was induced after a month's course of splenin, group 6 contained guinea pigs in which Bordet's phenomenon was induced on the 49th day after infection by injection of 1 ml of a 24-h culture of *E. coli* (10 units based on an optical standard), and group 7 consisted of guinea pigs in which Bordet's phenomenon was induced after a course of splenin.

Spontaneous mortality and mortality due to tuberculin or *E. coli* among the animals was counted. Five surviving animals from each group were sacrificed 24 h after induction of tuberculin shock or Bordet's phenomenon. Macroscopic lesions in the viscera were assessed in points, the population composition of thymus, spleen, and inguinal lymph node cells was determined in squash preparations (in 5000 cells counted), and the number of E and EAC rosette-forming cells (RFC) in the above-mentioned organs and bone marrow was determined by the method of Sandberg et al. [3]. The peripheral blood lymphocyte blast transformation test (LBTT) also was studied in cultures with phytohemagglutinin (PHA) and tuberculin, and titers of complement and antituberculous antibodies in the serum were determined in microtitrators of "Takats" type; for convenience of evaluation, $\log_2$ of dilution was calculated in both tests. The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS

The degree of involvement of the viscera in guinea pigs infected with tuberculosis and treated with splenin (group 3) was indistinguishable from that in the control (group 2; Fig. 1). In tuberculin shock preliminary injection of splenin (group 5) reduced the severity of the pathological changes, but it had the opposite effect on induction of Bordet's phenomenon.

Injection of tuberculin evoked a severe general reaction (tuberculin shock), which ended in death of seven of the 20 animals. A heteroallergic reaction to intraperitoneal injection of *E. coli* was the cause of death of two of 14 guinea pigs with typical signs of Bordet's phenomenon.

Preliminary treatment with splenin increased the number of animals surviving after tuberculin shock: Of 14 guinea pigs receiving tuberculin only three died. Bordet's phenomenon ended lethally in eight of 14 guinea pigs treated with splenin ($P < 0.05$).

Cytological investigation of squash preparations from guinea pigs treated with splenin showed a tendency for the number of juvenile and, in particular, of mature plasma cells in the spleen to rise. Gaseous changes in the regional lymph nodes were less marked, and under the influence of splenin the number of blast cells in the contralateral lymph nodes

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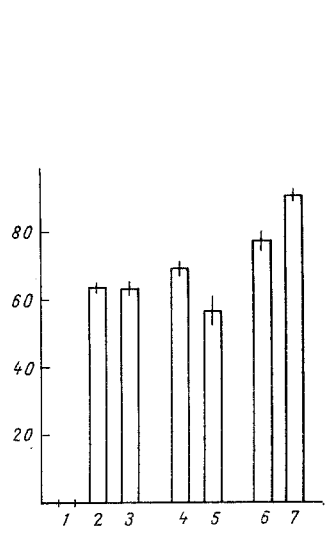

Fig. 1

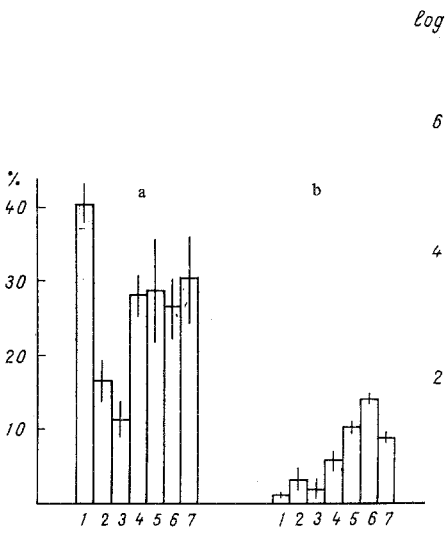

Fig. 2

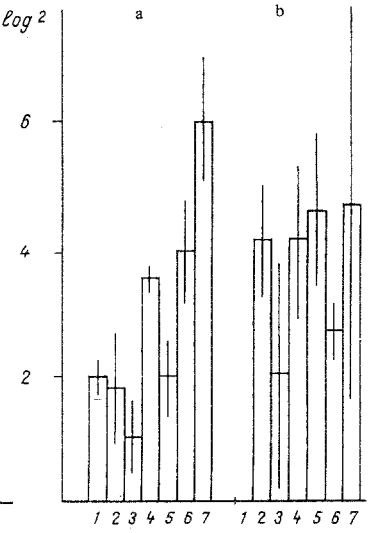

Fig. 3

Fig. 1. Severity of tuberculous foci in viscera of guinea pigs. Abscissa, group of animals; ordinate, severity of lesions (in points).

Fig. 2. Blast-transformation of peripheral blood lymphocytes of guinea pigs in cultures with PHA (a) and tuberculin (b). Abscissa, group of animals; ordinate, number of blast cells (in %).

Fig. 3. Titers of complement (a) and antituberculous hemagglutinating antibodies (b). Abscissa, group of animals; ordinate, $\log_2$ of titers.

increased to 20.7 ± 0.7 (in the control animals of group 2 the number was 10.0 ± 2.8; $P < 0.02$), and the number of prolymphocytes increased to 40.0 ± 7.6 (15.0 ± 6.7 in group 2).

The number of blast cells in the contralateral lymph nodes of the animals in which tuberculin shock was induced (group 4) was only 1.0 ± 1.0 per 5000 cells. Preceding treatment with splenin (group 5) increased the number of blast cells to 11.7 ± 4.4 ($P < 0.05$), but had no appreciable effect on the number of prolymphocytes. These findings are evidence of a definite stimulating effect of splenin on lymphoid proliferation.

The study of LBTT with PHA (Fig. 2) showed differences in intensity in different groups. By the 36th day after infection significant ($P < 0.001$) inhibition of the blast response of the T lymphocytes to PHA was observed in the animals of group 2, and this did not recover after injection of splenin. Injection of tuberculin (group 4) and *E. coli* (group 6) increased the reactivity of the lymphocytes to PHA. Preceding splenin therapy (groups 5 and 7) had no additional stimulating effect on this parameter.

The response of the lymphocytes *in vitro* to tuberculin in the infected animals during this period was low (3.7 ± 1.7%). In animals receiving tuberculin (group 4), specific blast formation amounted to 5.8 ± 1.1%, and splenin therapy potentiated the response ($P < 0.01$). Induction of Bordet's phenomenon (group 6) evoked a sharp response of the lymphocytes to tuberculin, but preliminary injection of splenin (group 7) significantly depressed it (Fig. 2).

Tuberculin shock caused a decrease in the number of E-RFC in the spleen to 22.8 ± 2.3% (35.0 ± 2.3% in the intact animals; $P < 0.01$). Preliminary injection of splenin increased the number of E-RFC to higher figures (44.0 ± 1.6%; $P < 0.01$). The development of Bordet's phenomenon did not reduce the number of E-RFC in the spleen (40.2 ± 3.4%), and splenin therapy given before induction of Bordet's phenomenon did not affect this parameter (40.7 ± 5.2%). No regular changes in the number of E-RFC depending on the type of experimental procedure performed on the animals could be found in the thymus and bone marrow. In the lymph nodes of the guinea pigs of group 7, however, the number of E-RFC was considerably greater (52.7 ± 2.7; $P < 0.01$) than in the animals of group 6 (37.2 ± 3.4%), in which Bordet's phenomenon was induced without a preliminary course of splenin therapy. No EAC-RFC were found in the thymus, and in the spleen, lymph nodes, and bone marrow their number was reduced, especially so in animals exposed to tuberculin shock (11.0 ± 2.5%, 6.3 ± 0.9%, and 4.6 ± 1.1% respectively compared with 32.6 ± 2.1%, 14.6 ± 1.6%, and 7.5 ± 0.6% in intact animals). Preliminary treatment with splenin prevented this fall to some extent in the spleen (17.3 ± 6.1%) and lymph nodes (11.7 ± 4.8%), but not in the bone marrow (3.4 ± 1.0%).

Bordet's phenomenon also reduced the number of EAC-RFC in the spleen (25.0 ± 2.4), lymph nodes (8.0 ± 1.0%), and bone marrow (5.2 ± 0.6%). Preliminary splenin therapy led to normalization of their number (39.7 ± 1.9%, 14.0 ± 3.6%, and 6.3 ± 2.0% respectively).

It will be clear from Fig. 3 that the high complement titer indicates the existence of considerable alternative changes, for it increases considerably in tuberculin shock and during the development of Bordet's phenomenon (groups 4 and 6). Preliminary administration of splenin lowered the complement titer in tuberculin shock (group 5, P = 0.05) and sharply increased it in the animals of group 7 (P = 0.01), in which the lesions in the viscera were most severe (Fig. 1).

No significant changes in titers of antituberculosis hemagglutinins depending on the nature of the experimental procedure were discovered (Fig. 3).

Splenin thus induced considerable changes in the course of the general tuberculin reaction and of Bordet's phenomenon, the pathogenetic mechanisms of which differ significantly, the reason being probably that splenin has different effects on the cellular and humoral reactions in animals infected with tuberculosis. Treatment with splenin proved to be effective for the correction of immunologic disturbances due to tuberculin shock, but not to Bordet's phenomenon.

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