MODIFICATION OF HUMORAL IMMUNITY IN MICE BY SCORPION VENOM

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It has now been shown that the venoms of various species of scorpions possess marked immunotropic properties. Research undertaken in this direction has been mainly concerned with the preparation of antisera [6, 9, 11], the formation of immunity [8, 12], and determination of the antigenic properties of the toxin [5].

With its high biological activity [4], scorpion venom is of considerable interest in connection with its possible effect on immunologic reactivity *in vivo*.

The action of venom of the scorpion *Buthus caucasicus* on the state of humoral immunity in mice was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 470 albino mice weighing 18-20 g, immunized with a 10% suspension of sheep's red blood cells and kept on the ordinary animal house diet. The venom was injected intraperitoneally in doses of 0.25, 0.5, 1, 2, and 3 mg/kg body weight, 48 h before immunization, simultaneously with, and 48 h after immunization (at different stages of the primary immune response). Sheep's red blood cells and physiological saline were injected into the control mice. The immunoreactivity of the animals was assessed as the number of antibody-forming cells (AFC) in the mouse spleen by a modified method [7] on the 3rd, 5th, 7th, and 10th days of the primary immune response. Antibody titers in the blood serum were determined by the hemagglutination test. The number of rosette-forming lymphocytes (EAC) was counted in the peripheral blood [10]. Statistical analysis of variance was carried out by Student's t test at the $P = 0.05$ level on the M 7000 computer. The program was written in FORTRAN-IV language.

EXPERIMENTAL RESULTS

Scorpion venom has a modifying action on immunologic responses *in vivo*. The character of its effect on the parameters studied depends on dose. In doses of 0.5-2 mg/kg, injected 48 h after immunization, the venom caused a well-marked increase in the number of AFC. Under the influence of the toxin in a dose of 1 mg/kg the number of cells on the 5th day of the primary response increased to $304.87 \pm 34.76/10^6$ spleen cells, or 135.50% of the control value (Fig. 1b).

Injection of the venom in a dose of 3 mg/kg after immunization caused a distinct fall in the number of AFC (Fig. 1c). Comparison of the agglutinin titers in the experimental and control animals revealed similar changes.

A single injection of the venom in a dose of 1 mg/kg at different periods of immunogenesis appreciably modified the character of the response to the antigen (Fig. 2). Attention is drawn to the fact that scorpion venom did not affect the immunologic response when injected 48 h before immunization (Fig. 2b). Injection of the toxin simultaneously with immunization inhibited antibody formation from $267.68 \pm 8.35/10^6$ cells in the control to $173.38 \pm 6.70/10^6$ spleen cells ($P < 0.001$; Fig. 2c). Considerable stimulation of the immune response was observed when the venom was injected in the productive phase of immunogenesis. The number of AFC increased from 239.74 ± 10.53 to $324.87 \pm 34.76/10^6$ spleen cells ($P < 0.05$; Fig. 2d).

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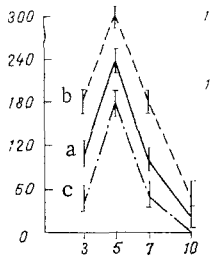

Fig. 1

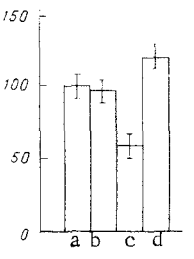

Fig. 2

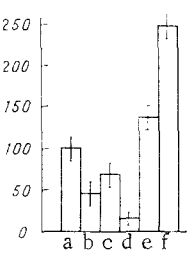

Fig. 3

Fig. 1. Effect of scorpion venom on time course of changes in number of splenic AFC in mice during primary immune response: a) control, b) venom in a dose of 1 mg/kg, c) in a dose of 3 mg/kg. Abscissa, days of investigation; ordinate, number of AFC per 10^6 nucleated spleen cells.

Fig. 2. Dependence of action of scorpion venom in a dose of 1 mg/kg on time of injection: a) control, b) venom injected before immunization, c) simultaneously with immunization, d) after immunization. Ordinate, number of AFC on 5th day of immune response (in % of control).

Fig. 3. Effect of various doses of scorpion venom on number of EAC in peripheral blood of mice: a) control, b) venom in dose of 0.25 mg/kg, c) 0.5 mg/kg, d) 1 mg/kg, e) 2 mg/kg, f) 3 mg/kg. Ordinate, EAC level (in % of control).

These results show that the scorpion venom did not affect immunocompetent cells in the G_0 phase of the cell cycle and that it activated processes developing after the antigenic stimulus and the proliferative stage of the immune response.

In the next series of experiments the action of scorpion venom on the number of EAC was studied. The smallest of the doses of toxin tested (0.25-1 mg/kg) caused a marked depressor effect on the level of B cells (Fig. 3b-d).

An increase in the dose to 2 and 3 mg/kg led to an increase in the number of EAC (Fig. 3e, f).

To study the effect of the venom, depending on the time of its injection, on the EAC level only one dose was used, namely 1 mg/kg. The first result be noted was the inhibitory action of the toxin on EAC at all times of administration. A more than 90% decrease in the number of cells (from 12.42 ± 0.45 to 1.82 ± 0.11%; $P < 0.001$) was observed when the toxin was injected after immunization. Smaller changes were found when the venom was injected before the antigen and simultaneously with it (to 4.21 ± 0.18%, $P < 0.001$ and 7.32 ± 0.26%, $P < 0.001$ respectively).

The immunotropic action of the venom was thus determined both by its concentration and by the time of its administration. As regards the opposite direction of the changes in the humoral immunity system under the influence of high and low doses of the venom, an explanation can be postulated on the basis of its chemical composition and the known mechanisms of its action on the body. Scorpion venom has been shown to interact actively with adrenergic and cholinergic structures [1, 4]. Depending on the dose of the venom, predominant activation of one or other mediator systems takes place, and this leads to the characteristic changes in the parameters observed. This suggestion thus appears to be soundly based because, in the modern view, during regulation of immunogenesis the influences of the cholinergic and adrenergic systems are interdependent [2, 3].

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IMMUNE COMPLEXES IN THE THYMUS IN RHEUMATIC FEVER

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Germinal centers — focal concentrations of lymphoid cells in the corticomedullary and medullary zones of the lobules, are found in the thymus in certain diseases. These formations are particularly frequent in the thymus of patients with myasthenia, but they are also found in systemic lupus erythematosus (SLE), rheumatic fever, and thyroiditis [2, 3, 5-9]. According to some investigators, these concentrations of lymphoid cells consist of "prohibited clones" of lymphocytes which arise in the thymus under pathological conditions and are immunologically competent with respect to the antigens of this gland [2, 5]. According to another point of view, concentrations of lymphoid cells in the parenchyma of the thymus are analogous to lymphoid infiltrations which may be formed in any injured organ as a result of penetration of lymphoid cells from the blood stream [5]. Injury to the tissue of an organ is known to be accompanied by the appearance of bound immunoglobulins in the damaged cells and by the deposition of immune complexes in the internal medium of the organ. Deposition of immune complexes containing IgM, IgA, IgG, and complement have been found in the medullary zone of the thymus in patients with myasthenia [1]. Immune complexes also have been found in the thymus tissues of patients with SLE [4].

In the investigation described below a search was made for immune complexes in the thymus tissues of patients with rheumatic fever.

EXPERIMENTAL METHOD

Immune complexes were detected by the use of fluorescein isothiocyanate-labeled globulin fractions isolated from the blood serum of animals (rabbit, sheep) immunized with myeloma IgM and IgA or normal human IgG (from the N. F. Gamaleya Institute of Epidemiology and Microbiology). The presence of complement in thymus sections of rheumatic fever patients was determined by means of a fluorescein isothiocyanate-labeled globulin fraction isolated from the serum of a sheep immunized with the C3 component of human complement (from Hyland, USA). Thymus sections from rheumatic fever patients undergoing operations for valve implantation at the age of 8-18 years (27 cases) and from persons dying from acute trauma at the age of 8-22 years (16 cases) were studied. Frozen sections 5-6 μ thick were prepared from unfixed thymus tissue frozen in petroleum ether at −86°C. Unfixed sections from the thymus of a rheumatic fever patient and from a control thymus, placed on the same slide, were washed for 30 min in a current of buffered physiological saline (BPS), pH 7.5, and treated with the labeled preparations for

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