CYTOSEROLOGIC INVESTIGATION OF RAT LYMPHOID TISSUE AFTER THERMAL TRAUMA

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The question of the effect of burn trauma on the immunobiological state of the organisms has recently engaged the attention of investigators. It has been shown that the pathological proteins formed in burns possess the properties of autoantigens and that they circulate for a long time in the blood of burned patients, causing autosensitization [7-9]. Investigators [2-6, 13, 14] using serologic methods have demonstrated "antiburn antibodies" in the blood of burned patients and of experimental animals receiving burn injuries, and this has been regarded as proof of the presence of autoimmune processes in burns.

Meanwhile, however, many aspects of the problem of the immunology of burns have remained unsolved and debatable [10, 11, 12], notably the problem of the state of the lymphoid tissue of the burned organism.

In the present investigation the lymphoid tissue of albino rats was studied after burn trauma by means of an immunomorphological test — the plasma-cell reaction.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing about 250 g. A thermal burn was inflicted on the carefully shaved skin over the posterior part of the spine by means of the flame from cotton wool soaked in spirit. Two groups of animals were used. The rats of group 1 received a burn covering 20% of the body surface with an exposure of 60 sec (severe burns) and those of group 2 a burn of 10% of the body surface with an exposure of 30 sec (mild burns). At various times after infliction of the burn, the rats were sacrificed (3 rats at each time), and a cytoserologic investigation made of the immunologic activity of the lymphoid tissue.

The animals were lightly anesthetized with ether and opened up, after which the organs were perfused through the left ventricle. The cytological investigation of the lymph glands and spleen was carried out by the impression method from freshly cut organs as described by G. A. Gurvich [1]. The impression preparations were dried at room temperature, fixed in methyl alcohol for 5-10 min, and stained with azure II eosin.

To estimate the plasma-cell reaction quantitatively, the cells of the plasma cell series were counted in 50 fields of vision (using an immersion objective). The counting was done in accordance with the classification of antibody-forming cells adopted at the Prague symposium in 1960: group 1 included young forms (plasmablasts, transition forms, and immature plasma cells), and group 2 mature plasma cells.

EXPERIMENTAL RESULTS

In preliminary experiments, to determine the regional distribution of lymph drainage from the burned area, a solution of ink was injected into the animals at the site of the burn. Maximal accumulation of the ink was found in the inguinal lymph glands. Subsequently, therefore, the cytoserologic investigation was carried out on the inguinal lymph glands, as the regional glands, and on the spleen.

The results of the cytological investigation of the rats of group 1 with severe burns are given in Fig. 1.

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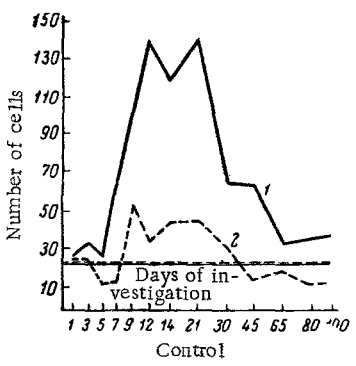

Fig. 1

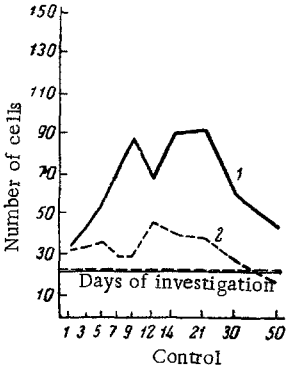

Fig. 2

Fig. 1. Changes in plasma cell reaction in the lymphoid organs of rats with "severe burns." 1) Inguinal lymph glands; 2) spleen. Abscissa — days of investigation, ordinate — number of cells.

Fig. 2. Changes in the plasma cell reaction in the lymphoid organs of rats with "mild burns." Legend as in Fig. 1.

In the unburned control rats the number of cells of the plasma cell series was comparately small — 23/32 in the inguinal lymph glands and 24/25 in the spleen, where the numerator represents the number of cells of group 1 (immature forms), and the denominator the number of mature plasma cells.

Cytological investigation of the impression films from the rats on the 1st, 3rd, and 5th days after trauma showed no essential changes in the plasma cell reaction either in the lymph glands or in the spleen.

It was only on the 7th day after burning that the number of cells of group 1 began to increase in the inguinal lymph glands — they consisted mainly of large basophilic cells with a large, indistinctly outlined nucleus. The number of these cells showed a sharp increase in the impressions obtained from the rats on the 7th-9th day after burning, and on the 12th day it reached 140 cells in 50 field of vision. The cell reaction remained well defined until the 21st day, after which it diminished abruptly, and by the 30th day only 65 cells were counted. Subsequently, the plasma cell reaction fell gradually; starting with the 65th day, the number of cells of group 1 remained approximately constant and within normal limits.

In contrast to the well defined plasma cell reaction in the inguinal lymph glands, the plasmablast reaction in the spleen was much weaker and was only slightly above the control level. At the same time, the general pattern of the changes in the curves in the inguinal lymph glands and in the spleen was very similar.

In the rats of group 1, the serologic activity of extracts of the lymph glands and spleen was investigated in the early stages by the complement fixation reaction (the cold modification). The antigens used were saline extracts from thermally denatured skin taken from the rats on the 2nd, 12th, and 21st days after burning. In every case, however, negative results were obtained, evidently on account of the weak immunogenicity of the thermally denatured breakdown products, and the correspondingly low titer of antibodies, and also of the inadequate "burn specificity" of the antigens used.

The results of the cytological investigation of the rats of group 2 with mild burns are given in Fig. 2.

The first point to be noted is that in the animals of this group the same general principles apply as in the rats of group 1. The plasmablastic response reaction was well marked in the regional lymph glands, but only very weak in the spleen.

However, unlike in the animals of group 1, in this case the appearance of young forms of plasma cells was observed earlier (on the 3rd-5th day), and their number reached its maximum on the 9th day and continued as a plateau until the 21st day. The number of cells then fell and reached its initial level on the 50th day.

Meanwhile, the general plasmablastic response reaction in the regional lymph glands of the animals of this group was much weaker than in the rats of group 1, a result presumably reflecting the different degrees of activity of the immunologic changes in response to different intensities of burn trauma.

The cytological changes observed in the animals, expressed as an increase in the number of young forms of plasma cells, must evidently be regarded as a morphological reaction reflecting immunologic changes taking place in the organism under the influence of burn trauma.

As a result of these investigations a definite correlation was observed between the plasmablastic reaction and the severity of the burn trauma. It was noted that the intensification of the plasmablastic reaction coincided in time with the beginning of attachment of the scab (preceded it slightly). The maximum of the reaction remained at about the same level throughout the period of detachment of the scab, and this was evidently due to the circulation in the body of breakdown products of thermal denaturation from the burn wound at this period. Next followed a sharp fall in the intensity of the reaction, coinciding in time with complete detachment of the scab. Since in a mild burn the tissue breakdown was less intensive and correspondingly less of the breakdown products entered the blood stream, the plasmablastic response reaction of the organism was weaker.

As the regards the mature plasma cells, no definite correlation could be found between the accumulation of these forms and the dynamics of the plasmablastic reaction. A sharp increase in the number of mature plasma cells was observed in the early period after the burn, and it persisted almost to the end of the investigation. This reaction was particularly intensive in the rats with severe burns; the mature plasma cells filled whole fields of vision. Bearing in mind reports in the literature this fact may be regarded as a response reaction to the circulation of large quantities of toxic breakdown products in the body.

Special attention should be paid to the fact indicating inhibition of the response reaction in severe burns, evidently reflecting the stressor reaction developing in the organism under the influence of the burn trauma. Since the number of cells of the plasma cell series in the inguinal lymph glands in normal conditions in rats is small, the cytological changes characteristic of the alarm reaction could not be clearly defined. Special experiments are therefore required, in which the plasma cell reaction in the cervical lymph glands is analyzed (normally these glands contain large numbers of cells of the plasma cell series) and the involution of the thymicolymphatic apparatus is studied in detail. In this way the special features of the adaptation reaction could be assessed in burn trauma.

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