THE USE OF IMMUNOAUTORADIOGRAPHY TO STUDY THE ANTIGENIC STRUCTURE OF BURNED HUMAN SKIN

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An antigenic component not found in the tissues of clinically healthy persons or in extracts from the leukocytes, pleural exudates, pus from the wounds of burned patients and hemolyzed serum was discovered by the use of a highly sensitive immunoautoradiographic technique in burned human skin (the burn scab).

The antigenic structure of burned human skin has been studied by the agar diffusion test earlier in the author's laboratory. Antigenic components found in the burn scab differed from antigens of bacteria infecting burn wounds and they were not found in normal skin and in most tissues of clinically healthy persons [2, 3, 5-7].

To study the nature of antigens detected in the burn scab the antisera were exhausted with antigens from the spleen, lung, and liver under the control of the agar diffusion test. In subsequent research with these exhausted sera technical difficulties interfered with the further study of the nature of the antigenic components: the antisera after exhaustion with a pool of tissues became viscous, they were highly contaminated with antigens and blood, and the halos formed around the wells containing antigens and serum made it very difficult, and sometimes almost impossible, to record and interpret the results.

With these facts in mind, it was decided to use the method of indirect immunoautoradiography, previously suggested by Rowe [10] for simple diffusion in agar, in order to study the antigenic structure of burned skin; this method could eliminate the difficulties mentioned above and could considerably increase the sensitivity of the agar diffusion test (by 30-50 times), while retaining the high specificity of the method [1, 8, 9].

EXPERIMENTAL METHOD

A variant of the indirect immunoautoradiographic method developed by Él'gort and Abelev [9] was used. In the present investigation the immunoautoradiographic stage of the work was carried out by D. A. Él'gort in the laboratory of immunochemistry and diagnosis of tumors (Head, Professor G. I. Abelev), N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR.

Saline extracts of various tissues were used as the antigens. Antigens from burned human skin (burn scab) were prepared as follows: material was obtained from patients with severe thermal burns during sloughing or excision of areas of burned skin (usually within 2-3 weeks after burning). The burn scab was carefully freed from necrotic areas and pus, cut into small pieces, ground in a mortar with quartz sand, and then homogenized in the proportion of 1 g scab to 9 ml physiological saline. The homogenate was allowed to stand overnight in the refrigerator and then centrifuged (TsLR-1) at 2500-3000 rpm for 30 min. The resulting supernatant was used as the antigen. Antigens from the liver, spleen, and lung were prepared from the organs of clinically healthy persons dying from accident. Material for the antigens from the burned skin of patients with severe thermal injuries was obtained from the cadavers of persons who died from their burns. Preparation of the antigens from the organs of skin was carried out as

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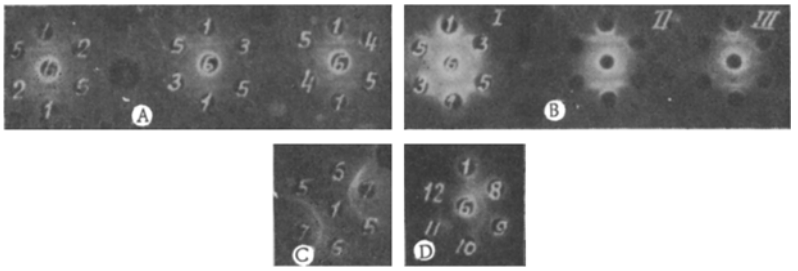

Fig. 1. Antigenic structure of burned human skin (burn scab) studied by immunoautoradiography. A) Peripheral wells contain antigen from burn scab (1) and antigens from liver (2), spleen (3), and lung (4); other wells contain physiological saline (5); central well contains exhausted serum against burned human skin (6). B) I) test system; II) test system diluted 4 times; III) test system diluted 8 times; wells contain antigen (3) and physiological saline (5). C) Peripheral wells contain antiserum (6), serum against human spleen (7), and physiological saline (5); central well contains antigen (1). D) peripheral wells contain antigen (1); protein 10 mg/ml, and antigen in dilutions of 1:4 (8), 1:8 (9), 1:16 (10), 1:32 (11), and 1:64 (12); central well contains antiserum (6).

described above. All solutions of antigen were concentrated, when necessary, in a current of air. The protein concentration in the solutions of antigen from the burn scab varied within wide limits (5-77 mg/kg) depending on the object of the investigation. The protein content in solutions of antigens from the liver, spleen, and lungs was 50-60 mg/ml, and in solutions of antigens from intact skin from patients dying from burns it was 20-25 mg/ml.

Extracts from leukocytes were used as possible sources of "inflammatory" antigens. Leukocytes were isolated from the buffy coat by the addition of 10% gelatin and separated on the centrifuge. After repeated freezing and thawing (on dry ice at −70°C) and grinding with quartz sand in a mortar saline extracts with a protein content of 10-15 mg/ml were obtained. Serous and purulent pleural exudates from patients with cancer and tuberculous of the lungs (protein content 40-50 mg/ml), pus obtained from the wounds of burned patients (protein content 10-15 mg/ml), and hemolyzed human serum also were tested.

Hyperimmune sera against burned human skin were obtained by long-term immunization of rabbits with saline extracts of burn scab with the addition of Freund's adjuvant. Immune sera were exhausted with normal human serum, with a "pool" of bacteria (Staphylococcus, Proteus, Bacillus pyocyaneous), and with staphlococcal toxoid, and also with a "pool" of organs of clinically healthy persons (liver, spleen, and lung) in previously selected optimal doses. Completeness of exhaustion was verified by the agar diffusion test. Lyophilized sera, concentrated twice to give a concentration of 160 mg/ml and then exhausted, and also the γ-globulin fraction isolated by the alcohol method [4] from previously exhausted sera were used for the experiments. Sera against human spleen also were used as a control.

EXPERIMENTAL RESULTS

In the preliminary experiments the antigenic structure of the burn scab and the organs of clinically healthy subjects (liver, spleen, and lung) was compared. The exhausted serum aginst burn scab reacted with antigen from burn scab to form a single clearly defined precipitation line, but it did not react with antigens from the liver, spleen, and lung (Fig. 1A). The exhausted antiserum likewise did not react with antigens of normal skin, microorganisms isolated from burn wounds (Staphylococcus, Proteus, B. pyocyaneus), or with staphylococcal toxoid. Later serial dilutions were made on the antigen from the scab (initial protein content 20 mg/ml) and the antiserum, while the organ antigens were used undiluted. The antigenic component in the burn scab was clearly detected when the test system was diluted 4-8 times; i.e., in this modification the antigenic component studied was found in saline extracts from the scab containing 2.5 mg protein/ml but was not found in extracts from the liver, spleen, and lung containing 50-60 mg protein/ml (Fig. 1B). These results were confirmed by comparative tests to study the reaction between antigen from burn scab and sera against the burn scab and human spleen. The antispleen serum did not reveal any antigenic components in the burn scab detectable by serum against the burn scab (the "absence of identity" reaction; Fig. 1C).

An antigenic component not found in the organs of clinically healthy subject was thus detected in burned human skin (burn scab). In these investigations five sera against burned human skin and two against human spleen were used. The antigenic component studied was detected by four antiscab sera, while one antiserum did not detect any other antigenic components in the burn scab than those present in human parenchymatous organs.

To determine the maximal dilution of the saline extract from the scab in which it was still possible to detect this antigenic component, the antigen was titrated. The results showed that when tested with undiluted antiserum this antigen was discovered in extracts with a protein content of 1.25-12.5 mg/ml (Fig. 1D). This range of protein concentrations was evidently explained by the fact that the antigen studied was present in different quantities in different scabs. Altogether 22 specimens of burn scabs with a protein content in their antigens of 5-77 mg/ml were investigated; in four extracts, with a protein content below 20 mg/ml, the test antigen could not be found.

Comparative tests with a test system obtained from various materials which were possible sources of the inflammatory antigens failed to detect the antigenic component found in the burn scab in extracts from leukocytes, in pleural exudates, in pus from wounds of burned patients, in hemolyzed serum, or in the intact skin from patients dying from thermal burns.

An antigenic component not found in the organs of clinically healthy persons was thus demonstrated in burned human skin (burn scab) by means of an immunoautoradiographic method, and a test system was developed for use in the further study of the nature of this newly discovered antigen.

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LITERATURE CITED

1. G. I. Abelev and R. D. Bakirov, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 271.
2. S. M. Vul' and I. I. Kolker, Lab. Delo, No. 1, 33 (1970).
3. S. M. Vul' and I. I. Kolker, Pat. Fiziol., No. 3, 62 (1972).
4. L. A. Zil'ber and G. I. Abelev, The Virology and Immunology of Cancer [in Russian], Moscow (1962).
5. I. I. Kolker, Pat. Fiziol., No. 6, 78 (1969).
6. I. I. Kolker and S. M. Vul', Byull. Éksperim. Biol. i Med., No. 4, 64 (1971).
7. I. I. Kolker, S. M. Vul' and A. P. Nevinnaya, Pat. Fiziol., No. 5, 52 (1970).
8. S. D. Perova, D. S. Él'gort, and G. I. Abelev, Byull. Éksperim. Biol. i Med., No. 3, 45 (1971).
9. D. A. Él'gort and G. I. Abelev, Byull. Éksperim. Biol. i Med., No. 2, 118 (1971).
10. D. S. Rowe, Bull. World Health Org., 40, 613 (1969).