SIMPLIFICATION OF ANTIGENIC STRUCTURE OF BURNED HUMAN SKIN COMPARED WITH NORMAL SKIN

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By comparative immunochemical analysis using various modifications of the agar diffusion method, the following results were obtained: 1) in burned human skin (burn scab) additional antigens differing qualitatively from those of normal skin are found; 2) organ-specific antigens present in normal human skin are lost (antigenic simplification).

Investigations using L. A. Zil'ber's anaphylaxis with desensitization test and serological methods have yielded results indicating that burned and normal skin are not antigenically identical [5, 7-9, 11]. Immunochemical investigations [4] have demonstrated the presence of additional antigens in burned human skin compared with normal skin.

Thermal denaturation modifies the antigenic properties of autoglobulins [10], and heating proteins reduces their antigenic activity [6]. It can therefore be postulated that, as a result of thermal denaturation, not only do additional antigenic components appear in the burn scab, but some of the antigens present in normal human skin disappear.

In the present investigation, changes in the antigenic structure of the burned skin were studied by an immunochemical method.

EXPERIMENTAL METHOD

To compare the antigenic structure of burned and normal human skin, Ouchterlony's double diffusion method in gel was used in the modification of Gusev and Tsvetkov [2]. Saline extracts from various tissues were used as antigens. Skin and other tissues (spleen, lung, liver, kidney, heart) were obtained from clinically healthy persons dying accidentally; the burn scab was taken from patients with severe thermal burns of the skin. All antigens were standardized relative to protein, the content of which in the specimens was determined by Lowry's method. To exclude isoantigenic differences as far as possible, all antigens used in the work were prepared from a tissue pool obtained from persons of different blood groups.

Hyperimmune sera against burned and normal human skin were obtained by prolonged immunization of rabbits with saline extracts from the corresponding tissues, with the addition of Freund's adjuvant. For the comparative immunochemical analysis a pool of two or three tissue antisera was used in order to exclude individual differences between rabbits as regards antibody production. To increase the serological activity of the antisera, a twice-concentrated serum against human burn scab was used (the lyophilized serum was diluted in distilled water to a concentration of 160 mg/ml), and the γ-globulin fraction was precipitated from antiserum against normal skin with alcohol, and the specimens were concentrated by five times relative to the initial volume of antiserum [3].

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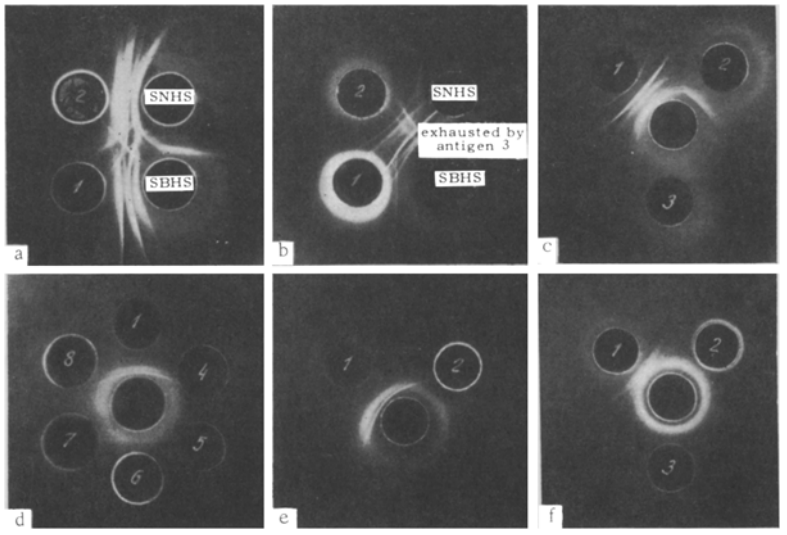

Fig. 1. Comparative immunochemical analysis of antigenic structure of burned and normal human skin: a) comparison of two systems (G. I. Abelev's modification): antiserum against normal human skin (SNHS)—antigen from normal skin (1) and antiserum against burned human skin (SBHS)—antigen from burned skin (2); b) the same modification, SNHS and SBHS exhausted by normal human serum (antigen 3); c) central well contains SNHS exhausted by antigen 3; d) central well contains SNHS exhausted by antigen 3 and by pool of organs; peripheral wells contain antigens from normal skin (1), liver (4), kidney (5), heart (6), lung (7), and spleen (8); e) central well contains SNHS exhausted by antigen 3 and by pool of organs; peripheral wells contain antigens 1 and 2; f) central well contains SNHS exhausted by antigens 3 and 2; peripheral wells contain antigens 1, 2, and 3.

EXPERIMENTAL RESULTS

Initially Abelev's [1] modification of the agar diffusion reaction was used. This test enables qualitatively different antigens to be detected in test systems even with the use of unexhausted antisera. The results shown in Fig. 1a demonstrate that when the two systems (serum against burn scab—antigen from burn scab and serum against normal skin—antigen from normal skin) were tested by Abelev's "cross" method, the human burn scab was found to contain antigens qualitatively different from those of normal skin, and the normal skin to contain an antigen qualitatively different from those obtained in the burn scab. These findings suggest that the burn scab has lost a certain antigen (or antigens) present in normal human skin. This phenomen on of antigenic simplification of the human burn scab was subsequently confirmed by the use of serum against burn scab and the γ-globulin fraction from serum against normal human skin, previously exhausted by the "pool" of lyophilized sera of donors of different blood groups in the same modification of the method. It is clear from the results shown in Fig. 1b that the tested systems contained several (2-4) qualitatively different antigens present in the burn scab and two antigens present in normal human skin.

Hence, by the use of this modification of the agar diffusion method, common antigens were found in the test systems, together with antigens specific for each system. This indicates that normal human skin contains antigens which are absent in burned skin (in the burn scab).

These results obtained by Abelev's "cross" method were subsequently confirmed by a comparative study of normal and burned human skin using antiserum against skin (γ-globulin fraction) exhausted with normal human serum. It is clear from Fig. 1c that the skin contained additional antigens not present in the burn scab (the reaction of partial identity).

In subsequent tests, to study the nature of the antigens lost by the human burn scab, the γ-globulin fraction from a hyperimmune serum against normal human skin was used. After removal of antibodies against serum proteins, the γ-globulin fraction of the antiserum, together with the skin, actively reacted with antigens from other organs of clinically healthy persons, and also from the burn scab. During further exhaustion of the γ-globulin fraction by the pool of organs from clinically healthy persons, for which a pool from the spleen, liver and lung was used in pre-selected proportions of optimal neutralizing doses, antibodies against heterologous tissues were removed; the γ-globulin fraction did not react with antigens from the tested tissues but continued to form two or three precipitation lines with antigen from normal human skin (completeness of exhaustion was verified by testing with antigens in a concentration of 60-80 mg/ml; Fig. 1d).

These investigations thus revealed the presence of two or three organ-specific antigens in skin. Meanwhile, the γ-globulin fraction did not react with antigen from human burn scab (Fig. 1e). On the basis of these results it can be postulated that organ-specific skin antigens have been lost by the human burn scab, or at least, they are present in quantities not detectable by the agar diffusion test.

In another series of experiments, after removal of antibodies against serum proteins, the antiserum against normal skin was exhausted with antigen from the burn scab. These tests showed that the γ-globulin fraction of these sera did not react with antigens from the burn scab or from the tissues of clinically healthy persons, but it formed two or three precipitation lines with an antigen from normal skin (Fig. 1f). Consequently, the results of this series of experiments confirm the view that by thermal denaturation, the human burn scab loses organ-specific antigens which are present in normal human skin.

This comparative immunochemical analysis using the agar diffusion reaction in its various modifications thus showed that human burn scab, on the one hand, contains additional antigens compared with normal skin, and on the other hand, it has lost organ-specific antigens present in normal human skin (antigenic simplification).

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