DETECTION OF TISSUE ANTIGENS IN THE BLOOD OF PATIENTS WITH BURNS

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Immunochemical tests on the sera of patients with burns showed that tissue antigens having common components with burned and normal human skin appear in the blood as early as on the 1st-2nd day after trauma. These antibodies are not found in the sera of clinically healthy persons and are not identical with C-reactive protein. These antigens were found to circulate in the patients' blood stream until 2-3 months after trauma.

KEY WORDS: burns; toxemia; tissue antigens.

Investigations in the writers' laboratory have shown that burned human skin (the burn scab) contains extra antigenic components that are not found in normal skin or in most tissues of clinically healthy subjects [7-9]. The results so far obtained are direct evidence that the burn scab is the main source of the immunopathological processes taking place in thermal injuries, but this does not rule out the possibility of additional sources of autosensitization in patients with burns connected with the higher rate of catabolism not only in burned but also in intact tissues [5].

The presence of tissue antigens in the blood of patients with burns was studied by immunodiffusion methods.

EXPERIMENTAL METHOD

Ouchterlony's double diffusion in gel technique, in the modification of Gusev and Tsvetkov [3] and Grabar's method of analytical immunoelectrophoresis in 1% agarose, in the modification of Abelev and Tsvetkov [1], were used for the immunochemical investigation of patients' sera. More than 50 sera obtained at different times after injury from patients with thermal burns of varied severity were studied. Sera of clinically healthy persons – blood donors (25 sera), patients with spinal cord injuries (25 sera), patients with acquired heart disease (20 sera) – were used as the controls.

The antigens consisted of saline extracts of burned and normal skin and also of certain parenchymatous organs (spleen, lungs, liver) from clinically healthy persons dying from accidents[2, 8]. The hyperimmune sera obtained against burned and normal human skin [9] were preliminarily absorbed with normal human serum. To prevent the detection of antigens of bacterial nature in the blood of the burned patients, sera against burn scab were additionally absorbed with a "pool" of microorganisms most frequently isolated from burn wounds, and with staphylococcal toxoid [9]. After absorption, the γ-globulin fraction was isolated from the antisera by alcoholic precipitation [6] and by concentrating the preparations by 4-5 times relative to the initial volume of antiserum.

EXPERIMENTAL RESULTS

To detect tissue antigens in the sera of patients with burns two test systems previously developed in the writers' laboratory for the comparative immunochemical analysis of burned and normal human skin

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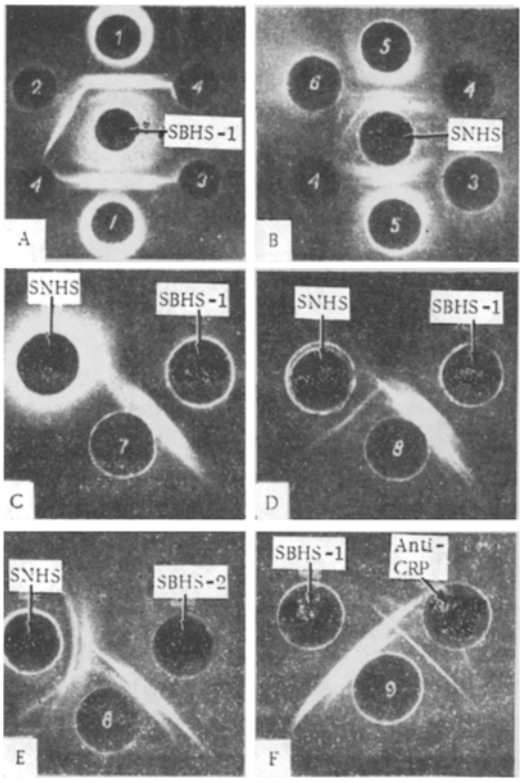

Fig. 1. Immunodiffusion tests of sera from patients with burns. A: central well contains serum against burned human skin (γ-globulin fraction), exhausted with normal human serum and with "pool" of microorganisms (SBHS-1); peripheral wells contain antigens: from burned human skin (1), serum of patient with burns (2), donor's serum (3), and physiological saline (4). B: central well contains serum against normal human skin (γ-globulin fraction), exhausted with normal human serum (SNHS); peripheral wells contain antigens: from normal human skin (5), serum of patient with burns (6), and antigens 3 and 4. C: central well contains antigen 7 (serum from patient with burns); peripheral wells contain SBHS-1 and SNHS. D: central well contains antigen 8 (serum from patient with burns); peripheral wells contain SBHS-1 and SNHS. E: central well contains antigen 8; peripheral wells contain SBHS-1 exhausted with antigen 5 (SBHS-2) and SNHS. F: central well contains antigen 9 (serum from patient with burns); peripheral wells contain SBHS-1 and serum against C-reactive protein (anti-CRP).

were used: serum against burn scab+antigen from burn scab and serum against normal skin+antigen from normal skin.

The comparative study of the burn scab and sera from patients with burns by means of the first test system showed that the patients' sera contained antigenic components common with antigens of the burn scab ("burn" antigens; Fig. 1A). Parallel tests with the second test system showed that sera from patients with burns contained a common antigenic component with antigens of normal human skin ("skin" antigens; Fig. 1B). Control tests of the serum from clinically healthy persons gave no response to the test systems used.

Further investigations showed that the antigens detectable in the patients' sera appeared in the blood very soon after burning (1st-2nd day) and continued to be detectable in the patients' blood for a long time, until 2-3 months after burning. In the late periods after trauma, "skin" antigens were found in the patients' sera much less frequently than "burn" antigens.

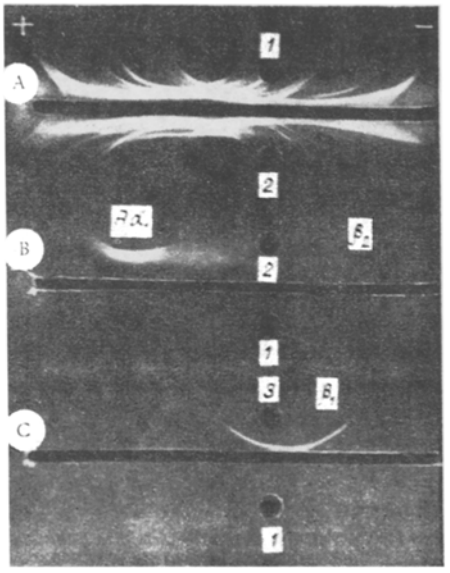

Fig. 2. Immunoelectrophoretic investigation of sera from patients with burns. A: gutter contains serum against normal human serum; wells contain antigens: donor's serum (1) and serum from patient with burns (2). B: gutter contains serum aginst burned human skin, exhausted with normal human serum, with "pool" of microorganisms, and with normal human skin; wells contain antigens 1 and 2. C: gutter contains serum against normal human skin exhausted with normal human serum; wells contain antigens: serum from patient with burns (3) and antigen 1.

Comparative study of the patients' sera by means of the two test systems showed that although many of the sera studied were active in both test systems, some "burn" sera reacted only with antiserum against burn scab (Fig. 1C) and, on the other hand, some patients' sera reacted only with serum against normal human skin. On the basis of these results it can be postulated that antigens of different origin were detected in the patients' sera by means of the test systems used. Comparative investigation of active patients' sera by means of the two antisera showed that one antigen detectable in the sera of patients with antiserum against burn scab was identical with the antigen detected in the same sera by antiserum against normal skin (Fig. 1D). For that reason, serum against burn scab was additionally exhausted with normal human skin to remove antibodies against normal tissues from the antiserum against burn scab. After absorption the spectrum of precipitation bands obtained by testing this antiserum with the patients' sera was narrowed and the comparative study of the test sera by means of the two antisera showed that antigens detectable in the patients' sera by means of this additionally exhausted "scab" antiserum were not identical with the antigen found in the patients' sera by means of "skin" antiserum (Fig. 1E). These results thus showed that different antigenic components are detected in the sera of patients with burns by the two antisera used.

The results obtained by the immunodiffusion test were later confirmed by immunoelectrophoresis. "Burn" antigen (or antigens) detected by the "scab" antiserum lay in the zone of albumin–α_1-globulins (Fig. 2B), whereas the "skin" antigenic component detected by "skin" antiserum possessed relative electrophoretic mobility corresponding to β_1-globulins* (Fig. 2C).

The use of the test systems in the control investigation of sera from patients with traumatic spinal injuries and acquired heart disease gave no reaction with the "skin" antiserum, indicating absence of "skin" antigens in the blood of clinically healthy persons and patients with other pathological states. Meanwhile five of the sera from the "spinal" patients tested, despite the presence of deep trophic lesions consisting of indolent, infected bedsores, reacted with "scab" antiserum, although the latter did not react with the sera from patients with acquired heart disease. These results suggest that the "scab" antiserum may possibly reveal certain "inflammatory" or perhaps "necrotic" [4] antigens formed in the course of chronic suppurative and necrotic processes, such as take place after severe burns and traumatic lesions of the spinal cord.

In patients with chronic suppurative and necrotic lesions C-reactive protein (CRP) is found in the bloodstream, as the present writers have shown in relation to patients with burns [10]. Comparative immunochemical investigations of sera from patients with burns by means of antisera against burn scab and CRP showed that serum against CRP reveals an antigenic component in the sera of patients with burns, but it differs qualitatively from antigens detectable in the same patient's serum by the "scab" antiserum (the "absence of identity" reaction; Fig. 1F). The immunoelectrophoretic studies also showed that antigens detectable by "scab" antiserum lie in the albumin–α_1-globulin zone whereas CRP lies in the β_2-globulin zone. Consequently, "inflammatory" antigens detected in the sera of patients with burns are not identical with CRP.

These immunochemical investigations thus showed that at least two types of tissue antigens not detectable in the blood of clinically healthy persons and not identical with CRP circulate in the bloodstream

* The relative electrophoretic mobility was calculated relative to the electrophoretic mobility of normal human serum albumin, subjected to electrophoresis in the same experiment (Fig. 2A).

of patients with burns. The principal source of these pathological antigens is the burn scab ("burn" antigens). Meanwhile the severe disturbance of protein metabolism after thermal injuries, accompanied by a marked increase in permeability, leads to the appearance of normal tissue antigens ("skin" antigens) in the bloodstream as well.

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