EFFECT OF TEMPERATURE ON THE ANTIGENIC PROPERTIES OF THE SKIN

I. I. Kolker, S. M. Vul',
and M. A. Grigor'eva

UDC 612.79.017.1.014.43

It was shown by immunochemical analysis of normal human skin and skin heat-treated in vitro that of the four or five organ-specific skin antigens no fewer than two are thermostable, for they withstand heating to 100°C for 3 min, and two or three antigens are thermolabile. The thermostable antigens have the electrophoretic mobility of α_1- and γ-globulins. In burned human skin (burn scab) the thermolabile and one of the two thermostable organ-specific antigens are lost.

KEY WORDS: normal and burned human skin; antigens.

Previous investigations in the writers' laboratory have shown simplification of the antigenic structure of the burned human skin (burn scab), i.e., the loss of its organ-specific skin antigens [6]. In this connection a problem requiring special attention is whether all organ-specific antigens of the skin are lost in the burn scab (complete antigenic simplification) or whether some organ-specific skin antigens are preserved (partial antigenic simplification).

The effect of temperature on the antigens of human skin was investigated by means of immunochemical methods.

EXPERIMENTAL METHOD

A comparative study of normal human skin and of skin treated by exposure to heat in vitro at different temperatures, and also of burned human skin (burn scab) was carried out by Ouchterlony's agar diffusion method in the modification of Gusev and Tsvetkov [4] and by immunoelectrophoresis by the method of Grabar and Williams in the modification of Abelev and Tsvetkov [1].

Saline extracts obtained from normal skin by the method described previously [3], exposed for 3 min to temperatures of 50, 60, 70, 80, and 100°C, were used as the antigens. Antigens from burned human skin (burn scab) were prepared from material obtained from patients with severe thermal burns during sloughing or excision of areas of burned skin (usually 2-3 weeks after burning). The burn scab was carefully freed from necrotic areas and pus and cut into small pieces. The subsequent stages of preparation were similar to those during the preparation of antigens from normal skin.

Hyperimmune sera against normal human skin were obtained by prolonged immunization of rabbits with saline extracts of skin with the addition of Freund's adjuvant.. Native antiserum, antiserum absorbed with lyophilized human serum (at the rate of 100-120 mg to 1 ml antiserum), and also antiserum absorbed with lypohilized serum and tissue antigens, for which purpose a mixture of antigens was used from the spleen, liver, and lung (organ-specific serum) [2], were used in the experiments. To increase the serologic activity of the antisera, γ-globulin fractions obtained from native and the corresponding absorbed antisera

Laboratory of Microbiology and Immunology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Vishnevskii.*) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 204-206, February, 1976. Original article submitted March 4, 1975.

* Deceased

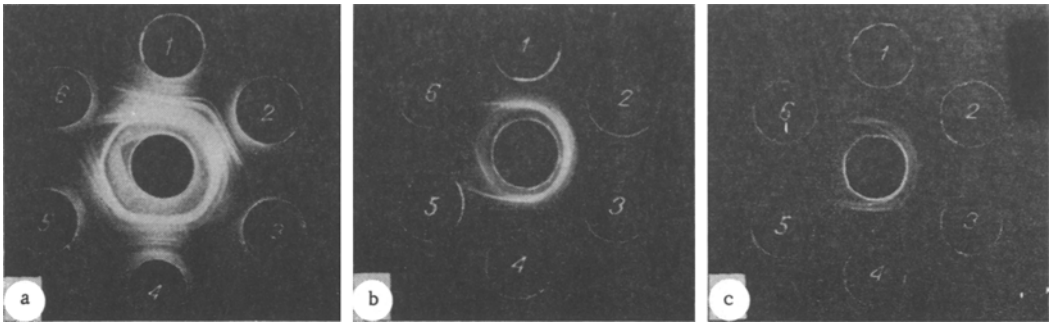

Fig. 1. Comparison of antigenic structure of normal human skin and skin heat-treated in vitro. Peripheral wells contain antigens from normal skin (1) and antigens from skin heated to 50°C (2), 60°C (3), 70°C (4), 80°C (5), and 100°C (6). Central wells contain serum against normal human skin, unabsorbed (a), or absorbed with normal human serum (b), and organ-specific serum (c).

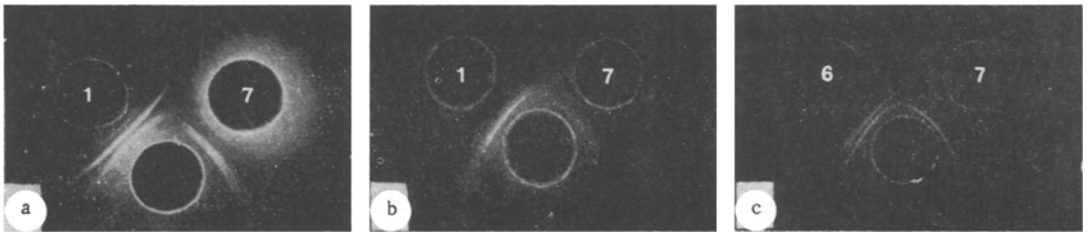

Fig. 2. Comparison of antigenic structure of normal and burned human skin (burn scab) and of skin exposed to a temperature of 100°C for 3 min. Peripheral wells contain antigens from normal skin (1) and from skin heated to 100°C (6), and antigen from burn scab (7). Central wells contain serum against normal human skin absorbed by serum (a), and organ-specific serum (b and c).

by alcoholic precipitation [5] were used; the preparations were concentrated fivefold relative to their initial volume.

EXPERIMENTAL RESULTS

In the initial experiments loss of part of the antigens in the skin when heated to 70-80 or 100°C was established with the aid of native antiskin serum in the agar-diffusion test (Fig. 1a). Immunoelectrophoresis revealed a decrease in the number of precipitation bands, and the decrease was particularly clearly visible at 80 and 100°C.

The use of antiskin serum from which antibodies against serum proteins had been removed showed that some of the antigenic components of the skin were lost in extracts heated to 80 and 100°C, but the thermostable components, forming bands with antiserum that merged as in the reaction of identity (Fig. 1b), were preserved in all the samples tested. Immunoelectrophoretic investigation also showed the presence of three or four thermostable tissue antigens in the test system.

In the experiments in which organ-specific antiskin serum was used four or five organ-specific antigens were discovered by the agar diffusion test in normal human skin, of which two or three antigens were thermolabile and were not found in saline extracts heated to 80 and 100°C, but at least two antigens were thermostable and were discovered in extracts heated to 80 and 100°C, for they formed bands with antiserum that merged as in the identity reaction (Fig. 1c). A study of the electrophoretic mobility of the thermostable skin antigens thus discovered showed that they migrated in the zones of the α_1- and γ-globulins.

The use of highly active hyperimmune sera thus revealed four or five organ-specific antigens in normal human skin, of which two or three were thermolabile and at least two, with the mobility of α_1- and γ-globulins, were thermostable and tolerated heating to 100°C for 3 min.

The study of the fate of the thermostable organ-specific skin antigens in burn scab was continued in the next series of experiments in which normal human skin was compared with skin heated to 100°C for

3 min and with burned human skin (burn scab). Experiments with antiskin serum absorbed with normal human serum confirmed again the fact, discovered previously in the writers' laboratory, that some of the antigens characteristic of normal human skin are lost in burn scab (Fig. 2a).

The study of antigens from burn scab and normal skin with the aid of organ-specific antiskin serum revealed the presence of one of the organ-specific antigens of normal skin in burn scab (Fig. 2b). A comparative study of burn scab and of skin heated to 100°C showed that the antigen remaining in the burn scab is thermostable and that, at the same time, one of the two thermostable organ-specific antigens still present in skin heated to 100°C is lost in burn scab (Fig. 2c). On the basis of these results not only is the phenomenon of antigenic simplification of burned human skin established previously in the writers' laboratory confirmed, but ideas regarding the complex changes taking place in the antigenic structure of human burn scab are considerably widened. The loss of not only thermolabile, but also of one thermostable organ-specific skin antigen in burn scab suggests that the constant presence of tissue and bacterial enzymes in the scab may in turn evoke additional (besides the action of the temperature factor) changes in the antigenic structure of burned skin. It is possibly through the action of these factors that one thermostable organ-specific skin antigen in the scab is lost. Meanwhile, besides simplification of the antigenic structure, one thermostable organ-specific human skin antigen remains in burned skin (burn scab).

The authors are grateful to Professor G. I. Abelev for valuable comments.

LITERATURE CITED

1. G. I. Abelev and V. S. Tsvetkov, Vopr. Onkol., No. 6, 62 (1960).
2. S. M. Vul' and M. A. Grigor'eva, Vestn. Dermatol., No. 10, 34 (1974).
3. S. M. Vul' and I. I. Kolker, Pat. Fiziol., No. 3, 62 (1972).
4. A. I. Gusev and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
5. L. A. Zil'ber and G. I. Abelev, The Virology and Immunology of Cancer [in Russian], Moscow (1962).
6. I. I. Kolker and S. M. Vul', Byull. Éksperim. Biol. Med., No. 4, 64 (1971).