ANTIGENIC SPECIFICITY OF MACROMOLECULAR PROTEINS OF BURNED SKIN

B. E. Movshev

UDC 617-001.17-07:616.5-008.939.6-097.2-078

Antigens detectable by burn antiserum only are found in the fraction of high-molecular-weight proteins extracted from the burned skin of rats. The content of serum proteins in this fraction is much greater than in extract obtained from normal skin.

Analysis of the antigenic structure of burned skin is an important task in the noninfectious immunology of burns. Antigenic differences between the skin of normal and burned animals were first demonstrated experimentally by N. A. Fedorov et al. [7-9], using the method of anaphylaxis with desensitization, and the autoantigenic properties of products of burned skin were postulated. Later, the nosologic specificity of the burn antigen and of burn antibodies was subsequently confirmed completely or partially by immunologic tests: complement fixation [3], Boyden's passive hemagglutination [10, 13], immunofluorescence [4], etc., but the immunochemical nature of the antigen remained unknown.

If antisera obtained by heteroimmunization with extract of burned skin are used in the usual manner for this purpose, great technical difficulties arise because of the need to analyze many antigen–antibody systems. Preliminary fractionation of the complex mixture of antigens extracted from burned skin is therefore an essential condition for the successful elucidation of antigenic structure and for obtaining antibodies of high specificity.

The antigenic composition of the fraction of macromolecular proteins of burned skin was investigated in the present study.

EXPERIMENTAL METHOD

The experimental animals were 102 Wistar rats weighing 80-100 g. An area of skin on the dorsum of the animals was shaved and burned with the flame of a spirit lamp (area of burn 20% of the body surface, exposure 30-35 sec). The animals were decapitated 48 h after burning. The area of skin was excised, freed from adipose tissue, cut into pieces with scissors (0.2×0.2 cm), and frozen in liquid nitrogen at −196°. The tissue was weighed, thawed at 37°, treated with 3 volumes (by weight) of 0.1 m NaCl, pH 7.0, and minced in a knife homogenizer at 8000 rpm 5 times for 1 min each time with intervals for cooling. The homogenate was pressed through a Kapron sieve and the residue treated in the same manner two more times. The supernatants were pooled, lipids were extracted with ether at between −10 and −12°, the product was dialyzed against 20 volumes of 0.01 M ammonium acetate buffer, pH 6.8, and this was followed by sublimation.

The dry preparation was dissolved in 0.01 M tris-HCl buffer, pH 8.0, with the addition of 0.1 M NaCl solution, and the fraction with molecular weight greater than 200,000 was isolated by gel-filtration on Sephadex G-200 in equilibrium with the same buffer (column 2.5×200 cm). Some of the material of this fraction, after determination of its protein content, was used to immunize 15 rabbits in accordance with

Central Research Laboratory, Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 9, pp. 58-61, September, 1970. Original article submitted February 16, 1970.

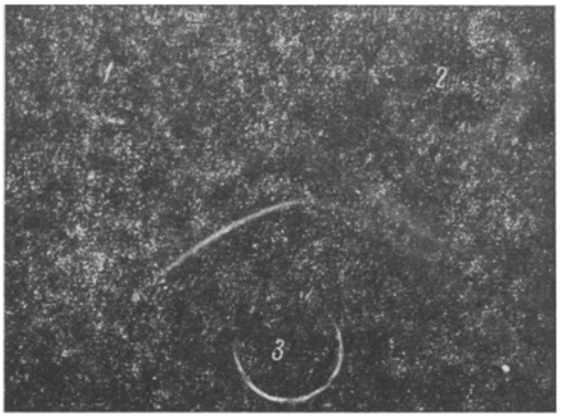

Fig. 1. Antigens of macromolecular fraction from burned skin of Wistar rats. Agar-diffusion reaction. Unstained specimen in 15% NaCl solution: 1) hyperimmune antiserum against burned skin; 2) antiserum against serum proteins; 3) fraction pH 4-I of burned skin.

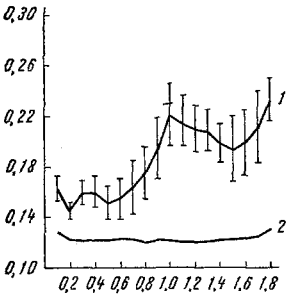

Fig. 2. Photomicrograph of antigen–antibody reaction between fraction pH 4-I (on left) and antisera (on right). Abscissa, distance between wells (in mm); ordinate, optical density; 1) reaction with burn antiserum; 2) reaction with serum against normal tissues.

the scheme used at the N. F. Gamaleya Institute of Epidemiology and Microbiology, with the addition of Freund's adjuvant [5]. The first injection of antigen was given intradermally, and the total number of reimmunization cycles was increased to six. After each cycle of reimmunization blood was taken from the animals, serum was obtained and inactivated at 56° for 30 min, and the γ-globulin fraction was isolated by salting out with ammonium sulfate. The product was dialyzed, concentrated, and treated with merthiolate in the ratio of 1:10,000. The different antisera contained at least 7 types of antibodies detectable in double immunodiffusion reactions.

The rest of the material from the macromolecular fraction was separated into subfractions. After dialysis and concentration, the pH of the fraction was adjusted to 3.85 (measured with a glass electrode) with 0.2 M acetate buffer, and this was followed by centrifugation for 100 min at 40,000 g and 0-2°. The supernatant (fraction pH 4-I) was used for analysis of its antigenic composition by a micromodification of the agar diffusion reaction [2], using 0.75% Difco agar in 0.14 M NaCl solution, pH 7.0, on a cover slip measuring 32×40 mm, the thickness of the layer being 1 mm. The reaction time from adding the reagents to the wells was 24 h. Photography was carried out in 15% NaCl solution to eliminate haloes around the wells [1]. The cover slips with agar were washed in several changes of physiological saline, pH 7.0, dried, and stained with amido black.

Densitometry of the specimens was carried out with a type MF-2 nonrecording microphotometer. The counting interval was 0.1 mm and the total magnification of the instrument 30×. The width of the aperture was $^1/_{200}$ of the distance between the wells.

EXPERIMENTAL RESULTS

In the agar diffusion reaction, 3 visible precipitation lines were formed between fraction pH 4-I and burn antiserum (Fig. 1). Two of these were identical with the lines of serum proteins of Wistar rats. This was confirmed by reactions between rat serum and burn antiserum. Meanwhile, in some experiments a definite antigenic difference was discovered (incomplete identity in immunodiffusion) between serum protein with electrophoretic mobility of α_2 globulin, detected in fraction pH 4-I of burned skin and the normal serum component. It was provisionally assumed that one of the macromolecular serum proteins of burned skin possesses a more complex antigenic spectrum than the corresponding normal protein, and besides common antigenic determinants it also contains others which are antigenically different. Evidence in the literature also suggests anomalies in the α_2-globulin fraction in burns [6, 11, 12], but these facts as a whole require further clarification and elucidation.

The present experiment showed that serum protein admixtures found in fraction pH 4-I of burned skin are also present in an extract of soluble proteins from normal skin, although in much smaller amounts.

Analysis of the antigenic specificity of the 3rd component of fraction pH 4-I is the most important aspect of this investigation. The formation of this precipitation line was shown to be the result of interaction between fraction pH 4-I of burned skin and the corresponding burn antiserum. No analogous precipitation line was formed with other antisera: against rat serum proteins, or against normal skin. The corresponding antigen could not be found in comparable amounts (the absolute limit of sensitivity of the agar diffusion reaction) with the aid of burn antiserum either in normal serum or in normal rat skin.

These results indicate that burned skin contains a macromolecular protein with different antigenic properties from normal. The possibility cannot be ruled out that the observed differences are due to quantitative differences in the content of this antigen in normal and burned skin.

For a more detailed analysis of antibodies existing in burn antiserum against specific antigen, the antiserum was successively exhausted (absorbed) with serum proteins and with extract of normal skin of Wistar rats. Absorption was carried out by adding various doses of antigens to the antiserum, keeping the mixture for 30 min at 37° and for 24 h at 4°, and separating the resulting precipitate by centrifugation. All operations were carried out under immunodiffusion control. Since absorption of burn antiserum by extract of normal skin was not followed by the formation of a visible precipitate, before the agar diffusion reaction was carried out the immune antiserum was centrifuged for 30 min at 20,000 g.

Altogether 7 agar diffusion tests were carried out between exhausted antiserum and fraction pH 4-I of burned skin, 3 tests with serum proteins, and 2 tests with extract of normal skin as antigen. The experimental results are shown as arithmetic mean values and standard errors in Fig. 2.

Using the principle of objective recording, and taking advantage of the high sensitivity of the method, 2 zones of colored precipitate were clearly distinguished. A diffuse, wide precipitation band occupied the middle position between the wells. The optical density of this zone was low, but sufficient for visual assessment. A thin (about 0.15 mm) precipitation line, detectable only on the photomicrograph, was found nearer to the well containing antigen. The ratio between these precipitation zones, based on the quantity of dye bound with the protein, was 5 : 1. The increase in optical density near the edges of the graph was due to staining of the halo zones around the wells.

It is worth noting that no precipitation lines were formed between exhausted burn antiserum and the serum or skin of normal rats.

The results thus show that fraction pH 4-I of the macromolecular proteins from burned skin contains at least two antigens not detectable in comparable amounts in normal serum or skin. The study of the immunochemical nature of these antigens thus discovered and examination of their nosologic or other specificity will be the subject of future research.

LITERATURE CITED

1. A. I. Gusev, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 99.
2. A. I. Gusev, and V. S. Tsvetkov, Lab. Delo, No. 2, 43 (1961).
3. V. N. Kazakova, Pat. Fiziol., No. 2, 34 (1964).
4. I. I. Kolker, Vestn. Akad. Med. Nauk SSSR, No. 3, 42 (1964).
5. L. A. Lyudogovskaya, in: Immunochemical Analysis [in Russian], Moscow (1968), p. 5.
6. V. A. Mazurenko, in: Burns [in Russian], Kiev (1966), p. 35.
7. N. A. Fedorov and S. V. Skurkovich, Khirurgiya, No. 9, 48 (1955).
8. N. A. Fedorov, in: The Problem of Trauma [in Russian], Moscow (1960), p. 94.
9. N. A. Fedorov et al., in: Current Problems in Immunology [in Russian], Moscow (1964), p. 312.
10. L. Pavkova et al., Chekhosl. Med. Obozr., 2, 92 (1965).
11. R. Cervetti and A. Franceschelli, Minerva Med., 54, 3264 (1963).
12. C. Fossoul, Acta Chir. Belg., 61, 791 (1962).
13. O. Haferkamp, Arch. Klin. Chir., 301, 118 (1962).