CHARACTERISTICS OF THE SPECIFIC ANTIGEN OF BURNED SKIN

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Antisera obtained by hyperimmunization of rabbits with preparations of high-molecular-weight proteins of burned and normal rat skin were tested in the agar diffusion test before and after exhaustion by normal skin and serum proteins. A single precipitation line was found in the composition of the specific test system on visual investigation. Increasing the sensitivity of the test by using microphotometry of stained preparations revealed the polycomponent nature of the system, in which, besides the specific antigen present in the highest titer, other antigen–antibody complexes are present. The specific antigen of burned skin has relatively weak immunogenic properties.

KEY WORDS: burns; specific antigen of burned skin; immunogenicity.

The fact that normal and burned tissues differ antigenically, first shown by Fedorov and co-workers [8, 11, 12] and subsequently confirmed repeatedly [3, 4, 14-16], is a milestone in the study of noninfectious immunology of burns and in the development of recent ideas concerning antitoxic immunity in burns [9, 10].

For several years now the antigenic composition and properties of the proteins of burned skin have been studied in the writer's laboratory as possible sources of autoimmunization. Methods have been developed for fractionating skin extracts [6], studying the antigenic specificity of high-molecular-weight protein antigens [5], and isolating them in the purified form [7].

The object of this investigation was to continue the immunochemical study of high-molecular-weight antigens of burned skin experimentally.

EXPERIMENTAL METHOD

Skin of normal and burned Wistar rats 46-48 h after burning by the flame from cotton wool soaked in spirit was used as the material. Preparation and fractionation of the extracts and isolation of high-molecular-weight proteins were carried out by methods described earlier [6, 7]. Ten noninbred rabbits weighing 2-2.5 kg [1] were immunized with the high-molecular-weight fractions of burned and normal skin mixed with Freund's complete adjuvant (Difco, USA) [1]. Antigenic material also was injected without adjuvant in a dose of 1.5 mg/kg on the 14th day intramuscularly, into two different regions of the body, and on the 21st day, into four different regions, subcutaneously. Blood serum was obtained on the 6th, 8th, 10th, and 12th days after the last injection of antigen, and subsequently after each of four cycles of reimmunization (interval between cyles 1.5-2 months). After determination of the antibody titer the antisera were pooled and the γ-globulin fraction isolated [13].

The fraction of high-molecular-weight proteins of burned skin, soluble at pH 4.0, was chromatographed on anion-exchange cellulose DE-32 (Whatman, England) in 0.01 M tris-HCl buffer, pH 8.3, using a sodium chloride concentration gradient. Fractions eluted in salt concentrations of 0.2-0.3 M were pooled, concentrated, and dialyzed against 0.14 M sodium chloride solution, pH 7.0.

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TABLE 1. Concentration of Antibodies in Sera of Rabbits after Immunization by Preparations of Normal and Burned Skin

Antigen	Content of antibodies against high-molecular-weight proteins*				
	from burned skin			from normal skin	
Normal serum (α_2-macroglobulin)	1:8	1:4	1:8	1:2	1:1
Ditto (transferin)	1:16	1:32	1:32	1:16	1:8
Normal skin (acid protein)	1:64	1:32	1:16	1:16	1:16
Specific from burned skin	1:8	1:8	1:2	—†	—

* Maximal dilution of antiserum at which precipitation line still appears with corresponding antigen is shown.
† No precipitation line appeared.

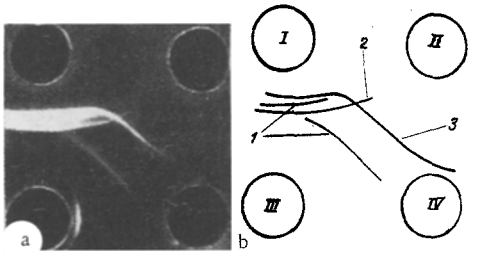

Fig. 1

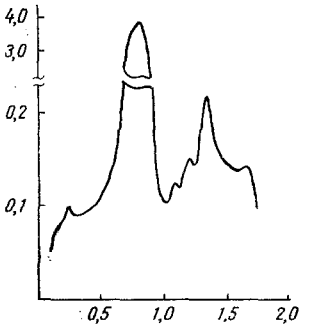

Fig. 2

Fig. 1. High-molecular-weight antigens of burned skin: a) unstained preparation; b) scheme of immunoprecipitation analysis: I) high-molecular-weight proteins of burned skin; II) fraction of proteins of burned skin after chromatography; III) antiserum against high-molecular-weight proteins of burned skin; IV) the same antiserum, exhausted by proteins of normal rat skin and serum. 1) Precipitates of antigens specific for blood serum proteins; 2) precipitate of antigen specific for normal skin proteins; 3) specific antigen of burned skin.

Fig. 2. Densitogram of precipitation reaction between proteins of high-molecular-weight fraction of burned skin after chromatography and corresponding antiserum exhausted by proteins of normal skin and serum (right). Abscissa, distance between wells (in mm); ordinate, optical density. Counting interval 0.05 mm, effective width of slit 0.01 mm.

The composition of the original preparations and of their fractionation products was studied by double immunodiffusion in agar [2]. Weak precipitation lines were detected on stained plates by means of a microphotometer as described earlier [5].

EXPERIMENTAL RESULTS

Comparative analysis by the precipitation test showed that antisera obtained by hyperimmunization with preparations from burned and normal skin contain antibodies of four and three types, respectively (Table 1). The maximal dilution of antiserum with which it was still possible to find a precipitation line with one of the antigens of the original mixture can be used to assess the immunizing effect of the corresponding component indirectly. As the writer showed previously, the high-molecular-weight protein fraction of burned skin contains not only a specific component not found in normal skin, but also traces of α_2-macroglobulin, transferin, and acid skin protein. In the course of immunization, very different immunogenic properties of each of these components were found, and in addition, there were considerable individual differences in the immune response of the rabbits. Despite the fact that the specific component was predominant

in the composition of the original fraction of burned skin used for immunization, the content of the corresponding antibodies to it was low in all the antisera tested (1 : 2-1 : 8). The immunizing effect of the specific protein of burned skin is evidently less marked than that of the other components. It was therefore impossible to prepare a test system againt the specific antigen of burned skin by maximal dilution of the preparations.

A solution of γ-globulin against burned skin was exhausted by a freshly prepared serum of normal rats by incubating the mixture at 37°C for 40 min and at 4°C for 2 h, followed by centrifugation for 20 min at 17,000 rpm. The high-molecular-weight fraction of normal skin was used for exhaustion under identical conditions. During tests for the completeness of exhaustion in the precipitation reaction by the square scheme suggested by G. I. Abelev, only antibodies against the specific component of burned skin were found in low titer in the composition of the immune γ-globulin (Fig. 1). Visually a single precipitation line, not found during the investigation of normal skin and serum proteins or by the use of antiserum against the high-molecular-weight proteins of normal skin, was found in the composition of the test system.

However, on analysis of the test system using stained preparations by the microphotometric method, its polycomponent nature was demonstrated (Fig. 2). Sensitive measurements showed at least five different antigen–antibody complexes in the composition of the test system, among which the precipitate of specific antigen was absolutely predominant. The nature of the remaining components remains uncertain.

The results of immunoprecipitation analysis in a heterologous system suggest that the antigenically demonstrable difference between normal and burned skin is determined by a high-molecular-weight protein regularly found in burned skin. If this protein belongs to the group of normal serum or skin components, which cannot be ruled out on the basis of the available data, its content under normal conditions must be below the limit of sensitivity of the agar precipitation test. For future investigations in this direction it would be useful to increase the resolving power of immunoprecipitation analysis by means of microphotometry of stained preparations.

The weak immunogenic properties of the specific antigen of burned skin provide indirect confirmation of the species nonspecificity of the burn antigen postulated previously [12].

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