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IMMUNOCHEMICAL STUDY OF WATER-SOLUBLE CORNEAL ANTIGENS

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UDC 617.713-022.7-092:612.017.1]-078.33

KEY WORDS: cornea, water-soluble antigens.

Corneal antigens are found in considerable isolation from the immunogenesis system and they exhibit organ-specificity. Injury to the cornea as a result of exposure to infectious agents, trauma, or surgery creates conditions for the resorption of the corneal proteins and promotes the development of autosensitization [4, 6]. There is no doubt that the development of effective methods of prevention and treatment of cases of keratitis with an autoimmune component, and also of severe postoperative complications such as uveitis, epithelial-endothelial corneal dystrophy, or rejection of a graft depends on our knowledge of the localization, specificity, and physicochemical properties of the corneal antigens.

Nine antigens were found in a corneal extract by the immunodiffusion method [3]. However, the immunochemical properties of these proteins remained virtually unstudied.

The aim of this investigation was a detailed study of water-soluble antigens of the bovine cornea.

EXPERIMENTAL METHOD

Tissues of the eyes and other organs of persons dying from trauma and from the corresponding bovine organs were used. The tissues were minced mechanically, ground with quartz sand, treated with 3-4 volumes of Tris-glycine buffer, pH 8.3, and additionally homogenized. The resulting homogenate was frozen 3 times to −10°C and thawed at room temperature, after which it was centrifuged at 8000 rpm for 30 min. The supernatant was used to isolate and study the antigens. A specific antiserum was obtained by immunizing rabbits with tissue extract from bovine cornea with the addition of Freund's complete adjuvant by the method of intradermal injections at 10 to 12 sites on the trunk, at the rate of 1 ml of extract per kilogram body weight, 4 times with intervals of 9 days between the injections. The protein concentration in the extracts, measured by Lowry's method, was 20-30 mg/ml. Reimmunization was carried out 1 month after the last immunization. Blood was taken form the marginal vein of the animals' ear on the 7th, 9th, and 11th days after reimmunization. Serum obtained from the blood was exhausted with dried human blood plasma. Precipitating antibodies, corresponding to the corneal antigens, were detected in the immune sera in a microversion of the immunodiffusion method in a layer of gel [10]: immunodiffusion was carried out in 1.5% agar gel for 24 h, the volume of the sample being 10 μl. The organ-specificity of the corneal antigens thus found was tested by the same method

Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 9, pp. 294-295, September, 1990. Original article submitted August 10, 1989.

in a standard test system [7]. To determine the spectra of the corneal antigens and their electrophoretic mobility, the method of crossed immunoelectrophoresis [9] in a layer of 1% agarose (Difco) on 0.05 M Veronal-Medinal buffer, pH 8.6, ionic strength 0.05, voltage 10 V/cm in direction 1 and 3 V/cm in direction 2, was used. In the last case, the agarose contained 20% of antiserum. Separation of the proteins by molecular weight was carried out by the gel-filtration method [2] on a column measuring 1.2 × 46 cm, packed with Sephadex G-200 (Pharmacia), with the rate of elution from 8 to 16 ml/h. Extract of bovine corneal endothelium, epithelium, or stroma in 0.01 M Na^+,K^+-phosphate buffer was applied in a volume of 0.5 ml to the column. Blue dextran (Serva, West Germany) was used as the marker. Gel-filtration was carried out with the aid of a chromatographic system (LKB, Sweden). The optical density of proteins passing through the column was recorded on a "Uvicord" instrument. Protein fractions eluted from the column were collected in test tubes in volumes of 1-2 ml and studied by immunodiffusion and immunoelectrophoresis. To determine the organ- and species-specificity of the antigens thus revealed, the antiserum obtained was subjected to the immunodiffusion test with various tissue extracts of the bovine and human eye, and also of human organs such as the adrenals, kidneys, spleen, brain, liver, muscles, lungs, stomach, skin, and bone tissue.

EXPERIMENTAL RESULTS

The free precipitation arcs were obtained by immunodiffusion in gel between the immune serum obtained as described above and extract of bovine cornea. A reaction of partial immunochemical identity was found between antigens of the cornea and other structures of the bovine eye, such as the lens, vitreous body, aqueous humor, iris, ciliary body, choroid plexus, and retina. No crossed reactions were found between antigens of the bovine cornea and tissues of the human eye or other organs.

Immunoelectrophoresis revealed nine antigens in the bovine cornea, three of them with the mobility of γ-globulins being located in the epithelium, two with the mobility of γ-globulins in the endothelium, and four (two with mobility of β-globulins and one each with mobility of α_1- and α_2-globulins) in the stroma. The molecular weights of the epithelial γ-proteins, as shown by the results of gel-filtration, was approximately 108 ± 30 kD, of the endothelial γ-proteins 155 ± 30 kD, and of the stromal proteins 375 ± 90 kD.

Thus the stroma of the bovine cornea consists of large macromolecules which, on immunization, induce antibody production; the endothelium and epithelium have smaller proteins, but all, unlike the stroma, possess γ-mobility.

The stroma of the cornea consists of large protein macromolecules organized into highly regular architectural assemblages [8]. It is this construction which allows the cornea to perform its basic role ascribed to it by nature, namely protection of the internal medium of the eye and enabling light to be refracted.

The different antigenic composition of the epithelium and endothelium is a rather unexpected fact. It will be noted that the endothelium is poor in its antigenic composition. However, this also may be biologically advantageous, for the appearance of autoantibodies in response to injury would aggravate the state of this most vulnerable layer of the cornea even more [3]. Nevertheless, it can be tentatively suggested that dystrophy of the corneal endothelium as a postoperative complication will take place by an autoimmune mechanism, for immunization, even with a small quantity of corneal tissue extract, induces the production of antibodies to endothelial proteins.

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