PHYSICOCHEMICAL PROPERTIES OF KIDNEY-SPECIFIC ANTIGEN

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The writer previously [1, 2, 4] found specific kidney antigens in man and CBA mice. These antigens were shown to be positively charged proteins with electrophoretic mobility in the γ-globulin zone. On passive immunization of CBA mice by injection of antikidney serum containing antibodies against the specific antigen into them, they developed an autoimmune nephroso-nephritis, characterized by involvement predominantly of epithelial structures of the nephron, i.e., those in which this antigen is located [3, 6]. There are scattered items of information in the literature to the effect that antigens with this localization can induce autoantibody formation in man, for in patients with protracted forms of nephroso-nephritis, antigen–antibody complexes have been found by the immunofluorescence method in the epithelium of the convoluted tubules [7, 8]. This antigen is not found in the blood serum of urine of healthy individuals. In mice with experimentally induced nephroso-nephritis it was found in the urine by immunodiffusion methods [5]. These observations suggest that this antigen may be an autoallergen. Sensitization to it may play a definite role in the pathogenesis of autoimmune nephroso-nephritis.

In connection with the facts described above, an investigation was carried out to study the physicochemical properties of kidney-specific antigen.

EXPERIMENTAL METHOD

Immune sera against human and CBA mouse kidney were obtained in Chinchilla rabbits (n = 6) by prolonged (for 1 year and 9 months) immunization, consisting of a basic cycle and four reimmunizations. As a result, antibodies against the specific kidney antigen were clearly demonstrated in the resulting immune sera.

The following products were obtained from kidneys of CBA mice and clinically healthy persons dying from accidental causes: 1) kidney extract in cold buffered sucrose solution (0.25 M sucrose, 0.05 M $MgCl_2$, and 0.35 M Tris-buffer, pH 7.5) in the ratio of 1:10; 2) mitochondrial fraction — the residue suspended in buffered sucrose solution; 3) the supernatant after centrifugation of the kidney extract on the L-265B ultracentrifuge at 15,000g for 40 min at 4°C; 4) microsomal fraction — the residue suspended in buffered sucrose solution; 5) the supernatant after centrifugation of fraction 3 at 70,000g for 90 min at 4°C. Fraction 5 was subjected to further fractionation by means of Centriflo CF-50, Minicon A-75, and Amicon S-125 filters. As a result another four fractions were obtained, containing proteins with molecular weights (mol. wt.) of: 6) <50,000 daltons (D); 7) >50,000 D; 8) >75,000 D, and 9) >125,000 D.

The specific antigen was detected in all kidney fractions by methods of immunodiffusion in gel, with the use of specific antikidney sera. The physicochemical properties of the kidney-specific antigen also were studied.

EXPERIMENTAL RESULTS

The results showed that specific kidney antigen is present in kidney extracts and supernatants after centrifugation at 15,000 and 70,000g, and also in fractions containing proteins with mol. wt. >50,000 and >75,000 D. It was not discovered in residues suspended in buffered sucrose solution, in fractions containing proteins with mol. wt. <50,000 and >125,000 D (Fig.

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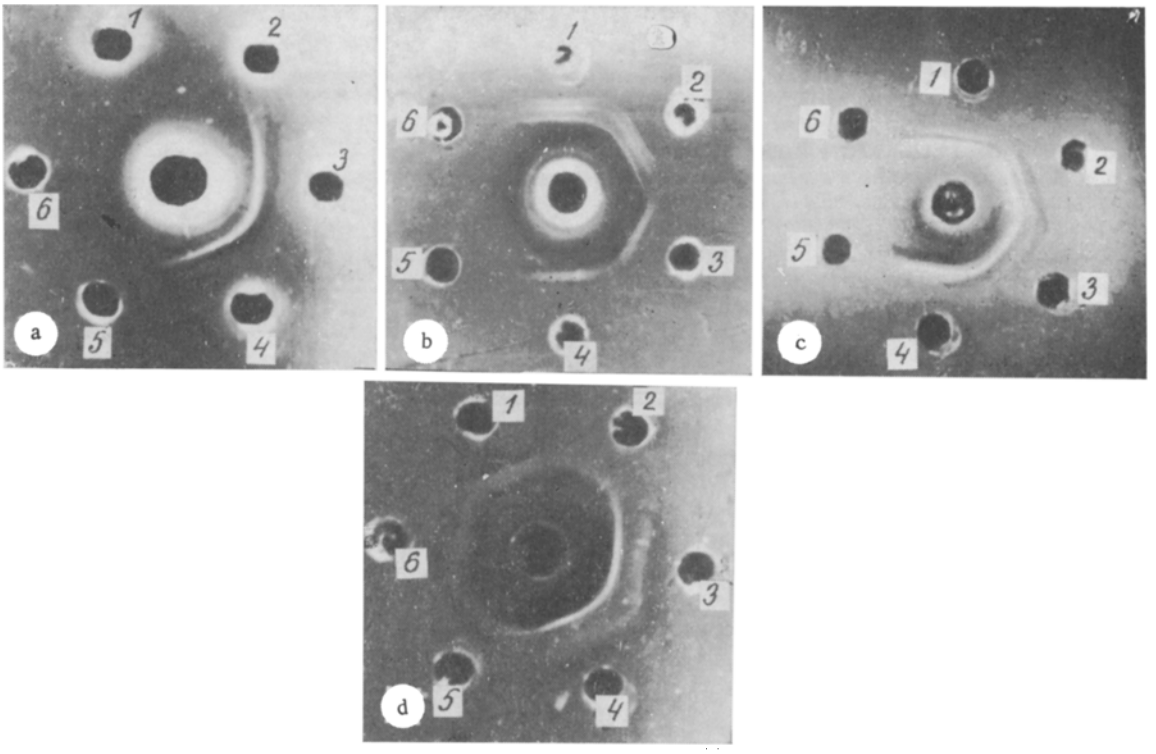

Fig. 1. Results of agar diffusion test with adsorbed antikidney serum (central well) and different fractions of kidney extract (peripheral wells). Fractions of kidney extract containing proteins with mol. wt. of: a) <50,000 D (1 and 2), >50,000 D (3 and 4); >125,000 D (5 and 6); b) 75,000 D (1 and 2); >50,000 D (3 and 4), mixture of extracts from heterologous organs — liver, heart, lung, spleen (5 and 6); c) fractions salted out with $(NH_4)_2SO_4$ at 50% saturation of kidney extract (1 and 2), at 70% saturation (3 and 4), and 20% saturation (5 and 6); d) kidney extract heated at 75°C (1 and 2), 65°C (3 and 4), and 85°C (5 and 6).

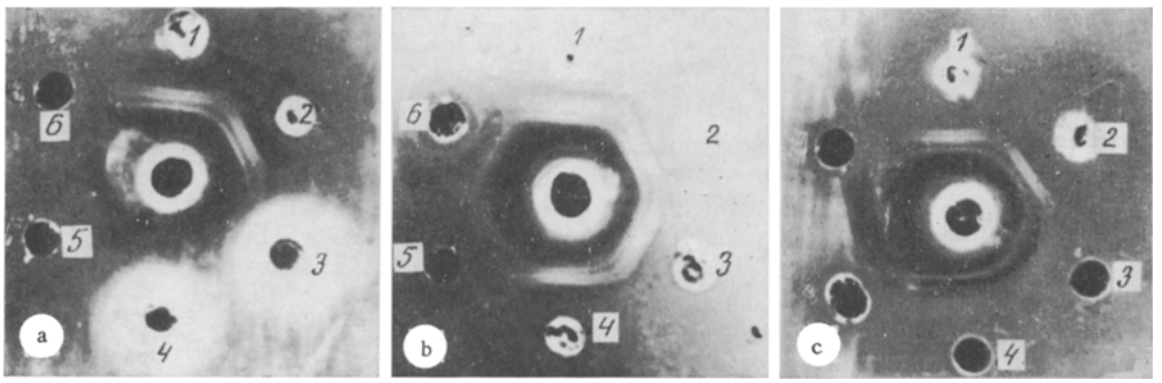

Fig. 2. Results of agar diffusion test with adsorbed antikidney serum (central well) and with kidney antigen after exposure to the action of enzymes (in peripheral wells). Fractions of kidney extract containing proteins with mol. wt. >75,000 D: a) not subjected to treatment (1 and 2), after treatment with trypsin in a dose of 50 mg/ml for 1 day (3 and 4), mixture of extracts from heterologous organs (5 and 6); b) not treated with enzymes (1 and 2), after treatment with hyaluronidase (3 and 4), mixture of extracts from heterologous organs (5 and 6); c) not treated with enzymes (1 and 2), after treatment with RNase in a dose of 1 mg/0.3 ml for 1 day (3 and 4), mixture of extracts from heterologous organs (5 and 6).

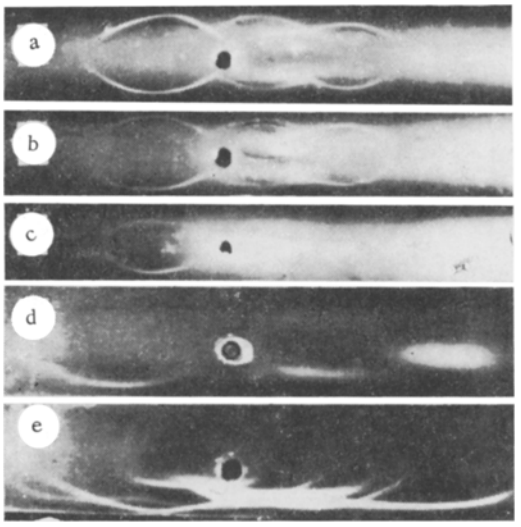

Fig. 3. Results of immunoelectrophoresis. Wells (from top to bottom) contain kidney extracts treated with DNase in a dose of 50 μg (a), RNase in the same dose (b), papain in a dose of 50 mg for 24 h (c), papain in a dose of 50 mg for 1.5 h (d); e) human blood serum. Bottom gutter contains serum precipitating human proteins; all others contain antikidney serum.

1a, b). Consequently, mol. wt. of kidney-specific antigen >75,000 D but <125,000 D, i.e., it is about 100,000 D.

Kidney-specific antigen was found in fractions precipitated with ammonium sulfate by 50 and 70% saturation of the kidney extract, but it was not found in fractions precipitated at 20 and 30% saturations (Fig. 1c).

On heating in a waterbath at 45, 56, and 65°C for 30 min the kidney-specific antigen preserved its immunologic activity, although it disappeared at 75 and 85°C (Fig. 1d).

In reactions with extracts treated with trypsin or papain for 24 h the specific antigen was no longer detectable (Figs. 2a and 3). In reactions with extracts treated with the above enzymes for 1.5 h the character of the precipitation band formed by kidney-specific antigen and the corresponding antibodies changed. It became wide, with indistinct outlines (Fig. 3d). Consequently, kidney-specific antigen is sensitive to trypsin and papain. However, it was resistant to hyaluronidase (Fig. 2b).

Kidney-specific antigen was resistant to RNase and DNase provided that these enzymes were used in a dose of 50 μg/ml extract (Fig. 3a, b), but it was partially destroyed by the use of these enzymes in a larger dose (1 mg/0.3 ml of extract) and during an exposure of not less than 24 h (Fig. 2c).

The results thus show that the kidney-specific antigen demonstrated by these experiments is evidently not a glycoprotein, but it may be a ribonucleoprotein or deoxyribonucleoprotein.

To continue the study of the physicochemical and immunologic properties of kidney-specific antigen and also to develop methods of detecting sensitization to specific kidney antigen in patients with various forms of autoimmune nephritis, this antigen must be isolated in the purified form.

LITERATURE CITED

1. R. F. Averkina, Byull. Éksp. Biol. Med., No. 6, 92 (1969).
2. R. F. Averkina, Byull. Éksp. Biol. Med., No. 4, 74 (1972).
3. R. F. Averkina, Arkh. Patol., No. 3, 56 (1975).

4. R. F. Averkina, Byull. Éksp. Biol. Med., No. 6, 566 (1979).
5. R. F. Averkina, in: Data on Current Problems in Modern Histopathology [in Russian], Moscow (1971), p. 68.
6. E. S. Mashkina, R. F. Averkina, and I. M. Yarovaya, Arkh. Anat., No. 3, 85 (1980).
7. P. Freedman et al., Arch. Intern. Med., 105, 524 (1960).
8. D. Koffler and F. Paronetto, J. Clin. Invest., 44, 1665 (1965).