IMMUNOCHEMICAL STUDY OF THE CBA MOUSE KIDNEY

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Seven antigens, one of which (with a relative electrophoretic mobility of 0.35) is specific for the kidney, were detected in the CBA mouse kidney by immunoelectrophoresis and the agar precipitation test. The remaining kidney antigens were common to the kidney and other mouse organs and tissues. Antigens with the mobility of albumin and α globulin were serum in origin; an antigen with close to zero mobility was common to the kidney and liver; an antigen with the mobility of α_2 globulin was evidently not homogeneous and, besides wide interorgan specificity, most closely resembled lung antigen.

KEY WORDS: kidney; antigens; organ specificity.

An important role in the pathogenesis of several diseases, including nephritis, is played by autoimmune mechanisms [8, 9]. To study these mechanisms and also ways of reducing the frequency of allergic states, an important step is to determine the sensitizing properties of organ antigens, notably those of the kidney. However, before these problems can be solved, it is first necessary to undertake an immunochemical analysis of the kidney tissue of healthy individuals. This problem has received insufficient attention in the literature [3, 7].

Accordingly the aim of the present investigation was to make an immunochemical study of the kidney of healthy CBA mice, animals on which the writers had previously [4, 5] developed an experimental model of autoimmune tubulonephritis.

EXPERIMENTAL METHOD

Immune sera were obtained against antigens of CBA mouse kidneys in 6 rabbits by two methods. In the first method, kidney homogenate was injected intraperitoneally into three rabbits in a dose of 10 ml per animal, and two weeks later a saline extract was injected intravenously in a dose of 1 ml, eight times at intervals of 3 days. The course of intravenous injections was repeated three times at monthly intervals and a 4th time after 6 months. As a result, immune sera were obtained with titers of antikidney antibodies of 1:1000 two months, 1:2000 8 months, and 1:4000 15 months after the beginning of immunization.

Another three rabbits were immunized with kidney extract together with Freund's adjuvant in accordance with the scheme described previously [1]. As a result, after two months, sera with an antibody titer of 1:8000 and 1:4000 were obtained.

The agar precipitation test on slides and immunoelectrophoresis were used, with both unadsorbed and adsorbed antikidney sera. The experimental conditions and the methods of adsorption of the sera were described previously [2, 6, 10].

Extracts of the kidney, liver, lung, heart, spleen, skin, stomach, intestine, uterus, placenta, thymus, lymph nodes, testis, subcutaneous connective tissue, muscle, brain, and eye and also blood serum were used as antigen.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that seven antigens with a relative electrophoretic mobility (compared with the mobility of human serum proteins) of albumin and of α, β, and γ globulins, were detected in a saline extract of the mouse kidney.

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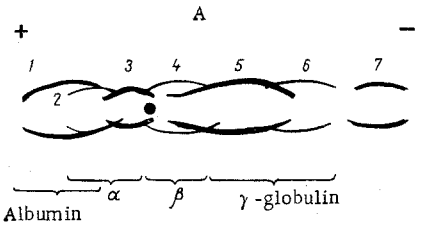

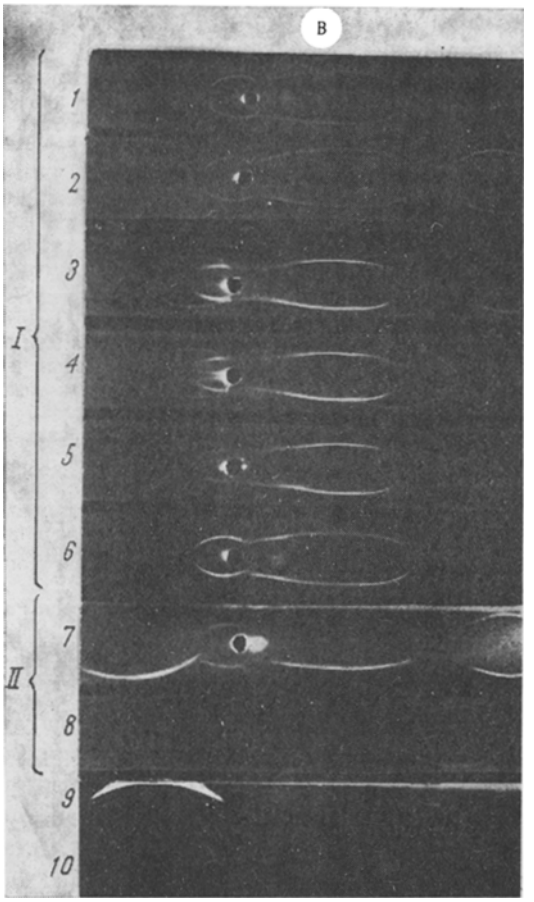

Fig. 1. Results of immunoelectrophoresis. A) Composite scheme; B) results of individual tests. Antikidney sera in troughs. I) Nos. 1-6 obtained by first method (number 1, 2 months; number 2, 8 months; number 3, 15 months after beginning of immunization). II) Nos. 7 and 8 obtained by second method, No. 9, serum precipitating human protein. Immune sera Nos. 1-6 and No. 7 (below wells) not adsorbed; No. 7 (above) adsorbed with liver powder; Nos. 8, adsorbed with liver and lung extract. Wells contain following extracts: Nos. 1-3, 7, and 8) kidney, No. 4) liver, No. 5) spleen, No. 6) lung, No. 9) human albumin; No. 10) dextran.

Antigens 1 and 2 were found both in the kidney and in extracts of other mouse organs, and also in blood serum. Antigens 3-6 were shown to be interorgan antigens and to have no similarity with serum proteins. Antigen 3 was found very clearly in lung extract as well as in kidney extract (Fig. 1, No. 6). Antigen 6 was found in the liver as well as in the kidney (Fig. 1, No. 4). Antigen 7 was found in kidney extract only (Fig. 1, Nos. 2, 3, 7).

Precipitin arcs corresponding to antigens 1, 3, 5 and 7 (Fig. 1) were dense, whereas the precipitin arcs corresponding to the other antigens were thin and less marked.

TABLE 1. Antigens of CBA Mouse Kidney and Their Similarity to Antigens of Other Organs (values of relative electrophoretic mobility)

Kidney antigens	Zone of mobility	Electrophoretic mobility	Zones of distribution
1	Albumin	1,02 (1,14—0,90)	Blood serum and many organs
2	α-Globulin	0,85 (0,88—0,82)	The same
3	α_2 Globulin	0,72 (0,78—0,70)	Many organs, especially lung
4	β_2 Globulin	0,38 (0,44—0,32)	Many organs
5	γ Globulin	0,22 (0,25—0,16)	The same
6		0,05 (0,10—0,12)	Kidney, liver
7		—0,35 (from —0,20 to —0,53)	Kidney only

Legend. Results of 154 immunoelectrophoresis tests, including 84 with kidney extract and 70 with extracts from other organs and blood serum, are given in this table.

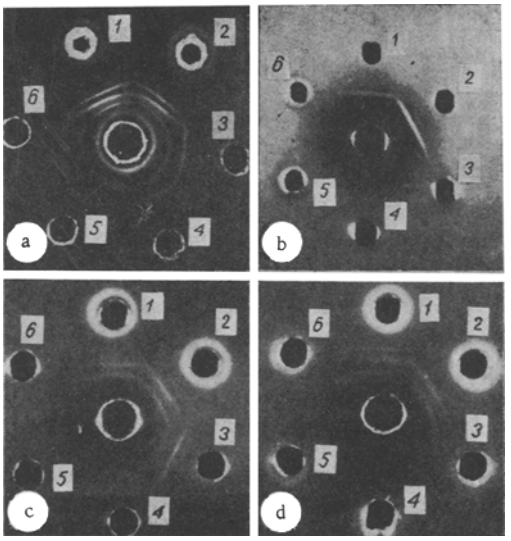

Fig. 2. Results of agar precipitation test. Central wells contain antikidney serum adsorbed with mouse blood serum (A), and additionally with a mixture of liver and lung extracts (B), and liver powder (C and D). Peripheral wells contain: 1 and 2) kidney extract; 3 and 4 in A, B, and C) lung extract; 3 and 4 in D) spleen extract; 5 and 6) liver extract.

Immune sera obtained by the first method revealed 2 or 3 antigens (the 3rd-5th) in kidney extract two months after the beginning of immunization (Fig. 1, No. 1); after 8 months they revealed 3 or 4 antigens, antigen 7 having been found in addition (Fig. 1, No. 2); after 15 months they revealed 5 to 7 antigens (Fig. 1, No. 3).

Immune sera obtained by the second method revealed antigens 1, 3, 5, and 7 in kidney extract (Fig. 1, No. 7 below the well), i.e., basically the same as sera obtained by the first method after 8 months.

Antikidney sera, adsorbed twice with liver powder, continued to react with kidney extract, with the formation of two precipitin arcs (Fig. 1, No. 7 above the well). These sera did not react with extracts of liver, spleen, or heart but formed a weak precipitin arc with lung extract in the zone of mobility of α_2 globulin. Sera adsorbed with liver and lung extract formed only one precipitin arc in the zone of mobility 0.26 (Fig. 1, No. 8).

Since antibodies against kidney antigens 3 and 5 appeared sooner (2 months) in the rabbits during immunization, these antigens were evidently strong; antigen 7 was of average strength, for it was detected by sera obtained as the result either of longer immunization (after 8 months) or with the use of an adjuvant; other kidney antigens, not counting the serum antigens, were weak and antibodies were formed against them only after 15 months.

The agar precipitation test revealed 5 or 6 antigens in mouse kidney extract. Antikidney sera, adsorbed with mouse blood serum, continued to react with kidney extract with the formation of four precipitin bands, one

of which was formed only with kidney extract (Fig. 2A); the other three precipitin bands were common to the kidney and other organs. Lung and kidneys tissues were found to possess the greatest similarity in antigenic properties, followed by the uterus, stomach, intestine, and liver. The least similar were tissues of the kidney and brain, eye, and muscles; other organs occupied an intermediate position as regards the degree of their antigenic similarity with the kidney.

Antikidney serum, adsorbed twice with liver powder, reacted with kidney extract with the formation of 2 or 3 precipitin bands, and with lung extract with the formation of one or two bands, whereas with other heterologous extracts only one precipitin band was formed or no reaction was present (Fig. 2C, D). Adsorption of the antikidney serum with liver and lung extract removed antibodies against antigens common to the kidney and other organs, and such a serum reacted only with kidney extract, with the formation of one precipitin band (Fig. 2B).

Clearly the results of the agar precipitation test agree on the whole with those of immunoelectrophoresis and are evidence that mouse kidney tissues contain a specific kidney antigen besides various antigens common to several organs.

According to data in the literature [7], the specific human kidney antigen has been identified as an α_2 macroglobulin. Previously [3], the present writer showed that the human kidney contains a specific, positively charged antigen, with marked cathodal mobility. The results of the present investigation confirm these findings and show that mouse kidney, like human kidney, contains a specific antigen with similar mobility.

Since specific antigens have been detected in the kidneys, it will be possible in future to study their chemical nature, their sensitizing properties, and their role in the pathogenesis of autoimmune nephritis.

LITERATURE CITED

1. R. F. Averkina, in: Laboratory Methods of Investigation of Noninfectious Immunology, in: Laboratory Immunology, O. E. Vyazov (ed.)[in Russian], Moscow (1967), p. 55.
2. R. F. Averkina, Byull. Éksp. Biol. Med., No. 4, 74 (1972).
3. R. F. Averkina, in: Principles of Immunoembryology [in Russian], O. E. Vyazov and V. M. Barabanov, eds, [in Russian], Moscow (1973), pp. 83 and 104.
4. R. F. Averkina, Arkh. Patol., No. 3, 56 (1975).
5. R. F. Averkina, in: Current Problems in the Immunology of Reproduction [in Russian], Novosibirsk (1977), p. 142.
6. R. F. Averkina and O. E. Vyazov, Byull. Éksp. Biol. Med., No. 6, 92 (1969).
7. P. G. Prokopenko, Byull. Éksp. Biol. Med., No. 8, 210 (1977).
8. V. V. Serov, Morphological Basis of Immunopathology of the Kidneys [in Russian], Moscow (1968).
9. T. G. Trayanova, V. V. Sura, M. K. Mazharov, et al., Ter. Arkh., No. 7, 39 (1974).
10. A. H. Coons and M. M. Kaplan, J. Exp. Med., 91, 1 (1950).