IMMUNOCHEMICAL IDENTIFICATION OF AN α_2-MACROGLOBULIN SPECIFIC FOR THE HUMAN KIDNEY

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An α_2-macroglobulin specific for the kidney (RS α_2-macroglobulin) with a molecular weight of 2×10^6, was identified by immunochemical analysis. The antigen was shown not to be immunochemically identical with α_2-H-globulin (ferritin), renal-pancreatic α_2-globulin, uromucoid, or serum α_2-macroglobulin. The content of RS α_2-macroglobulin in kidney tumor tissue was lower than in the normal kidney and none whatever was found in 16 of 23 tumors. At the level of sensitivity of the monospecific test system for RS α_2-macroglobulin it could not be found in healthy human blood or in the blood and urine of patients with nephrological diseases.

KEY WORDS: human kidney; organ-specific antigen; cancer of the kidney.

The study of organ-specific antigens responsible for the chemical and functional individuality of an organ is an urgent task in modern cancer immunochemistry because malignant change in a tissue is accompanied by the loss of predominantly its organ-specific antigens [1, 3]. The existence of antigens specific for the human kidney has been demonstrated by only a few investigations [2, 11, 12]. However, the fact that these antigens have not been identified in various other organs or with the cytoplasmic antigens of the genitourinary tract, the absence of details of methods for their isolation, and the narrowness of the specificity control (only liver, lung, spleen, heart, and small intestine have been represented in the specificity control) prevent any reliable conclusions regarding their specificity for the kidney. Meanwhile the writer has observed that the human kidney contains an antigen of narrow specificity, namely renal-pancreatic α-globulin (RP α-globulin), which has been found only in the kidney, pancreas, and stomach [7]. The possibility cannot be ruled out that the kidney is closer antigenetically speaking to these latter organs than to the liver, spleen, and lung.

The object of this investigation was the immunochemical identification of a human renospecific α_2-macroglobulin, to study some of its physicochemical properties and to determine its content in normal and tumor tissue from the kidney.

EXPERIMENTAL METHOD

Tissue extracts were prepared in Tris-glycine buffer (pH 8.3) with Triton X-100. Under standard conditions extracts were obtained from the organs of adults dying from trauma, from fetuses obtained by termination of pregnancy on medical grounds, and from tumors of the kidneys obtained after operations for malignant neoplasms of the kidneys with a histologically established diagnosis of "carcinoma of the kidney." Extracts were prepared from two regions of the kidney tumors: from the tumor tissue itself and from tissue of the same kidney not visibly affected by the tumor process. Extracts from animal kidneys were obtained under similar conditions.

Antisera were prepared by immunizing eight rabbits with a fraction of pooled extract of kidneys insoluble at 30% saturation with ammonium sulfate. These antisera were exhausted with lyophilized donors' plasma and serum, and with extracts of adult organs (liver, spleen, lung, thyroid gland, brain, heart, adrenal, pancreas,

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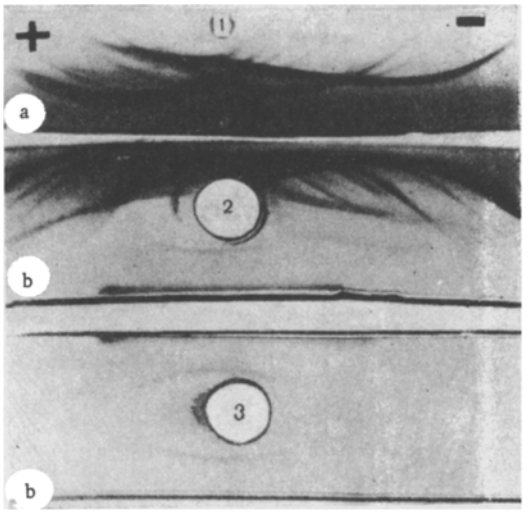

Fig. 1

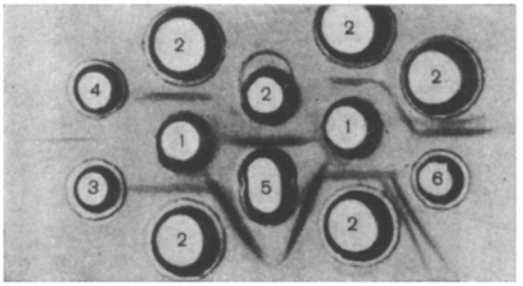

Fig. 2

Fig. 1. Immunoelectrophoretic analysis of RS α_2-macroglobulin. 1) Donors' blood serum; 2, 3) kidney extract. Antiserum against RS α_2-macroglobulin before (a) and after (b) exhaustion with donors' plasma and serum and with extracts from adult human organs (liver, spleen, lung, thyroid gland, brain, heart, adrenal, pancreas, gastric mucosa, bladder, small and large intestines, renal pelvis, ureter, prostate gland, uterus, skin, ovary, and muscle) and fetal thymus.

Fig. 2. Immunochemical identification of RS α_2-macroglobulin. 1) Monospecific antiserum against RS α_2-macroglobulin; 2) kidney extract (standard test antigen); 3) antiferritin serum; 4) ferritin (standard test antigen); 5) antiserum against RP α_2-globulin [7]; 6) antiserum against uromucoid.

gastric mucosa, bladder, small and large intestine, renal pelvis, ureter, prostate gland, uterus, skin, ovary, and muscle) and fetal thymus. In some cases the antiserum was additionally exhausted with extracts from normal kidney and tumors of the kidney.

A modified method [9] of immunodiffusion dialysis [13], immunoelectrophoresis [10], gel filtration [5], disk electrophoresis [6], and methods of specific detection of glyco-, lipo-, and ferroproteins [5, 8] were used.

EXPERIMENTAL RESULTS

Antisera (Nos. 6 and 18) exhausted with lyophilized plasma, donors' serum, and extracts from the organs listed above continued to react in the precipitation test with kidney extract in a single precipitation line. On immunoelectrophoresis the protein migrated in the α_2-globulin zone (Fig. 1). Its electrophoretic mobility relative to serum albumin was 0.78.

A monospecific test system obtained for kidney α_2-globulin was used to study its identity in tests with organs and with known cytoplasmic proteins of the α-globulin zone and with uromucoid (Fig. 2). As Fig. 2 shows, the kidney α_2-globulin was not identical with α_2-H-globulin (ferritin), RP α_2-globulin, or uromucoid (antiserum against uromucoid was obtained from Behringwerke, West Germany). Monospecific antiserum against kidney α_2-globulin had no antibodies against serum α_2-macroglobulin (from Behringwerke, West Germany).

Partially purified renal α_2-globulin, on gel filtration on a Sephadex L-200 column, was eluted with the blue Dextran peak (molecular weight 2×10^6), it migrated in the α_2-macroglobulin zone during disk electrophoresis in 7.5% polyacrylamide gel, it was unstable when kept for 5 min at 80°C, and it was insoluble in trichloroacetic and sulfosalicylic acids and on 35% saturation of the protein solution with ammonium sulfate; it stained for protein but not for glyco-, lipo-, and ferroproteins.

At the level of sensitivity of the standard test system the kidney α_2-macroglobulin could not be detected in 159 samples of extracts from adult human organs as listed above, in 53 samples of fetal organs (liver, spleen, lung, thymus, adrenal, pancreas, stomach, large and small intestines) at different periods of gestation,

TABLE 1. Content of Kidney α_2-Macroglobulin in Embryonic and Definitive Human Kidneys and in Kidney Tumors

Material examined	Total number of samples tested	Number of positive samples	Mean titer and limits of variation
Fetal kidney	8	8	1 : 3 (1 : 2—1 : 8)
Adult kidney	43	43	1 : 3,7 (1 : 1—1 : 8)
Carcinoma of the kidney:			
tumor tissue	23	7	1 : 1,9 (1 : 1—1 : 4)
surrounding normal kidney	23	23	1 : 6,2 (1 : 1—1 : 16)
Carcinoma of the renal pelvis:			
tumor tissue	2	0	
surrounding normal kidney	2	2	1 : 3 (1 : 1—1 : 4)

in the serum of 33 blood donors, in the blood and urine of 24 patients with carcinoma of the kidney, and in 80 samples of blood and urine from 12 recipients of a cadaveric kidney. On the other hand, it was constantly found in adult and fetal kidneys (Table 1), but never in mouse, rabbit, ox, and pig kidney. On the basis of these results the α_2-macroglobulin can be regarded as a specific antigen for the human kidney.

The content of renospecific α_2-macroglobulin (RS α_2-macroglobulin) in the kidney tumor tissue was lower than in embryonic and definitive kidneys, and none could be detected in 16 of 23 tumors (Table 1). Its content in the tissue of the visually unaffected kidney tissue around neoplasms of the kidney was a little higher than in the normal kidney, evidently as the result of compensatory hypertrophy of the differentiated kidney cells.

Exhaustion of the monospecific antiserum against RS α_2-macroglobulin with a kidney tumor extract giving a negative reaction for RS α_2-macroglobulin did not affect the activity of the antiserum; exhaustion of the antiserum with kidney extract did inactivate it. The RS α_2-macroglobulin evidently disappeared from the kidney cell parallel with the degree of loss of its functional characteristics.

It would be interesting to attempt to identify RS α_2-macroglobulin by more sensitive methods in the blood serum and urine of patients with nephrological diseases and also to estimate it quantitatively in biopsy material from the kidneys, when it evidently could reflect the functional integrity of the kidney cells.

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