Renal Cell Carcinoma Neoplastic Antigens

by
Carmela Patricia Morales

University Undergraduate Fellow, 1986-1987.

Texas A&M University

Department of Pathology and Laboratory Medicine

APPROVED

Fellows Advisor: John Kerkvliet MD

Honors Director: Louise Caldwell

Acknowledgements

I wish to express my sincerest gratitude to my advisor, Dr. John Kochevar, for his time and patience. Working with him was an invaluable experience and his constant support was truly inspirational.

I also wish to thank Dr. Joyce Davis and the entire staff at the department of Pathology and Laboratory Medicine. They were always helpful and I enjoyed working with them.

I would like to thank my parents for their love and friendship not only during this project, but throughout my years at Texas A&M.

Lastly, thanks to Mike, who made my senior year unforgettable.

Table of Contents

Table of Illustrations

ABSTRACT

A renal cell carcinoma neoplastic antigen was purified from a freeze-thaw extract of cultured tumor cells using immunoaffinity chromatography. In addition, renal cell carcinoma immune complexes were also isolated from the serum of a patient with metastatic disease. This was done using a previously described renal carcinoma selective monoclonal antibody covalently linked to Sepharose 4B. The crude samples were passed through the column and the antigens and immune complexes were eluted with an acidic buffer. The neoplastic antigen was represented as a single protein band with a molecular weight of 61kd on SDS polyacrylamide slab gel electrophoresis under reducing conditions. The blood serum eluate showed four distinct bands with molecular weights of 70, 50, 23 and 61 kd. Using immunoblotting assays, the first three bands were found to be IgG heavy, IgM heavy and IgG light antibody chains, respectively. The 61kd band was the neoplastic antigen. These results suggest that the antibody may be useful in a clinical assay for the detection and monitoring of renal cell carcinoma.

INTRODUCTION

Renal cell carcinoma is newly diagnosed in about 20,000 Americans annually (1). By the time that the intial dignosis is made, metastases will be present in about 33 per cent of the cases (2). Renal cell carcinoma is a common tumor, being 13th on the list of most frequently occurring malignant neoplasms (3). This high incidence as well as some unusual features of the tumor system have made its study especially challenging.

Renal cell carcinoma, also known as renal adenocarcinoma, is a type of cancer which has its origin in the proximal convoluted tubular lining cells of the kidney. The classical symptoms of presentation are hematuria, pain and a flank mass (4). There is substantial variation in this triad and hematuria is the most important indicator of ~~malignant~~ renal carcinoma. The presence of a palpable mass is a later feature that is associated with advanced pathologic stage of disease. At the present time, there is no early detection test for renal cell carcinoma. Ultrasonic and roentgenographic techniques are currently used as diagnostic tools. Both of these, however, rely on the presence of a relatively large tumor mass and are thus limited to diagnosing later stages of the disease, when the tumor has almost inevitably metastasized. At autopsy, 95 per cent of renal carcinoma victims display metastases, most commonly to the lungs, liver, bone and lymph nodes (5). There are two important routes by which metastases

can occur, direct extension and vascular invasion. The renal vein is the most common route for the release of tumor cells into the circulatory system. There are several reasons for which the high rate of metastases can be attributed, the most important being delay in seeking medical attention. Most patients seek medical care when their normal routine is disrupted by disease. Pain is a common reason for seeking medical care and is often the first sign of renal carcinoma. A lack of pain is not uncommon with early growth of renal carcinoma and as a result the average delay from onset of symptoms to treatment is 1.8 years (6).

The prognosis of patients with renal carcinoma is relatively poor. Advanced stages of the disease are associated with a five year survival rate of less than 10 per cent (7). The ten year survival rate for patients after nephrectomy of a stage A disease is about 90 per cent, while the same rate for nephrectomy at a late stage of the disease is about 20 per cent. These rates suggest that early diagnostic tests would be of extreme importance in improving the survival rate by allowing treatment to begin at an earlier stage.

Our approach to the development of a clinical assay to detect the presence of renal carcinoma has focused on the host-tumor interaction. Reports of spontaneous tumor regression, although rare, have been documented (8). Even more importantly, previous studies have reported the presence of circulating antibodies in the serum of patients with

metastatic renal carcinoma (9). These observations suggest that humans have a primary immune response to the tumor and have prompted a variety of studies of renal carcinoma neoplastic antigens including immunotherapy and development of tests for circulating or excreted antigens as markers for disease.

Monoclonal antibodies have provided powerful tools for studying renal carcinoma neoplastic antigens. There have been numerous reports of anti-renal cell carcinoma monoclonal antibodies (12-15). Generally, these studies have described a complex pattern of antigen cross reactivity with other tumors and in some cases with adult and fetal normal tissues. In 1981, Ueda and associates reported a panel of seventeen monoclonal antibodies that immunoprecipitated three glycoproteins of 160, 120, and 115 kilodaltons (12).

In 1985, Finstad and associates performed further studies on these antibodies (13). It was reported that monoclonal antibody S4 reacted with a variety of frozen tissue sections, specifically 16 of 20 renal cell carcinomas, an osteosarcoma, a spindle cell carcinoma and a mesothelioma by immunofluorescent staining. In addition, it also reacted with the glomerulus, proximal tubule, and interstitial matrix of adult and fetal kidney. Sedmak and Tubbs reported that the specificity of the S4 monoclonal antibody for renal adenocarcinoma was 87.5 per cent after immunoperoxidase staining frozen sections which included lung, breast, colo-

rectal, ovarian, renal and uterine primary tumors (14). These results suggest that monoclonal antibody S4 may be useful in determining the primary location of adenocarcinomas of unknown origin.

In 1986, Oosterwijk and associates described a monoclonal antibody that reacted with an antigen on the membrane of renal cancer cells as well as with a variety of normal tissues (15). Attempts to isolate the antigen with affinity chromatography and immunoblotting were met with little success.

Most of these reports have shown cross reactivity with normal tissues and thus are not suitable for specifically directed immunotherapy or marking. It is the purpose of this project to isolate and identify a tumor protein unique to renal carcinoma. The following report describes the purification of a renal cell carcinoma neoplastic antigen by immunoaffinity chromatography using a monoclonal antibody reactive with renal cell carcinoma.

MATERIALS AND METHODS

Two different sources of antigens were subjected to the analysis described below. The first was a freeze-thaw extract of cultured renal carcinoma cells (ACHN) obtained from the American Type Culture Collection (Rockville, Maryland). ACHN was developed from a 22 year old patient with widely disseminated renal cell carcinoma. The second antigen source was human serum taken from a patient with metastatic renal cell carcinoma.

PREPARATION OF A RENAL CARCINOMA SPECIFIC MONOCLONAL ANTIBODY.

The monoclonal antibody was developed by the student's advisor and was available at the onset of this project (10). Briefly, a BALB/C mouse was hyperimmunized with the cultured tumor cells. The spleen cells were fused with myeloma cells by the addition of polyethylene glycol. Dot immunoblotting assays were used to detect antibody producing clones reactive with tumor cell extracts and nonreactive with normal kidney cell extracts. The hybridoma selected on the basis of the immunoblotting results was subcloned twice by limiting dilution. The antibody has shown to be selective for renal cell carcinoma by immunoperoxidase staining using paraffin tumor sections. It has reacted with all of about 90 cases of renal cell carcinoma tested to date. It also occasionally reacts with other neoplasms, notably malignant melanoma but it is nonreactive with normal tissues.

PROTEIN A CHROMATOGRAPHY FOR ANTIBODY PURIFICATION.

The monoclonal antibody was purified from hybridoma cell supernatant by protein A affinity chromatography by the manufacturer's instructions (AffiGel,Protein A, (MAPS), Biorad Laboratories Richmond, California). As directed, the supernatant was first concentrated fourfold in Aquacide II (Behring Diagnostics, La Jolla, California). The concentrate was diluted 1:1 in MAPS binding buffer, pH 9.0. The sample was then loaded onto the prepared column and washed with binding buffer until the absorbance at 280nm approached that of the pure binding buffer. The antibody was eluted with MAPS elution buffer, pH 3.0, and the absorbance of each fractionat 280nm identified the fractions with the highest protein concentration. These were combined, dialyzed in 10^8 volumes of phosphate buffered saline (PBS) and concentrated fivefold in Aquacide II (Behring Laboratories, Richmond, California). Sodium dodecyl sulfate polyacrylamide gel electrophoresis verified antibody purityand the protein concentration was determined by the Lowry method.

LOWRY METHOD FOR PROTEIN CONCENTRATION DETERMINATION (11).

The protein concentration of the column eluate and unpurified samples was determined using a modification of the method of Lowry. The first step was the preparation of five solutions labelled A,B,C,D and E. Solution A was composed of 2% (w/v) Na_2CO_3 in 0.01N NaOH. Solution B was 1% (w/v) $CuSO_4$ in distilled water. Solution C contained 2% (w/v) sodium tartrate in distilled water. Solution D was composed

of 1 part solution A:0.01 part solution B:0.01 part solution C. Solution E was 1N Folin's reagent.

A standard protein curve using 1mg/ml bovine serum albumin (BSA) and the unknown samples were set up in 10x75mm test tubes according to table 1. Each tube was vortexed and the tubes were set for 10 minutes. 100 ul of solution E was added to each tube and immediately vortexed. The panel was allowed to set for 30 minutes. The absorbance readings of tubes 2 through 9 were taken at 670nm using tube 1 as the correction blank.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR BUFFER DETERMINATION (16).

ACHN cells were grown to confluence in tissue culture flasks then removed from the flask surface by gentle scraping with a syringe plunger. The cells were washed and resuspended in PBS and then frozen. After thawing, the suspension was ultracentrifuged to pellet and remove cellular debris. The protein concentration of the supernatant (usually 1mg/ml) was adjusted to 0.01mg/ml with PBS. 100ul of the solution was added to each well of a polystyrene microtiter plate and allowed to dry overnight. The protein was fixed onto the well by adding 50% methanol to each well for 10 minutes, followed by a wash with PBS. The non-specific protein binding sights were blocked by adding 300ul of 1% bovine serum albumin in PBS for 30 minutes. Each well was then rinsed thrice with 0.05% Tween-20 in PBS. 100ul of a 0.1mg/ml monoclonal

antibody solution was added to each well for 45 minutes. The wells were rinsed with PBS and 100ul of each of the prospective buffers was added to an individual well for 45 minutes (table 2). The wells were thoroughly rinsed with PBS and incubated with 100ul of a 1:500 dilution of goat antiserum to mouse IgG labelled with peroxidase (Cooper-biomedical, Malvern, Pennsylvania) for 45 minutes. The wells were washed with PBS and 100ul peroxidase substrate solution (Bio-Rad laboratories, Richmond, California) was added to each well. A deep green color indicated a positive reaction. The most strongly positive and strongly negative reactions determined binding and eluting buffers, respectively.

ELISA FOR VERIFYING IMMUNOREACTIVITY OF THE PURIFIED ANTIGEN.

The procedure was identical to the ELISA described above except that 0.1ug of the purified antigen was added to each well and no buffers were added to any of the wells.

IMMUNOAFFINITY CHROMATOGRAPHY FOR ANTIGEN PURIFICATION.

Thepurified antibody was prepared for linkage by overnight dialysis against 10^8 volumes of coupling buffer (0.1M $NaHCO_3$, pH 8.3, plus 0.5M NaCl). Activated Sepharose 4B (Pharmacia, Uppsala, Sweden) was swelled and washed with excess 1mM HCl over a sintered glass filter. The slurry was rinsed and reswollen with coupling buffer. Antibody (5mgs) in coupling buffer was mixed with each ml of slurry and gentlyrocked in a capped tube for 2hours at room temperature. The unbound

antibody was rinsed away with coupling buffer over a sintered glass filter.Thegel was reswollen in 0.1mMTris-HCl, pH 8.0, and rocked for two hours to block any unbound protein binding sights. The slurry was then washed thrice in alternating cycles of 0.1M acetate, pH 4.0, plus 0.5 M NaCl and 0,1M Tris, pH 8.0, plus 0.5M NaCl over a sintered glass filter. The gel wasreswollen and stored inPBS at 4C after adding 0.01% thimerosal.

The prepared column was equilibrated with 15 bed volumes of MAPS binding buffer. A 1:1 mixture of the cultured tumor cells and binding bufferwas then loaded onto the column (1ml total volume). Unbound protein was then washed away with excess binding buffer until the absorbance at 280nm approached that of binding buffer alone.

The antigen waseluted with elutionbuffer into 1ml fractions. Absorbance readings at 280nm identified the fractions with the greatest protein concentrations. These were combined and dialyzedagainst 10^8 volumesof PBS overnight at room temperatureand concentrated twofold with AquacideII. The protein concentration was determined by the Lowry method and eluate purity was verified by a SDS-PAGE as described below.

SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS (17).

Aliquots of the column eluate, unpurified samples and molecular weight standards were electrophoresed under reducing conditions in a discontinuous buffer system on a 12% slab gel. The separating gel was prepared by mixing 16ml of 30%

acrylamide and 0.8% bis acrylamide in distilled water (bis 30:0.8),10ml of 0.5M Tris-HCl, pH 8.6, and 13.6ml distilled water and 0.4ml of 10% sodium dodecyl sulfate (SDS). Polymerization was initiated by the addition of 0.02ml N,N,N',N'-tetramethylethylenediamine (TEMED) and 0.35ml 10% ammonium persulfate. The mixture was poured into a Hoefer vertical gel slab unit (model SE400, San Fransisco, California) and set for 15 minutes.

The stacking gel was prepared by mixing 1.3ml bis 30:0.8, 2.0ml 1.5M Tris-HCl, pH 6.8, 4.6ml 50% glycerol and 0.8ml 10% SDS. Polymerization was initiated by adding 0.005ml TEMED and 0.075ml 10% ammonium persulfate. The mixture was immediately poured over the separating gel. A 15 well comb of 1.5mm thickness was inserted into the unpolymerized stacking gel, overlayed with water and allowed to set for 30 minutes.

Samples were prepared for electrophoresis by mixing 100ul of a 1mg/ml sample and 100ul of a buffer composed of 20% glycerol, 10% 2-mercaptoethanol, 4.6% SDSand 25% 0.5M Tris, pH 8.6, in distilled water. 0.5ul of 0.1% bromophenol blue was added to all samples and all were heat denatured for 3 minutes at 90C.

The samples were loaded onto lanes with a 215ul capacity and electrophoresed for 5 hours at 50mA in a running buffer composed of 0.025M Tris(hydroxymethyl) Aminomethane, 0.192M glycine and 0.1% SDS in distilled water. The gel was stained for protein in 0.125% Coomassie blue in 50% methanol,

destained in 7% acetic acid and preserved as described below.

POLYACRYLAMIDE GEL PRESERVATION (18).

A 70cm by 34cm sheet of cellophane (BCC America, Chadds Ford, Pennsylvania) was soaked in water for five minutes. The sheet was then draped over a PVC frame (inner dimensions 17 x 21cm, outer dimensions 24 x 28cm). The gel was layed onto the sheet and saturated with 7% acetic acid. All air bubbles were removed and the sheet was carefully folded over to sandwich the gel. The apparatus was overlayed with a second, identical frame and secured with clamps on all four sides. The film was dried overnight under a fume hood with a uniform stream of air on both sides. The gel was then cut to an appropriate size.

TRANSFER OF ELECTROPHORESED PROTEINS TO NITROCELLULOSE SHEETS (19).

Identical gels with all lanes containing the purified serum were run and transferred to nitrocellulose sheets by electrophoresing for one hour at 100V in running buffer plus 20% methanol. Using a Hoefer Transphor unit (model TE42), a sheet of nitrocellulose and an electrophoresed gel were sandwiched between a 5cm thick foam sponge and a plastic grid on either side. The apparatus was inserted into the transfer unit such that the nitrocellulose sheet was on the anode side and electrophoresed as described above.

IMMUNOBLOTTING ASSAYS FOR ANALYSIS OF PURIFIED TUMOR CELLS (19).

The nitrocellulose sheets were cut into lengthwise

strips. The non-specific protein binding sights on three identical strips were blocked by soaking them in a 5% dry milk (Carnation, Los Angeles, California) in 0.1M Tris solution plus 0.1M NaCl and 0.2% Triton X-100 (blocking solution). The first strip was incubated with a 0.1mg/ml solution of purified monoclonal antibody for one hour. The second and third strips were the positive and negative controls. These were treated with polyvalent mouse serum to the cultured tumor cells and an irrelevant IgG1 monoclonal antibody (MOPC 2, Sigma, St. Louis, Missouri), respectively. The strips were washed twice in the blocking solution for 30 minutes each and then incubated with goat antiserum to mouse IgG labelled with peroxidase diluted 1:10 for one hour. The strips were washed twice for thirty minutes each with 0.1M Tris-HCl, 0.1M NaCl (TBS,pH 7.4) and developed with 0.5mg/ml 4-chloro-1-naphthol in TBS with 20% methanol and 0.01% hydrogen peroxide.

IMMUNOBLOTTING ASSAYS FOR ANALYSIS OF PURIFIED SERUM.

The nitrocellulose strips containing the purified patient serum were treated exactly as those containing the purified tumor cells with the exception of the secondary antibody. The first strip was incubated with rabbit antiserum to human immunoglobulins, the second with rabbit antiserum to human IgM heavy antibody chains and the third with rabbit antiserum to human IgG antibody chains (DakoPatts, Santa Barbra, California) all diluted 1:250 for one hour. The strips were then washed with

the blocking solution and incubated in peroxidase labelled goat anti-rabbit antiserum diluted 1:500 for one hour. The strips were washed twice for thirty minutes each in 0.1M Tris-HCl,pH 7.4 (TBS) and developed in 0.5mg/ml 4-chloro-1-napthol in TBS with 20% methanol and 0.01% H_2O_2. No control strips were run in this assay.

RESULTS

The protein A column purified from 0.01 to 0.1mg of the antibody from each ml of culture supernatant. The gel electrophoresis verified antibody purity, showing only two distinct bands which corresponded to the heavy and light antibody chains. The purified antibody was concentrated to obtain a 0.5mg/ml solution.

Results from the modified ELISA determined that MAPS binding and MAPS elution buffers were most effective in the binding and releasing of the monoclonal antibody from the fixed antigen. The most positive well indicated that the antibody was still bound to the antigen, while a strong negative reaction suggested that the antibody had been removed from the well by the buffer. These criteria identified the binding and elution buffers, respectively.

The final concentration of the immunoaffinity column was 5mgs of antibody per ml of slurry and column lengths were limited to 1ml for all runs. Typically, 30mls was sufficient to remove the excess protein.

In the columns using cultured tumor cells as the antigen source, the protein concentrations of the unpurified tumor cells and the purified antigen were found to be 1.0mg/ml and 0.1-0.5mg/ml, respectively. Correspondingly, in the columns using patient serum as the antigen source, the protein concentrations of the unpurified and purified serum were 66.7mg/ml and 0.18mg/ml, respectively.

The polyacrylamide gel containing the purified cultured tumor cells showed a single band with a molecular weight of 61 kilodaltons (figure 1, lane b). The gel containing the purified patient serum showed 4 distinct bands with molecular weights of 70, 61, 50 and 23 kilodaltons (figure 2, lane a). The lane containing the unpurified cultured cells contained an array of proteins with varying molecular weights (figue 1, lane d). The 61 kilodalton band, however, could not be identified, since this region was obscured by the presence of large quantities of albumin (a 66 kilodalton protein) extending into the area.

The immunoblotting assays performed on the purified cultured tumor cells produced nitrocellulose strips containing no bands on either the negative control strip or the purified cell strip. The positive control strip contained a variable number of bands. Thenitrocellulose strip treated with rabbit antiserum to human IgG produced two bands at 50 and 23 kilodaltons. The strip treated with rabbit antiserum to human IgM produced a single band at 70 kilodaltons. The strip treated with rabbit antiserum to human immunoglobulins yielded two bands at 50 and 23 kilodaltons. None of the strips contained a band at 61 kilodaltons.

DISCUSSION

A previously described murine monoclonal antibody shown to be reactive with renal cell carcinoma was used to purify a unique renal cell carcinoma protein from cultured tumor cells. The immunoaffinity chromatography column containing the antibody was then used to purify renal carcinoma immune complexes from human serum. The isolation of a unique tumor protein using a one-step technique is important in developing a clinical assay by which small quantities of the circulating antigen could be identified.

The purified monoclonal antibody was attached to sepharose beads by a covalent linkage. The effect of the covalent linkage on the antibody's tertiary structure (and thus possibly its binding capability) could not be determined.

The most important factor in optimizing the efficiency of the column was the selection of binding and eluting buffers. Similar studies have shown substantial variation in the types of buffers used for binding and eluting of proteins (20,21). It was from these reports as well as manufacturer suggestions that the panel of prospective buffers was chosen. The MAPS binding and MAPS elution buffers were found to be the optimal choices. The MAPS binding buffer was effective in removing the excess protein while its high ionic strength prevented the non-specific binding of undesirable proteins. The MAPS elution buffer promoted the release of the antigen without appreciable trailing. This is important in minimizing the number of

fractions necessary to pool in order to retain a majority of the protein.

The optimal volume of tumor extract or serum for the maximum extraction of the protein was approximately 1/2ml per ml of column. This corresponded to about 33.3mgs of crude protein per 5mgs of the monoclonal antibody. At this ratio, it was found that the amount of antibody exceeded the amount of antigen, and it was thus assumed that all of the antigen was removed from the crude samples.

The protein contents of the crude tumor cell extract on SDS-PAGE showed numerous proteins with varying molecular weights (figure 1,lane d). Comparison with the purified samples showed a substantial amount of purification with virtually no background (figure 1,lane b; figure 2, lane a).

Repeated use of the column resulted in progressive non-specific protein binding as shown by Coomassie blue staining of SDS-PAGE gels (figure 1;lane c). These findings indicate that the elution buffer damaged the antibody or antibody-sepharose complexes. Consequently, each column was used only once to optimize the quality of the purification.

The yield of the pure antigen from either the tumor cell extract or the patient serum should not be considered with quantitative emphasis. Although column flow rates, application elution techniques in all of the experiments were uniform, it is likely that there were uncontrollable factors such as handling, that introduced an element of variation. In addition,

the amount of antigen initially present in the samples could not be determined.

The column eluate containing the purified tumor cell extract yielded a protein with a molecular weight of 61kd. This was represented as a single band on a SDS-PAGE stained with Coomassie blue. Specific reactivity with the antibody using an ELISA verified that the protein was the neoplastic antigen. However, repeated attempts to use immunoblotting techniques for further verification were unsuccessful. Amido black staining confirmed that the protein was transferred to the nitrocellulose, suggesting that the immunoreactivity of the protein was destroyed, most probably by SDS-denaturation.

The column eluate containing the purified serum contained four bands at 70, 50, 23, and 61kd. The first three bands identified by the immunoblotting assay corresponded to IgM heavy, IgG heavy and IgG light antibody chains respectively. The band at 61kd again represented the neoplastic antigen.

Apparently, the binding of the monoclonal antibody to the immune complex does not affect the immunoreactivity of the antigen in vivo. It is thus possible to isolate circulating complexes and monitor their presence qualitatively as a means to study the effect of treatment in the immune response to renal carcinoma. In addition, by testing for the presence of the antigen in a patient after radical nephrectomy, the phenomenon of tumor recurrence could be monitored.

The successful application of antigen purification from blood serum could be developed for use in the antigen purification from other body fluids, such as urine. The immunoaffinity technique could form the basis for an inexpensive and highly selective assay for antigen detection before the presence of a tumor mass could be detected. This would be of enormous value in improving the low prognosis of renal cell carcinoma victims. The inability to detect early tumor growth is a major reason for the low survival rates of persons with metastatic renal cell carcinoma.

The immunoaffinity chromatography technique is useful in that it allows small quantities of the antigen to be obtained in a highly purified form. As a result, it is would be possible to determine the amino acid sequence of the protein. From this sequence, a nucleic acid probe could be developed. Use of the probe could further genetic studies on the gene responsible for the antigen production.

In conclusion, it appears that this antibody can be used to identify and isolate the neoplastic antigen from samples of cultured tumor cells and human serum. In addition, immune complexes circulating in the system of renal cell carcinoma patients can also be purified. It is hoped that the purification of the neoplastic antigen and the immune complexes will aid in future study of renal carcinoma. Most importantly, we hope that the antibody will be useful in the early detection of this serious tumor system.

REFERENCES

1.Jones,G.W.Ca-A Journal for Clinicians,32:280-285,1985.

2.DeKernion, J.B.Progress and Controversies in Oncological Urology.Alan R. Liss,New York,New York,1984.

3.Churg,J.,Spargo,B.,Mostofi,F.,Abel,M (eds.).International Academy of Pathology Monograph, Kidney Diseaes: Present Status.Williams and Wilkins Company,1979.

4.Bennington,J.,Beckwith,J.Tumors of the Kidney,Renal Pelvis, and Ureter.Second series,fascicle 12 of Atlas of Tumor Pathology.Armed Forces Institute of Pathology, Washington,D.C.,1975.

5.Bennington,J.L., and Kradjian,R.Renal Carcinoma.W.B. Saunders Co.:Phiadelphia,Pa., 1967.

6.Graham,A.P.Malignancy in the kidney,survey of 195 cases. J. Urol.,58:10-21,1947.

7.McNichols,D.W.,Segura,J.W.,DeWeerd,J.H. J. Urol.,126:17-23,1981.

8.Fairlamb,D.J.Spontaneous regression of metastases of renal cancer.Cancer,47:2102-2106,1981.

9.Wright,G.L. Jr.,Schellhammer,P.R.,Rosato,F.,FaulconerR.J. Cell-mediated immunity in patients with renal carcinoma as measured by leukocyte inhibition test.Urology,12:525,1978.

10.Kochevar,J.A renal cell carcinoma neoplastic antigen detectable by immunohistochemistry is defined by a murine monoclonal antibody. Cancer,1987.

11.Lowry,O.H.,Rosenbrough,N.J.,Farr,A.L.,Randall,R.J. Protein Measurement with the Folin Reagent.J.Biol. Chem.,193:265,1951.

12.Ueda,R.,Ogata,S.,Morrissey,D.M.,Finstad,C.L.,Szkudlarek,J, Whitmore, W.K.,Oettgen,H.F.,Lloyd,K.O., and Old,L.J. Cell surface antigens of human renal cancer:identification of tissue-specific kidney glycoproteins.Proc.Natl.Acad. Sci.USA,78:5122-5126,1981.

13. Finstad,C.L.,Cordon-Cardo,C.,Bander,N.H.,Whitmore,W.F., Melamed,M.R., and Old,L.J.Specificity analysis of mouse monoclonal antibodies defining cell surface antigens of renal cancer.Proc. Natl. Acad. Sci. USA, 82:2955-2959, 1985.

14. Sedmak,J.D.,and Tubbs,R.R.The sensitivity and specificity of monoclonal antibodies UR0-2 and 19-9 in the immunohistochemical classification of primary adenocarcinoma.Cleveland Clinic Quarterly, 53: 277-282, 1986.

15. Oosterwijk,E.,Ruiter,D.J.,Hoedemaker,P.J.,Pawels, E.K.J.,Jonas,U.,Zwartendijk,J.,and Warnaar,S.O. Monoclonal antibody G 250 recognizes a determinant present in renal cell carcinoma and absent from normal kidney. Int.J.Cancer, 38: 489-494, 1986.

16. Engvall,E., and Perlmann,P.Enzyme-linked immunosorbent assay, ELISA.J.Immunol., 109:129-135, 1972.

17. Laemmli,U.K.Cleavage of structural proteins during the assembly of the head of Basteriophage T4.Nature, 227: 680-685, 1970.

18. Wallevik,K., and Jensenius,J.C.A simple and reliable method for the drying of polyacrylamide slab gels. J.Biochem.Biophys.Methods,6(1): 17-21, 1982.

19. Towbin,H.,Staehelin,T.,and Gordon,J.Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc.Natl.Acad.Sci. USA,76: 4350-4354, 1979.

20. Weintraub,B.Concentration and purification of human chorionic somato-mammotropin (HCS) by affinity chromatography: application to radioimmunoassay. Biochem.Biophys.Res.Comm.,39: 83-89, 1970.

21. Avrameas,S., and Ternynck,T.Use of iodide salts in the isolation of antibodies and the dissolution of specific immune precipitates.Biochem.J.,102: 37c-39c, 1967.

FIGURE LEGEND

Figure 1: Lanes from 12% SDS-PAGE gels stained with Coomassie blue. (a) molecular weight markers as indicated in the margin. (b) 61kd band which represents the neoplastic antigen purified from cultured tumor cells with the antigen purification column. (c) 61kd band along with weaker bands of non-specifically bound proteins as a result of repeated column use. (d) multiple proteins in 30ug of crude tumor extract.

Figure 2: A lane from 12% SDS-PAGE as well as strips from the immunoblotting assays on the purified human serum. (a) Proteins purified from human serum using the antigen purification column. (b) 50kd band on a nitrocellulose strip which represents IgG heavy antibody chains identified by immuno-blotting assays. (c) 70kd band on a nitrocellulose strip which represents IgM heavy antibody chains identified by immuno-blotting assays. (d) IgG heavy antibody chains and a 23kd band which represents IgG light antibody chains identified by immunoblotting assays.

ILLUSTRATIONS

Table 1

Experimental setup for the Lowry method of protein concentration determination.

tube	1	2	3	4	5	6	7	8	9
BSA (ul)	-	5	10	15	20	-	-	-	-
sample (ul)	-	-	-	-	-	5	10	15	20
H_2O (ul)	200	195	190	185	180	195	190	185	180
Solution D (ml)	1	1	1	1	1	1	1	1	1

Table 2

Panel of prospective binding and elution buffers subjected to enzyme-linked immunosorbent assays (ELISA).

---

phosphate buffered saline (PBS)

6M Guanidine-HCl, pH 3.1

MAPS elution buffer, pH 3.0

0.1M acetate plus 0.5M NaCl, pH 4.0

0.1 M tris plus 0.5M HCl, pH 8.0

saturated NaCl

MAPS binding buffer, pH 9.0

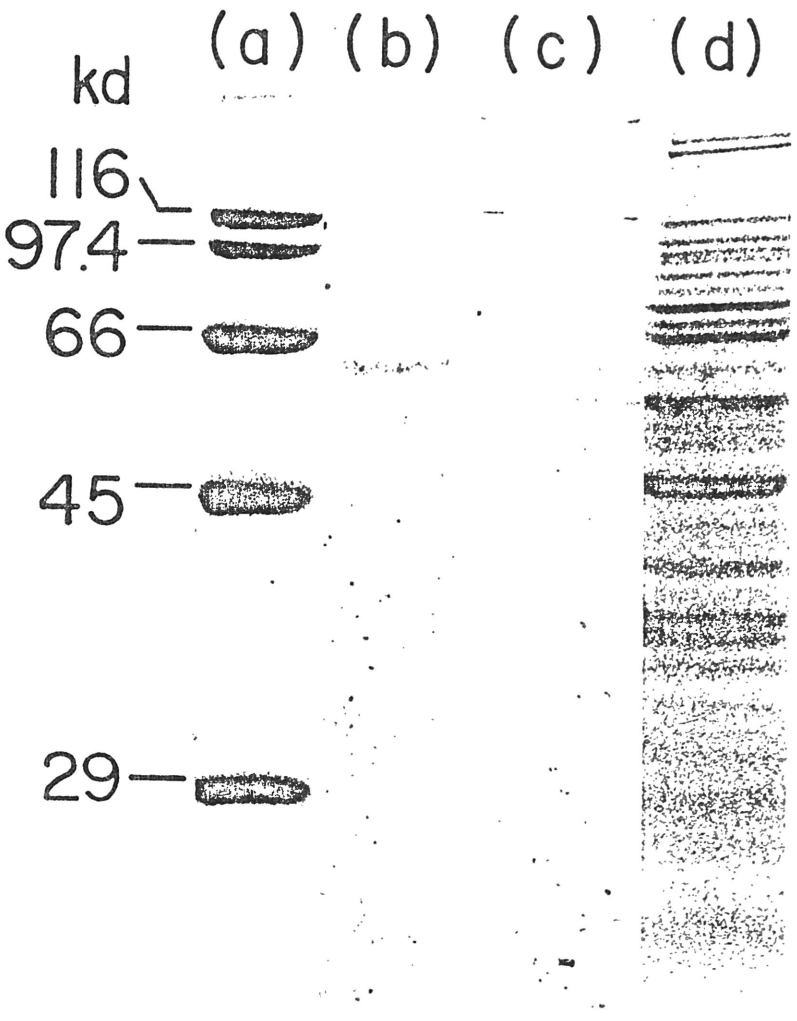

Figure 1: Note the 61 kd band in lane (b). (c) shows non-specific protein binding which occurred after repeated column use.

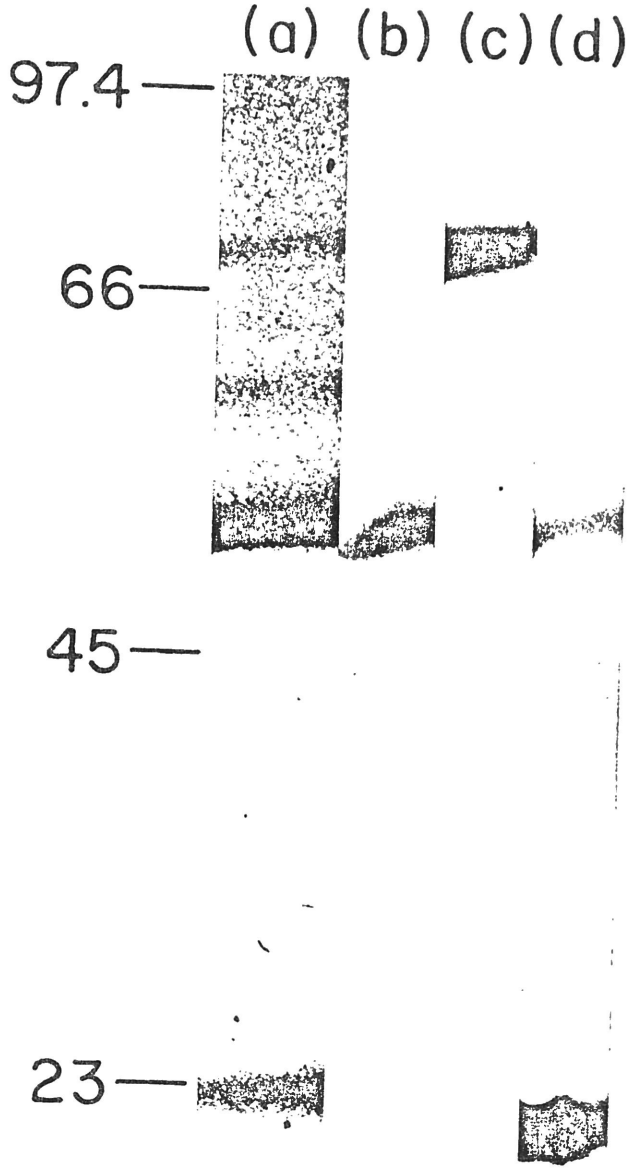

Figure 2: Note the 62 kd band in lane (a). The other bands represent human immunoglobulins that were bound the antigen in circulating immune complexes.