Immunohistochemical Analysis of Monoclonal Antibodies to Renal Antigens

Application in the Diagnosis of Renal Cell Carcinoma

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Four mouse monoclonal antibodies reacting with renal proximal epithelium were characterized on normal adult tissues, fetal kidneys, and various malignancies. These four antibodies were found to react with renal cell carcinomas (RCCs) but not with a variety of other tumors. Three antibodies react only with some primary RCCs and not with metastatic RCCs. The fourth antibody, RC 38, reacts with 46 of 47 primary RCCs tested and with 8 of 13 RCC metastases. No reaction was seen with 179 tumors of various origin, which indicates that this antibody is very useful in diagnosing RCC. (Am J Pathol 1986, 123:301-309)

SINCE the introduction of the hybridoma technique by Köhler and Milstein;[1] efforts have been made to isolate monoclonal antibodies (Mab's) with a high specificity for certain tumors.[2-5] Such Mab's can be useful diagnostic and therapeutic tools.

Renal cell carcinoma (RCC) is a tumor that can be difficult to differentiate from clear-cell tumors of other origins. Mab's specific for RCC may therefore contribute in establishing a final diagnosis. Recently several Mab's to kidney antigens and RCC-associated antigens were described.[6-9] These Mab's have been tested on tissue culture cells[6,9] and on frozen tissue sections[7,8,10]; but have not yet been applied to a large variety of tumors as diagnostic reagents.[6,8,10]

In our laboratory a large number of Mab's to various renal antigens have been produced and tested on a panel of normal tissues, RCC, and other tumors. Here we describe four Mab's that react with proximal tubular epithelium, the presumed tissue of origin of RCC.[11,12] We present data indicating that the four Mab's described have a high specificity for primary RCC and that one of these Mab's is useful in the diagnosis of metastatic RCC. The distribution and the time of first appearance in fetal kidneys of the antigens recognized was also studied, so that we could better understand the staining results obtained on primary and metastatic RCC.

Materials and Methods

Source of Tissues

Tumor tissue samples, excluding necrotic and hemorrhagic areas, were taken from surgical specimens of various malignancies. In 3 cases of RCC both primary and metastatic tumor was obtained at surgery. In 2 cases of RCC autopsy material was used. In these 2 cases primary tumor and corresponding metastases were obtained. From each RCC several nonadjacent tumor samples were taken. Renal adenomas were obtained at autopsy.

Normal tissues used for specificity tests of Mab's were from autopsies performed within a few hours after

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death or from uninvolved parts of surgical specimens. These included kidney, ureter, bladder, stomach, jejunum, colon, testis, cervix, pancreas, prostate, lung, liver, breast, skeletal muscle, brain, lymph node, uterus, thyroid gland, adrenal gland, and skin.

All Mab's described here were tested on at least three different tissue sections of the aforementioned normal tissues, obtained from different patients.

Fetal kidney tissues of 11, 13, 14, 15, 18, and 20 weeks' gestation as estimated from body length measurements were obtained from abortions.

All tissues were snap-frozen and stored at −70 C until used.

Preparation of Cell Homogenates

Cell homogenates were prepared from adult renal cortex or medulla and from RCC. Tissue was homogenized with a Potter Elvehjem homogenizer (5 strokes at 1500 rpm) in three volumes of phosphate-buffered saline, pH 7.4 (PBS). After centrifugation for 10 minutes at 1000*g*, the supernatant was used for immunization purposes, for coating nitrocellulose filters, or for ELISA.

Immunization

Balb/c mice were used for immunization purposes. Each animal was immunized intraperitoneally at least three times with homogenates prepared as described above. For the immunization with RCC, homogenates from three different patients were used. For the first injection the tissue homogenate was mixed with an equal volume of Freund's complete adjuvant (Sigma, St. Louis, Mo). Each animal was injected with 0.5 ml of the mixture of tissue homogenate and Freunds adjuvant. Booster injections with Freunds incomplete adjuvant (1:1, Sigma, St. Louis, USA) were given at two weeks intervals. Three days after the last injection the mice were killed and the spleen cells were used for fusion.

Fusion of Spleen Cells with Sp2/0 Cells and Detection of Hybridomas Producing Relevant Antibody

The spleen cells of immunized mice were fused with Sp2/0 cells essentially according to Köhler and Milstein.[1] After fusion, the cells were plated into 20 Petri dishes, 5 cm in diameter, in soft agar (0.4%) and incubated for 10 days at 37 C in a CO_2 incubator. Then the agar was overlaid with nitrocellulose filters saturated with a cell homogenate corresponding to the homogenate used for immunization and a second filter; saturated with 0.75% gelatin dissolved in Hanks' balanced salt solution (HBSS) or saturated with a homogenate made from a normal adult human liver, as a first screen to discriminate between kidney relevant and irrelevant antibody-producing colonies. These filters were prepared as follows: Cell homogenates were diluted 20-fold in HBSS and sonified. The filters were soaked in HBSS and put on a sintered glass funnel 47 mm in diameter. Then 10 ml of the cell homogenate was sucked through, and the filters were air-dried and sterilized by ultraviolet light (30-watt Philips TUV at 90 cm, 2 times, 20 minutes each side). To reduce toxicity of the filters, the filters were washed in sterile water. Thereafter the filters were soaked in HAT medium containing fetal calf serum to block remaining protein binding sites. After overnight incubation on the soft agar, the filters were removed and incubated for 1 hour with rabbit anti-mouse Ig conjugated to horseradish peroxidase. After extensive washing in 10 mM Tris-HCl, pH 7.4, containing 0.02% sodium dodecylsulfate and 0.5% sarcosyl NL 30 (Ciba-Geigy B.V., Arnhem, The Netherlands), the filters were developed with 0.05% diaminobenzidine and 0.03% H_2O_2 in 50 mM Tris-HCl, pH 7.4. The procedure followed was adapted from Sharon et al.[13]

Colonies producing antibodies that reacted with the filters coated with the relevant cell homogenate, but not with the filters coated with liver cell homogenate or gelatin were picked and grown in suspension in microtiter plates. Undiluted culture media of these cells were tested on cryostat sections of several tissues. Colonies producing antibodies positive on adult renal tissue sections or RCC sections and negative on liver and lung tissue sections were subcloned and further analyzed on frozen sections of other tissues.

Staining and Scoring Procedure for Frozen Tissue Sections

To test the specificity of the Mab's and to identify the structures stained, indirect immunoperoxidase staining was performed on frozen sections of various normal tissues, fetal kidneys, and tumors as described by Van Muijen et al,[14] with the exception that 3,3'-diaminobenzidine was used as substrate. Sections were counterstained with hematoxylin.

Sections of normal tissues were scored negative when not a single cell was stained. Tumors were scored as negative when no tumor cells were stained; eg, tumor sections in which only blood vessels were stained were considered to be negative.

The subsite of the nephron stained by the Mab's was established by using generally accepted morphologic criteria such as presence of brush border and width of tubule lumen. The position of the tissue section in the kidney was also taken into consideration. Rabbit anti-

human Tamm Horsfall protein (RAH-THP) was used to identify the ascending limb of Henle's loop and the distal convoluted tubule.[15,16] Double immunofluorescence staining was performed to identify any overlap of THP-containing cells and the cells stained with the Mab's.

Test Set of Poorly Differentiated Malignant Tumors for Which the Diagnosis of RCC Had Been Considered

To confirm the diagnostic potential of Mab RC 38, a test set of diagnostically difficult tumors was selected. Tumors of this test set included three cytologic aspirates which present additional diagnostic difficulties and poorly differentiated malignant tumors with a histologic appearance and clinical presentation that mimicked RCC. The possibility of RCC had therefore been considered by the pathologist in the differential diagnosis. The histologic appearance of these cases included adenocarcinoma with clear-cell, cribriform, or acinar patterns, undifferentiated large-cell malignant tumors, and spindle-cell malignant tumors. None of these cases was included in the series of tumors used to test the specificity of the Mab's.

Tumors of the test set were stained and scored by E.O. without knowledge of the final diagnosis.

ELISA

Renal cortex and liver homogenates were diluted in 0.1 M $NaHCO_3$ pH 9.0, to 0.5 mg of protein/ml; and 100 µl of diluted extract was added to microtiter wells (Sterilin Limited, Teddington, UK). The contents were allowed to evaporate at 37 C. After blocking remaining binding sites on the plastic with 3% ovalbumin, the wells were incubated for 2 hours at 37 C with 100 µl of dilutions of culture medium. After washing and incubation with rabbit anti-mouse Ig conjugated to horseradish peroxidase, the plates were developed with O-diphenyl-amine and H_2O_2 for 20 minutes. The reactions were stopped with 50 µl 2.5 M H_2SO_4 and optical density readings at 492 nm were taken.

Results

Origin and Subclass of Monoclonal Antibodies

Mab RC 38, subclass IgG_1, was derived from fusion of the spleen cells of a mouse immunized with RCC homogenates. RC 3, subclass IgG_1, was developed with spleen cells from a mouse immunized with normal adult renal cortex homogenates. Mabs RC 69 (IgG_{2b}) and RC 154 (IgG_{2a}) were derived from fusion of the spleen cells of a mouse immunized with normal adult renal medulla homogenates.

Table 1—Immunohistochemical Staining Results With Monoclonal Antibodies in Frozen Sections of Nonrenal Tissues

Normal tissues	RC 3	RC 69	RC 154	RC 38
Ureter	–*	–	–	–
Urine bladder	–	–	–	–
Prostate	–	–	–	–
Testis	–	–	–	–
Uterine cervix/corpus	–	–	–	–
Adrenal gland	–	–	–	–
Thyroid gland	–	–	Follicles	–
Stomach	–	–	–	Mucous cells
Jejunum	–	–	–	Crypts, villi†
Colon	–	–	–	Crypts†
Pancreas	–	–	–	–
Liver	–	–	–	Sinuses
Breast gland	–	–	Ducts	–
Skin	–	–	–	Acini of sweat glands
Lymph nodes	–	–	–	Sinuses
Smooth/striated muscle	–	–	–	–
Brain	–	–	–	–

* Not a single cell stained.

† In jejunum and colon only the surface epithelium of the crypts or villi was stained with RC38.

All Mab's are only applicable on cryostat sections. When tested on formalin-fixed tissue, no staining reaction was observed.

Staining of Normal Human and Fetal Tissue Sections

RC 38

In tissue sections of adult kidney RC 38 stained the glomerular visceral epithelium and the epithelial cells of the proximal tubules up to the thin descending part of Henle's loop. Staining of the proximal tubular cells was mainly localized within the cytoplasm. No staining of THP-containing cells—present in the distal tubules—was seen, as indicated by double immunofluorescence.

In sections of the fetal metanephros, RC 38 stained differentiating visceral glomerular epithelial cells at the capillary loop stage and the most proximal part of the tubules connected to these regions.

In nonrenal tissue sections, RC 38 stained the surface of the epithelium in jejunum and colon, the mucous cells of the foveolar and glandular layer of the stomach mucosa and acini of sweat glands. In addition sinusoidal lining cells in the liver and lymph nodes were stained. Other tissues tested were negative (Table 1).

RC 3 and RC 69

In tissue sections of adult kidney RC 3 and RC 69 stained the proximal tubules up to the descending part of Henle's loop. No staining of more distal parts of the nephron was observed. The staining of proximal tubular cells was mainly associated with the brush border. RC 3 faintly stained the parietal epithelial cells of Bow-

Table 2—Immunohistochemical Staining of Malignant Lesions From the Urogenital Tract

Tumor type	RC 3	RC 69	RC 154	RC 38
Primary RCC	36/51*	30/44	20/44	44/45
Metastatic RCC	1/10	0/7	0/7	6/9
Wilms' tumor	0/2	0/2	0/2	0/2
Pelvic carcinoma	0/2	0/2	0/2	0/2
Bladder carcinoma	0/8	0/6	0/6	0/6
Prostatic carcinoma	0/1	0/1	0/1	0/1
Testicular carcinoma	0/6†	0/6†	0/6†	0/10†
Ovarian carcinoma	0/18	0/2	0/2	0/10

* Number positive over number tested.
† Six seminomas.

man's capsule in the region adjoining the outgoing tubule.

In sections of the fetal metanephros the middle limb of the S-shaped stage, the part that will eventually develop into the proximal tubule,[17,18] was stained with RC 69. Developing proximal tubules and differentiating parietal epithelium were heavily stained with both RC 3 and RC 69.

All nonrenal tissues tested were negative with RC 3 or RC 69 (Table 1).

RC 154

In adult kidney tissue sections a weak basolateral staining of the proximal tubular epithelium was observed with RC 154 in addition to intense cytoplasmatic basolateral staining of the distal tubules and small collecting ducts. In double immunofluorescence, the distal tubular epithelium was positive with both RC 154 and RAH-THP, whereas the proximal tubular epithelium was weakly positive with RC 154 only.

In the fetal metanephros essentially the same distribution was seen: more intense staining of the distal parts of the differentiating nephron as compared with the more proximal parts. Nephrons were stained after the development of the capillary loop stage.

In nonrenal tissue sections, weak staining of ducts in breast gland and follicles in thyroid gland tissue sections was observed. Other tissues tested were negative (Table 1).

ELISA With Normal Tissue Extracts

In a preliminary study to evaluate whether the Mab's of this study can be used to detect the corresponding antigens in crude extracts of kidney, an ELISA was set up in which wells in microtiter plates were coated with kidney or liver extracts. No reaction was found with the liver-extract-coated wells, whereas the reaction with the kidney-extract-coated wells gave optical density readings at 492 nm, ranging from 0.5 for RC 154 to 1.5 for RC 38. Further studies are necessary to establish whether any of these antibodies is useful for detecting RCC antigens in blood or urine.

Table 3—Immunohistochemical Staining of Non-Urogenital Tract Malignancies

Tumor type	RC 3	RC 69	RC 154	RC 38
Mammary carcinoma, primary	0/9*	0/9	0/7	0/17
Mammary carcinoma, metastatic	ND†	ND	ND	0/9
Pulmonary carcinoma, primary	0/15	0/9	0/8	0/20
Pulmonary carcinoma, metastatic	ND	ND	ND	0/4
Colonic carcinoma, primary	0/11	0/9	0/7	0/17
Colonic carcinoma, metastic	ND	ND	ND	0/4
Adrenal cortical carcinoma	ND	ND	ND	0/6
Gastric carcinoma	ND	ND	ND	0/5
Liver cell carcinoma	ND	ND	ND	0/2
Gallbladder carcinoma	ND	ND	ND	0/1
Salivary gland tumors	0/6	0/6	0/6	0/6
Sarcoma‡	0/9	0/6	0/6	0/20
Melanoma	0/7	0/2	0/2	0/19
Non-Hodgkin's lymphoma, histiocytic	ND	ND	ND	0/6
Total number of tumors tested	57	41	36	136

* Number positive over number tested.
† Not done.
‡ Sarcomas tested with RC 38 were osteosarcoma (4), chondrosarcoma (4), leiomyosarcoma (3), histiocytic sarcoma (2), synovial sarcoma (2), fibrosarcoma (2), neurosarcoma (2), and rhabdomyosarcoma (1).

Staining of Tumors

The staining results on RCC and other tumors from the urogenital tract are summarized in Table 2. RC 38 stained 95% of primary and 60% of metastatic RCC lesions. RC 3 reacted with 70% of primary RCC and 10% of metastatic RCC. RC 69 and RC 154 reacted with 70% and 40% of primary RCCs, respectively. RC 69 and RC 154 did not stain the sections of metastatic RCCs tested.

Table 4—Heterogeneity of Staining of RCC With RC 3, RC 69, RC 154, and RC 38

Estimated percentage of tumor cells stained	RC 3	RC 69	RC 154	RC 38
0%	15*	14	24	1
<1%	1†	1†	1	1
1–20%	9	9	9	8
21–50%	7	7	3	2
>50%	19	13	7	33
Total	51	44	44	45

* Number of tumors stained.
† Same tumor.

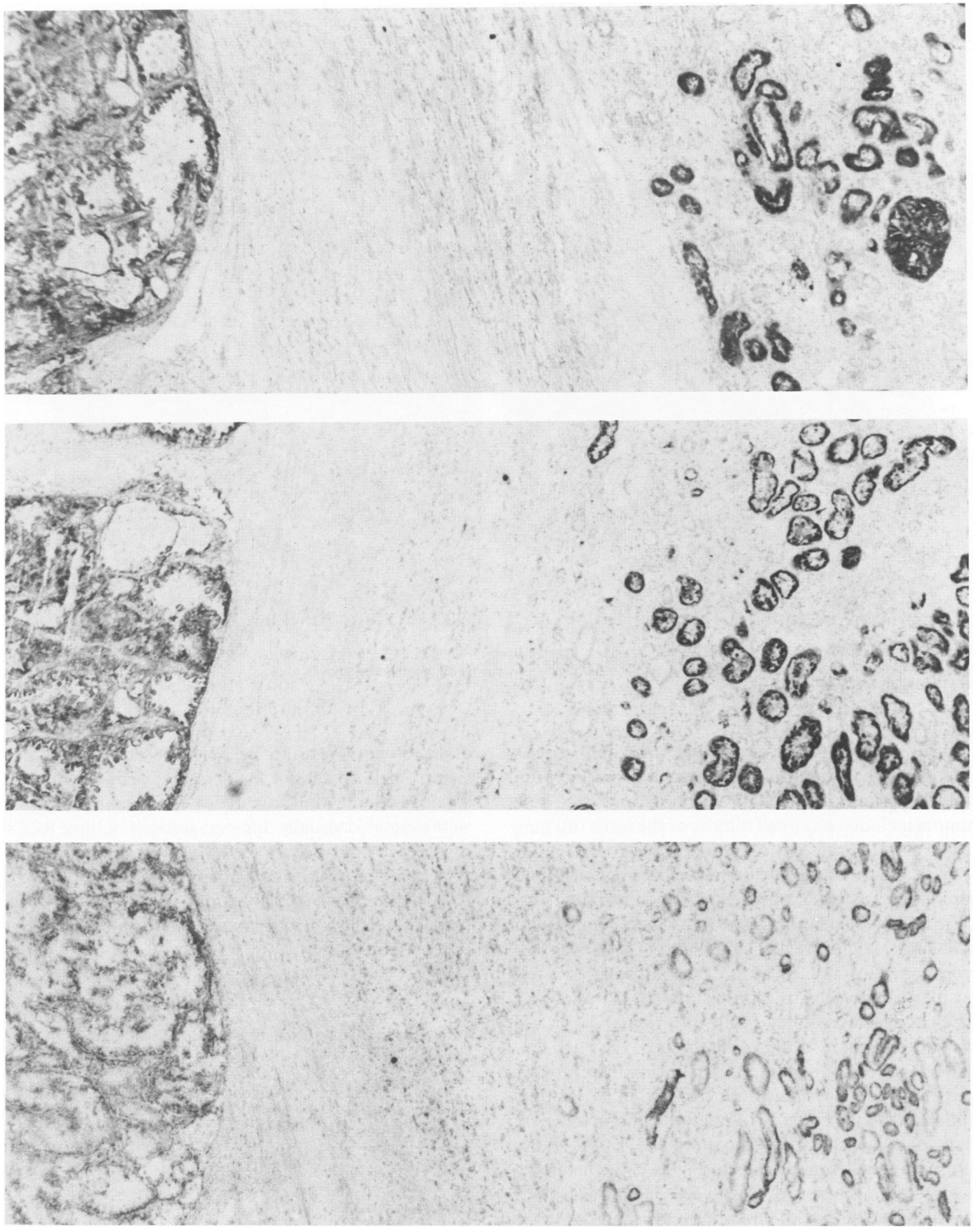

Figure 1—Frozen sections of primary RCC (left) and uninvolved kidney tissue (right). Stained with RC 38 (**A**): a glomerulus, proximal tubules, and tumor cells are positive. With RC 3 (**B**): proximal tubules and tumor cells are positive. With RC 154 (**C**): proximal and distal tubules and tumor cells are positive. (Counterstained with hematoxylin, ×60)

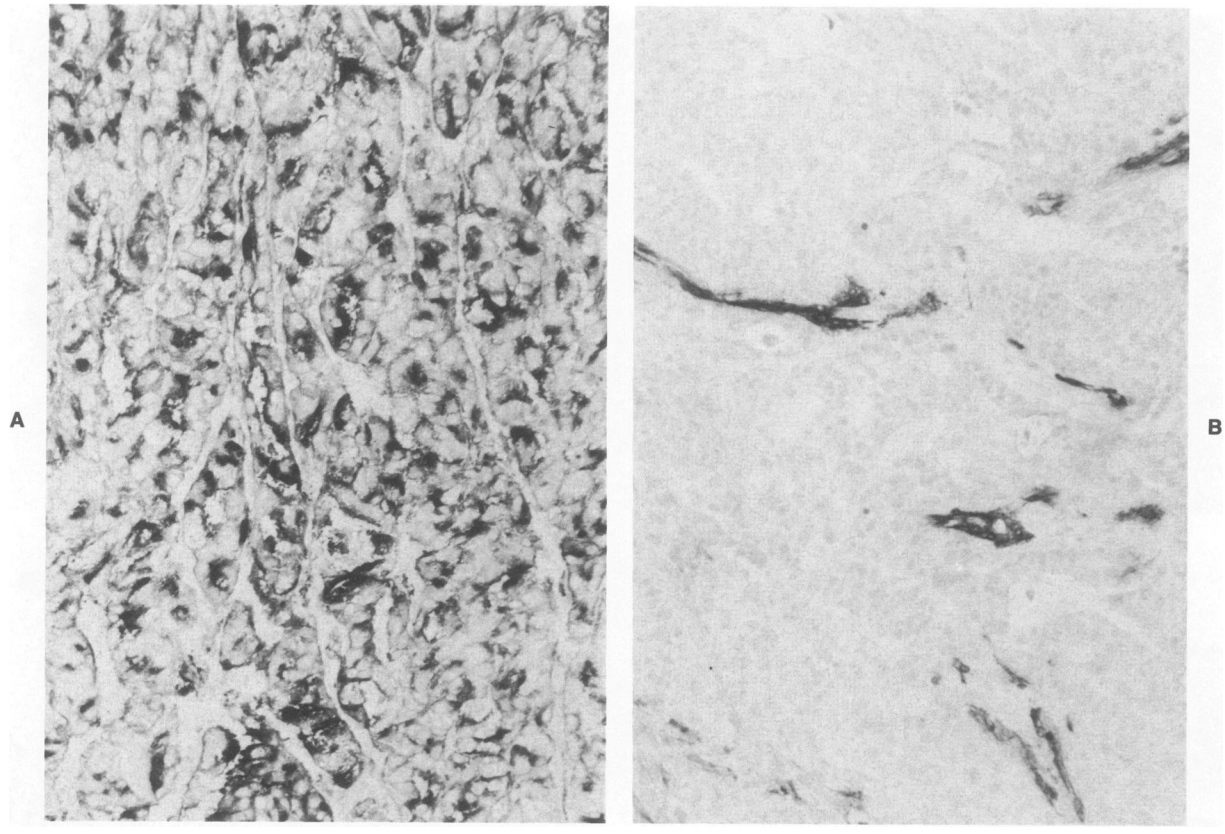

Figure 2—Frozen section of RCC (**A**) and colonic carcinoma (**B**) stained with RC 38 and counterstained with hematoxylin. In the RCC tissue section tumor cells are unevenly stained. In the colonic carcinoma a positive reaction with the endothelial cells of blood vessels is seen, whereas the tumor cells are negative. (×160)

Other tumors of the urogenital tract and various tumors originating outside the urogenital tract were not stained by any of the Mab's (Table 2 and 3). These tumors included clear-cell tumors of the testis (6), lung (2), ovary (3), and soft tissue (1).

With RC 38 staining of endothelial cells of blood capillaries was often observed in all tumors.

In Table 4 the percentages of tumor cells stained with the Mab's are indicated. The staining of RCC was heterogeneous for all four Mab's and ranged from a few positive tumor cells to diffuse staining of tumor sections. A typical staining result on primary RCC and the adjoining uninvolved kidney tissue is shown in Figure 1. With RC 38 a glomerulus, the proximal tubules, and tumor cells are stained. With RC 3 and RC 69 (data not shown) the proximal tubules and tumor cells are stained, and with RC 154 proximal and distal tubules and tumor cells are stained. In Figure 2 the heterogeneous staining of an RCC with RC 38 is shown (left) and also the staining of endothelial cells but not of tumor cells in a colonic carcinoma (right).

The percentages of tumor cells stained were quite similar in all pieces of one tumor studied except for two tumors where RC 3 failed to stain one tissue block and a few positive tumor cells were observed in another tissue block. In Table 4 the percentages of tumor cells stained with the Mab's are indicated. These percentages were estimated visually. In tissue sections of three RCCs only 50–100 positive tumor cells/sq cm of tumor section were seen with one or two Mab's corresponding to less than 1% of cells positive (see Table 4), while higher percentages of tumor cells were stained with the other Mab's tested.

Table 5—Reaction of Primary RCC With RC 3, RC 69, and RC 154

Tumor phenotype	Number of lesions
RC 3⁺/RC 69⁺/RC 154⁺	12
RC 3⁺/RC 69⁺/RC 154⁻	14
RC 3⁺/RC 69⁻/RC 154⁺	1
RC 3⁺/RC 69⁻/RC 154⁻	2
RC 3⁻/RC 69⁻/RC 154⁺	5
RC 3⁻/RC 69⁻/RC 154⁻	7
RC 3⁻/RC 69⁺/RC 154⁺	0
RC 3⁻/RC 69⁺/RC 154⁻	0
Total number of RCC tested	41

These RCCs are the subset of the RCCs of Table 2, which have been tested with all four Mab's. All 41 tumors in Table 5 are positive with RC38.

Table 6—Immunohistochemical Staining Results With RC 38 on Tissue Sections From Test Set of Malignant Tumors for Which the Diagnosis of RCC Was Considered*

Case	Sex/age	Location	Histologic appearance	RC38	Ultimate Diagnosis	Remarks
1	M72	Liver†	Cl	+	Metastasis of RCC‡	
2	M65	Lymph node	Cl	+	Metastasis of RCC‡	
3	M72	Kidney†	Cl	+	Primary RCC‡	
4	M71	Kidney†	Cl	+	Primary RCC‡	
5	F53	Femur	Sp	–	Metastasis of RCC‡	
6	F49	Subcutis	Sol	–	Metastasis of RCC‡	
7	M60	Lymph node	Ac	–	Adenoca, origin unknown	No renal tumor
8	M67	Lung	Ac/Cr	–	Adenoca of lung	
9	F53	Thoracic wall	Ac	–	Angiosarcoma	Factor VIII rel ag*
10	M78	Scapula	Ac/Cr	–	Follicular ca, thyroid	thyroglob*
11	M33	Thoracic wall	Sol	–	Non-Hodgkin's ly, Histiocytic	LCA*
12	F56	Subcutis	Cl	–	Clear-cell sarcoma	NKI/C3*
13	F52	Foot	Ac/Sol	–	Extraskeletal myxoid chondrosa	
14	M72	Neck	Sp	–	Synovial sarcoma	
15	F24	Scapula	Sol	–	Non-Hodgkin's ly, Histocytic	LCA*
16	M44	Abdomen	Sol	–	Adenoca, colorectal	CEA*
17	F91	Breast	Ac/Cr	–	Ductal ca of breast	
18	F42	Thoracic wall	Sol	–	Undifferentiated malignant tumor, origin unknown	No renal tumor

Abbreviations used: M, male; F, female; Cl, clear-cell appearance; Ac, acinar appearance; Cr, cribriform appearance; Sol, solid appearance; Sp, spindle-cell appearance; LCA, leukocyte common antigen[26]; NKI/C-3, Mab recognizing a melanoma-associated antigen[27]; ca, carcinoma; sa, sarcoma; ly, lymphoma; rel, related; ag, antigen.

* None of the tumors of the test set is included in Tables 2, 3, 4, or 5.

† Cytologic aspirate.

‡ Renal tumor present.

In metastatic RCC stained with RC 38 the same heterogeneity was seen as in primary RCC.

In primary RCC various combinations of the antigens recognized by RC 3, RC 69, and RC 154 are expressed, as shown in Table 5. Twenty-seven of 41 primary RCCs tested with these three Mab's stained with two or more of the Mab's; 7, with none. Note that some of the possible combinations of antigen expression were not observed.

In 5 cases primary RCC as well as a corresponding metastatic lesion was available. RC 3 and RC 69 stained 2 and RC 154 stained 1 of these primary RCCs and none of the metastatic lesions, while RC 38 stained all 5 primary RCCs and 3 metastases.

Staining of Test Set Tumors

Four of the tumors (Cases 1–4) in the test set of 18 tumors were stained with RC 38 antibody (Table 6). In these 4 cases the ultimate diagnosis, based on additional clinicopathologic findings, was RCC. Two other tumors, which were compatible with RCC on histologic and clinical grounds, were negative with RC 38. The other 12 tumors which were negative for RC 38 were eventually found to be nonrenal on the basis of clinical or other histochemical studies.

Staining of Renal Adenomas

In one adenoma, 10 mm in diameter, a few cells were stained with RC 38; whereas RC 3, RC 69, and RC 154 failed to stain any cell. In another adenoma, 4 mm in diameter, no cells were stained by any of the Mab's. Five other adenomas—four from one patient—ranging in size from 2 to 4 mm were tested with RC 38 only and were found to be negative.

Discussion

In this study we describe the isolation of a number of Mab's that recognize kidney-related antigens and the localization of such antigens on normal and malignant human adult tissues and fetal kidneys. The results indicate that these Mab's are highly specific for RCC. Other investigators have also described Mab's with a high specificity for RCC.[7,8,10]

A comparison between the Mab's described in this study with Mab's against renal antigens described by others is difficult, partly because of different assay methods. Most Mab's initially described by Ueda et al[7] and later used by Bander[19] appear to recognize other epitopes than the Mab's described here. S4 and our Mab RC 38 may recognize the same protein, because the over-

all staining results are quite similar. However, when one compares the data of Finstad et al[8] with our results, RC 38 and S4 show different staining results on a few normal tissues, which indicates that both Mab's recognize different epitopes. Also, Uro 1–6 and EC1[20] and PHM 5[21] probably recognize determinants other than the Mab's described in this study. For the Mab's TN1–10, described by Müller and Müller,[22] we cannot exclude that TN1 recognizes an antigen similar to RC 3 or RC 69. Vessella et al[9] described a number of Mab's that were initially characterized on a panel of tumor cell lines and were therefore difficult to compare with our Mab's. One of these Mab's, D5D, was more extensively characterized[10] and appeared to recognize an RCC-associated antigen absent in normal kidney, unlike the Mab's of this study.

In RCC, the expression of the antigens corresponding to the four Mab's was variable, with respect both to numbers of antigens expressed and to percentages of positive RCC tumor cells. This variability was also found by others[8] and in our previous study with other Mab's to renal antigens.[23] The combinations of antigens RC 3^+/RC 69^+/RC 154^+ and RC 3^+/RC 69^+/RC 154^- were frequently observed in primary RCC, whereas no tumors were found to have a RC 3^-/RC 69^+/RC 154^+ or RC 3^-/RC 69^+/RC 154^- phenotype. Although 5 RCCs displayed a RC 3^-/RC 69^-/RC 154^+ phenotype, which might at first glance suggest that these tumors originated from the distal tubular epithelium, these tumors also reacted with RC 38 antibody, which does not stain distal tubular epithelium; this argues against a distal tubular origin. Bander et al[6] suggested that some RCC tumors may originate from the distal tubular epithelium, also based on immunohistochemical findings.

The antigens recognized are renal differentiation markers. The observation that RC 38 antigen, which appears at a later stage of nephron development than RC 69 antigen, is retained in all primary RCCs where RC 69 antigen is sometimes lost, illustrates that tumor differentiation does not necessarily reflect normal differentiation.

Antigen expression corresponding to Mab's RC 69 and RC 154 was not found in RCC metastases, whereas RC 3 stained only 1 of 10 of the metastases tested. Thus, there is a clear difference with regard to the occurrence of these antigens in primary versus metastatic RCC. These data contrast with the data of Finstad et al,[8] who reported that in their study no consistent differences were found between primary and metastatic RCC. In the 5 patients from whom primary and metastatic RCC was available for study, we observed the loss of the antigens corresponding to all four Mab's in a number of cases (see Results). Our data indicate that RCC cells with metastasizing capacity have lost the antigens corresponding to RC 3, RC 69, and RC 154.

Of the renal adenomas, only 1 expressed RC 38 antigen, while 6 others did not express RC 38 antigen. RC 3, RC 69, and RC 154 antigen were not expressed in the 2 renal adenomas tested. The surrounding proximal tubular epithelium was clearly stained in all sections. Renal adenomas are often considered precursor lesions of RCC, and the main distinguishing point between adenoma and carcinoma is considered to be the size of the lesion, a diameter of 3 cm constituting the borderline between these lesions.[24,25] Our limited data on small renal adenomas only suggest that a subset of these adenomas do not represent a transitional stage from normal proximal tubules to RCC, because proximal tubular epithelium and primary RCC always express RC 38 antigen. The data are compatible with an origin from distal tubular epithelium.

RC 38 did not stain tumor cells of breast tumors, carcinomas of the gastrointestinal tract, or angiosarcomas, although a staining reaction was seen in the corresponding normal tissues. In most tumors, RC 38 stained the endothelium of small vessels, whereas in normal tissues, except in the liver and lymph nodes, the capillary endothelium did not stain. Therefore, RC 38 might be useful in studies on angiogenesis or vascularization in human tumors. However, mouse endothelial cells in human tumors transplanted in nude mice were not stained by RC 38.

Combining the data of Tables 2 and 6, we found that tumor cells of 46 of 47 primary RCCs and 8 of 13 metastatic RCCs were stained with RC 38. These tumors include 4 tumors of the test set, which consisted of tumors for which the diagnosis of RCC had been contemplated. Also, RC 38 did not stain tumor cells of a wide variety of other tumors that included 12 clear-cell tumors of different origins. These data indicate that RC 38 is useful for diagnostic purposes provided snap-frozen tissue is available.

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