CELL SURFACE ANTIGENS OF HUMAN RENAL
CANCER DEFINED BY AUTOLOGOUS TYPING*

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The two key questions of human cancer immunology remain unresolved. These relate to whether tumor-specific antigens exist in human cancer and, if so, whether there is immunological recognition of these antigens in humans. To date, candidate human tumor-specific antigens defined by heterologous sera have, on further analysis, turned out to belong to the category of differentiation antigens, antigens characterizing normal cells at some phase of differentiation, rather than antigens that are restricted to neoplastic cells. The tumor antigens that are detected by reactions with human sera fall into several categories, with alloantigens (particularly products of the *HLA* complex and *ABO* locus), antigens related to Epstein-Barr virus, and antigens related to the heterologous sera used in culturing human cancer cells representing the best studied examples. In general, however, most surveys of human populations for humoral or cell-mediated immunity to cell surface or intracellular antigens of human cancer cells have not provided the sort of evidence that would permit the distinction of tumor-specific reactions from reactions directed to other categories of antigens.

To develop as direct an approach as possible to the question of whether patients with cancer recognize tumor-specific antigens on the surface of their cancer cells, we have developed a serological approach, referred to as autologous typing, that has been applied to the study of cell surface antigens of malignant melanoma (1–3), acute leukemia (4), and astrocytoma (5). Autologous typing has several features: (*a*) direct tests are restricted to reactions between sera and tumor cells from the same patient, eliminating the need to consider reactions that are a result of conventional alloantibodies and assuring the detection of antigens restricted to autologous tumor cells, i.e., class 1 antigens, see below; (*b*) several serological tests are used in parallel, reducing the possibility that antibody belonging to a particular class might be missed; (*c*) target cells from solid tumors are provided by serially passaged tissue culture lines, permitting

repeated tests to be performed (in the case of leukemia, where adequate numbers of viable cells can be obtained directly from the patient, target cells are stored by cryopreservation for repeated sampling); and (d) absorption tests are relied on to analyze the specificity of the reactions observed, providing a way to classify the antigens detected. Three classes of surface antigens have been detected by autologous typing of melanoma, astrocytoma, and leukemia. Class 1 antigens are restricted to autologous tumor cells and cannot be detected by absorption tests on normal or malignant cells of any other type, autologous, allogeneic, or xenogeneic. Three class 1 antigens of melanoma (AU, BD, and BI) and two class 1 antigens of astrocytoma (AC and BC) have been identified in our initial survey of 36 melanoma patients (1–3) and 30 astrocytoma patients (5). Class 2 antigens are found on autologous as well as certain allogeneic tumors, but cannot be detected on normal cells. Three class 2 antigens have been defined: the AH antigen of melanoma (2), the serologically related AJ antigen of astrocytoma (5), and a leukemia cell surface antigen detected by autologous antibody in one patient in our initial survey of 21 patients with acute leukemia (4). On the basis of current evidence, class 1 and class 2 antigens can be considered tumor-restricted antigens capable of eliciting humoral immunity in the autologous host. In contrast, class 3 antigens are not restricted to tumors; they can be detected on both normal and malignant cells of human and animal origin. The complex pattern of antigen occurrence revealed by absorption analysis indicates that class 3 antigens are a diverse and complex group, with some antigens showing differentiation characteristics, some alloantigenic characteristics, and some heterospecific characteristics (3). Antibodies that detect class 3 antigens are the ones most commonly found in patients with cancer and, because in direct tests these antibodies can appear to react only with tumor cells, rigorous absorption analysis is necessary to distinguish reactions of this sort from reactions detecting class 1 and class 2 antigens.

Autologous typing of human renal cancer is the subject of the present report. Renal cancer can be serially cultured from the tumors of 10–20% of patients, and this has permitted us to apply autologous typing to the study of a significant number of renal cancer patients. An important feature of these studies with renal cancer is the availability of cultured normal kidney cells for absorption tests. This is a unique advantage in the study of renal cancer as compared to melanoma and astrocytoma, where it has not yet been possible to analyze the normal cellular counterpart.

Materials and Methods

Tissue Culture

RENAL CANCER CELLS.   Sterile tumor specimens were dissected free of adherent normal tissue and finely minced in Earle's balanced salt solution (EBSS).[1] The released cells and small tissue fragments were treated with 0.25% trypsin (Bacto-trypsin, Difco Laboratories, Detroit, Mich.) and 0.02% collagenase (Worthington Biochemical Corp., Freehold, N.J.) for 15–30 min at 37°C with continuous mixing using a magnetic stirrer. The resulting cell suspension was washed three times in EBSS and resuspended in Eagle's minimum essential medium supplemented with 1% nonessential amino acids, 2 mM glutamine, 0.01 U/ml insulin (Eli Lilly & Co.,

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[1] *Abbreviations used in this paper:* EBSS, Earle's balanced salt solution; MEM, minimum essential medium; FBS, fetal bovine serum; NRK, normal rat kidney; MHA, mixed hemadsorption; IA, immune adherence; C3-MHA, anti-C3 mixed hemadsorption; PA, protein A; VBM, Veronal buffer medium; EBV, Epstein-Barr virus.

Indianapolis, Ind.), 100 U/ml penicillin, 100 μg/ml streptomycin, and 20% fetal bovine serum (primary minimum essential medium [MEM]). The cells were seeded in Falcon No. 3013 (25 cm^2) plastic tissue culture flasks (Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, Calif.) and fed with primary MEM twice a week. When cultured epithelial cells became confluent, they were detached and dissociated with 0.25% trypsin and transferred into new flasks. Initial subculturing began 2-12 wk after seeding the original specimen. After the third subculture, cells were fed twice a week with Eagle's MEM supplemented with 1% nonessential amino acids, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum (complete MEM). The frequency of subculturing different renal cancer cultures ranged from once every week to once every 4 wk. Cultures were repeatedly tested for mycoplasma, fungi, and bacteria. Contaminated cultures were discarded.

Cell lines of renal cancers from 13 patients have been passaged for >10 generations. The designations of these renal cancer cell lines are; SK-RC-1 (patient AA), SK-RC-2 (AB), SK-RC-3 (AC), SK-RC-4 (AE), SK-RC-5 (AF), SK-RC-6 (AG), SK-RC-7 (AX), SK-RC-8 (BE), SK-RC-9 (BM), SK-RC-10 (BT), SK-RC-11 (BZ), SK-RC-12 (CA), and SK-RC-13 (CH). Cultures of renal cancer cells that have been passaged <10 times are designated by the patient code.

NORMAL KIDNEY CELLS.   Normal kidney tissue adjacent to the renal cancer or from nephrectomies performed for reasons other than malignancy were cultured in the same way as renal cancers. As normal kidney cells attach and start to proliferate much faster than renal cancer cells, initial subculturing was usually possible within 1-2 wk. Cultures of fetal kidney cells were purchased from Microbiological Associates (Bethesda, Md.) or were established in our laboratory from kidneys of aborted first trimester fetuses.

FIBROBLASTS.   Fibroblasts were cultured from biopsied abdominal or arm skin as previously described (1).

OTHER CELL LINES.   For derivation of other cell lines see references 1-6. A line of normal rat kidney (NRK) was supplied by Dr. P. O'Donnell, Sloan-Kettering Institute. VERO and BGMK, two cell lines derived from monkey kidney, were obtained from Microbiological Associates.

Serological Procedures.   The mixed hemadsorption assay (MHA), immune adherence assay (IA), anti-C3 mixed hemadsorption assay (C3-MHA) and protein A assay (PA) were performed as previously described (1-3, 5). To reduce the requirement for reagents (serum, target cells, and absorbing cells) we have recently adapted these serological assays to Falcon 3034 plates (Falcon Labware, Div. of Becton, Dickinson & Co.). As the modified assay requires five times less serum than the standard assay in Falcon 3040 plates (10 μl rather than 50 μl per well) and correspondingly less cells for absorption tests, extensive analysis is possible even when the supply of sera and cultured cells is limited. Qualitative and quantitative absorption tests were performed according to procedures developed in our past work (1-5). Cultures are harvested for absorption tests by mechanical scraping rather than by exposure to enzymes or EDTA. Serum is absorbed at three doubling dilutions below its endpoint (titer). One aliquot of diluted serum remains unabsorbed, whereas other aliquots are mixed with the cells to be tested. In qualitative absorption tests, 0.15 ml of appropriately diluted serum is mixed with 0.1 ml of washed packed cells. (For assays in Falcon 3034 plates, 75 μl of serum and 50 μl of packed cells are used.) In quantitative absorption tests, the diluted serum is absorbed with a series of increasing numbers of viable cells. Absorption is carried out on ice for 1 h with occasional shaking. After absorption, the cells are removed by centrifugation, and the absorbed and unabsorbed sera are tested for residual reactivity with the target cell.

Rosette Formation with Rat Erythrocytes.   Subconfluent monolayers of cultured cells growing in Falcon 3040 plates were prepared. After removal of the medium, 50 μl of a 0.2% (vol/vol) suspension of rat erythrocytes in Veronal buffer medium with 5% fetal bovine serum (VBM-FBS) were added to each well and the plates left undisturbed for 45 min at 24°C. The plates were then agitated gently, washed once in VBM-FBS, and the attachment of rat erythrocytes to the cultured cells was determined by light microscopy.

Results

Growth Characteristics and Morphology of Renal Cancer and Normal Kidney in Tissue Culture.   Renal cancers from 73 patients were studied. 80% of the renal cancers were
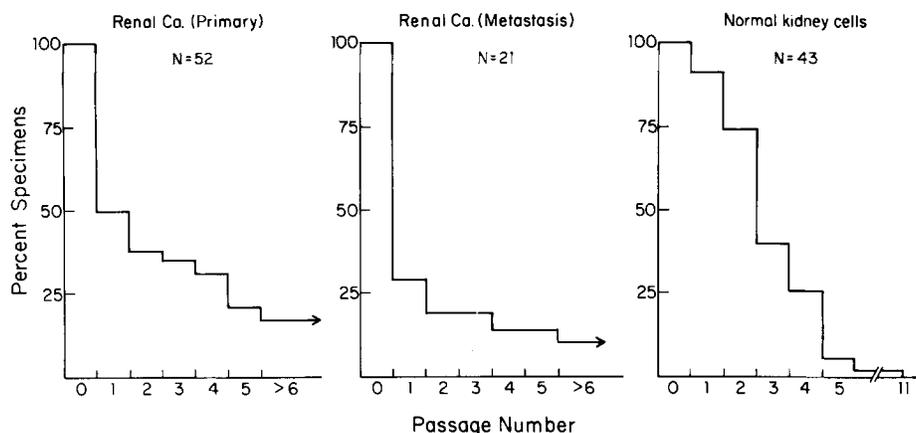
Fig. 1.   Growth characteristics of renal cancer and normal kidney in vitro. 73 specimens of primary or metastatic renal cancer and 43 specimens of normal kidney were studied over a 2-yr period (October, 1976 to December, 1978).

classified as predominantly clear cell type, the remainder showing mixtures of clear cells and granular or spindle cells. In 52 cases, primary renal cancers were cultured; in 21 cases, renal cancer tissue came from metastatic sites, most commonly lung. Cultures of normal kidney cells were derived from normal kidney tissue adjacent to renal cancer in the surgical specimens (40 cases) or from kidneys removed for reasons other than malignancy (3 cases). As shown in Fig. 1, 50% of the primary renal cancers could be subcultured at least once and 17% were successfully passaged more than six times. With metastatic renal cancer, 29% of the specimens could be subcultured at least once and 10% were passaged more than six times. Two factors appear to contribute to the poorer growth of cultures from metastatic renal cancer; more tissue is generally obtained from primary renal cancer, resulting in a larger yield of viable cancer cells to initiate cultures, and fibroblast overgrowth was a greater problem with specimens from metastatic sites than from the primary tumor. Renal cancers that could be subcultured for more than six times, whether from primary or metastatic sites, continued to grow vigorously on subsequent passage. Fig. 2 illustrates the three main cell types that can be distinguished in established cultures of renal cancer. The first type grows as islands of compact epithelial cells with irregular, sharply defined edges (Fig. 2 A). The second type exhibits a globular shape with pseudopod-like structures (Fig. 2 B). Although some cultures contained a preponderance of one cell type or the other, most cultures were mixtures of both cell types and, after reaching a confluent state, the two cell types became indistinguishable. The third and least common cell type showed a well-defined spindle shape and these cells tended to grow in mounds several layers deep (Fig. 2 C). Lack of contact inhibition was a characteristic feature of established renal cancer cultures.

The growth and morphology of cells derived from specimens of normal kidney were clearly distinguishable from cultures of renal cancer. Normal kidney cells tend to grow more rapidly than renal cancer cells during initial culture and a high percentage of normal kidney cultures could be passaged two to three times (Fig. 1). However, after passage 2, the growth rate of normal kidney cells fell sharply, and only one of 43 cultures could be passaged more than five times. In contrast to the frequent occurrence of granules and vacuoles in the cytoplasm of renal cancer cells, the cytoplasm of
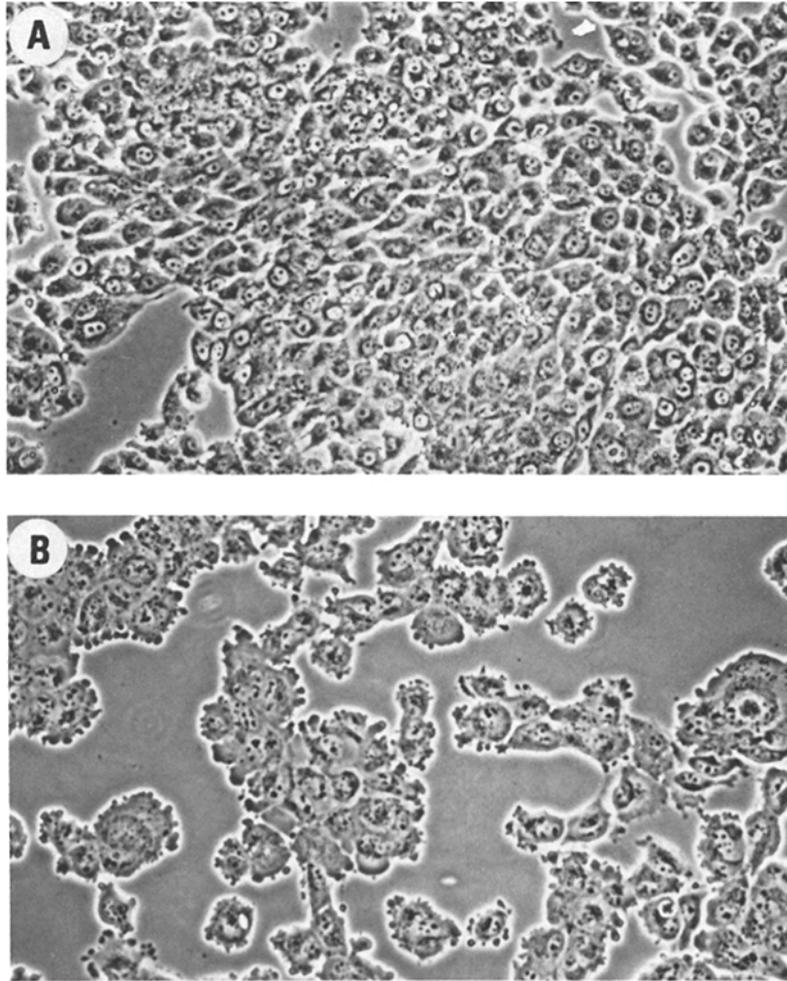
FIGS. 2 A, B.

FIG. 2. Morphology of renal cancer cells and normal kidney cells in vitro (X 160). A–C, cell types that can be distinguished in cultures of renal cancer. D, characteristic appearance of cultured normal kidney cells. (See text for description.)

normal kidney cells has few granules or vacuoles. Cultured normal kidney cells have a characteristic crescent shape with smooth edges, tend to grown in a whorled pattern, and show contact inhibition after a confluent monolayer is formed (Fig. 2 D).

Presence of Receptors for Rat Erythrocytes on the Surface of Renal Cancer and Normal Kidney Cells. During a search for discriminant binding of heterologous erythrocytes to the surface of cultured human cells, we found that renal cells, both normal and malignant, have receptors for rat erythrocytes. Table I shows the range of human cell lines tested for rosette formation with rat erythrocytes. All cultures of renal origin bound rat erythrocytes, whereas 46 lines derived from other sources did not. Two of 10 astrocytoma lines were exceptional in having receptors for rat erythrocytes. Erythrocytes from other species (chicken, mouse, cat, dog, horse, sheep, monkey, and human) did not show specific binding to these cultured cell lines.
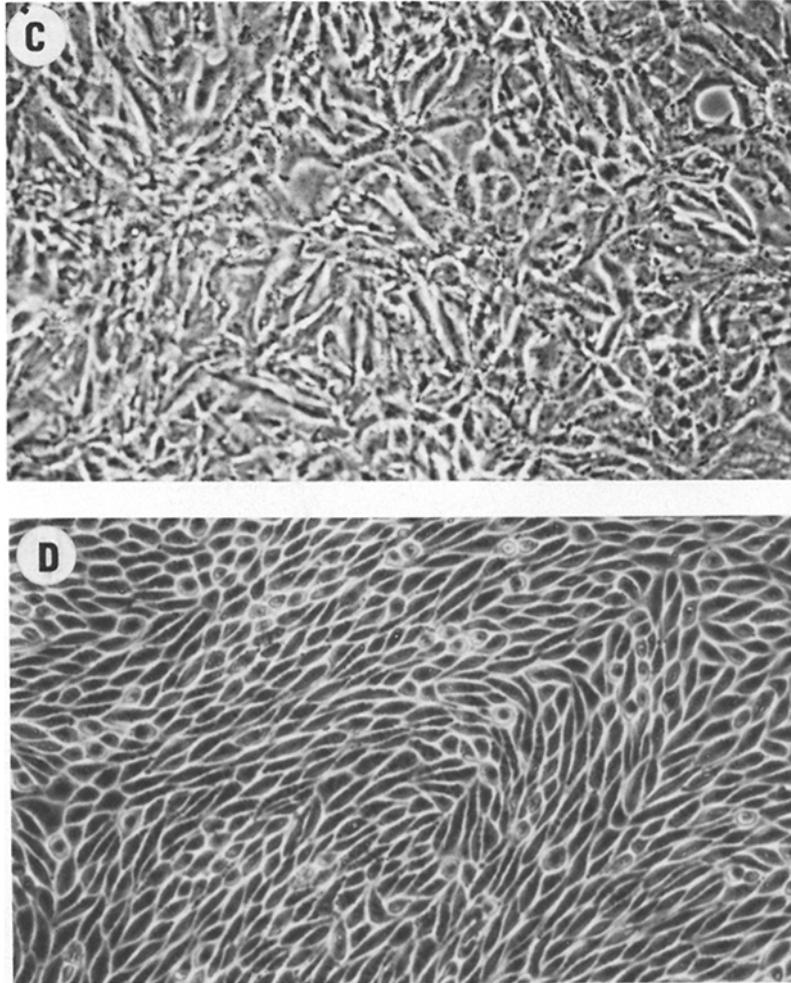
FIGS. 2 C, D.

Reactions of Autologous Sera with Surface Antigens of Cultured Renal Cancer Cells. Sera from 28 patients were tested for reactivity with cell surface antigens of autologous renal cancer cells. Table II summarizes the results of these tests. IA assays showed that sera from 23 of 28 patients gave positive reactions, with titers ranging from 1/2 to 1/128. MHA assays detected a low titered reaction in 1 of 22 patients. C3-MHA assays revealed autologous reactions in all 26 patients tested, with titers ranging from 1/4 to 1/256. PA assays detected antibodies in 14 of 15 patients, with titers ranging from 1/2 to 1/160. Autologous reactions detected by IA and C3-MHA assays were generally stronger and higher titered with an incubation of serum and target cells at 4°C rather than 24°C. In contrast, maximal reactivity in PA assays occurred at 24°C.

13 of the renal cancers in this series propagated sufficiently well in culture to permit further study of autologous serum reactivity. Six of these were chosen for detailed analysis. To illustrate this analysis, the results of autologous typing with sera from two patients, AG and AX, will be presented.

TABLE I

Tests for Rat Erythrocyte Receptors on Cultured Normal and Malignant Cells of Human Origin

Rosette formation	No rosette formation	
Renal cancer	Astrocytoma	Carcinoma
SK-RC-1, 2, 3, 4, 6, 7, 8, 9, 10,	AN, AS, BAR, BD,	Bladder, T-24
11, 12, BL, CP, DA, DY	U138MG, U178MG,	Breast, AlAb, CaMa, MCF-7,
A-498	U373MG	SK-BR-3
CaKi-1	Neuroblastoma	Cervix, ME-180
Normal kidney	SK-NMC	Colon, HT-29
AX, BL, CA, CJ, DB, DF, DH,	SK-NSH	Larynx, HEp-2
DV, DY	Melanoma	Lung, SK-LC-LL
Fetal kidney	SK-MEL-13, 14, 19, 25, 27,	Ovary, SK-OV-3
Astrocytoma	28, 29, 31, 37, 40, 57, 59	Fibroblasts
AJ, BB	Sarcoma	AI, AP, AR, AT, AU, AV,
	SAOS-2	AX, BL, BM, CA
	U2OS	PHEL-6 (fetal)
		WI-38 (fetal)

Analysis of Autologous Reactivity of AG and AX Sera. The reactivity of AG and AX sera for autologous renal cancer cells as determined in the four serological test systems is shown in Fig. 3. In both cases, antibody was detected by PA, C3-MHA, and IA assays, but not by MHA assays. The reactivity of AG serum was highest in PA assays and AX reactivity was highest in C3-MHA assays. These assays were chosen, therefore, to analyze AG and AX sera.

Reactivity of Sequential Serum Samples for Autologous Renal Cancer Cells. Simultaneous titrations of individual sera from AG and AX collected over a 2-yr period are shown in Fig. 4. The reactions of both AG and AX sera remained essentially unchanged over this time period.

Tests of AX Sera for Reactivity with Autologous Normal Kidney Cells and Fibroblasts. Fig. 5 shows that AX serum has reactivity for autologous normal kidney cells as well as renal cancer cells. Tests with autologous fibroblasts were essentially negative. Comparable tests have been possible with the sera of eight other patients and a similar pattern of reactivity for autologous renal cancer cells and normal kidney cells, but not fibroblasts, was seen.

Qualitative Absorption Analysis of AG and AX Sera. The reactivity of AG and AX sera for autologous renal cancer cells was analyzed by qualitative absorption tests. Fig. 6 illustrates individual absorption tests and Tables III and IV summarize the results of the analysis. Three patterns of absorption were observed; complete absorption, partial absorption, and no absorption. In the case of AG, only autologous renal cancer cells gave complete absorption. In the case of AX, autologous as well as one allogeneic renal cancer (SK-RC-4) completely absorbed reactivity. A limited number of cell lines showed partial absorption of AG and AX reactivity; these lines were derived from cancers of renal as well as nonrenal origin. Astrocytoma AJ partially absorbed both AG and AX reactivity; other cell lines (T-24, ME-180, A-498, and SK-RC-9) showed partial absorption of either AG or AX reactivity. No absorption of AG or AX reactivity for autologous renal cancer cells was found with a large number of other cell types; these tests included allogeneic renal cancer cells, allogeneic and xenogeneic normal kidney cells, fetal kidney cells, and a range of normal and malignant nonrenal cells. Absorption tests with AG and AX autologous normal kidney cells could not be

TABLE II

Survey of Sera from Patients with Renal Cancer for Autologous Antibody to Surface Antigens of Cultured Renal Cancer Cells: Summary of Serological Tests

Patient	Age	Sex	Stage‡ of disease	Culture generations studied	Maximal antibody titer*						
					IA		MHA		C3-MHA		PA
					4°C§	24°C	4°C	24°C	4°C	24°C	24°C
AA‖	41	M	II	51–65	−¶		−	−	>1/64	−	1/40
AB‖	59	M	IV	6–31	1/16	1/8	−	−	1/256	1/40	1/40
AC	69	F	III	4	1/2	1/2	−	−	1/8	1/4	
AE‖	48	M	II	3–30	1/64	1/32	−	−	1/128	1/32	1/160
AF	50	M	I	2–7	1/32	1/4	−		1/64	1/8	1/40
AG‖	72	M	II	1–36	1/4	−	−	−	1/32	1/4	1/160
AI	55	M	III	1–3	>1/64		−		>1/64		
AQ‖	53	F	III	1–14	1/16	1/16	−		>1/64	1/16	
AR‖	46	M	IV	2–5	−	−	−	−	1/16	1/16	
AX‖	64	M	II	1–40	1/128	1/64	−	−	1/256	1/64	1/32
AZ	69	M	I	1	1/8				1/32	1/16	
BB	34	F	II	1	−		−				
BE	47	M	IV	2–15	1/4	1/4			1/4	1/4	1/8
BI	47	F	II	1–2	1/4	−	1/4	−	1/16	1/16	
BK	27	M	IV	1–2	1/4	1/8	−	−			
BL	73	F	IV	2–13	1/16	1/8	−	−	1/16	1/8	−
BM‖	56	M	IV**	2–28	1/4	1/2	−	−	1/64	1/32	1/2
BU	61	F	III	1	1/8				>1/64		
BZ	60	M	III	1–13	−	−	−	−	1/16	1/4	1/160
CA	68	M	IV	2–9	1/32		−	−	1/64	1/64	1/5
CB	57	M	II	1–2	>1/64		−		>1/64		
CD	56	F	IV	1–3	1/8	1/2	−	−	1/16	1/2	1/20
CG	62	F	II	1–3	1/32	1/4			1/64	1/32	
CH	63	M	IV**	1–11	1/64	1/32			1/64	1/64	1/40
CI	71	M	II	1–4	−		−		1/8		>1/64
CP	64	M	IV	1–6	1/40				1/60		1/20
CU	50	M	II	1	1/16		−		1/32	1/4	
CV	67	F	II	1–3	1/16		−		1/64		

‡ Stage I: tumor within true renal capsule; stage II: tumor invades perinephric fat but is within Gerota's fascia; stage III: tumor involves major renal vein and/or regional lymph nodes without involvement of perinephric fat or inferior vena cava; stage IV: tumor involvement of adjacent organs (other than adrenal) or distant metastasis, or both.

* Titer: highest serum dilution showing 10% positive cells in IA and MHA; 20% positive cells in C3-MHA and PA.

§ Incubation temperature of target cells and serum.

‖ 10^7 cultured renal cancer cells (>passage 9) were implanted subcutaneously into nu/nu mice. Progressive tumor growth was observed in the case of AA, AQ, and AR.

¶ Signifies no reaction at 1/2 serum dilution.

** Culture of renal cancer derived from metastatic sites: lung (BM) and brain (CH).

performed; a specimen of normal kidney was not obtained from patient AG, and an insufficient number of normal kidney cells were cultured from patient AX.

To exclude the participation of antigens related to fetal bovine serum (FBS) in the reactions observed, the cell lines of AG and AX renal cancer were grown for five passages in medium supplemented with normal human serum rather than FBS. These cells absorbed autologous reactivity as efficiently as cells grown in FBS.

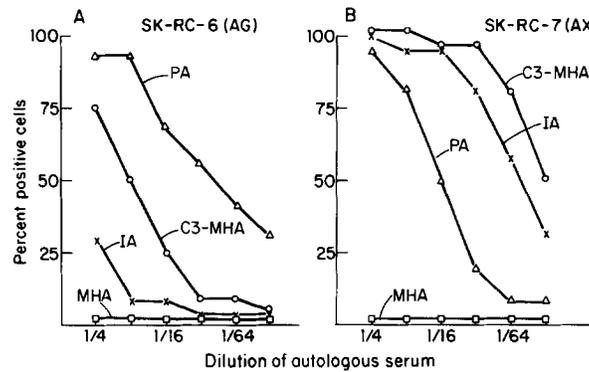*Quantitative Absorption Analysis of AG and AX Sera.* To explore the significance of the

Fig. 3. Reactivity of AG and AX sera for autologous renal cancer cells as determined by four serological assays: IA, MHA, C3-MHA, and PA assays. SK-RC-6 and SK-RC-7 are the designations for the established renal cancer cell lines from patient AG and patient AX, respectively.
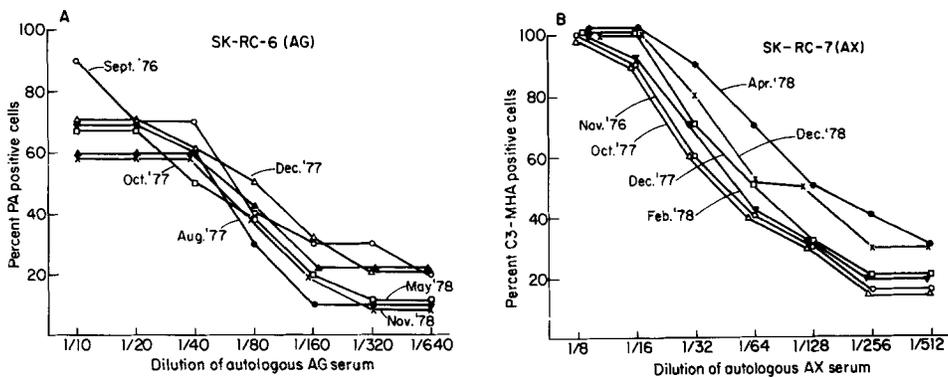

Fig. 4. Reactivity of sequential samples of AG and AX sera for autologous renal cancer cells. (4 A) AG sera collected over a 26-mo period and tested for autologous reactivity with SK-RC-6 cells (passage 35) by PA assays. (4 B) AX sera collected over a 25-mo period and tested for autologous reactivity with SK-RC-7 cells (passage 21) by C3-MHA assays.

partial absorption observed with certain cell lines, AG and AX sera were analyzed by quantitative absorption tests (Fig. 7). Reactivity of AG sera for autologous renal cancer was completely absorbed by $\doteqdot$ 1.8 × 10^6 autologous renal cancer cells. Two cell lines (SK-MEL-27 and T-24) that failed to absorb AG reactivity in qualitative absorption tests were also negative in quantitative absorption tests over the range of cell numbers examined. The line of renal cancer cells (A-498) that showed partial absorption in qualitative absorption tests reduced AG reactivity to the same fixed level with 0.12 × 10^6 cells as with 8 × 10^6 cells. This pattern of partial absorption indicates that A-498 cells lack one or more of the determinants recognized by AG serum on autologous renal cancer cells.

Quantitative absorption analysis of AX serum showed that autologous AX renal cancer cells (SK-RC-7) and the allogeneic renal cancer cell line (SK-RC-4) express comparable amounts of the determinant or set of determinants recognized by AX sera on autologous renal cancer cells. VERO and DM normal kidney cells absorbed little or no AX reactivity over the range of cell numbers tested. Astrocytoma AJ,
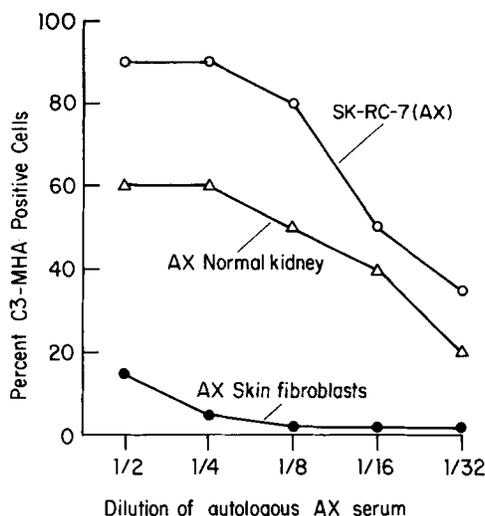
Fig. 5. Reactivity of AX serum for cultured autologous renal cancer cells (SK-RC-7), normal kidney cells, and skin fibroblasts, as determined by C3-MHA assays.
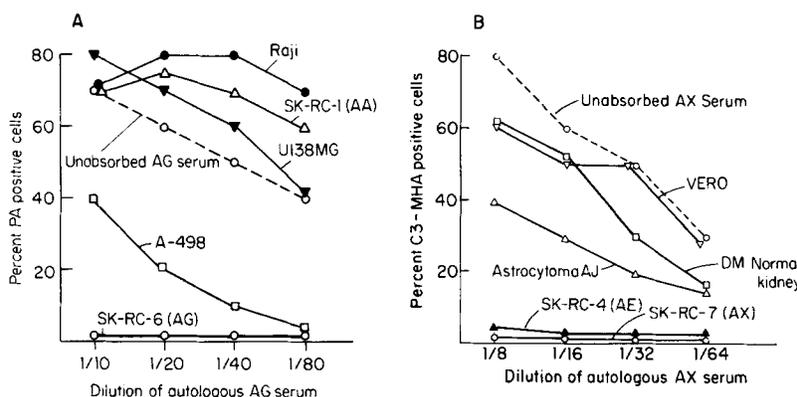

Fig. 6. Qualitative absorption analysis of AG and AX sera. (6 A) Individual absorption tests with AG sera. AG reactivity for autologous renal cancer cells (SK-RC-6) was completely absorbed by SK-RC-6 and partially absorbed by allogeneic renal cancer A-498. Astrocytoma U138MG, Burkitt lymphoma Raji and allogeneic renal cancer SK-RC-1 did not absorb AG reactivity. (6 B) Individual absorption tests with AX serum. AX reactivity for autologous renal cancer cells (SK-RC-7) was completely absorbed by SK-RC-7 and allogeneic renal cancer SK-RC-4, and partially absorbed by astrocytoma AJ. VERO, a cell line derived from monkey kidney, and normal kidney cells from patient DM did not absorb AX reactivity.

comparable to the results obtained in qualitative absorption tests, reduced but did not remove AX reactivity at the highest number of AJ cells tested. This capacity of astrocytoma AJ to reduce the reactivity of AX sera could be eliminated by preabsorbing AX serum with astrocytoma AJ. Fig. 8 represents a qualitative absorption test with AX sera that had been preabsorbed at a 1:1 ratio of undiluted AX serum and packed AJ cells. Astrocytoma AJ no longer lowered reactivity of the preabsorbed serum, whereas SK-RC-9 continued to show partial absorption. Fig. 9 shows a

TABLE III

Analysis of the PA Reactivity of AG Serum for Autologous Renal Cancer Cells: Summary of Absorption Tests

Complete absorption	No absorption		
Autologous cultured cells SK-RC-6 (AG)	Autologous cultured cells: Fibroblasts AG	Ca bladder T-24 Ca breast AlAb Ca cervix ME-180	Allogeneic noncultured cells: AB erythrocytes
Partial absorption	Allogeneic cultured cells:	Ca larynx HEp-2	Xenogeneic cells and serum:
Allogeneic cultured cells:	Renal cancer:	Ca lung SK-LC-LL	
SK-RC-7 (AX)	SK-RC-1 (AA)	Melanoma SK-MEL-13	Cultured monkey
Ca kidney A-498	SK-RC-2 (AB)	" -27	kidney cells:
Astrocytoma AJ	SK-RC-9 (BM)	" -28	VERO
	SK-RC-10 (BT)	" -40	Rat erythrocytes
	CaKi-1	Sarcoma U2OS	Sheep erythrocytes
	Normal kidney CI	Sarcoma SAOS-2	Guinea pig kidney
	" " CJ	Burkitt lymphoma Raji	Guinea pig spleen
	" " CK	EBV-transformed B cells	cells
	" " CP	BT, CV	Guinea pig brain
	" " CQ	Fetal skin fibroblasts F5	Fetal bovine serum
	" " DB	Fetal kidney cells (6447)	
	" " DF		
	Astrocytoma U138MG		

TABLE IV

Analysis of the C3-MHA Reactivity of AX Serum for Autologous Renal Cancer Cells: Summary of Absorption Tests

| Complete absorption	No absorption		
Autologous cultured cells:	Autologous cultured cells:	Normal kidney AN	Sarcoma U2OS
SK-RC-7 (AX)	Fibroblasts AX	" AT	Burkitt lymphoma Raji
Allogeneic cultured cells:	EBV-transformed B-	" AU	EBV-transformed B-
SK-RC-4 (AE)	cells AX	" AY	cells CV, CX,
	Autologous noncultured cells:	" CI	LM
Partial absorption		" CJ	Fetal skin fibroblasts F2
Allogeneic cultured cells:	Erythrocytes	" DB	Fetal skin fibroblasts F5
SK-RC-9 (BM)	Lymphoid cells	" DJ	Fetal kidney cells 19
Astrocytoma AJ	Allogeneic cultured cells:	" DM	Allogeneic noncultured cells:
Ca bladder T-24		" DT	
Ca cervix ME-180	Renal cancer:	Fibroblasts AG	AB erythrocytes
	SK-RC-1 (AA)	Astrocytoma U138MG	Xenogeneic cells and serum:
	SK-RC-2 (AB)	" U178MG	
	SK-RC-6 (AG)	" AT	Cultured monkey kidney cells:
	SK-RC-8 (BE)	Ca breast AlAb	
	SK-RC-11 (BZ)	Ca larynx HEp-2	VERO, BGMK
	AR	Ca lung SK-LC-LL	Cultured rat kidney cells:
	CaKi-1	Melanoma SK-MEL-13	
	CaKi-2	" -28	NRK
	A-498	" -37	Rat erythrocytes
		" -40	Sheep erythrocytes
			Guinea pig kidney
			Guinea pig spleen cells
			Fetal bovine serum
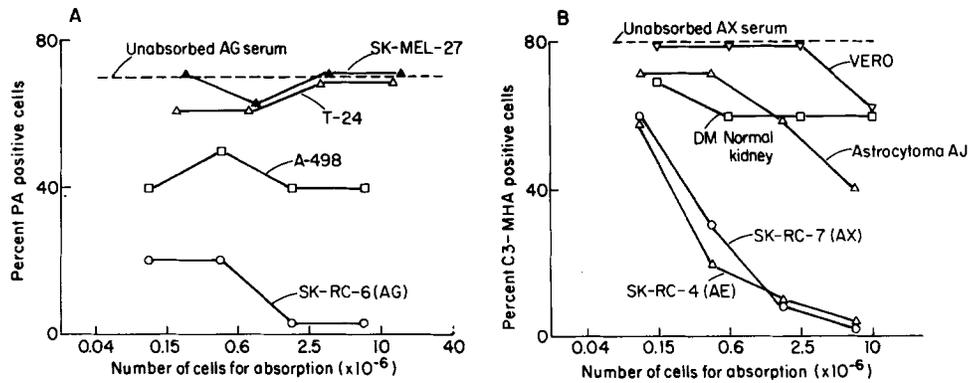
Fig. 7. Quantitative absorption analysis of AG and AX sera. (7 A) Aliquots of 1/10 diluted AG serum were absorbed with counted numbers of SK-RC-6, A-498, T-24, and SK-MEL-27 cells. The absorbed sera were then tested for residual PA reactivity for autologous SK-RC-6 cells. The dotted line represents reactivity of unabsorbed 1/10 diluted AG serum. In contrast to complete absorption by SK-RC-6, A-498 reduced reactivity to the same fixed level over a broad range of cell numbers. (7 B) Aliquots of 1/8 diluted AX serum were absorbed with counted numbers of SK-RC-7, SK-RC-4, astrocytoma AJ, DM normal kidney, and VERO cells. The absorbed sera were then tested for residual C3-MHA reactivity for autologous SK-RC-7 cells. The dotted line represents reactivity of unabsorbed 1/8 diluted AX serum. On the basis of absorption capacity per cell, autologous SK-RC-7 and allogeneic SK-RC-4 renal cancers express comparable amounts of antigen. Astrocytoma AJ significantly reduced AX reactivity at the highest cell number tested, in contrast to little or no absorption by DM normal kidney or VERO cells.

quantitative absorption analysis of the preabsorbed AX serum. Astrocytoma AJ does not absorb any further reactivity over the range of cell numbers tested. Autologous renal cancer cells showed significant absorption with 0.12 × 10⁶ cells and complete absorption with 8 × 10⁶ cells. Absorption with 1.2 × 10⁶ SK-RC-9 cells reduces AX reactivity by approximately one-half: increasing the number of SK-RC-9 cells by a factor of four does not reduce reactivity further. This absorption analysis of AX sera indicates that at least three determinants are detected by the reactions of AX sera with autologous renal cancer cells. The complete set is shared with SK-RC-4, the allogeneic renal cancer that completely absorbs autologous AX reactivity. Cells that show a fixed partial absorption lack one (SK-RC-9) or more (astrocytoma AJ) of these determinants.

Autologous Typing of Renal Cancer: Summary of Results. Table V summarizes the results of autologous typing of cultured renal cancer cells from six patients. In addition to the analysis of AG sera by PA assays and AX sera by C3-MHA assays (see above), these sera were also analyzed with one other serological assay (C3-MHA in the case of AG and IA in the case of AX). Absorption tests of AX sera gave a similar pattern with both C3-MHA and IA assays. Absorption tests of AG sera by C3-MHA assays, as compared to PA assays, detected a broader range of cells, including normal cells, showing partial or complete absorption of autologous reactivity. Complete absorption of autologous reactivity of AB and AE sera was found with certain allogeneic renal cancer cells, resembling in this respect the reactions of AX sera rather than AG sera. (No allogeneic renal cancer showed complete absorption of autologous reactivity of AG sera tested by PA assays, see above). A comparison of the capacity of different allogeneic renal cancer cells to absorb autologous reactivity of AB, AE, AG, and AX sera indicates the detection of different sets of determinants in each of these autologous
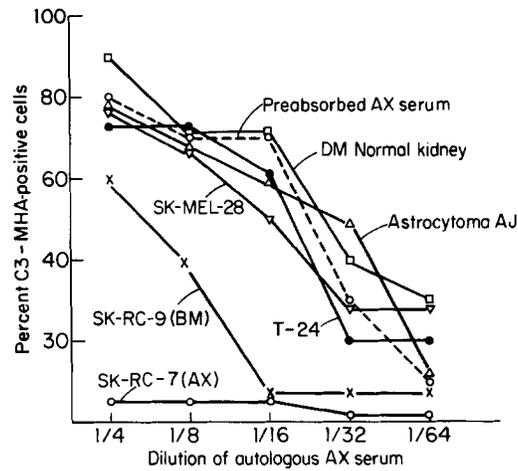
Fig. 8. Qualitative absorption tests of AX sera preabsorbed with astroctyoma AJ. Undiluted AX serum was first absorbed with an equal volume of packed astrocytoma AJ cells. Aliquots of this preabsorbed serum (diluted 1/4) were further absorbed with the cell types indicated and retested for residual C3-MHA reactivity for autologous SK-RC-7 cells. SK-RC-7 and SK-RC-9 showed the expected complete and partial absorption, respectively, of AX reactivity. Astrocytoma AJ absorbed no further reactivity from preabsorbed AX serum.
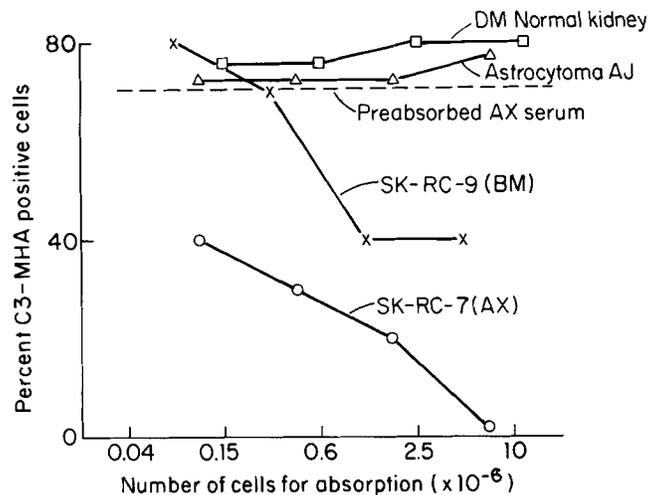

Fig. 9. Quantitative absorption analysis of AX serum preabsorbed with astrocytoma AJ (see legend, Fig. 8). Aliquots of preabsorbed 1/3 diluted AX serum were further absorbed with counted cell numbers of the tumor indicated. The dotted line represents reactivity of preabsorbed 1/3 diluted AX serum without further absorption. 8.0 × 10⁶ SK-RC-7 cells absorbed autologous AX reactivity completely. 1.25-5 × 10⁶ SK-RC-9 cells showed a fixed partial reduction of AX reactivity. Absorption with astrocytoma AJ did not cause a further reduction of AX reactivity.

systems. The autologous reactivity of sera from two other patients, AR and BM, was completely absorbed by a variety of cell types, both normal and malignant, and in the case of AR, even monkey kidney cells absorbed autologous reactivity.

In terms of classifying the antigens recognized by autologous typing of renal cancer cells, AR and BM sera detect class 3 antigens, as indicated by the capacity of normal

TABLE V

Cell Surface Antigens of Cultured Human Renal Cancers Defined by Absorption Tests with Autologous Sera

Patient	AG	AG	AX	AX	AB*	AE	BM	AR
Serological assay	PA	C3-MHA	C3-MHA	IA	C3-MHA	C3-MHA	C3-MHA	C3-MHA
Absorbed with:								
Autologous cells:								
Renal cancer	+	+	+	+	+	+	+	+
Fibroblasts	−	−	−	−	−	−	P	
Allogeneic cells:								
Renal cancer	P or −	+ or P	+, P or −	+, P or −	+, P or −	+, P or −	+ or P	+
Normal kidney	−	P	−	−	−	−	+ or P	
Fibroblasts	−	P	−	−	−	−	+ or P	
Nonrenal cancer	P or −	+, P or −	P or −	P or −	P or −	P or −	+, P or −	+ or P
Fetal kidney		P	−	−	−		P	
Fetal fibroblasts	−		−		−	−	P	
Erythrocytes	−	−	−	−	−	−	−	−
Xenogeneic cells and serum:								
Monkey kidney	−	P	−	−	−	−	P or −	+
Rat kidney			−		−	−		
Sheep erythrocytes	−	−	−	−	−	−	−	−
Fetal bovine serum	−	−	−	−	−	−	−	−
Class of antigen	1, 2	3	2	2	2	2	3	3

+, complete absorption; P, partial absorption; −, no absorption.
* Cultured autologous normal kidney cells were available for absorption tests; no absorption of autologous serum reactivity for AB renal cancer cells was observed.

cells to absorb autologous reactivity. AB, AE, and AX sera detect a range of class 2 antigens, as indicated by partial or complete absorption with renal and nonrenal cancer cells, but not by normal cells. AG sera, analyzed by PA assays, detect both a class 1 antigen (restricted to autologous cancer cells) and class 2 antigens (shared with certain allogeneic cancer cells). By C3-MHA assays, however, AG sera show predominantly class 3 reactivity.

Discussion

The pattern of reactivity of autologous sera with cultured renal cancer cells observed in the present study shows a general similarity to our past findings with melanoma (1–3), leukemia (4), and astrocytoma (5). It seems likely that a similar pattern will emerge as other tumor types are analyzed in the same way. Before this can be done, however, particularly in the case of some of the most common human malignancies such as breast and lung, our ability to propagate these cancers in vitro will have to be improved. The culturing of the corresponding normal cell type is another challenge that will need to be met before the general significance of humoral immune reactions to autologous tumor cells can be evaluated. With regard to the four tumor types (melanoma, leukemia, astrocytoma, and renal cancer) that have now been analyzed by autologous typing, the corresponding normal cell population is most readily available in the case of renal cancer. How closely related the cells cultured from normal kidney and the cells cultured from renal cancer are to one another remains to be determined, but quite clearly, epithelial cells derived from normal kidney represent a far more relevant cell population than fibroblasts or EBV-transformed B-lympho-

blast lines, two cell types that are commonly used as control cells in the serological study of human cancers. Despite vigorous initial growth, normal kidney cells can rarely be passaged more than four to five times and this restricted growth potential limits their usefulness. This behavior of normal kidney cells in culture most likely reflects exhaustion of required growth factors, a possibility we are now exploring.

By testing autologous combinations of tumor cells and sera, the contribution of conventional alloantibody to HLA and AB products can be excluded. Nevertheless, there still remains a complex array of antibodies recognizing cell surface antigens expressed by renal cancer cells. Similar to our findings with melanoma and astrocytoma, absorption analysis revealed three classes of renal cancer antigens detected by autologous sera; class 1 antigens, restricted to the autologous tumor; class 2 antigens, shared by certain allogeneic tumors of renal as well as nonrenal origin; and class 3 antigens, expressed by normal as well as malignant cells. The serum of one patient (AG) had antibodies to class 1, 2, and 3 antigens; other patients showed predominantly class 2 (e.g., AX) or class 3 (e.g., AR) reactivity by autologous typing. Partial absorption of autologous reactivity with certain allogeneic cancer cells was a consistent feature in the analysis of sera from renal cancer patients with class 1 or class 2 reactivity. This could be a result of a lower quantity of antigen on the allogeneic cell or to a qualitatively different spectrum of antigens expressed by the autologous and allogeneic cell. Quantitative absorptions and double absorptions are useful in distinguishing between these two possibilities. In the two cases studied most extensively (AG and AX), partial absorption was found to be a result of allogeneic cells lacking one or more of the determinants detected by autologous sera on the autologous target cell. Another possibility to account for results simulating partial absorption is that complexes of antigen and antibody are released from the cell surface during the absorption procedure and remain in the supernate after removing the absorbing cells by centrifugation. These complexes may bind to target cells because they are more sticky or because one of the antibody combining sites is free. Although this remains a theoretical possibility, we have not detected any difference in the reactivity of absorbed sera after differential ultracentifugation to remove antigen/antibody complexes.

The frequency of antibody to class 2 antigens in patients with renal cancer is higher than in our corresponding studies of patients with melanoma, leukemia, and astrocytoma. As the detection of class 2 antigens may be obscured by higher titered antibody to class 3 antigens, it is possible that class 2 reactivity to these other cancers is more prevalent than presently recognized. In the study of experimental tumors, the finding of class 2 antigens raises the suspicion of virus-coded or virus-related antigens and this possibility is being explored with regard to renal cancer by absorbing sera detecting class 2 antigens with cells infected with viruses suspected of having an association with human cancer, e.g., papovaviruses, herpesviruses, and oncornaviruses. Another clue that may link class 2 antigens to a virus is the presence of natural antibody to surface antigens of renal cancer cells in the sera of apparently normal individuals (L. Li, unpublished observations). The reactivity of such sera is removed by renal cancer cells, but not by normal kidney, fetal kidney, or a variety of other normal or malignant cells. Thus, the renal cancer antigens detected by certain normal human sera appear to resemble in part the class 2 antigens detected by autologous typing. Whether these natural antibodies recognize the same range of class 2 determinants as sera from renal cancer patients remains to be determined.

Summary

Sera from 28 patients with renal cancer were tested for reactivity with surface antigens of cultured autologous renal cancer cells. Four serological assays were used to survey sera for autologous antibody. Immune adherence, protein A, and C3-mixed hemadsorption assays detected reactivity in a high percentage of patients (80–100%), whereas mixed hemadsorption assays were negative with sera from all but one patient. Reactive sera from six patients were analyzed by absorption tests with autologous, allogeneic, and xenogeneic normal and malignant cells. The absorption analysis indicated the detection of three classes of surface antigens by autologous sera: class 1 antigens, restricted to autologous renal cancer cells; class 2 antigens, present on certain allogeneic renal and nonrenal cancer cells; and class 3 antigens, found on a wide variety of normal and malignant cell types. The sera of one patient detected class 1, 2, and 3 antigens, the sera of three patients detected class 2 antigens, and the sera of two patients detected class 3 antigens. This analysis of renal cancer, with the recognition of three classes of surface antigens recognized by autologous sera, resembles the results of autologous typing of three other human malignancies: malignant melanoma, acute leukemia, and astrocytoma. Evidence provided by autologous typing of these cancers indicates that class 1 and class 2 antigens are tumor-restricted and that under certain circumstances these antigens are immunogenic for the autologous host.

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