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Detection of Antigenic Differences and Similarities Between Human Transitional Cell Carcinoma Cell Lines Using Rabbit Antisera

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Summary. Rabbit antisera to cell lines derived from transitional cell carcinomas were examined by complement dependent cytotoxicity, absorption analysis, and inhibition assays and the staphylococcal protein-A assay. Test cells were from cell lines derived from transitional cell carcinomas (TCC-CL), other tumours, and normal cells. Following several absorptions of the antisera with normal tissues and insolubilized FCS, activity was undetectable against many carcinoma cell lines but remained against TCC-CL, a lung carcinoma derived line, and many of the non-carcinoma lines. Further absorption of anti-253J (a TCC-CL) antisera with non TCC lines resulted in specific activity against 253J. This selectivity was supported by SpA assay results. Inhibition and absorption experiments using cultured cells and supernatants disclosed a complex quantitative and/or qualitative antigenic relationship between cell lines. Absorption of antisera with tumour and normal tissue specimens and inhibition of cytotoxicity by patients'and controls'sera indicated possible clinical relevance of detected antigens.

Key words: Transitional cell carcinoma, Rabbit antisera.

INTRODUCTION

Human transitional cell carcinoma (TCC) of the urinary bladder is one of the more frequently used systems for immunological study. Tests for cell mediated immunity have indicated that TCC derived cell lines (TCC-CL) may express antigen(s) relevant to the disease (1, 6, 16, 21, 22). We have recently reported a serologically demonstrable relationship between 2 TCC-CL, and 2 surgical transitional cell carcinoma specimens (4). That conclusion was based on absorption analyses of an antiserum raised in rabbits against the T24 TCC-CL. This report presents data which show that the original problem is even more complicated than was indicated by preliminary studies. Xenogeneic antisera were raised against 2 other TCC-CL in addition to T24. Complement dependent cytotoxicity, absorption and inhibition techniques, and the Staphylococcal-protein-A antibody binding assay were used. It was shown that though certain TCC-CL may appear to share specificities when analysed by one technique, differences can also be detected using different methods and/or antisera.

These antisera were used to examine the possible relevance of these antigens to clinical bladder cancer. This approach was chosen to circumvent the problem of natural killing activity in tests for cell mediated immunity (7, 10, 30) which has complicated attempts to detect tumour associated antigens (TAA).

MATERIALS AND METHODS

Cell Cultures

Cell lines were used in this study for immunization, absorption, and target cells. Cell lines were tested for mycoplasma contamination by the method of Russel at al. (26). The DAPI was kindly supplied by Professor Otto Dann of the Insti-

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Table 1. Cell lines used in the present study

Cell Line	Derivation	Reference
253J	Transitional cell carcinoma of the bladder	11, 12
647 V	Transitional cell carcinoma of the bladder	11
J82	Transitional cell carcinoma of the bladder	23
T24	Transitional cell carcinoma of the bladder	8
SCaBER	Squamous cell carcinoma of the bladder	20
ChaGo	Adenocarcinoma of the lung	25
MAC21	Adenocarcinoma of the lung	9
HT144	Squamous cell carcinoma of the lung	24
SK-Mes-1	Squamous cell carcinoma of the lung	14
HeLa	Cervical carcinoma	15
HT29M	Carcinoma of the colon	5
TE671	Medulloblastoma	18
CHP100	Neuroblastoma	27
LAN-1	Neuroblastoma	28
SK-N-MC	Neuroblastoma	2
SK-N-SH	Neuroblastoma	2
MT	Sarcoma	17
FL	Amnion	13

tut für Angewandte Chemie der Friedrich-Alexander-Universität, West Germany. All lines except ChaGo were PPLO-free. The line referred to as MAC-21 was found to have G6PDase isoenzyme A, and HeLa marker chromosomes. (The chromosome studies were done by Dr. Robert Sparkes, UCLA Department of Medicine, and the isoenzyme determinations by the UCLA Cancer Center.) All cell lines included in this study are listed in Table 1. Cell lines were maintained in continuous culture on either Eagle's MEM with nonessential amino acids (Gibco, San Jose, California) with 10% fetal calf serum (Reheis, Armour Pharmaceuticals, Phoenix, Arizona, or Gibco) or RPMI-1640 with 15% fetal calf serum. Gentamycin (50 mg/l, Schering, Kenilworth, New Jersey) and fungizone (10%, Gibco) were added.

Antisera

Antisera were prepared by immunization of New Zealand white female rabbits (>3 kg). Cells (1-2 x 10^7) from three TCC-CL (T24, 253J, 647V) were each injected intravenously into one or two different animals. Rabbits were immunized twice with a two week interval and blood collected 1 and 3 weeks after the second immunization. They were boosted at the time of the second bleeding and blood was collected again two weeks later. A fourth antiserum was obtained from Dr. D. Kempner of this department and was raised against 2 cell cultures derived from human lung tumours. The rabbit was first immunized with HT144 and boosted with SK-Mes-1 cells. All sera was stored at -70°C.

Cytotoxicity Test

The microcytotoxicity test was performed as previously described (3). Briefly, cells were plated and incubated at 37°C in 5% CO_2 humidified air, to allow them to adhere (100-200/well) in Microtest I plates (Falcon 3034, Oxnard, CA). The next day, old media was removed and cells were incubated at 37°C with 10 µl of heat-inactivated (56°, 30 min) diluted antiserum for 1 h, followed by 10 µl of rabbit serum (diluted 1:5 in veronal buffered saline, as a complement source) for 2 h. Plates were flooded with medium, incubated overnight, washed, fixed, and stained. The 50% cytotoxic endpoint was determined relative to the identical dilution of pooled normal rabbit serum.

Inhibition Assay

Inhibition of cytotoxicity was determined using the basic cytotoxicity test described above. Five µl of potential blocking agent and 5 µl of a dilution of antiserum which would cause between 60% and 90% cytotoxicity were added together to the target cells, incubated for 1 h, and the complement added as explained. All blocking tests were done with an antiserum against 253J (C41), and using 253J target cells. After staining, cells were enumerated using the Quantimet 720 (Imanco, Cambridge, England, belonging to Dr. Mitsuo

Table 2. Antisera absorbed (1:2) with PBC, Luliki x2, FCS tested against multiple target cells

Target cells	C40[a] X253J d21	C41 X253J d33	C45 XT24 d33	C10 XHT144 and SK-Mes-1
Transitional cell carcinoma				
253J	1:160-320	>1:320	1:40	1:40
T24	1:20-40	1:80	-[b]	-
J82	1:40-80	1:80-160	1:40	1:80
Other carcinoma				
SK-Mes-1	1:80[c]	1:160[c]	1:40	1:40[c]
ChaGo	-	-	-	1:10
MAC21	-	-	-	-
HeLa	-	-	-	-
HT29M	-	-	-	-
SCaBER	-	-	-	-
Neuroblastomas				
SKNSH	1:160-320	≥1:320	1:40-80	1:160
CHP100	1:160-320	>1:320	1:80-160	1:160
SKNMC	-	-	1:20-40	1:80
LAN-1	1:10-20	1:20-40	-	-
TE671	1:60	≥1:320	1:160	1:320
Sarcoma				
MT	1:80	1:60	1:20-40	1:80
"Normal"				
FL	-	-	-	-

[a] Means that antisera from rabbit C40, against the 253J cell line was obtained on day 21 after the initial immunization

[b] Indicates no detectable cytotoxicity at a dilution of 1:10

[c] Had prozones with dilutions of 1:10-20

Takasugi, UCLA Department of Surgery). Percent cytotoxicity was calculated (5 or 6 replicates were used in each case):

$$\frac{\#\text{ cells w/NRS} - \#\text{ cells w/C41}}{\#\text{ cells w/NRS}} \times 100$$

The difference in % cytotoxicity caused be the added inhibitor was calculated:

$$\left(\frac{\#\text{ cells in well w/NRS} - \#\text{ cells w/C41}}{\#\text{ cells w/NRS}} - \frac{\#\text{ cells w/(NRS+I)} - \#\text{ cells w/(C41+I)}}{\#\text{ cells w/NRS+I}}\right) \times 100$$

Absorptions

Antisera were absorbed undiluted or diluted 1:2 with balanced salt solution (BSS). The sera were used this concentrated in order to avoid overlooking antibodies which could be in very low concentration. Sera were incubated with absorbent (tissue culture cells or homogenized tissue as indicated) at 4°C for 1 h, and recovered by centrifugation. Sera were first absorbed (1/1:v/v) with a mixture of peripheral blood cells (PBC) including red and white cells obtained from at least 3 donors of different blood groups (A, B, O). The second and third absorptions (serum/cells = 2/1: v/v and 5/1:v/v respectively) were then pooled homogenates of lung, liver, and kidney, obtained at autopsy (Luliki). At least 3 donors were used for each tissue. Additional sequential absorptions with Luliki were of no further benefit (unpublished observations). Further absorptions will be described along with the results.

Staphylococcal-Protein-A (SpA) Assay

This assay was performed according to Zeltzer and Seeger (31). The SpA assay is dependent upon

Table 3. Specificity of anti-253J antisera after repeated sequential absorptions[a]

Absorbed antiserum			Target cell					
Rabbit	Day	Antigen	T24	J82	253J	FL	SK-Mes-1	HT29M
C40	21	253J	-[b]	-	1:40-80	-	-	-
C41	33	253J	-	-	1:320	-	-	-
C45	33	T24	-	-	-	-	-	-
C10		HT144, SK-Mes-1	-	-	-	-	-	-

[a]Sera were diluted 1:2 and absorbed sequentially with ① PBC, ② and ③ Luliki x2, ④ FHMS (Fl:Ht29M:MAC21:SK-Mes-1 = 2.6:0.5:0.6:0.3, relative volumes, absorbed 10:1 = serum:cells = v/v), ⑤ FHS (Fl: HT29M:SK-Mes-1 = 1.0:0.5:0.5, relative volumes, absorbed 4:1 = serum:cells = v/v), ⑥ SK-Mes-1 (1.2:0.5 = serum:cells = v/v)

[b]A(-) indicates <1:10, or no detectable activity at 1:10 dilution

Table 4. Percent activity remaining by SpA assay in sera absorbed (undiluted) with PBC and Luliki x1

Antiserum	Antigen	Day	Dilution	Cell lines			
				T24	253J	647V	FL
C40	253J	33	1:100	52.1	57.5	53.8	35.1
C41	253J	33	1:100	51.7	70.1	46.9	49.0
			1:200	45.6	60.1	47.8	41.6
C42	647V	33	1:100	40.2	42.9	56.6	38.6
			1:200	40.2	39.9	49.1	37.5
C44	T24	33	1:200	43.4	39.8	37.8	28.6

the adherence of radio-iodinated-SpA to the Fc portion of bound IgG molecules. Results were expressed as percent activity remaining following absorption:

$$\frac{\text{SpA counts/5 mg cell protein for absorbed serum}}{\text{SpA counts/5 mg cell protein for unabsorbed serum}} \times 100$$

Patients' Sera

Sera from cancer patients and normal individuals were obtained from the UCLA Immunobiology Group Serum Bank. All sera were stored at -70°C.

Tissue Specimens

Prostatic tissue specimens were obtained from Dr. Theodore I. Malinin, University of Miami, through the National Prostatic Cancer Project. All other specimens were obtained from UCLA Surgical Pathology with the exception of one normal bladder, normal lungs, livers, and kidneys which were obtained at autopsy. (Bladder specimens were supplied by Dr. J. Waisman, Department of Pathology, UCLA). Tissues were either used immediately or stored at -70°C.

RESULTS

Cytotoxicity of Absorbed Antisera

All serum samples from all rabbits were cytotoxic to all cell lines when tested unabsorbed. Sera were diluted 1:2, absorbed with PBC, and twice with normal, pooled lung, liver and kidney homogenates (Luliki), as discussed in Materials and Methods. They were once more absorbed with glutaraldehyde fixed fetal calf serum (1:2 = FCS: antiserum = v/v). Table 2 shows cytotoxic titres after absorption. At this stage all sera were cytotoxic to most of the non-carcinoma but not carcinoma-derived cell lines (i. e., only to the transitional cell and lung carcinomas). Of the 3 anti TCC-CL sera, only C40 and C41 (both anti 253J) showed high (though not yet specific) activity against TCC-CL. In C45 (anti T24) all activity

Table 5. Blocking by supernates from cell lines of cytotoxicity by absorbed C41[a] against 253J

Supernatant source[b]	% cytotoxicity with supn't. - % without supn't. Dilution of C41	
	1:50[c]	1:100[c]
253J	10.8	27.3
J82	17.7	27.3
T24	-2.8	11.6
HT29M	-1.2	0.8
HeLa	5.1	9.8
SK-Mes-1	-0.5	21.4
FL	-0.5	18.9

[a] Absorbed with PBC, Luliki x2, FHMS, FHS, SK-Mes-1

[b] Cultures were not fed for 10 days before collection of supernate

[c] Experiments with 1:50 and 1:100 dilution of C41 were done on different days

Table 6. Absorption of preabsorbed[a] C40 day 21 (anti 253J) with transitional cell carcinoma cell lines

Antiserum	J82	Test on[b] 253J	SK-Mes-1
Preabsorbed	1:20-40	1:80	1:20-40
+ T24	1:10	1:40	-[c]
+ J82	-	1:20-40	-
+ 253J	-	-	-
+ 647V	-	1:20-40	-

[a] Serum was preabsorbed with PBC, Luliki x2, and FHMS - see footnote to Table 3 for details

[b] Preabsorbed antisera were also tested against FL and HT29M, but found to be negative. The titre against T24 was too low to interpret reduction by absorption

[c] (-) indicates a titre of <1:10

against the immunogen was removed by the non-specific absorption procedure. The anti-lung tumour cell line serum (C10) retained activity against 2 TCC-CL and 2 lung tumour lines, but this activity was lower than C40 and C41 against 2 of the 3 TCC-CL.

Selective Activity for Line of Immunization

Table 3 shows results of sera tested after further absorptions; twice with pooled non TCC cell lines and once with SK-Mes-1. SK-Mes-1 was the last cell line retaining activity in addition to TCC-CL prior to this last absorption (data not shown). The resultant antisera against 253J (C40 day 21 and C41 day 33) retained activity only against 253J with C41 having the greater titre. C45 and C10 were void of any detectable activity.

Corroborating results were obtained using the SpA assay. These data are shown in Table 4. In these experiments, 4 anti TCC-CL sera were absorbed undiluted, with PBC and then with Luliki. With each antiserum, the percent activity remaining was greatest against the immunogen. Both antisera against 253J and the one against 647V (647V cells were unusable for cytotoxicity assays due to complement sensitivity) showed this trend; the phenomenon was much less pronounced with the antiserum to T24.

Inhibition of Cytotoxicity of C42 Day 33 (anti 253J) by Tissue Culture Supernatants

Culture supernatant was collected and centrifuged after 10 days of not refeeding confluent cultures. The effect of supernatants on cytotoxicity of C41 (day 33) absorbed to be selective against 253J (see Table 3) is shown in Table 6. C41 was chosen because of its high titre. Supernates from cultures of 253J and J82 showed the greatest inhibition in 2 separate experiments.

Absorption of C41 Day 21 (anti 253J) with Cell Lines

C40 (anti 253J) was diluted 1:2 and pre-absorbed with PBC, twice with Luliki, and FHMS (non TCC-CL) as described in Table 3. Absorption was complete against FL and HT29M, but not against SK-Mes-1. The antiserum was then absorbed with each of the 4 TCC-CL. Absorption is considered significant only with a $\geq$ 2 doubling dilution reduction in titre. Results are shown in Table 6. J82, 253J, and 647V removed activity against J82; these TCC-CL plus T24 removed activity against the non TCC-CL, SK-Mes-1, and only 253J removed activity against 253J.

Absorption with Tumour Specimens and Normal Tissues

Sera were preabsorbed with PBC (1:1 = serum: cells = v/v) twice with Luliki (2:1 = serum:cells = v/v, and 5:1 = serum:cells = v/v respectively) and then with a mixture of cells from culture including FL, HT29M, MAC21 and SK-Mes-1 (10:1 = serum:cells = v/v, abbreviated FHMS). Absorption was complete against Fl, HT29M, and MAC21 but not SK-Mes-1. Sera were then absorbed (2:1 - cells:serum - v/v) with homogenates of the tissues listed in Table 7. The results of absorption of C40 (anti-253J) with tissue homogenates are shown in Table 8. The results were similar to and consistent with results with C41

(anti-253J) except that with C41 the titres against 253J were much greater, and therefore differences after absorption were less pronounced. A reduction in titre of two doubling dilutions is considered significant. Absorption of activity against J82 and 253J occurred only with two of the four TCC bladder tumour specimens and not with any of the other tissues. A similar result was also seen, with the additional absorption by an epidermoid bladder tumour (#9), in tests against SK-Mes-1, the epidermoid lung tumour-derived cell line.

Using the C10 antiserum (against lung tumour cell lines), activity against J82 was removed by specimens 4-13 including all TCC, benign bladder disease, epidermoid bladder carcinoma, and normal prostate and prostate with benign disease. Activity against T24 was significantly reduced only by TCC specimens 6 and 7, and against 253J by 6 and 7 and by the epidermoid carcinoma of the bladder, specimen 9, and against SK-Mes-1 only by TCC #6. However, in the case of SK-Mes-1, the initial titre was low (1:20) such that a reduction to <1:10 may not be a significant absorption. The results with the C10 antiserum are summarised in Table 9.

Inhibition of Cytotoxicity with Human Sera

In order to test the possibility that the C41 day 33 (anti-253J) antiserum absorbed to the point of reacting only with 253J detected an antigen relevant to human bladder cancer, patient and control sera were used to test for inhibition of the cytotoxic reaction. Human sera were diluted 1:2 in medium and 5 µl were mixed in the microtest wells (containing 253J cells) with 5 µl of C41 diluted to effect between 60-90% cytotoxicity. Table 10 gives results of two experiments in which sera from patients with bladder cancer, other cancers and normal individuals were tested for inhibition. In experiment 1 the bladder cancer patients were late stages and in two they were early. Also, in experiment 2 the sera from other cancer patients were collected later than the sera in experiment 1. The spread within each group is great and the difference between groups is not significant. However, in experiment 2 there is a trend for the bladder cancer patients' sera to inhibit more than the other sera.

Table 7. Tissues used for absorption

Number	Tissue
1-3	Normal bladder mucosa
4-7	Transitional cell carcinoma of the bladder
8	Epithelial atypia, cystitis, no tumour, pt. Rad TCC
9	Epidermoid carcinoma of the bladder
10-11	Normal prostate
12-13	Benign prostatic hypertrophy
14	Prostatic carcinoma
15	Breast carcinoma

Table 8. Absorption of Preabsorbed[a] C40 (anti-253J) with Fresh Tissue Specimens

	Test on:[b]		
Serum	J82	253	SK-Mes-1
Preabsorbed	1:20-40	1:80	1:20-40
+ 1[c]	1:10	1:40	1:10
+ 2	1:40	1:80	1:20
+ 3	1:40	1:80	1:20
+ 4	1:10	1:80	1:10-20
+ 5	1:20	1:40-80	1:10
+ 6	-[d]	1:10-20	-
+ 7	-	-	-
+ 8	1:20-40	1:40-80	1:10
+ 9	1:10-20	1:40-80	-
+ 10	1:20-40	1:40-80	1:20
+ 11	1:20	1:80	1:20
+ 12	1:10-20	1:40	1:10-20
+ 13	1:10-20	1:40	1:10-20
+ 14	1:10	1:40-80	1:10-20
+ 15	1:10-20	1:40-80	1:10-20

[a] Preabsorbed with PBC, Luliki x2, and FHMS, a mixture of non-TCC-CL

[b] Preabsorbed sera were also tested against FL and HT29M and found negative, and against T24, against which the titre was too low to be useful for reduction by absorption

[c] See Table 7 for identification of specimens

[d] A (-) indicates no detectable activity at a dilution of 1:10

DISCUSSION

In an earlier paper we reported that a rabbit antiserum against TCC-CL (T24) could be absorbed and shown to be functionally specific for T24 (4). The present report presents data obtained using antisera against T24 and also against other TCC-CL's which amplify and complicate the implications of the original finding. Results in Table 2 show that the inclusion of more target cells is essential: if SK-Mes-1 cells and the non-carcinoma cells were not tested, C40 and C41 would have appeared specific for the TCC-CL's.

In this study it was shown that TCC-CL's can be distinguished from each other using xenogeneic antisera. This is clear with the antisera to 253J (Table 3) which after limiting absorptions react

only with 253J, implying distinctive antigen(s) on that cell line. The results with the SpA assay (Table 4) confirm this and extend it to possible distinctions between other TCC-CL's. However, more target cells and absorption data would be needed with the Sp A assay to confirm the conclusion of distinctive antigens on 647V. The identity(s) of the distinctive antigen(s) on 253 J are quite unclear. They could reflect a qualitative or quantitative difference. They could be alloantigens (e.g., HL-A-although many donors were used for absorptions in order to minimize this – or tissue-associated antigens), fetal antigens, or tumour-associated antigens (TAA). The last possibility is most intriguing due to the probable connection of bladder cancer in humans with carcinogens (19) and the individual TAA-associated with chemically-induced tumours in animals. Unfortunately, it is not easily testable due to the unavailability of the patient.

The results shown with inhibition by culture supernatants of cytotoxicity by C41 day 33 (Table 5) indicate a sharing of some antigen(s) between 253J and J82. Similarities and differences between TCC-CL are also shown by the absorption of the C40 day 21 (anti-253J) antiserum. With this antiserum, J82, 253J, and 647V removed activity against J82, all four TCC-CL including T24 removed activity against the non-TCC-CL, SK-Mes-1; and only 253J removed against 253J. However, absorption with a mixture of non-TCC-CL followed by SK-Mes-1, also removed activity against all lines except 253J (Table 3). This implies a possible sharing of antigens between the four TCC-CL and SK-Mes-1 having quantitatively or qualitatively least or fewest, then T24, then J82 and 647V, and finally 253J with the greatest reactivity. Our earlier paper indicated that T24 and J82 share some specificity(s). The difference

Table 9. Absorption of Preabsorbed C10 (anti-HT144 and SK-Mes-1)

	Target Cells[a]			
	T24	J82	253J	SK-Mes-1
Preabsorbed serum titre	1:20-40	1:20-40	1:40	1:20[b]
Reduction of two doubling dilutions by:	6-7	4-13	6-7, 9	6[b]

[a] FL and HT29M were used but were negative with preabsorbed serum

[b] This reduction may not be significant, since the original titre of 1:20 does not allow for a reduction of $\geq$ 2 doubling dilutions in titre under experimental conditions

Table 10. Inhibition of Cytotoxicity of a 1:100 dilution of absorbed C41 by human sera

Exp.	Patients	Difference in % killing due to human sera	Mean ± SD	Significance[a]
1	Bladder cancer (late stage)	45.6 28.3 35.8	36.6 ± 8.7	p >.40 p'<.571
	Other cancers	67.6 53.9 16.8 -1.6	34.2 ± 32.1	
2	Bladder cancer (early stage)	35.5 106.2 71.5 73.7	71.7 ± 28.9	p <.20 p'<.114
	Other cancers (early stage)	19.7 58.5 66.8	48.3 ± 25.1	p <.10 p'<.114
	Normals	49.6 18.2 53.2	40.3 ± 19.2	p <.40 p'<.350

[a] By student's t test = p; by rank sum = p'

in findings probably reflects the different antisera. Clearly the earlier, stronger anti-T24 serum was different from C45 used here. It is extremely unlikely that all these antigens are TAA, especially since a lung carcinoma cell line is involved. However, it is nevertheless possible that shared (among TCC as well as between bladder and other tumours) and unique TAA do exist and may be found in human bladder cancer.

The occurrence of antigens defined by rabbit antisera to human TCC-CL's on tissue specimens of TCC is apparent (Table 8), and in agreement with previous findings with a rabbit serum against T24 (4), but the significance is unclear. Two of four specimens of TCC absorbed activity against TCC-CL (253J and J82) in the C40 (anti-253J) and C41 (anti-253J) antisera. No other "fresh" tissues, including three normal bladder specimens, were effective. The fact that two TCC specimens also significantly decreased activity in C40 and C41 against SK-Mes-1 (epidermoid lung carcinoma) is not surprising since the antisera were against TCC-CL but not found to be specific for TCC-CL at the stage of pre-absorption used. Furthermore, it is also not surprising that the TCC specimens also removed activity against TCC-CL from an (not specific) antiserum against lung carcinoma cell lines (C10). However, the removal of the anti-SK-Mes-1 activity from the C10 antiserum by the # 6 TCC specimen is more consistent with non-specific absorption. On the basis of this absorption information, it is likely that at least specimen #7 shares an antigen(s) with the TCC-CL, but the relevance to in vivo desease is not conclusive. This is especially true since activity in C40 and C41 against all targets except 253J can be removed by absorption with a sequence of non-TCC-CL and SK-Mes-1.

The reduction in titre of C4O against SK-Mes-1 (squamous cell carcinoma of the lung) by the one squamous cell carcinoma of the bladder (#9) is consistent with Sofen and O'Toole's finding of shared antigens between squamous cell carcinomas of different histologic sources (29).

The results obtained with inhibition of cytotoxicity experiments add another dimension of complexity. The experiments were done using the C41 antiserum which, after appropriate absorption, reacted only with 253J and not with T24, J82 or non-TCC-CL, presumably detecting a more restricted antigen, or one in greater quantity on 253J. Table 10 shows that sera from bladder cancer patients do not significantly inhibit cytotoxicity more than sera from patients with other cancers or normal individuals. Nevertheless, there is a trend in which the early stage TCC patients' sera inhibit more than later stage and other groups of donors. If the phenomenon is real, then the 253J antigen could be extremely important in the course of disease. Blocking in such a situation could be due to antigen-antibody complexes, rather than only antigen which should be found in greater concentration in later stage patients' sera. Blocking by immune complexes could result from specific inhibition or from an anti-complementary effect. However the latter possibility is less likely since sera from early stage patients with other cancers did not inhibit cytotoxicity.

Although not conclusive, these data therefore suggest the presence of antigens detected by rabbit antisera on human TCC-CL. The possible relationship of these antigens to the tumours in vivo and to the clinical course remains difficult to define.

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