

Complement-Fixation Reaction of Human Neoplastic Tissue with Sera From Hamsters Bearing Virus-Induced Tumors.* (32138)

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Neoplastic transformation of animal and human cells by oncogenic viruses is ordinarily accompanied by the appearance of new cellular antigens which are detectable by various procedures(1). One of these is the complement-fixing (CF) antigen which is usually referred to as T antigen but also as ICFA or neoantigen. Such antigen has been demonstrated in adenovirus, SV₄₀, polyoma, and Rous sarcoma virus-induced tumors(1-6). The CF antigen, except for cross-reactions within the adenovirus and avian leukosis groups, is specific to virus and not to host cell species from which it was derived. The CF antigen appears to be synthesized under the influence of viral genetic material but is not present in the intact virus particle except for the reported presence of C antigen in adenovirus type 12 tumors(7). The fact that the new antigen persists in the neoplastic cell on serial passage *in vitro* and *in vivo* is taken as strong evidence to support the concept of continued persistence in the tumor cells of at least a portion of the genetic material from the virus which caused the malignant transformation initially. If this be true, then the demonstration of CF antigen might be of value in defining the etiology in tumors of unknown origin, such as in man.

Investigations during the past several years in our laboratories have sought to demonstrate the etiology of human cancer by direct isolation of virus *in vitro* or *in vivo*(8-11) and by attempted induction of virus from human or animal tumor using various chemical, physical, or biological procedures(12,13). The findings were negative and the quest for virus in human cancer has since been extended to explore the possible usefulness of tests for specific tumor T antigens in human neoplastic tissues. A small portion of the tissue speci-

mens from cases of human neoplasia gave positive reactions in tests with sera from hamsters bearing adenovirus 7 or 12 tumor but not with adenovirus 18, SV₄₀, or Rous sarcoma tumor. However, tissue specimens from non-neoplastic disease of man also gave positive reactions and, in addition, there were apparent isoantibody-antigen reactions between the human and hamster materials. The findings are given here and their significance is discussed.

Materials and methods. Human specimens. The neoplastic and normal human tissues and sera were provided by the Human Tissue Procurement Program, National Cancer Institute, Bethesda, Md. Each tissue was accompanied by a histologic diagnosis and a brief clinical history of the patient from whom it was derived. The tissues were stored at -60°C to -70°C from the time of collection until processing and the sera were stored at -70°C or at -20°C . For complement-fixation (CF) testing, soft tissues were ground with alundum in a mortar to give a 20% homogenate (w/v) in Mayer's veronal buffer solution. Bone specimens were homogenized for 5 minutes at 16,000 rpm in an Omnimix cup held in an ice bath. The suspensions were clarified by centrifuging for 20 minutes at 1,500 rpm in the horizontal head in the cold and the supernatant fluid was stored at -60°C until tested. The initial 20% tissue extract is referred to as undiluted.

Hamster tumors and antisera. Random-bred Golden Syrian hamsters obtained from Lakeview Hamster Colony, Newfield, N. J., were used throughout the studies. Virus-free adenovirus 7, 12 and 18 and SV₄₀ tumor transplant or cell culture lines were established in this laboratory(3,14) and the Rous sarcoma hamster tumor lines were obtained from Drs. H. Koprowski and R. J. Huebner. Fortner's FSA3B tumor was obtained from Dr. J. J. Trentin in forty-fifth passage and was passed

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TABLE I. Source of Hamster Tumor Antisera.

Initial virus			Hamster tumor antiserum	
Kind	Strain	Pool	No. of sera in pool	Virus or tumor used to prepare antiserum
Adenovirus 7	Pinckney	A	18	Hamster transplant line C1, pass. 12
" "	" "	B	1	Virus-induced primary tumor
Adenovirus 12	Huie	A	10	Hamster transplant line A1, pass. 4, 7 and 9
" "	" "	B	4	Virus-induced primary tumor
Adenovirus 18	14606 (Chanock)	A	16	Tissue culture line A2, pass. 23-41
SV ₄₀	VA 45-54	A	25	Virus-induced primary tumor
Rous sarcoma	Schmidt-Ruppin	A	16	Hamster transplant line A1, pass. 16-18
—	—	A	14	Normal control serum

once in newborn hamsters. The hamster tumors were prepared for CF testing as described above for human soft tissues. The tumor antisera were prepared in hamsters by inoculating oncogenic virus or tumor cells which were maintained by serial transplantation in the same line of hamsters or by cell culture. The histories relating to the preparation of the various antisera are shown in Table I. The sera were collected from hamsters 10 to 134 days following detection of primary viral tumor or 41 to 133 days following tumor transplant. A portion of the tumor was removed surgically from hamsters bearing adenovirus 7 and 12 transplant tumor in order to prolong life and achieve high antibody titers. The adenovirus 18 tumor antisera were collected from hamsters bearing tumors following repeat inoculation with increasing numbers of adenovirus 18 tumor cells propagated in cell culture. *Complement-fixation test.* Prior to test, the antisera were inactivated at 56°C for 30 minutes and the thawed tissue antigens were reclarified by centrifuging for 20 minutes at 1,500 rpm. The micro-CF test was performed as described by Sever(15) using 2 exact units of guinea pig complement. The CF titer was the highest initial dilution of tissue suspension which gave 3+ or 4+ fixation (4+ equals no hemolysis). Hamster tumor antigens used to assay the hamster antisera and to test the patients' sera were diluted to contain 4 to 16 antigen units per test dose; F5a3B tumor antigen was used undiluted and at a dilution of 1:2. The hamster antisera used to test the human tissue preparations and the hamster tumor antigens were diluted 1:20 and contained 8 to 16 antibody units per test dose based on assays with

homologous antigen. *Characterization of CF antigens.* The human and hamster tumor extracts were diluted 1:2 and 1:4, respectively, and were reclarified by centrifuging at 1500 rpm for 20 minutes. The supernatant fluids were tested for CF activity without further treatment, following high speed centrifugation, or after treatment with ether or heat. High speed centrifugation was for 2 hours at 30,000 rpm in a number 40 Spinco rotor and the upper half of the supernate was collected for testing. Heating was at 56°C for 30 minutes in a water bath. Ether treatment was for 2 hours at 4°C employing a 20% concentration of peroxide-free ether and the aqueous fraction was collected for assay.

Results. Preliminary screening of human tissue extracts. As shown in Table II, a total of 123 tissues from 111 cases of human neoplastic disease of diverse type were examined for presence of CF antigen employing hamster adenovirus 7, 12, and 18, SV₄₀ and Rous sarcoma tumor antisera. Four of the tissues were from organs with normal morphology. Additionally, 22 tissue specimens were collected from 20 patients without signs of neoplastic disease. The pathologic diagnoses and the findings in tests with hamster antisera are presented in the Table. Ten neoplastic tissues, 3 morphologically normal tissues from cases of cancer, and 4 tissues from cases of non-neoplastic disease gave positive tests with one or more hamster tumor antisera and, in 6 instances, with serum from normal hamsters as well (Table III). The positive test results are dealt with more fully in the subsequent sections.

Human tissue extracts which gave positive

CF results. The CF antigen titers of the 13 tissue specimens from 12 patients with neoplastic disease and of the 4 tissues from 3 patients with non-neoplastic disease which gave positive results are shown in Table III.

All positive reactions were confined to adenovirus 7 or 12 tumor antisera or to normal serum except for the reaction of morphologically normal liver from patient no. 130 with adenovirus 18 tumor antiserum. Two neoplastic tissue specimens (patient no. 120 and 130), 2 normal appearing tissues from patients with neoplastic disease (no. 130 and 11) and 2 tissues from patients with non-neo-

plastic disease gave positive CF results with the normal hamster serum pool. Three normal-appearing tissues from cases of neoplastic disease (no. 130, 11 and 117) reacted with adenovirus 7 and 12 tumor antisera and 4 tissues from patients with non-neoplastic disease also gave positive reactions with either or both of these antisera. The morphologically normal liver and lung tissues of patients 11 and 117 gave positive results even though neoplastic tissues from the same individuals were negative. The CF test results with neoplastic tissues were positive about twice as often with adenovirus 12 tumor antiserum as with

TABLE II. Summary of Complement-Fixation Tests of Human Neoplastic and Non-Neoplastic Tissue Extracts with Sera from Tumor-Bearing Hamsters.

Tissue origin	Neoplastic tissue Pathologic diagnosis	No. cases	Findings in CF tests			
			Neoplastic tissue		"Normal" tissue*	
			No. tested	No. positive†	No. tested	No. positive†
<i>Neoplastic disease</i>						
Connective	Liposarcoma	4	5	1	2	1
	Sarcoma, unclassified	1	1	0	1	1
Endothelial	Leukemia					
	Myelocytic					
	Acute	4	5	0	0	0
	Chronic	4	4	0	0	0
	Lymphocytic					
	Acute	7	8	0	0	0
	Chronic	1	1	0	0	0
	Erythroleukemia	3	3	1	0	0
	Lymphosarcoma	5	5	2	0	0
	Multiple myeloma	2	3	0	0	0
	Reticulum cell sarcoma	6	9	2	1	1
	Hodgkin's disease	11	12	2	0	0
Muscle	Rhabdomyosarcoma	3	3	0	0	0
Epithelial	Carcinoma	51	51	1	0	0
Miscellaneous	Melanoma	6	6	1	0	0
	Wilm's tumor	2	2	0	0	0
	Mesodermal tumor	1	1	0	0	0
	TOTALS:	111	119	10(8.4%)	4	3
<i>Non-neoplastic disease</i>						
Normal liver or spleen	Fatty degeneration	3	—	—	3	0
	Infarct	1	—	—	1	0
	Congestion	3	—	—	3	0
	Extramedullary hemato- poiesis, normal morph.	1	—	—	2	2
	Central necrosis	1	—	—	1	0
	Hemosiderin deposition	1	—	—	1	0
	Normal morphology	10	—	—	11	2
	TOTALS:	20			22	4(18%)

* Normal appearing tissue from patient with neoplastic disease.

† Positive reaction at 1:2 or greater dilution in tests with one or more antitumor serum.

TABLE III. Complement-Fixation Titers of Human Neoplastic or Normal Tissue Extracts Which Gave Positive Results (See Table II) with One or More Hamster Serum Pools

Patient no.	Blood type	Tissue	Tissue tested	CF antigen titer of tissue extract in tests with hamster sera									
				Adeno 7 Pool A 1:20*	Adeno 12 Pool A 1:20	Adeno 18 Pool A 1:20	SV ₄₀ Pool A 1:20	Rous sarcoma Pool A 1:20	Normal Pool A 1:20				
<i>Neoplastic disease</i>													
130	B+	Liposarcoma	Primary tumor, abdomen Liver, normal morph.	0 4	2 8	0 2	0 0	0 0	0 0	0 0	2 4	0 0	0 0
11	B+	Sarcoma, unclass'd.	Liver, normal morph. Metastatic tumor, pleura	4-8 0	8-16 0	0 0	0 0	0 0	0 0	0 0	4-8 0	0 0	0 0
33	O+	Erythroleukemia	Spleen	0	8-16	0	0	0	0	0	0	0	0
122	A+	Lymphosarcoma	Primary tumor, spleen	0	16	0	0	0	0	0	0	0	0
126	O+	"	"	0	16	0	0	0	0	0	0	0	0
124	A+	Reticulum cell sarcoma	"	4	8	0	0	0	0	0	0	0	0
120	A+	"	"	8	8	0	0	0	0	0	4	0	0
117	A+	"	"	0	0	0	0	0	0	0	0	0	0
15	O+	Hodgkin's disease	Lung, normal morph. Spleen Lymph node	8 0 0	8 16 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
128	O-	"	Spleen	0	32	0	0	0	0	0	0	0	0
64	Unknown	Carcinoma	Metastatic tumor, liver	4	16	0	0	0	0	0	0	0	0
40	A+	Melanoma	"	8	8-16	0	0	0	0	0	0	0	0
<i>Non-neoplastic controls</i>													
147	A-	Non-malignant	Liver, normal morph. Spleen, extramedullary hematopoiesis	8 2	8 2	0 0	0 0	0 0	0 0	0 0	4 0	0 0	0 0
161	A+	"	Liver, normal morph.	0	2	0	0	0	0	0	0	0	0
115	A	"	"	4	8	0	0	0	0	0	0	0	2

* Dilution of serum pool used.

TABLE IV. Complement-fixation Test Results Summarized According to Kind of Tissue

Disease	Tissue tested Kind	Results	
		No. positive total tested	% Positive
Neoplastic	Liver*	4/32	12.5
	Spleen	7/33	21.2
	Other†	2/58	3.4
Non-neoplastic	Liver	3/15	20.0
	Spleen	1/7	14.3

* Includes 2 liver specimens which were morphologically normal.

† Includes tumor tissue of abdomen, peritoneum, lung, bone, kidney, lymph node, muscle, soft tissue, head and neck, breast, ovary and mediastinal. Two of these tissues were morphologically normal.

type 7 tumor antiserum and the antigen titers were usually greater when the former serum was employed. The tests with each tissue specimen shown in the Table were repeated 2 to 6 times and the findings were consistent with those which are presented.

Table IV shows the analysis of the CF test results according to whether the tissues were of neoplastic or non-neoplastic origin and according to kind of tissue. It is seen that 15 of 87 liver and spleen samples tested gave positive results (17.3%) contrasted with only 2 of 58 positive tests (3.4%) of tissues other than liver or spleen. The percentages of liver and spleen tissues which were positive were approximately the same, whether of

TABLE V. Representative Box Complement-Fixation Tests of Human Neoplastic and Normal Tissue Extracts with Sera from Tumor-Bearing Hamsters.

Patient no.	Pathologic diagnosis	Tissue	Dilution of tissue suspension	Degree of fixation* observed in tests of tissues with hamster sera																							
				Adeno 7						Adeno 12						Adeno 18		SV ₄₀	Rous sarcoma	Normal sera							
				Pool A		Pool B		Pool A		Pool B		Pool A	Pool B	Pool A	Pool A	Pool A	Pool A										
				20	40	80	160	10	20	40	80	160	20	40	80	160	20	20	20	20							
11	Sarcoma, unclassified	Liver, normal morphology	2	3	3	3	0	4	4	4	3	-	-	4	4	3	0	4	4	-	-	-	0	0	0	4	4
			4	4	4	4	3	4	4	-	-	-	-	4	4	3	1	4	4	-	-	-	0	0	0	4	3
			8	2	4	4	1	0	1	-	-	-	-	4	4	1	0	3	2	-	-	-	0	0	0	3	0
			16	0	0	0	0	0	0	0	0	-	-	3	0	0	0	0	0	-	-	-	0	0	0	0	0
		32	0	0	0	0	0	0	-	-	-	-	0	0	0	0	0	0	-	-	-	0	0	0	0	0	
		Metastatic tumor, pleura	2	0	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	0	0	0	0	-
			4	1	0	0	0	1	1	-	-	-	-	4	0	0	0	1	0	-	-	-	0	0	0	0	0
			8	1	0	0	0	0	0	-	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0
16	2		2	0	0	0	0	-	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0		
32	1	1	0	0	0	0	-	-	-	-	2	0	0	0	0	0	-	-	-	0	0	0	0	0			
33	Erythroleukemia	Spleen	2	1	0	0	0	1	1	-	-	-	4	0	0	0	1	0	-	-	-	0	0	0	0	0	
			4	1	0	0	0	0	0	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0	
			8	1	0	0	0	0	0	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0	
			16	2	2	0	0	0	0	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0	
		32	1	1	0	0	0	0	-	-	-	2	0	0	0	0	0	-	-	-	0	0	0	0	0		
		Lymph node	2	1	0	0	0	1	1	-	-	-	3	0	0	0	1	0	-	-	-	0	0	0	0	0	
			4	1	1	0	0	0	0	-	-	-	4	1	0	0	2	0	-	-	-	0	0	0	1	0	
			8	0	0	0	0	0	0	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0	
16	0		0	0	0	0	0	-	-	-	4	0	0	0	0	0	-	-	-	0	0	0	0	0			
32	0	0	0	0	0	0	-	-	-	0	0	0	0	0	0	-	-	-	0	0	0	0	0				
64	Carcinoma, poorly differentiated	Metastatic tumor, liver	2	0	0	0	0	0	0	-	-	-	1	0	0	0	0	0	-	-	-	0	0	0	0	0	
			4	3	1	0	0	3	0	-	-	-	3	0	0	0	3	1	-	-	-	0	0	0	1	1	
			8	0	0	0	0	0	0	-	-	-	4	1	0	0	3	1	-	-	-	0	0	0	0	0	
			16	0	0	0	0	0	0	-	-	-	4	0	0	0	2	0	-	-	-	0	0	0	0	0	
32	0	0	0	0	0	0	-	-	-	2	0	0	0	0	0	-	-	-	0	0	0	0	0				
40	Melanoma	Metastatic tumor, liver	2	4	4	2	1	0	0	-	-	-	4	3	0	0	0	0	-	-	-	0	0	0	0	0	
			4	3	4	0	0	0	0	-	-	-	4	3	0	0	0	0	-	-	-	0	0	0	0	0	
			8	4	4	4	0	0	0	-	-	-	4	4	0	0	0	0	-	-	-	0	0	0	0	0	
			16	0	0	0	0	0	0	-	-	-	0	0	0	0	0	0	-	-	-	0	0	0	0	0	
Control tests of hamster tumor antigens and sera																											
Line C1	Undifferentiated sarcoma	Adenovirus 7, hamster tumor	2	4	4	4	4	4	4	4	3	0	2	1	0	0	1	0	0	0	0	0	0	0	0	0	
			4	4	4	4	4	4	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0		
			8	4	4	4	4	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
			16	4	4	4	4	4	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
32	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						
Line A1	Undifferentiated sarcoma	Adenovirus 12, hamster tumor	2	2	2	1	0	0	0	0	0	0	4	4	4	4	4	4	4	4	4	4	0	0	0	0	
			4	2	1	0	0	0	0	0	0	4	4	4	4	4	4	4	4	4	4	0	0	0	0		
			8	0	0	0	0	0	0	0	0	4	4	4	3	4	4	4	4	4	4	0	0	0	0		
			16	0	0	0	0	0	0	0	0	4	4	4	3	4	4	4	4	4	4	0	0	0	0		
32	0	0	0	0	0	0	0	0	4	4	3	0	4	4	4	4	3	4	0	0	0	0					
Line B1	Undifferentiated sarcoma	Adenovirus 18, hamster tumor	8	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4	4	4	4	0	0	0	0	
Line P5-1	Fibrosarcoma	SV ₄₀ hamster tumor	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	
Line B1	Sarcoma	Rous sarcoma hamster tumor	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	
----	Normal	Hamster muscle	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

* Degree of fixation of complement, i.e., absence of hemolysis on 0-4+ scale.

† Reciprocal of serum dilution.

‡ - equals not done.

TABLE VI. Box Complement-fixation Titrations of Hamster Serum Pools with FSa3B Tumor Antigen.

Tumor antiserum Kind	Pool	Serum dilution (reciprocal)	Degree of fixation Dilution of FSa3B tumor antigen	
			1:1	1:2
Adenovirus 7	A	20	2	0
" "	B	20	4	3
		40	3	3
		80	2	2
Adenovirus 12	A	20	3	2
" "	B	20	3	3
		40	2	2
		80	0	0
Adenovirus 18	A	20	2	0
SV ₁₀	A	20	0	0
Rous sarcoma	A	20	0	0
Normal hamster serum	A	20	0	0

neoplastic (16.9%) or non-neoplastic (18.2%) origin.

Box titrations were carried out with tissue specimens from 5 of the patients with neoplastic disease employing all 8 hamster serum pools shown in Table I and the findings are given in Table V. Strong reactions were noted with these sera in tests of hamster tumor antigens, while weak reactions and prozone effects were commonly seen with human tissue extracts. Except for the liver specimen from patient 11, the tissues gave weaker reactions with adenovirus 7 or 12 primary tumor antisera (B pools) than with tumor transplant antisera (A pools).

Sabin(16) noted frequent appearance of high titer cross-reacting CF antibodies in hamsters for different virus induced tumors following immunization by transplant, especially with "Eddy" strain SV₄₀ tumor, but not following induction of primary tumor by virus. Such nonspecific cross-reactions were usually detectable in tests with what Sabin refers to as "nonviral" FSa3B tumor antigen. The hamster serum pools employed in the present study (Table I) were assayed for such nonspecific antibody in tests with FSa3B antigen and the results are shown in Table VI. The FSa3B antigen gave positive reactions at low dilution (1:1 or 1:2) only when tested with the adenovirus tumor antisera and there appeared to be little difference in reactivity with transplant (A pools) and primary (B pools) tumor antisera.

Characterization of CF antigen present in human tissue extracts. Animal tumor T antigens have generally been found(2,4,6) to be heat labile (56°C for 30 minutes), non-sedimentable upon high speed centrifugation (30,000 rpm for 2 hours), and ether stable (20% for 2 hours). The effect of these treatments upon 5 of the human tissue extracts is shown in Table VII. None of the human tissue extracts was active following any of the treatments. Both of the control hamster tumor antigens were reduced 4-fold in titer by high speed centrifugation. The CF activity of adenovirus 7 tumor antigen was destroyed by heat and by ether treatment while, by contrast, type 12 tumor antigen titers were reduced only 2-fold by these treatments.

CF tests of sera from human cancer patients. Sera were available from 3 of the patients (no. 11, 33 and 15) listed in Table V whose tissues contained detectable CF antigen. These 3 sera plus serum from patient no. 1, a case of Hodgkin's disease, were tested with 6 human neoplastic tissue extracts which, with the exception of patient no. 1, had given positive reactions with the hamster antisera, and with 6 hamster tumor or normal control tissue extracts. Only the serum from patient 33 gave positive reactions (1:10) and these were with heterologous neoplastic tissue extracts from patients numbers 15, 64 and 40.

Discussion. Direct attempts to demonstrate viral etiology in human cancer by inoculation

TABLE VII. Characterization of Antigens in Human Neoplastic Tissue Extracts Which Fix Complement with Sera from Tumor-bearing Hamsters.

Patient no.	Pathologic diagnosis	Tumor antigen Tissue	Tissue treatment	CF antigen titer obtained in tests with tumor antiserum		
				Adeno 7 Pool A 1:20	Adeno 12 Pool A 1:20	Normal Pool A 1:20
11	Sarcoma, unclassified	Liver with normal morphology	Original (1500 rpm sup't)	4-8	8-16	4-8
			30,000 rpm sup't	0*	0	0
			56°C-30 min	0	0	0
33	Erythroleukemia	Spleen	20% ether treated	0	0	0
			Original (1500 rpm sup't)	0	8-16	0
			30,000 rpm sup't	0	0	0
15	Hodgkin's disease	"	56°C-30 min	0	0	0
			20% ether treated	0	0	0
			Original (1500 rpm sup't)	0	16	0
64	Carcinoma, poorly differentiated	Metastatic tumor of liver	30,000 rpm sup't	0	0	0
			56°C-30 min	0	0	0
			20% ether treated	0	0	0
40	Melanoma	"	Original (1500 rpm sup't)	8	8-16	0
			30,000 rpm sup't	0	0	0
			56°C-30 min	0	0	0
Hamster control	Undifferentiated sarcoma	Adenovirus 7 tumor	20% ether treated	0	0	0
			Original (1500 rpm sup't)	16	4	0
			30,000 rpm sup't	4	0	0
"	"	Adenovirus 12 tumor	56°C-30 min	0	-	-
			20% ether treated	0	0	0
			Original (1500 rpm sup't)	4	64	0
"	"	"	30,000 rpm sup't	0	16	0
			56°C-30 min	-	32	-
			20% ether treated	0	32	0

* 0 titer in tests of human tumor = <1:2 and in tests of hamster tumor = <1:4.

TABLE VIII. Findings in Complement-fixation Tests Employing Sera from Cancer Patients and Human or Hamster Tissue Extracts.

Patient no.	Neoplastic tissue extracts* tested		Antibody titer, case no.			
	Pathologic diagnosis	Tissue	11	33	15	1
11	Sarcoma, unclassified	Liver with normal morphology	0†	0	0	0
33	Erythroleukemia	Spleen	0	0	0	0
15	Hodgkin's disease	"	0	10	0	0
1‡	" "	"	0	0	0	0
64	Carcinoma	Metastatic tumor of liver	0	10	0	0
40	Melanoma	Metastatic tumor of liver	0	10	0	0
Hamster	Undifferentiated sarcoma	Adenovirus 7 tumor	0	0	0	0
"	" "	" 12 "	0	0	0	0
"	" "	" 18 "	0	0	0	0
"	Fibrosarcoma	SV ₄₀ tumor	0	0	0	0
"	Sarcoma	Rous sarcoma tumor	0	0	0	0
"	Normal (control)	Muscle	0	0	0	0

* The human tissue and normal hamster muscle extracts were employed at a 1:2 dilution and all others were used at dilutions containing 4 to 16 antigen units.

† 0 titer = <1:10.

‡ Patient no. 1 tissue extract was previously found to be negative in tests with all the hamster sera.

of extracts of neoplastic tissues into animals or cell cultures have failed to yield a single causative agent of proved etiologic relationship. The search for virus-specific tumor antigens in human tumor offers an indirect approach to demonstration of viral etiology in human neoplasia. Armstrong *et al*(17) and Henle *et al*(18) reported positive CF and immunofluorescence in tests using human sera and cells derived from Burkitt lymphoma and acute leukemias but the nature of these reactions was not clearly defined. Klein *et al*(19) demonstrated immune reactions between Burkitt lymphoma cells and patients' sera by means of the membrane immunofluorescence reaction but the tumor-specific quality of the reaction was not proved conclusively. Rapp *et al*(20) failed to detect positive CF or immunofluorescence reactions when cell cultures derived from a variety of solid tumors in man were tested with serum from the same patient. Negative CF results were also reported by Sabin in tests in which cells derived from several kinds of human tumor were tested with autochthonous human serum(21) or with hamster SV₄₀ antiserum(22). Fink (23) reported positive immunofluorescence staining in tests with cells from human leukemia and antiserum prepared by immuni-

zation of rabbits or monkeys with purified virus-like particles from human leukemic plasma. The significance remains to be established.

This report presents the findings in studies of a large number of a variety of human neoplastic and non-neoplastic control tissues to detect virus-specific T antigens employing antisera from hamsters bearing adenovirus 7, 12, 18, SV₄₀ or Rous sarcoma tumor. The hamster serum pools which were used demonstrated the expected viral tumor specificity (2,24,25) and failed to reveal cross-reactions between the hamster antigens and antisera which would be indicative of isoantigenic reactions. Further, the hamster tumor antisera were relatively free of antibody which reacted with "nonviral" FSa3B tumor antigen and which was frequently present in large amounts in the hamster tumor antisera prepared by Sabin(16). The low titer positive reactions with FSa3B antigen were with antisera from hamsters bearing primary adenovirus tumor as well as with sera from hamsters with transplant tumor.

Positive CF test results which were obtained were not due to cumulative anti-complementary activity of antigen and serum. Titrations of complement which were carried

out in the presence and in the absence of each hamster serum pool showed no anticomplementary effect. The dose of complement used in the tests was routinely determined by titration in the presence of each antigen and back-titrations of complement in the presence of all antigen dilutions confirmed the presence of 2 units of complement. The titers of antigen in the reacting human neoplastic tissue extracts were generally low and were beset with prozone reactions which are characteristic of low titer or weak-reacting antigen and antibody.

Only a small portion (10/119) of the human neoplastic tissues gave a positive CF reaction. These were of diverse types including liposarcoma, erythroleukemia, lymphosarcoma, reticulum cell sarcoma, Hodgkin's disease, carcinoma, and melanoma; shared the common property of being highly malignant; and seven of these were of endothelial origin. The positive reactions were obtained with neoplasia of histologic types which in their animal counterparts, might have been expected to be caused by both DNA (carcinoma, sarcoma) and RNA (leukemia) viruses. The positive reactions, with a single exception (adenovirus 18) were obtained with sera from hamsters with adenovirus 7 or 12 tumor and not with adenovirus 18, SV₄₀ or Rous sarcoma tumor antisera. Based on these findings alone, one might conclude etiologic significance between human tumor and adenovirus(es) related to types 7 and 12. The finding, however, of positive reactions with adenovirus 7 and 12 antisera in as great frequency with non-neoplastic as neoplastic tissues, especially liver and spleen, raises serious questions as to the significance of the results in relation to cancer. However, if specific, the findings might suggest, alternatively, the possible occasional occurrence in human neoplastic or non-neoplastic tissues of adenovirus T antigens due to antigenic transformation caused some time previously by a related adenovirus. The demonstration in a small number of instances of cross-reaction of the normal hamster serum pool with neoplastic or non-neoplastic human tissue indicates that occurrence of isoantigen-antibody reactions between man and hamster might

also have played a role in the positive results which were obtained.

The positive CF reactions which were found with human tissues were clearly centered on adenovirus 7 and 12, especially the latter. In each instance, the strongest reactions were obtained with pool A sera obtained from hamsters given transplant tumor tissue. Only weak or trace reactions were obtained when the corresponding sera prepared by induction of tumor by virus were used, even though these same sera reacted strongly with the homologous hamster tumor antigen. The question of whether the greater reactivity of sera prepared by immunization with transplant tissue from another hamster was due to stimulation of nonspecific antibodies reactive with human tissue or to the stimulation of a stronger reacting antibody which was more capable of detecting virus-specific antigen in human tissue cannot be stated. The failure of Rous sarcoma antiserum to react with the human tissue, even though prepared by immunization with transplant tumor antigen, tends to reduce the likelihood of the first possibility.

The biophysical and biochemical characterization of tumor antigens is subject to variations relating to difference in initial titer, kind of tissue, method of tissue homogenization, degree of antigen dispersion, procedures used to characterize the antigen, and the like. The reactive antigens in the human tissues were readily destroyed by heat as described by others(2,4,6) for T antigens. They were, however, more readily sedimented and less stable to treatment with ether. The latter may be of little importance since the low initial titers of the human neoplastic antigens limited the range of detectability for retention of antigen after treatment, and since Sabin(16) has shown that the findings, even with hamster tumor antigens, may be quite variable.

Except for one instance, the sera from patients with cancer did not react with human or animal tumor antigens. Such lack of antibody against T antigen in cancer patients is in agreement with the findings reported by others(20,21).

The significance of the findings in the

present study are necessarily dependent upon the specificity of the reactions which were obtained. Perhaps the most positive conclusion which can be drawn is that no evidence for etiologic role of adenovirus 18, SV₄₀ and Rous sarcoma virus in human tumor was obtained. Further, if a virus related to adenovirus 7 and 12 plays a role in human neoplasia, it would appear to be relatively infrequent. The positive results which were obtained were as frequent with non-neoplastic tissue as with neoplastic and the findings were occasionally obscured by apparent isoantigen-antibody reactions between hamsters and man. Finally, the kinds of tumor types which gave positive reactions were of such diversity as to limit the credibility for significance in relation to human cancer. Without substantial improvement in sensitivity and specificity of the procedures used, search for T antigen in human tumor does not promise to be a fruitful avenue for attack on the virus in human tumor problem.

Summary. A total of 123 extracts of tissues from 111 cases of human cancer of diverse type and 22 extracts of tissues from 20 cases of non-neoplastic disease of man were examined for presence of antigens fixing complement with sera from hamsters bearing adenovirus 7, 12 and 18, SV₄₀, or Rous sarcoma tumor. Thirteen tissues from cases of cancer and 4 tissues from non-neoplastic disease gave positive CF reactions with adenovirus type 12 and sometimes with type 7 tumor antisera but not with the other tumor antisera. Interpretation was complicated in some instances by apparent isoantigen-antibody reactions between human and hamster tissues. No evidence for etiologic role of adenovirus 18, SV₄₀ or Rous sarcoma virus in human tumor was obtained and if a virus related to 7 and 12 plays a role in human neoplasia, it would appear to be relatively infrequent. Without substantial improvement in specificity and sensitivity, tests for T antigen in human neoplastic tissues would not appear worthy of further emphasis.

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