FURTHER STUDIES ON SPECIFIC TRANSPLANTATION ANTIGENS IN ROUS SARCOMA OF MICE*

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Tumor-specific antigens have been demonstrated in several types of experimentally induced tumors by the use of various methods. Many of these antigens have the characteristics of transplantation antigens (1). Thus, it is possible in experimental systems to build up a specific transplantation resistance against isografting of chemically or physically induced tumors as well as against neoplasms of viral origin. In the case of viral tumors, infection with polyoma, SV40, Gross, Graffi, and Moloney leukemia viruses and adenovirus 12 has been found to induce a specific resistance (VIR) against subsequent isografting of tumor cells induced by the virus in question (2–14). This resistance might be an effect of the antiviral immune response killing all cells carrying the virus capsid antigens. However, experiments with polyoma and SV40 tumors as well as Shope papillomas have demonstrated that these tumors possess specific cellular antigens which are not identical with the antigens of the mature virus particle (15–17). Therefore, where virus-infected animals are concerned one has to consider not only the possibility of an antiviral immune response but also an immune reaction against virus-induced cellular antigens.

Tumors induced by the Schmidt-Ruppin variant of Rous sarcoma virus (RSV-SR) also possess specific antigen(s) detectable in transplantation tests (18) and by complement-fixation tests as well (19, 20). These tumors appear to offer an especially interesting system for antigenicity studies. The mouse tumors induced by this RNA virus do not appear to release any infectious virus *in vivo* or *in vitro* but still contain the virus genome. This can be detected *in vivo* by inoculation into chickens (21) or *in vitro* as a transformation of chicken fibroblasts in mixed cultures of the tumor cells and susceptible chicken cells (22). Of course, the possible existence of helper virus must be considered in this connection (23).

The present paper describes further and more detailed studies on the specific antigen(s) of Rous sarcoma of mice detectable in transplantation tests.

Material and Methods

Mice.—The inbred strain A/Sn and its coisogenic resistant sublines A.CA, A.BY, and A.SW as well as C57Bl/Kl and different F_1 hybrids between these strains and C3H/Kl, C57L, and

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CBA were used. The breeding and maintenance of these animals have been described previously (2).

The animals were kept in a unit also used for polyoma tumor experiments and they might therefore be contaminated by polyoma virus. Some of the mice used in the present experiments actually had positive titers of antipolyoma HI antibodies (up to 1/30720). To exclude the possibility that this polyoma contamination might interfere with the transplantability of Rous tumors, several experiments included polyoma virus-inoculated recipients and uncontaminated controls. No difference was found between polyoma-infected mice and controls with regard to the frequency of takes or rate of growth of the Rous tumors.

Virus.—A crude virus suspension was prepared using a simplification of the method described by Bryan *et al.* (24). Rous chicken sarcomas induced by RSV-SR were suspended in 0.1 M potassium citrate solution and homogenized in a Waring blendor. After magnetic stirring for 1 hour at +37°C and overnight at +4°C the preparation was centrifuged in an MSE cold centrifuge at 2000 RPM and the sediment discarded. Virus pools containing 2 to 10×10^4 focus-forming units (FFU)/ml were kept at −65°C. The titer remained constant during several months' preservation.

Tumors.—Sarcomas were induced in the strains A/Sn, A.CA, A.BY, C57Bl/Kl, as well as in the A × DBA and A × C57Bl F_1 hybrids by inoculating a suspension of living Rous chicken sarcoma cells (induced by RSV-SR). Tumors developed at the site of inoculation after 16 to 70 days and showed the same histological picture as described earlier (21). The neoplasms were transplantable in genetically compatible mice by mechanically prepared crude cell suspensions or trypsinized cells. After 2 to 23 passages in mice, intramuscular inoculation of cell suspensions into chickens gave rise to characteristic Rous sarcomas. Transplantation tests with Rous sarcomas were performed with trypsinized cell suspensions. A known number of trypan blue unstained tumor cells (25) were inoculated subcutaneously into the flank of genetically compatible recipients. In preliminary experiments the minimal cell doses required for progressive tumor growth in 100 per cent of untreated genetically compatible controls (=Dm) and in 100 per cent of similar mice preirradiated with 400 r total body irradiation (=Dmx) were determined for each tumor. Dm varied between 10^2 and 5×10^4 cells and Dmx between 10^2 and 5×10^3 for the 9 different tumors used. The recipients consisted of groups of mice pretreated with 2 to 8 subcutaneous inoculations of mechanically prepared cell suspensions of (*a*) allogeneic Rous sarcomas and (*b*) allogeneic non-Rous control tumors derived from the same foreign mouse strain as the Rous sarcomas. The last allograft was given 5 to 7 days before the challenge isograft. The control non-Rous tumors included 2 "spontaneous" mammary carcinomas, 2 methylcholanthrene-induced sarcomas, and 3 polyoma tumors. Some experiments included animals pretreated with heavily irradiated (8000 r) syngeneic or allogeneic Rous tumor cells.

Other types of recipient mice were inoculated 2 to 6 times intraperitoneally with 0.1 cc of the virus pool at adult age with the last injection being given 5 to 8 days prior to the test.

Finally, some experiments included recipient mice, pretreated with xenografts of Rous chicken sarcoma (induced by the Schmidt-Ruppin and Mill Hill virus strain, respectively). Mechanically prepared cell suspensions were inoculated subcutaneously 4 times at fortnight intervals. The first suspension was given in Freund's complete adjuvant. Control animals were inoculated with suspensions of normal chicken tissues derived from the tumor-bearing animal.

Chicken Antiserum.—Immunization of chicken against RSV-SR was performed according to the method of Fink and Rauscher (26) using either formalin-killed or heated virus. Sera were collected from birds subsequently challenged with live virus and carrying regressing or slowly growing tumors. Sera from several birds were pooled and stored at −20°C.

Mouse Serum was obtained from blood collected by puncturing the retroorbital sinus with a glass capillary.

Virus Assay.—The technique of Temin and Rubin (27) and Rubin (28) was used with

slight modifications. About 90 per cent of the White Leghorn chicken embryos were uniformly susceptible to RSV-SR. Primary cultures were prepared in 200 ml serum bottles. Chicken serum was omitted from the medium and replaced by calf serum. The secondary cultures were infected on the day of seeding on plastic Petri dishes by adding 0.1 ml of each virus dilution. Foci were counted after Giemsa staining.

Antiviral antibodies were assayed according to the technique of Rubin *et al.* (28, 29). After inactivation at 56°C for 30 minutes serum dilutions of 1:2 to 1:5 and 1:25 were incubated for 30 minutes at 37°C with an appropriate virus dilution, (giving 100 to 200 foci per dish in the absence of serum). At least 4 dishes were seeded for each virus-serum mixture. Every test included chicken immune serum as a positive control. A 90 per cent reduction of the number of foci was regarded as specific neutralization.

TABLE I

Results of 2 Transplantation Tests with Tumor RYC (7th and 8th Passages)

Recipients	Whole-body x-irradiation*	No. of takes‡ after inoculation of cell number				
		10^2	10^3	10^4	10^5	2×10^5
	r					
Untreated controls	—	6/13	4/4	4/4	—	—
Untreated controls	400	4/5	4/4	4/4	—	—
Allografted with Rous tumors	—	0/1	0/4	0/4	0/4	3/4
Allografted with Rous tumors	400	0/4	1/4	0/3	0/3	2/4
Allografted with non-Rous tumors	—	—	4/4	—	—	—
Allografted with non-Rous tumors	400	4/4	4/4	—	—	—

* 24 hours prior to test challenge.

‡ Figures denote fraction of mice with progressively growing tumors. The mice were observed for at least 90 days.

Irradiation Procedures.—Groups of *mice* received total body x-ray irradiation in a dose of 350 or 400 r. For irradiation of tumor cells a dose of 8000 r was used. X-Rays were generated at 200 kv, 15 ma, and filtered by 0.5 mm Al + 0.5 mm Cu for the mice and by 1 mm Al for the tumor cells.

The test animals were inspected once a week for the appearance of tumors. Three diameters of the tumors were measured and the geometrical mean estimated.

RESULTS

Resistance against Isotransplantation after Pretreatment with Rous Tumor Allografts.—Nine different Rous sarcomas were transplanted to genetically compatible recipients of the following 3 categories: untreated controls, mice pretreated with Rous sarcoma allografts, and mice pretreated with allografts of non-Rous tumors. Several experiments were performed with each test tumor, as a rule including 3 different cell doses. Recipients were either unirradiated or x-irradiated 24 hours prior to test challenge. Several experiments included both

TABLE II

Pooled Results of 28 Tests with 6 Different Test Tumors. Unirradiated Mice

Test tumor	Strain of origin	No. of takes* after inoculation of cell number‡											
		(0.1–0.2) × Dm			Dm			10 × Dm			100 × Dm		
		U§	R§	Non-R§	U	R	Non-R	U	R	Non-R	U	R	Non-R
RSC	A/Sn	9/12	0/11	2/7	17/20	3/16	8/8	—	4/4	—	—	—	—
RSD	A/Sn	9/18	5/18	9/15	20/22	3/18	6/8	—	0/8	—	—	—	—
RSE	A/Sn	3/8	0/8	1/8	8/8	1/8	6/7	—	5/8	—	—	3/4	—
RCB	A.CA	—	—	—	8/8	0/8	5/8	8/8	0/7	5/7	4/4	0/4	—
RYC	A.BY	6/13	—	—	18/18	1/18	8/8	16/16	5/17	4/4	—	3/8	—
RC57A	C57Bl/Kl	5/13	0/16	6/10	16/20	4/21	11/11	—	3/3	—	—	—	—
Total		32/64 (50%)	5/53 (9%)	18/40 (45%)	87/96 (91%)	12/89 (13%)	44/50 (88%)	24/24 (100%)	17/47 (36%)	9/11 (82%)	4/4 (100%)	6/16 (38%)	— —
X^2‖		13.61			70.81			5.78					
P		<0.001			<0.001			$0.02 > P > 0.01$					

* Figures designate the fraction of mice with progressively growing tumors.

‡ The cell number is indicated as multiples of the minimal cell dose required for progressive growth in 100 per cent of the controls (= Dm), as determined in preliminary experiments.

§ Designation of recipient groups: U, untreated controls; R, allografted with Rous tumor; non-R, allografted with non-Rous tumor.

‖ Calculated for animals allografted with Rous tumor, compared with animals allografted with non-Rous tumor.

TABLE III

Pooled Results of 28 Tests with 9 Different Tumors. Animals X-Irradiated (400 r) 24 Hours before Test

Test tumor	Strain of origin	No. of takes* after inoculation of cell number‡											
		(0.1 –0.2) × Dmx			Dmx			10 × Dmx			100 × Dmx		
		U§	R§	Non-R§	U	R	Non-R	U	R	Non-R	U	R	Non-R
RSC	A/Sn	6/7	2/7	7/8	11/11	4/12	8/8	—	4/4	—	—	—	—
RSD	A/Sn	—	—	—	4/4	8/14	12/12	14/14	4/14	14/14	—	—	—
RSE	A/Sn	4/5	2/5	2/6	16/16	12/19	19/19	11/11	12/14	13/13	—	5/5	—
RCB	A.CA	—	—	—	1/1	2/4	4/4	2/2	0/4	4/4	—	0/4	—
RYC	A.BY	—	—	—	6/7	4/9	7/8	12/12	6/13	15/15	—	8/12	6/6
RC57A	C57Bl/Kl	—	—	—	9/11	6/12	8/8	12/12	17/19	7/7	—	3/3	—
RDAA	A × DBA/Kl	—	—	—	9/9	3/4	—	8/8	3/4	5/5	—	—	—
RDAB	A × DBA/Kl	—	—	—	7/7	4/4	5/5	8/8	7/9	9/9	—	—	—
RSC57A	A × C57Bl/Kl	—	—	—	5/5	—	—	5/5	2/4	5/5	—	—	—
Total		10/12 (83%)	4/12 (33%)	9/14 (64%)	68/71 (96%)	43/78 (55%)	63/64 (98%)	72/72 (100%)	55/85 (65%)	72/72 (100%)	—	16/24 (67%)	6/6 (100%)
X^2‖					32.59			29.16					
P					<0.001			<0.001					

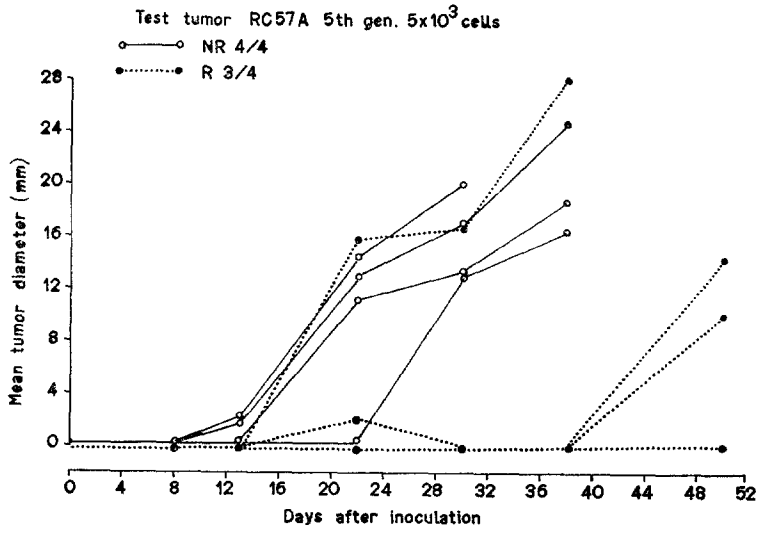
* Figures designate the fraction of mice with progressively growing tumors.

‡ The cell number is indicated as multiples of the minimal cell dose required for progressive growth in 100 per cent of the controls (= Dmx), as determined in preliminary experiments.

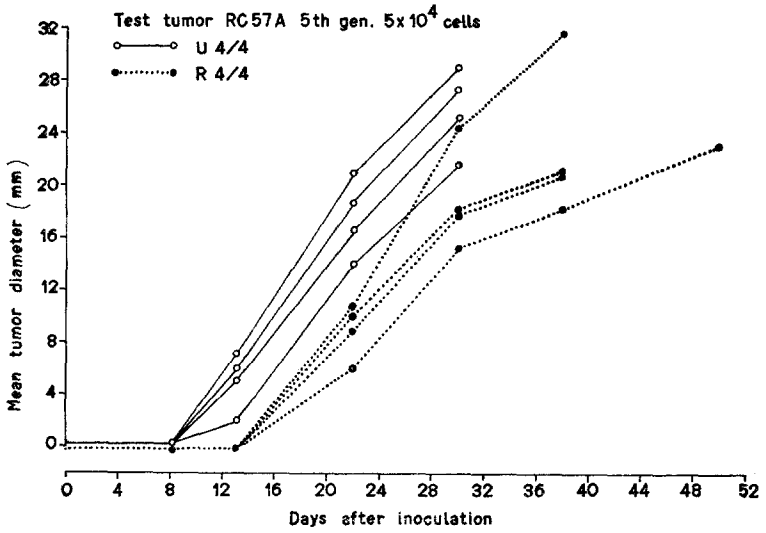
§ Designation of the recipient groups: U, untreated controls; R, allografted with Rous tumor; non-R, allografted with non-Rous tumor.

‖ Calculated for animals allografted with Rous tumor, compared with animals allografted with non-Rous tumor.

1 *a*



1 *b*



FIGS. 1 *a* and 1 *b*. Growth of 5×10^3 and 5×10^4 cells from fourth transfer generation of Rous tumor RC57A after transplantation into preirradiated (400 r) syngeneic mice untreated (*U*) or pretreated with allografts of the polyoma tumor SECX (*NR*) and the Rous tumor RCB (*R*), respectively. Figures denote fraction of mice with progressively growing tumors over total number inoculated. Each curve represents tumor growth in one recipient.

unirradiated and irradiated recipients of the different categories. As an example, Table I summarizes the results of 2 experiments with 5 different inoculum doses of tumor RYC (induced in the A. BY strain). Recipients pretreated with Rous tumor allografts showed a strong resistance against the isografting of cell doses

ranging between 10^2 and 10^5 cells. Not until 2×10^5 cells were inoculated was the resistance breaking down. Whole body x-irradiation with 400 r prior to the challenge did not abolish resistance. The recipients allografted with a non-Rous tumor (a polyoma-induced osteogenic sarcoma) had no demonstrable transplantation resistance against RYC.

Table II summarizes the results of 28 tests with 6 tumors in unirradiated recipients. For each tumor the inoculum doses are given as multiples of the minimal cell number required for growth in all untreated controls (=Dm) in preliminary tests. These pooled results clearly demonstrate the strong resistance of Rous tumor allograft pretreated recipients, when compared to untreated mice or animals pretreated with allografts of non-Rous origin. In mice pretreated with allografts of non-Rous tumors a weak transplantation resistance was sometimes expressed in a slightly reduced frequency of takes as compared to untreated controls. This weak resistance was not significant and could be completely abolished by irradiation.

The pooled results of 28 tests with 9 different Rous tumors isografted to recipients x-irradiated 24 hours before test are presented in Table III. Dmx was found to be $\frac{1}{10}$ to $\frac{1}{20}$ of the Dm for the same tumor except for the tumor RCB, for which Dmx was equal to 10^2 cells=Dm. The transplantation resistance of Rous tumor allografted recipients was only slightly reduced as compared to the corresponding unirradiated animals. The weak resistance of the unirradiated non-Rous tumor allografted mice, however, was completely abolished by x-irradiation, and the frequency of takes was the same as in preirradiated controls. The difference between Rous tumor and the non-Rous tumor allografted animals is highly significant.

The resistance of the Rous tumor pretreated animals was manifested not only by a reduced frequency of takes but also by markedly prolonged latency periods and delayed tumor growth rate as compared to untreated controls or mice pretreated with control tumors. This is demonstrated in Fig. 1, giving the growth curves of tumor RC57A with 2 different doses of inoculation. Although the frequency of takes is 3/4 and 4/4 for 5×10^3 and 5×10^4 cells, respectively, in the Rous allograft-pretreated group the tumors appear later and the rate of tumor growth is markedly reduced. The same results were regularly obtained also with the other tumors tested. Thus, the degree of resistance was higher than what is expressed by the rough take figures in the above tables.

In order to prove the specificity of the isograft resistance further, 2 polyoma tumors were isografted to untreated controls, to mice previously allografted with polyoma tumor or Rous tumor and to polyoma virus-inoculated animals, respectively. Mice pretreated with polyoma virus or polyoma tumor showed the expected resistance, while no such resistance could be demonstrated in the Rous tumor allografted recipients.

The tests for cross-reactivity between the different tumors used are summarized in Table IV. A clear-cut cross-resistance could be demonstrated be-

TABLE IV

Cross-Resistance between Rous Tumors and Various Other Mouse Tumors*

Test tumors	Tumors used for pretreatment																					
	Rous tumors						Rous tumors (HR-cells‡)					Polyoma tumors							Non-viral tumors			
	RSB	RSC	RSD	RSF	RCB	RYC	RSC	RCB	RDAA	RDAB	RSC57A	SECBT	SECX	SEYF	SESO-C11	SEWA-C10	TCSB-A3	TCSB-C2	MCA	MC57L	MWE	S14S
Rous tumors																						
RSC					+	+	+	+				−	−						−			
RSD					+																−	
RSE					+	+								−		−				−		
RCB	+	+		+													−	−				−
RYC	+	+		+											−	−						
RC57A	+		+	+	+			+										−				
RDAA					+		+		+							−						
RDAB					+	+				+						−						
RSC57A					+						+					−						
Polyoma tumors																						
SES3PT§													+									
SEWA-C10§		−		−																		

* + indicates a clear-cut cross-resistance, − the absence of detectable cross-resistance.

‡ X-irradiated 8000 r.

§ Polyoma virus immunized mice tested in parallel showed a clear-cut transplantation resistance.

TABLE V

*Isograft Resistance Induced by Heavily Irradiated Syngeneic or Allogeneic Rous Tumor Cells**

Tumor	Cell dose	Untreated		Isografted with Rous tumor (HR-cells)		Allografted with Rous tumor (HR-cells)		Allografted with untreated Rous tumor		Allografted with untreated non-Rous tumor	
		—	400 r‡	—	400 r	—	400 r	—	400 r	—	400 r
RSC	5×10^3	—	4/5	—	—	—	0/5	—	2/4	—	—
	5×10^4	—	5/5	—	1/4	—	—	—	—	—	—
RC57A	5×10^3	6/10	8/9	—	—	0/5	2/5	0/5	0/4	—	—
	5×10^4	7/10	6/6	—	—	2/4	1/4	0/5	3/4§	—	—
RDAA	$5 \times 10^2 - 10^3$	5/5	9/9	—	—	—	1/5	—	—	—	—
	$5 \times 10^3 - 10^4$	5/5	8/8	0/5	2/5	—	1/5	—	3/4§	2/4	5/5
RDAB	10^4	4/5	5/5	—	—	—	—	—	—	—	—
	10^5	5/5	5/5	0/5	0/6	—	—	—	3/4§	4/4	4/4
RSC57A	10^2	1/5	5/5	—	—	—	—	—	—	—	—
	10^3	5/5	5/5	2/5	0/4	—	—	—	2/4§	5/5	5/5
Total	Dm and Dmx, respectively	22/25	31/33	2/10	—	2/4	3/15	0/5	2/8	9/9	—
	10 × Dm and 10 × Dmx, respectively	5/5	29/29	0/5	3/19	—	2/9	—	11/16	2/4	14/14

* Figures denote fraction of mice with progressively growing tumors.

‡ 24 hours prior to test.

§ Prolonged latency period and delayed tumor growth.

tween Rous tumors, in all the combinations, while no cross-reactions were found between Rous tumors and polyoma tumors or various non-viral neoplasms.

Isograft Resistance Induced by Heavily Irradiated Rous Tumor Cells or by Crude Fractions of Ultrasonically Disrupted Rous Sarcoma Cells.—Since x-irradiation has been shown to abolish the immunizing capacity of polyoma tumor cells (15) but not that of methylcholanthrene- and SV40-induced neoplasms (30, 31), it seemed desirable to investigate the effect of x-irradiation on the antigenicity of Rous tumor cells. Table V summarizes the results. It is quite clear that the irradiated (8000 r) Rous tumors are capable of inducing a clear-cut transplanta-

TABLE VI

Results of Isotransplantation of Tumor RSC (5×10^3 Cells) to Recipients Pretreated with Sonically Disrupted or Heavily Irradiated Allogeneic Rous Tumor Cells. Recipients X-Irradiated (400 r) 24 hours before Test

Pretreatment	No. of takes*			Total
	Experiment 1	Experiment 2	Experiment 3	
Untreated controls	4/5	4/4	5/5	13/14
Supernatant‡	1/4	4/5§	4/4§	9/13
Sediment‡	3/5	4/5	4/4§	11/14
HR-Rous tumor cells (8000 r)	0/5	3/5	—	3/10
Unirradiated Rous tumor cells	1/4	4/5§	2/5	7/14
Non-Rous tumor cells	—	5/5	—	5/5

* Figures denote fraction of mice with progressively growing tumors.

‡ Ultrasonic treatment 2 minutes (ultrasonic power unit MSE). Centrifugation 4000 RPM 10 minutes.

§ Means prolonged latency period and/or slower tumor growth than in control animals.

tion resistance, which appears to be somewhat stronger than in the animals pretreated with non-irradiated Rous allografts. This might be due to the fact that the unirradiated cell suspension was diluted 1:10 in comparison with the irradiated suspension. This difference was introduced in an attempt to compensate for the multiplication of the non-irradiated cells prior to regression. Pretreatment with non-Rous tumor allografts gave no resistance. The results of pretreatment with heavily irradiated syngeneic or allogeneic Rous tumor cells are also included in Table IV.

In order to investigate whether integrity of the whole cell is essential for immunization, a series of experiments was performed with ultrasound disrupted cell fractions. After 2 minutes' sonication (ultrasonic power unit MSE, 18 to 20 kc per sec.) the mechanically prepared cell suspension was centrifuged at 4000 RPM for 10 minutes. The upper half of the supernatant was cautiously removed. The sediment was resuspended in the rest of the supernatant. Groups of mice

TABLE VII

*Results of Isografting of Different Rous Mouse Sarcomas to Mice Pretreated with RSV-SR Virus Pool or Xenografts of Rous Chicken Sarcoma**

Test tumor	Cell dose	Untreated controls		Xenografted with normal chicken tissue		Xenografted with Rous chicken sarcoma (MH strain)		Xenografted with Rous chicken sarcoma (SR strain)		Virus pool (RSV-SR) i.p.		Allografted with Rous tumor	
		—	400r‡	—	400r	—	400 r	—	400 r	—	400r	—	400 r
RSC	5×10^3	10/12	18/18	1/4	4/4	—	10/10	2/3	12/13	12/13	4/4	0/12	8/16§
	5×10^4	—	5/5	—	—	—	5/5	—	5/5	—	—	—	4/5§
RSE	10^3	3/8	4/4	—	—	—	—	—	—	3/8	4/4	0/8	1/4
	10^4	8/8	4/4	—	—	—	—	—	—	3/8	3/4	1/8	3/4§
RCB	10^2	8/8	1/1	—	—	—	—	—	—	3/7	4/4	0/8	2/4§
	10^3	8/8	2/2	—	—	—	—	—	—	6/7§	4/4	0/7	0/4
RYC	10^2	1/4	5/5	—	—	—	—	—	—	3/4	2/3	—	0/4
	10^3	8/8	6/6	—	—	—	—	—	—	4/4§	3/4	0/8	2/8
	10^4	4/4	—	—	—	—	—	—	—	3/4§	2/2	0/4	4/5§
RC57A	5×10^3	5/13	9/11	3/5	5/5	—	—	—	—	7/17	13/13	0/21	8/17§
	5×10^4	8/12	11/11	5/5	3/3	—	—	—	—	7/12	6/6	4/17	11/16
RDAA	5×10^2	—	9/9	—	—	—	5/5	—	3/4§	—	4/4	—	7/9§
	5×10^3	—	10/10	—	—	—	5/5	—	5/5	—	4/4	—	8/8
Total	Dm and Dmx, respectively	34/36	46/48	1/4	9/9	—	15/15	2/3	15/17	22/32	31/32	1/36	26/54
	10 × Dm and 10 × Dmx, respectively	12/12	38/38	—	3/3	—	10/10	—	10/10	9/11	20/22	0/11	28/45

* Figures denote fraction of mice with progressively growing tumors.

‡ 24 hours prior to test.

§ The resistance was expressed also as a markedly prolonged latency period and slower tumor growth than in the corresponding untreated controls.

pretreated with 4 subcutaneous inoculations of the supernatant or sediment revealed a weak but clear-cut transplantation resistance on subsequent isografting of tumor RSC (Dmx given to the preirradiated recipients) (Table VI). The resistance was indicated by a slightly reduced frequency of takes and a slower tumor growth than in untreated animals or mice pretreated with control tumor. It was weaker than the resistance induced by intact or lethally irradiated Rous tumor cells.

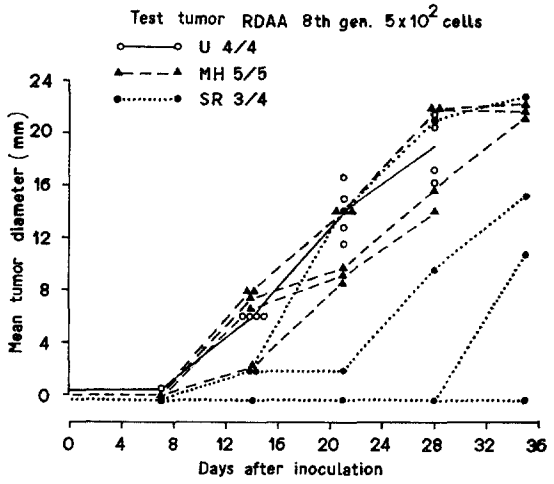


FIG. 2. Growth of 5×10^2 cells from the seventh transfer generation of Rous tumor RDAA after transplantation into preirradiated (400 r) syngeneic mice, untreated (*U*) or pretreated with xenografts of Rous chicken sarcoma, strain Mill Hill (*MH*), or Rous chicken sarcoma, strain Schmidt-Ruppin (*SR*). Figures denote fraction of mice with progressively growing tumors over total number inoculated. The tumor growth curve of the untreated mice represents the mean volume of all 4 tumors (the open circles giving the individual values), while the curves of the xenografted animals represent the growth of individual tumors.

Transplantation Tests in Mice Pretreated with RSV-SR Virus Pool or Xenografts of Rous Chicken Sarcoma.—In analogy with the virus-induced transplantation resistance (VIR) demonstrated for various virus-induced neoplasms (2–14), attempts were made to induce isograft resistance against Rous tumors by pretreatment with RSV-SR–containing materials. Groups of mice were pretreated with (*a*) 0.1 ml of the RSV-SR pool inoculated at least 4 times intraperitoneally every second week, (*b*) 4 xenografts of Rous chicken sarcomas induced by RSV-SR or the Mill Hill–Rous virus strain (RSV-MH), respectively, and as a negative control to these, (*c*) xenografts of normal chicken tissues. The results of Rous tumor isografting into these groups are presented in Table VII.

At the Dm level, RSV-SR, chicken Rous sarcoma, and normal chicken cells all appeared to induce an isograft resistance in comparison with untreated recip-

ients, provided that none of the groups was irradiated. Whole body irradiation 24 hours before challenge abolished the resistance induced by the RSV-SR pool and normal chicken cells, and in most cases also that induced by Rous chicken sarcoma as shown in Table VII. Chicken sarcoma induced by the RSV-MH induced no demonstrable resistance in irradiated animals.

The experiment in which a slight resistance was demonstrated in recipients pretreated with chicken Rous sarcoma xenografts is presented in Fig. 2. The

TABLE VIII

Titer of RSV-SR-Neutralizing Antibodies in Sera of Mice after Inoculation of a Virus Pool or Pretreatment with Iso-, Allo-, or Xenografts of Rous Sarcoma

Pretreatment	Results of transplantation tests	No. of tested sera with titer		
		<1:3	1:3–1:5	≥1:20
Untreated	—	6	—	—
Iso- or allografted with Rous tumor	Resistant	11	—	—
	Non-resistant	5	—	—
Xenografted with Rous chicken sarcoma (when adult)	Resistant	2	—	—
	Non-resistant	11	—	—
Xenografted with Rous chicken sarcoma (when new-born)	Resistant*	4	1	—
	Non-resistant	1	—	2
Virus pool	Non-resistant	4	—	—

* The transplantation results have been published (32).

resistance was complete in 1 animal while in the other 3 recipients it was expressed as a clearly diminished growth rate compared to untreated and MH-sarcoma xenografted mice.

Tests for Virus-Neutralizing Antibodies in the Sera of Mice Used in the Transplantation Tests.—The antiviral activity of sera obtained from mice exhibiting a specific isograft immunity according to the transplantation tests described above, was tested with the focus-neutralization technique of Rubin *et al.* (28, 29). Each test included 2 serum dilutions (1:2, 1:3, or 1:5 and 1:20 or 1:25, respectively) and one chicken immune serum as a positive control. In order to exclude the possibility that mouse sera contained factors which inhibited virus-neutralization, a mixture of equal parts of normal mouse serum and chicken immune serum was tested. The mixture neutralized the virus as efficiently as the immune serum alone.

The results of the neutralization tests are given in Table VIII. No virus-neu-

tralizing activity was demonstrated in any of the animals, pretreated with RSV-SR or iso-, allo-, or xenografts of Rous tumors, no matter whether transplantation resistance could be demonstrated or not.

The neutralization tests also included sera from mice, inoculated with Rous chicken sarcoma material when newborn. Isograft resistance was demonstrable in animals not developing primary tumors (32). As shown in Table VIII, 2 of the 3 non-resistant mice tested had virus-neutralizing antibodies in a titre of 1:20, and one of the 5 tested sera from resistant mice neutralized in dilution 1:3 but not 1:30.

DISCUSSION

The present investigation clearly demonstrates that Rous sarcomas in mice possess common specific transplantation antigen(s). In all combinations tested the Rous tumors cross-reacted while no cross-resistance was demonstrable between Rous sarcomas and neoplasms induced by polyoma virus or of non-viral origin. All Rous sarcomas were capable to induce resistance and responded to the specific immunity induced by other Rous tumors. Non-Rous neoplasms showed neither of these qualities.

The controls included in the transplantation tests ruled out such non-specific effects as the stimulation of the immunological reactivity by allograft pretreatment. If this were the case, allografted mice would be expected to develop resistance against antigenic tumors of different origin, as well; this was not the case. Furthermore, allografting with non-Rous tumors derived from the same mouse strain as the Rous allografts did not induce resistance against Rous tumor isografts. These controls also exclude the possibility that the specific anti-Rous isograft resistance was directed against isoantigens due to possible residual heterozygosis in the animals used. Whole-body x-irradiation (400 r) of the isograft recipients 24 hours prior to challenge was another precaution introduced to rule out non-specific effects. This dose of irradiation is known to decrease subsequent primary immune responses markedly (33) while secondary responses are much less affected. Comparison between the results summarized in Tables II and III clearly shows that the isograft resistance of Rous tumor-allografted mice is only somewhat reduced by x-irradiation and the specificity of this resistance is thus established beyond doubt. In contrast, the slight resistance of unirradiated recipients pretreated with allografts of various non-Rous tumors is completely abolished by x-irradiation and can be best explained as a non-specific stimulation of the primary immune response. A similar non-specific isograft resistance against methylcholanthrene-induced mouse sarcomas is sometimes induced by tumorous or non-tumorous grafts (34).

A clear-cut transplantation immunity was induced not only by intact cells but by irreversibly x-ray damaged Rous sarcoma cells as well. The immunizing efficiency of irradiated cells has previously been demonstrated for chemically

induced tumors and SV40 hamster sarcomas (30, 31), while the polyoma cell antigen appeared to be radiosensitive (15). Irradiated Graffi leukemia cells induce specific isograft immunity (10) but in this case it is not known whether the resistance is induced by the cells themselves or by the virus that they always contain. The mouse Rous sarcomas do not release any detectable infectious virus *in vivo* or *in vitro* and therefore the immunity is most likely induced by radioresistant cellular antigen(s) peculiar to tumors induced by this agent.

The presence of common tumor-specific transplantation antigen(s) in Rous sarcomas of mice is analogous to the existence of specific antigens in a number of other virus-induced tumors. The antigens are common for various tumors induced by the same virus but different for neoplasms induced by different viruses. In most cases it is not known whether the antigens are identical with or different from viral antigens. However, in tumor systems where non–virus-releasing tumor cells are available, polyoma and SV40 tumors, it has been shown that specific antigen(s) having a specificity distinct from the complete virus antigen(s) are common for tumors induced by the same virus (15, 16).

Infection of adult animals with oncogenic viruses has been found to induce an isograft resistance (VIR) specific against tumors induced by the same virus in question in all systems so far studied. In several cases attempts have been made to differentiate between an antiviral immune response inhibiting cells carrying viral antigen(s) and an immune reaction directed against specific cellular antigen(s) developing in virus-infected cells and in established virus-induced tumor cells.

Attempts have been made to induce the VIR against Rous tumors by pretreatment with a RSV-SR virus pool or xenografts of virus-containing Rous chicken sarcoma assumed to release virus in the host animal. Unirradiated recipients pretreated with the virus pool showed only a weak resistance which was completely abolished by preirradiation and was thus probably non-specific. This resistance was of about the same order of magnitude as demonstrated in unirradiated virus-pretreated hamsters (35). Although the virus pool used for pretreatment had a relatively high titer the lack of immunization may be due to an insufficient antigen dose, since the Rous virus cannot multiply in adult mice or does so to a very limited extent only. The absence of an antiviral immune response in the form of virus-neutralizing antibodies (Table VIII) supports this explanation.

Similarly, most mice xenografted with Rous chicken sarcoma tissue failed to develop any specific resistance (Table VII). Only one experiment (Fig. 2) revealed a weak resistance. No virus-neutralizing activity could be demonstrated in the sera of xenografted animals including those exhibiting the slight isograft resistance.

The isograft immunity demonstrated in the absence of antiviral antibodies in mice allografted with Rous tumors speaks against an identity between the trans-

plantation antigen(s) and viral antigen(s). However, such an identity cannot be excluded since humoral virus-neutralizing antibodies are not readily induced after virus infection of mice. Even if directed against the same antigens the development of a transplantation resistance must not necessarily be accompanied by the development of humoral virus-neutralizing antibodies. Relevant in this connection are some recent transplantation tests in mice pretreated with Rous chicken sarcoma when newborn (32). The animals that failed to develop primary tumors within 6 weeks showed an isograft resistance, while recipients pretreated with the same chicken tumor material as adults showed only a very weak resistance as in the present study. As indicated in Table VIII it was found that the resistant mice contained no detectable virus-neutralizing antibodies while 2 non-resistant mice did show positive titers. Thus there seems to be no correlation between the antiviral immune response and the development of isograft resistance. This appears to be a strong argument against the possible identity between the transplantation antigen(s) and viral antigen(s). The reason why newborn mice responded better than adults may be explained in various ways. Rous chicken sarcoma cells or the Rous virus may survive longer in newborns than in adults. The newborns would be exposed to a heavier virus infection affecting a larger number of mouse cells with subsequent induction of the specific antigen(s). The fact that adults do not respond well suggests that the resistance is not due to an immunization against antigen(s) fully expressed in the chicken sarcoma cells inoculated, since adult mice are more immunologically competent than newborns. It would follow that the postulated specific antigen of mouse Rous sarcomas is not identical with that of chicken sarcomas.

Another point favouring the non-identity of the tumor-specific antigen and virus antigen(s), is the finding that cell-free mouse Rous tumor extracts were capable of inducing isograft immunity. The extracts did not contain any infectious Rous virus according to the test performed *in vitro* and *in vivo*. Since no resistance was induced by similar extracts of Rous chicken sarcomas which certainly contain relatively large amounts of virus, the conclusion must be drawn that the resistance was most probably induced by cellular antigen(s) present in the mouse tumor extract.

The demonstration of specific antigen(s) in mouse Rous sarcoma extracts capable of inducing isograft resistance contrasts with the results of complement fixation tests (19, 20). The latter revealed specific common antigen(s) in cell-free extracts of sarcomas induced in hamsters and guinea pigs by RSV-SR and a corresponding antibody activity in tumor-bearing animals. The CF antigen(s) appeared to be identical with viral antigen(s) since sera obtained from tumorous animals reacted not only with extracts of Rous sarcomas induced in different species but also with pools of various related viruses such as myeloblastosis virus and lymphomatosis virus (RIF). In order to obtain an integrated picture of the RSV-SR carcinogenesis, it appears to be necessary to clarify the relation-

ship between the transplantation antigen(s), the CF antigen(s), and the viral antigen(s) further.

SUMMARY

Mice allografted with different sarcomas, induced by the Schmidt-Ruppin variant of Rous sarcoma virus (RSV-SR), showed a resistance against subsequent isografting of 9 different Rous sarcomas. Transplantation resistance could also be induced by Rous mouse tumor cells x-irradiated with 8000 r or with cell-free tumor extracts, containing no demonstrable virus. No transplantation resistance could be demonstrated after allograft pretreatment with various polyoma tumors or non-viral tumors. Allograft pretreatment with Rous tumors induced no demonstrable resistance against isografting of polyoma tumors. Inoculation of RSV-SR or Rous chicken sarcoma suspension into adult mice gave no clear cut resistance against isografting of mouse sarcomas. Neither after allografting of Rous tumors nor after virus or chicken sarcoma inoculation into adult mice could virus-neutralizing activity be demonstrated in the sera.

The results demonstrate the presence of common, specific transplantation antigen(s) in different Rous sarcomas in mice and speak against an identity between the transplantation antigen(s) and viral antigen(s).

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