SPECIFIC TRANSPLANTATION ANTIGENS OF MOUSE SARCOMAS INDUCED BY ADENOVIRUS TYPE 12

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All viral tumors investigated have been found to contain specific antigens common for all the neoplasms induced by the same virus but usually different for tumors induced by different viruses (1, 2). This pattern was demonstrated first for transplantation antigens, and has subsequently been demonstrated for the so called neoantigens detected by the complement fixation (CF) technique (3–5). These latter antigens are probably identical with those detected by fluorescent antibody (FA) tests on fixed cell preparations (6). The specific surface antigens detected by the FA technique in living leukemia cells are probably identical with the transplantation antigens of these cells (7).

The human adenovirus type 12 induces tumors in hamsters, rats, and mice (8, 9). The hamster neoplasms have been extensively studied for their content of virion antigens and neoantigens. Infectious virus has never been recovered from tumors induced by adeno 12 virus (8, 10, 11), although indirect indications have been obtained for the presence of the type-specific C virion antigen in hamster tumors (12, 13), by use of the CF and immunodiffusion techniques. Sera of hamsters carrying primary tumors were found to contain antibodies against the C antigen although this antigen was never detected when tumor cell preparations were tested directly. Indications for the existence of another virion antigen (called antigen D) in hamster tumor cells has been obtained by immunodiffusion tests (13).

Extracts of hamster adeno 12 tumors have been found to contain a common specific antigen (the so called neoantigen) detectable by the CF and immunodiffusion techniques (10, 13). This antigen appears to be identical with an antigen synthesized early during the in vitro infection cycle of human cells (14). In addition the neoantigen as detected by FA technique has been reported to appear in other mammalian cells (monkey, hamster, mouse, rabbit, rat, and chick) infected in vitro (15). In CF tests the adeno 12 neoantigen cross-reacts with a similar antigen present in neoplasms induced by adenovirus types 18 and 31 and with the antigens produced in human cells infected by these viruses. It

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does not cross-react with any of the corresponding antigens produced during the lytic infection cycle of other adenovirus types; e.g. type 7 (16). The adeno 7 neoantigen on the other hand cross-reacts with the corresponding antigens induced by types 3, 4, 11, 14, 16, and 21. A somewhat different cross-reactivity has been reported by use of the fluorescent antibody test (23). Serum of hamsters bearing adeno 12 tumors reacted not only with adeno 12 and 18 tumor cells but also with hamster and human cells infected with adenovirus types 1 and 7.

It has been found with neoplasms induced by polyoma virus, SV40 virus, and Rous sarcoma virus, that their common specific transplantation antigens can be demonstrated as specific isograft immunity in mice treated by (*a*) any tumor cells of the same viral origin, and (*b*) infection with the corresponding virus (2). The mechanism for the induction of immunity by virus infection is probably the following: the virus infects some host cells and induces in them the synthesis of an antigen identical with the tumor-specific transplantation antigen. The host animal will react immunologically against this antigen and would therefore reject isografts having the antigen.

Infection of mice with adeno 12 virus has been shown to induce a specific isograft immunity against adeno 12 tumors (17). The present results demonstrate a common specific antigenicity of the transplantation type in adeno 12 mouse sarcomas and suggest a cross-reaction between adeno 12 and adeno 7 tumors.

Materials and Methods

Mice.—Mice of the inbred strains C3Hf/KL, CBA, A/Sn, and A.CA and the F_1 hybrids between these strains were used. Their breeding and maintenance have been described (18).

Virus.—Adenovirus types 18, 12, 7, and 5 were kept by passage on the stable human cell line MAS (19). Types 18 (D.C.), 12 (Huie), and 7 (Gomen) were kindly supplied by Dr. Kjellén in Malmö, who had obtained types 18 and 12 from the National Institutes of Health, Bethesda, Md., in 1962 and type 7 from Dr. Pereira. Adenovirus type 5 was supplied by Dr. E. Norrby in Stockholm. Virus preparations used for tumor induction and immunization were prepared by disrupting packed MAS cells infected with adenovirus when CPE (cytopathic effect) was completed, by freezing and thawing five times and subsequent Freon treatment. Plaque titration of the viruses was performed on MAS cells by use of standard techniques with an observation period of 21 days (19). Titers of virus type 12 varied between 2.6×10^5 and 7.3×10^6 PFU/0.1 ml, for type 7 the range was $1.3 \times 10^6 - 2.1 \times 10^7$ PFU/0.1 ml. Adenovirus type 18 was not titrated. The polyoma virus used was originally obtained from Dr. B. E. Eddy. For control immunization purposes 128 HA units of virus were inoculated per animal.

Sera of mice which had been treated repeatedly with adenoviruses and tested for transplantation immunity were titrated with regard to antiviral antibodies (against type 7 and 12, respectively) by use of gel-precipitation technique and the hemagglutinin-inhibition (HI) test. HI antibodies against adeno type 12 were demonstrated by the technique of Schmidt et al. (20) using rat erythrocytes in the presence of heterotypic immune serum (rabbit anti-adeno 5). Mouse sera were adsorbed with kaolin and test red cells before the HI test against 8 HA units of virus.

Tumors.

Adeno 12 tumors: Adenovirus type 12 in aliquots of 0.1 ml (containing $2.6 \times 10^5 - 7.3 \times 10^6$ PFU) was inoculated subcutaneously into mice (less than 24 hr old) of the strains C3H/KL, A/Sn, and the F_1 hybrids between A/Sn and C3H, CBA, and C57BL. Tumors developed in 9 out of 47 animals surviving for more than 1 month (Table I). 8 neoplasms were obtained in the 22 C3H and (A × C3H) F_1 mice inoculated (=36%) while no tumors developed in 24 A/Sn and (A × C57BL) F_1 animals. The latency period varied between 58 and 270 days with an average of about 5 months. The tumors were of a poorly differentiated sarcomatous type located in muscle or subcutaneous connective tissue at the site of inoculation.

Four adeno 12 tumors were passaged serially in syngeneic mice. They were designated A12.HA, A12.HC, A12.HD (all of C3H/KL origin), and A12.SBA (developed in a (A × CBA) F_1 mouse) (cf. Table I). These tumors required high cell doses for growth upon transfer (more

TABLE I

Induction of Sarcomas by Infection of Newborn Mice (less than 24 hr old) with Adenovirus Type 12

Mouse strain	No. inoculated	No. surviving after 1 month	No. of tumor positives	Latency periods
				days
C3H	18	14	5	58, 125*, 130, 165‡, 180§
(A × C3H)F_1	8	8	3	135, 195, 270
(A × CBA) F_1	3	1	1	150‖
A/Sn	21	17	0	
(A × C57BL) F_1	8	7	0	
Total	58	47	9	2–9 months

* Tumor was designated A12.HD of male origin and was passaged serially in vivo.

‡ Tumor was designated A12.HA of female origin and was passaged serially in vivo.

§ Tumor was designated A12.HC of male origin and was passaged serially in vivo.

‖ Tumor was designated A12.SBA of female origin and was passaged serially in vivo.

than 10^7 cells). As previously observed with polyoma and Rous tumors the threshold dose decreased during the first four to five passages, but was then stable during the subsequent five passages studied. Each of the tumors was dose titrated determining the threshold dose necessary for growth in all untreated recipients and in mice whole-body X-irradiated with 350 R 24 hr previously.

Control tumors: Three mouse sarcomas induced by the RSV-SR virus were used for control purposes. Two were induced in vivo: RSC of A/Sn origin and RCB of A.CA origin (21). The third tumor designated TCRBA was induced by RSV-SR infection of CBA mouse embryo cell cultures in vitro. All these cell lines have previously been found to contain transplantation antigen common for RSV-SR mouse sarcomas (21).[1] A "spontaneous" mammary carcinoma designated SH2 developed in a C3H female carrying the MTV virus.

Transplantation tests: Crude suspensions of tumors were used for immunization purposes. The tumor tissue was pressed through a 60-mesh stainless steel screen into sterile basal salt solution (BSS), containing 100 μg streptomycin and 100 IU penicillin per ml to make a 10%

[1] N. Jonsson and H. O. Sjögren, unpublished data.

suspension. In tests for immunogenicity 0.1 ml doses of such suspensions were inoculated subcutaneously in the flanks four to eight times with 1–3 wk intervals. Immunity tests were performed 1–2 wk after the last dose. When using tumors which were genetically compatible with the animals to be treated, the cell suspensions were X-irradiated with 8000 R immediately

TABLE II

Results of three Experiments with Tumor A12.SBA Inoculated into Syngeneic (A × CBA) F_1 Hybrid Recipients Immunized with 4 Adeno 12–Induced Tumors and Adenoviruses 5, 7, 12 or 18, Respectively

Pretreatment*	Takes‡ in syngeneic recipients										
	Unirradiated Experiment 1			Irradiated (350R)§ Experiment 2				Irradiated (350 R) Experiment 3		
	10^6c	10^5c	TPD 50‖	10^6c	10^5c	10^4c	TPD 50	10^6c	10^5c	TPD 50
Untreated	5/5	3/10	1.6×10^5	5/5	4/4	0/5	3.2×10^4	4/4	2/5	1.3×10^5
SH2-HR	5/5	2/5	1.3×10^5	5/5	3/5		8×10^4	5/5	2/5	1.3×10^5
A12.SBA-HR	0/5	0/5	$\geq 3.2 \times 10^6$	0/5	0/5		$\geq 3.2 \times 10^6$	1/5¶	0/5	$\geq 2 \times 10^6$
A12.HA-HR	1/5	0/5	2×10^6	0/5			$\geq 3.2 \times 10^6$	0/5	0/5	$\geq 3.2 \times 10^6$
A12.HC-HR								2/5	2/5	5×10^5
A12.HD-HR								3/5	0/5	8×10^5
MAS								5/5	0/5	3.2×10^5
Ad.12 virus	—	1/5	$\geq 2 \times 10^5$	—	0/5		$\geq 3.2 \times 10^5$	1/5	0/5	$\geq 2 \times 10^6$
Ad.18 virus				—	0/4		$\geq 3.2 \times 10^5$			
Ad. 7 virus	—	1/5	$\geq 2 \times 10^5$					3/5	1/5	5×10^5
Ad. 5 virus								5/5	3/5	8×10^4
Polyoma virus	5/5	4/5	5×10^4	5/5	5/5	0/5	3.2×10^4			

* Pretreatment was carried out with four to eight doses of crude tumor cell suspensions (irradiated with 8000 R) of a SH2 spontaneous mammary carcinoma, four different adeno 12 mouse tumors (including the test tumor) and MAS human cells used for virus production. Treatment was also performed with three to four doses of adeno 5, 7, 12 and 18 viruses and polyoma virus intraperitoneally and subcutaneously with the last dose 1 wk prior to test.

‡ Figures denote the fraction of mice developing progressively growing tumors.

§ X-Ray–irradiation performed 24 hr prior to the test isografting.

‖ TPD 50 = the 50 per cent end points for tumor production calculated by the method of Kärber (22).

¶ Two other recipients developed distinct tumors which subsequently regressed (cf. Fig. 1).

before inoculation. This is denoted by "HR" (heavily irradiated) after the designation of the tumor in Fig. 1 and the tables. This dose is known to "sterilize" a sufficient fraction of the cells to inhibit any tumor outgrowth even from very large numbers of cells.

The pretreated groups of mice were challenged by subcutaneous inoculation of known numbers of trypan blue–unstained cells obtained by trypsinizing tumor tissue harvested in vivo. In the majority of the experiments (as specified in Results) the recipients were whole-body irradiated with 350 R 24 hr prior to challenge isografting. This treatment has previously been found to abolish any nonspecific stimulation of subsequent primary immune responses (2)

and would thus secure any demonstrated immunity as a true anamnestic response. This type of irradiation also decreases a subsequent secondary response although to a much lesser degree. Low levels of immunity might accordingly be missed when irradiated animals are used. Challenges were performed with at least two different cell doses in each experiment and the 50% end points for tumor production (TPD 50) were calculated by the method of Kärber (22).

RESULTS

Isograft Immunity Induced by Adeno 12 Tumor Cells.—Three different mouse tumors induced by adeno 12 virus were isografted into mice pretreated with four to eight doses of X-ray-killed or genetically incompatible adeno 12 tumor

TABLE III

Results of three Experiments with Tumor A12.HA Inoculated‡ into Syngeneic Recipients Immunized with Two Different Adeno 12-Induced Tumors or Adeno-viruses 7 and 12

Pretreatment*	Takes‡ in genetically compatible recipients											
	Irradiated (350R)§ Experiment 1				Irradiated (350R) Experiment 2				Unirradiated Experiment 3			
	3×10^6 c	3×10^5 c	3×10^4 c	TPD 50‖	3×10^6 c	3×10^5 c	3×10^4 c	TPD 50	3×10^7 c	3×10^6 c	3×10^5 c	TPD 50
Untreated	4/4	4/4	3/5	2.5×10^4	5/5	5/5	1/5	6.3×10^4	4/4	3/10	0/5	2.5×10^6
SH2-HR	4/4	4/4	—	$\leq 10^5$	4/4	4/4		$\leq 10^5$	5/5			$\leq 10^7$
A12.HA-HR	2/4	0/4	—	3×10^6	0/4	0/5		$\geq 10^7$	4/4			$\leq 10^7$
A12.SBA-HR	1/4	1/4	—	3×10^6	1/5	0/5		6.3×10^6				
A12.SBA					4/5	0/5		1.6×10^6	4/4			$\leq 10^7$
Ad. 12 virus	—	1/2	—		4/4	3/5		2.5×10^5	5/5	5/10		3×10^6
Ad. 7 virus	—	4/4	—	$\leq 10^5$	1/2	3/5		8×10^5	5/5	3/10		5×10^6
Polyoma virus					5/5	5/5		$\leq 10^5$		3/8		4×10^6

*, ‡, §, and ‖ compare corresponding footnotes to Table II.

cells in parallel with two kinds of controls: untreated mice or animals pretreated with a "spontaneous" mammary carcinoma. The recipients were usually whole-body irradiated with 350 R to abolish nonspecific effects of the pretreatments. Tables II, III, and IV summarize the results. Recipients treated with adeno 12 tumor material showed a very clear-cut transplantation immunity when compared to the simultaneously tested controls. The immunity was clearly demonstrated in all the combinations studied and was usually seen as a markedly reduced take frequency (TPD 50 usually being 10–100 times higher than in controls) but also as a prolonged latency period, decreased growth rate, and in one experiment with tumor A12.SBA as regression of initially growing tumors (cf. Fig. 1). The regressions occurred in one animal rapidly after the tumor had appeared and in another after the tumor had grown for about 5 wk with a maximum mean diameter of 14 mm.

In order to exclude further any nonspecific immunogenicity of the adeno 12 tumors the following experiments were performed: Mice immunized with adeno 12 tumor cells were challenged with genetically compatible Rous sarcomas. Serving as controls were mice treated with Rous sarcoma cells (expected to be immune), animals treated with polyoma virus or the spontaneous mammary carcinoma SH2 (expected to be nonimmune), and finally untreated recipients. The results are presented in Tables V and VI and show that pretreatment with the adeno 12 tumors A12.HA and A12.SBA did not induce any immunity

TABLE IV

Results of Two Experiments with Tumor A12.HD Inoculated into Syngeneic or Semisyngeneic F_1 Hybrid Recipients Immunized with Three Different Adeno 12–Induced Mouse Tumors or Adenoviruses 5, 7, and 12

Pretreatment*	Takes‡ in genetically compatible, irradiated§ (350R) recipients					
	Experiment 1			Experiment 2		
	10^6	10^5	TPD 50‖	10^6	10^5	TPD 50
Untreated	5/5	2/5	1.3×10^5	5/5	4/4	$\leq 3.2 \times 10^4$
SH2-HR	3/3		$\leq 3.2 \times 10^5$	5/5	5/5	$\leq 3.2 \times 10^4$
A12.HD-HR				1/5	0/5	2×10^6
A12.HA-HR	0/5		$\geq 3.2 \times 10^6$			
A12.SBA-HR	3/5		8×10^5	1/5	0/5	2×10^6
MAS cells				5/5	5/5	$\leq 3.2 \times 10^4$
Ad. 12 virus				2/5¶		1.3×10^6
Ad. 7 virus				2/4		10^6
Ad. 5 virus				5/5		$\leq 3.2 \times 10^5$
Polyoma virus	5/5		$\leq 3.2 \times 10^5$			

*, ‡, §, and ‖ compare corresponding footnotes to Tables II and III.

¶ Another recipient developed a distinct tumor which subsequently regressed (cf. Fig. 2).

against Rous sarcomas. The simultaneously challenged mice pretreated with Rous sarcoma cells did show an immunity as expected, thus proving that the experiments were performed in a way suitable for detection of graft immunity if there was any.

Isograft Immunity Induced by Adeno 12 and Adeno 7 Virus Infection.—It has been demonstrated with other viral tumors (induced by polyoma, SV40, and Rous virus) that infection of animals with an oncogenic virus causes a specific immunity to isografts of tumors induced with this virus, but does not affect the growth of similarly grafted tumors of other viral origin. Trentin et al. (17) reported that adenovirus type 12 also induces this type of specific isograft immunity.

In order to take advantage of the unusual possibility of studying the

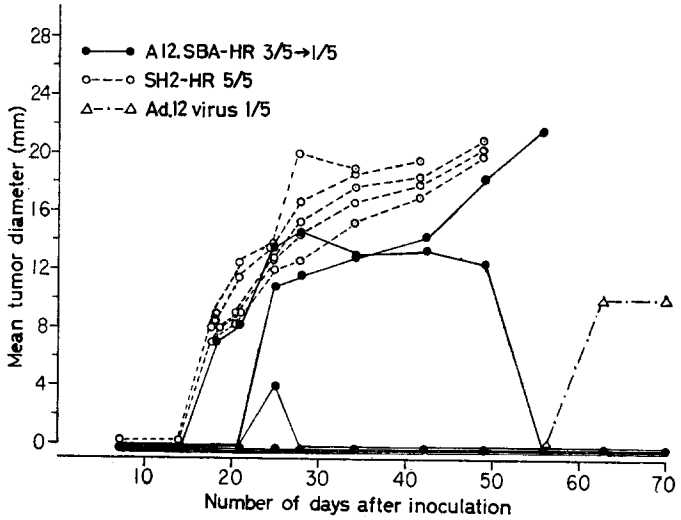

FIG. 1. Growth of 10^6 viable A12.SBA tumor cells in syngeneic (A × CBA) F_1 hybrids, whole-body-irradiated 350R 24 hr previously. One group (○-----○) was pretreated eight times with X-ray-killed SH2 cells (a spontaneous mammary carcinoma of C3H origin); another group (●——●) treated by seven inoculations of similarly killed A12.SBA tumor cells; and a third group (△—·—△) pretreated with four doses of adeno 12 virus subcutaneously. Figures denote the take frequencies. Each curve represents the growth of one tumor. Note the regression of two tumors in the group pretreated with A12SBA cells and the very small variation of tumor size within the group immunized with SH2 cells. (Compare Table II, Experiment 3).

TABLE V

Result of an Experiment with Rous Sarcoma RSC Inoculated into Whole-Body-Irradiated (350R) Semisyngeneic (A × CBA) F_1 Hybrid Recipients Immunized with Two Different Adeno 12-Induced Tumors

Pretreatment*	Takes‡ after inoculation of		
	10^4 cells	10^3 cells	TPD 50§
Untreated	4/5	1/5	3.2×10^3
SH2-HR	4/4	—	$\leq 3.2 \times 10^3$
RCB	2/5	0/5	1.3×10^4
A12.HA-HR	5/5	3/5	8×10^2
A12.SBA-HR	5/5	3/5	8×10^2
Polyoma virus	5/5	2/5	1.3×10^3

* Pretreatment was performed with crude irradiated suspensions of a spontaneous mammary carcinoma SH2 and with two adeno 12 mouse sarcomas, A12.HA and A12.SBA. As positive control mice pretreated with the Rous sarcoma RCB were included.

‡ Compare corresponding footnote in previous tables.

§ Compare corresponding footnote,‖, Table II.

TABLE VI

Result of an Experiment with Rous Sarcoma RCB Inoculated into Whole-Body-Irradiated (350R) Semisyngeneic (ACA × CBA) F_1 Hybrid Recipients Immunized with the Adeno 12 Virus-Induced A12.HA Tumor

Pretreatment*	Takes‡ after inoculation of		
	10^5 cells	10^2 cells	TPD 50§
Untreated	5/5	5/5	$\leq 3.2 \times 10$
TCRBA-HR	0/5	1/5	2×10^3
A12.HA-HR	5/5	5/5	$\leq 3.2 \times 10$

* Pretreatment performed with irradiated cells of the adeno 12 tumor A12.HA and the in vitro induced mouse Rous sarcoma TCRBA, respectively.

‡ Compare corresponding footnote, ‡, Table II.

§ Compare corresponding footnote, ‖ Table II.

TABLE VII

Summary of the Immunogenicity and Immunosensitivity of the Neoplasms Tested in this Investigation

Immunogenicity	Immunosensitivity				
	A12.HA	A12.HD	A12.SBA	RSC	RCB
A12.HA	+	+	+	−	−
A12.HC			+		
A12.HD		+	+		
A12.SBA	+	+	+	−	
SH2	−	−	−	−	
RCB				+	
Ad. 12 virus	+	+	+		
Ad. 7 virus	+	+	+		
Ad. 5 virus		−	−		
MAS cells	−	−	−		
Polyoma virus	−	−	−	−	

+, positive reaction; and −, no reaction.

specificity of this immunity that the more or less closely related adenoviruses offer, experiments were carried out in which the effect of adenovirus 12 infection was compared with the effects of infection with adeno types 7 and 5 as well as control treatment with material from uninfected human cells prepared with the same technique as the virus materials. The virus-treated animals were tested for isograft immunity in parallel with cell-pretreated mice in order to be able to compare the strength of immunities induced. The results are presented in

Tables II–IV and Figs. 1 and 2. It appears quite clear that adenovirus 12 induces an isograft immunity against the adeno 12 tumors while adenovirus 5 and the control MAS cell preparation, and the polyoma virus do not. Interesting enough, infection with adenovirus type 7 also induced an immunity against adeno 12 isografts. This immunity was in some experiments as strong as that induced by adeno 12 (Experiment 2, Table III, and Experiment 2, Table, IV)

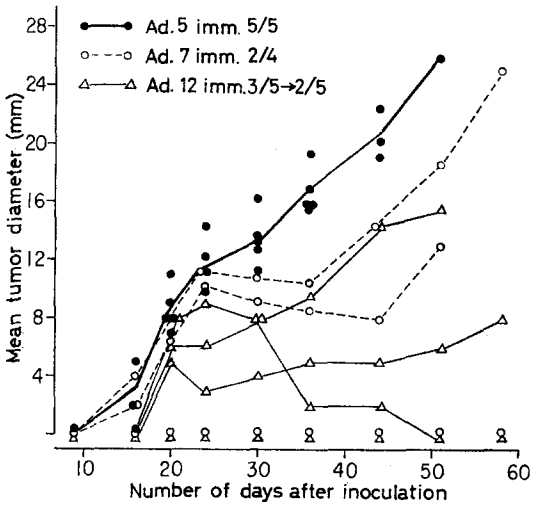

FIG. 2. Growth of 10^6 viable A12.HD tumor cells in semisyngeneic (A × C3H) F_1 hybrids, whole-body-irradiated 350R 24 hr previously. One group (●——●) was pretreated four times with adeno 5 virus subcutaneously; another group (○-----○) was treated with four inoculations of adeno 7 virus; and the third group (△——△) was similarly treated with adeno 12 virus. Figures denote take frequences. The tumors in the first group grew very similarly and are represented by an average curve. In the other groups each curve represents the growth of one tumor. Note the regression of one tumor in an adeno 12 virus-treated recipient and the very small variation of growth within the group treated with adeno 5 virus.

and in other experiments weaker (Experiment 3, Table II). It was demonstrable with all the three tumors used for isograft challenge (A12.SBA, A12.HA, and A12.HD).

In order to exclude the possibility that the demonstrated immunity of adeno 7 virus-infected mice against adeno 12 isografts was due to accidental contamination with adeno 12 virus, the serum of the resistant mice were tested for antibody activity against adeno 12 virus. For orientation, the gel-precipitation technique was first used. Antibodies against adeno type 7 virus were detected in sera from adeno 7-treated mice but none were found against adeno type 12. The same sera also contained HI antibodies against type 7 in a titer of 1:512. They contained no HI antibody activity against adeno type 12. Serum of adeno

12–infected mice, on the other hand, were found to contain high antibody titers against adeno 12. These results appear to rule out a contamination of the adeno 7–treated animals with adeno type 12 virus.

DISCUSSION

The results demonstrate that mouse tumors induced by the human adenovirus type 12 possess common specific transplantation antigen(s). These antigen(s) could be demonstrated as specific immunosensitivity of the tumor cells and as immunogenicity as well (Table VII).

The relationship between the demonstrated specific transplantation antigen(s) and the antigens previously detected by other techniques is unknown. Although the mouse tumor cells do not produce infectious virus it is quite possible that virion antigens are present in association with the cell membrane in such a way that they might be demonstrated as specific transplantation antigens. The neoantigen as detected by the FA technique on fixed cells appears to be localized exclusively intracellularly (23), which makes it unlikely that it is identical to the specific transplantation antigen(s). The demonstration that adeno 7 virus infection induces an immunity to adeno 12 tumor isografts indicates that the transplantation antigens induced by adeno 12 and adeno 7 are at least partially cross-reacting. In contrast, the neoantigens of adeno 7 and adeno 12 tumors do not cross-react (16). This difference in cross-reactivity is a further indication that the transplantation antigen(s) are not identical with the neoantigens. The single experiment in which adeno 18–infected mice were included also indicated, although not conclusively, that this virus is capable of inducing an immunity. This is not surprising since adeno virus 18 is in many respects closely related to adeno 12 virus, expressed also as a partial cross-reaction between their neoantigens.

Also other questions are raised by the demonstration that the adeno virus types 7, 12, and 18 induce an isograft immunity against adeno 12 tumors while adeno 5 virus infection fails to do so. Might it be a general property of *oncogenic* types of adenovirus to induce transplantation antigens which at least cross-react partially with each other similarly to those induced by adeno 7 and 12 viruses? The *nononcogenic* virus types might, as adenovirus 5, either be incapable of inducing specific transplantation antigens or might cause analogous antigens although of a distinct specificity.

Similar to the neoantigens, the specific transplantation antigens are maintained in the course of serial passage of the tumor cells in vivo or in vitro. This makes it very likely that at least part of the virus genome is present in these cells and determines the continuous antigen synthesis. Direct proof for this has recently been obtained by DNA-RNA hybridization experiments both in the polyoma and the adeno 12 tumor systems. In both systems a portion of the rapidly synthesized RNA of "virus-free" tumor cells has been reported to be

complementary to the DNA of the virus in question (24, 25). Of interest in this connection is the finding of Green et al.[2] that the RNA isolated from adeno tumor cells hybridizes not only with the DNA of the inducing virus type but to an appreciable extent also with the DNA of some other adenovirus types. In these experiments the RNA of adeno 12 tumors hybridized with adeno 18 virus DNA but not with DNA of adeno type 7.

The specific immunity to adeno 12 tumors after treatment with adeno 12 tumor cells, or after infection with adeno 12 or 7 viruses can also be demonstrated in vitro by the colony inhibition (CI) technique (26). It was possible to demonstrate both specific humoral antibody activity and cellbound immunity.[3] These results fully confirm the in vivo findings presented. The in vitro experiments also showed a cross-reaction between mouse and hamster tumors induced by adeno 12. The fact that the CI method discovered antibodies active against adeno 12 tumor cells in the adeno 7–infected mice indicates that it measures the same antigen as the transplantation immunity tests and not the neoantigen. This in vitro technique makes it possible to study further cross-reactivity of the specific transplantation antigens in tumors of different species and might also allow a closer investigation of the relationship between these antigens and the neoantigens and virion antigens.

SUMMARY

A specific isograft resistance against three different mouse adeno 12 sarcomas was demonstrated in mice treated with four to eight doses of viable or X-ray–killed adeno 12 mouse tumors. Whole-body X-ray irradiation with 350 R 24 hr previous to the test challenge did not abolish the resistance, indicating that it was due to a true anamnestic immune reaction. This was further proven by the finding that similar treatment with tumors of other origin did not induce any immunity, nor did the treatment with adeno 12 tumor material induce any immunity against two neoplasms of Schmidt-Ruppin-Rous viral origin.

The previous report by Trentin et al. (17) that adeno 12 infection leads to a specific transplantation immunity was fully confirmed. When the specificity of this virus-induced immunity was studied it was discovered that besides adeno virus type 12, type 7 and probably type 18 also gave the same type of resistance while adenovirus type 5 did not. A contamination of the adeno 7–infected mice with adeno type 12 was excluded by testing pooled sera from these animals for anti–adeno 12 CF or HI antibodies.

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[2] M. Green, personal communication.

[3] I. Hellstrom and H. O. Sjögren, data to be published.

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