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Induction of Murine Tumors in Adult Mice by a Combination of Either Avian Sarcoma Virus or Human Adenovirus and Syngeneic Mouse Embryo Cells[1]

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ABSTRACT

Primary murine Rous sarcoma was produced in adult mice of seven strains, C57BL/6, DBA/2, BALB/c, C3H/He, CBAJ, AKR, and DDD, by s.c. inoculation of a mixture of 5×10^6 chicken tumor cells containing Schmidt-Ruppin Rous sarcoma virus and 9- to 12-day-old mouse embryo cells (MEC) (2×10^6) of the syngeneic strain. The sarcoma developed at the site of injection in almost all mice tested, but there were some differences in the latent period and the survival time among mouse strains. When the number of cells inoculated was reduced to 5×10^4 for chicken tumor cells induced by the Schmidt-Ruppin strain of Rous sarcoma virus (SR-CTC) and 2×10^4 for MEC, no tumor was produced in C3H/He mice. These tumors had strain specificity and the Schmidt-Ruppin strain of Rous sarcoma virus genome in masked form.

The tumor at the site of injection originated in the embryo cells injected along with SR-CTC. This was confirmed by CBAT6/T6 marker chromosome analysis of the tumor cells of CBA mice induced with SR-CTC plus CBAT6/T6 MEC and also confirmed by transplantation of a C57BL/6 × C3H/He F_1 tumor which had been induced with SR-CTC plus C3H/He or C57BL/6 MEC.

Tumor induction in adult mouse by a mixture of virus and syngeneic 9- to 14-day-old embryo cells was tested for human adenovirus serotype 12 (Ad12) and simian virus 40. Primary Ad12 tumor was also induced in adult CBA, C3H/He, and DDD mice by $4 \times 10^{5\text{ to }6}$ 50% tissue culture infective dose of Ad12 with 5×10^6 syngeneic embryo cells. This tumor contained Ad12 T-antigen-positive particles in cells. But in the case of simian virus 40, the tumor did not appear for about 300 days of observation.

INTRODUCTION

Primary animal tumors provide useful experimental models for cancer chemotherapy and immunochemotherapy (6). For the induction of primary tumors in experimental animals, there are many agents such as chemical carcinogens, radiation, and oncogenic viruses. In the cases of tumor induction by RSV,[3] the age of the animals is considered to be extremely important (1, 5, 7), because the tumor incidence decreases with age. Therefore, newborn or young animals have usually been used for tumor induction because of immunological considerations, although genetic factors also influence the susceptibility of mice to oncogenesis of RSV (5, 19, 20).

We reported previously that murine Rous tumors could be induced in adult mice by inoculation of SR-CTC or SR-RSV, together with syngeneic embryo cells without the aid of immunological suppression (18). We suggested that oncogenicity of SR-RSV would manifest itself in the adult mice under conditions in which suitable target cells exist.

Binger *et al.* (3) reported the usefulness of the primary rat brain tumor induced with RSV in neonatal Fischer rats as a model for chemotherapeutic or immunotherapeutic screening. Considering its convenience in handling, we attempted to use our system for primary tumor induction by oncogenic viruses in several strains of adult mice for studies of biological status and therapy of tumor-bearing mice.

MATERIALS AND METHODS

Mice. The following strains were used: C57BL/6; DBA/2; BALB/c; C3H/He; CBA; AKR; and DDD. Mice older than 5 weeks and pregnant mice were obtained from 2 sources: closed colony mice of C57BL/6, DBA/2, C3H/He, CBA, AKR, DDD, and C57BL/6 × C3H/He F_1 (hereafter called B6C3F_1) strains were obtained from the Animal Center, Institute of Medical Science, University of Tokyo; and specific-pathogen-free inbred mice of BALB/c and C3H/He strains were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan. They were given cubed chow (Oriental Co., Tokyo, Japan) and water *ad libitum* in an air-conditioned animal room.

MEC. Nine- to 12-day-old mouse embryos, aseptically removed from pregnant mice, were washed with PBS(−). After removal of their heads, legs, and intestines, they were washed again with PBS(−), minced with scissors, and digested with 0.1% trypsin in PBS(−) at room temperature for 15 to 20 min. The cell suspension was centrifuged after adding calf serum at 10% v/v, and the precipitate was mixed with approximately 5 volumes of tissue culture medium (MEM containing 10% calf serum). The cell suspension was filtered through a stainless steel mesh to remove clusters and was centrifuged at 800 rpm for 3 min. The cells were suspended in MEM, and the viable cells were counted by the dye exclusion test.

Chickens and Preparation of SR-CTC. One- to 2-day-old chickens were obtained from 2 sources: "rockhorn" chickens (Plymouth Rock × White Leghorn) from Goto Chicken Farm in Gifu Prefecture, Japan; and White Leghorn line S (leukemia, Mareck disease free) from Nippon Institute of Biological Science in Tokyo, Japan. Ascites cells taken at the seventh day after inoculation of SR-DDD or SR-C3H/He ascites sarcoma lines, both originally induced by SR-RSV (16, 17, 22), were washed and suspended in MEM at a density of 2×10^6 cells in 0.1 ml and were inoculated s.c. into the wing webs of 1- to 2-day-old chickens. The chicken tumor that developed in the wing web was harvested

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[3] The abbreviations used are: ASV, avian sarcoma virus; SR-CTC, chicken tumor cells induced by the Schmidt-Ruppin strain of Rous sarcoma virus; SR-RSV, Schmidt-Ruppin strain of Rous sarcoma virus; MEC, mouse embryo cells; PBS(−), calcium- and magnesium-free phosphate-buffered saline (0.2 g KCl-0.2 g KH_2PO_4-8.0 g NaCl-1.15 g Na_2HPO_4 per liter); MEM, Eagle's minimal essential medium; Ad12, human adenovirus serotype 12; SR-RSV, Schmidt-Ruppin strain of Rous sarcoma virus; $TCID_{50}$, 50% tissue culture ineffective dose.

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aseptically 3 to 4 weeks after the inoculation. A suspension of SR-CTC was obtained by trypsinization of the chicken tumor with 0.25% trypsin in PBS(−) at room temperature for 20 to 30 min after treatment with 200 units of hyaluronidase dissolved in 10 ml of PBS(−). The cell suspension was processed further in the same way as the last part of the preparation of MEC.

Ad12 and Antibody for Ad12 T-Antigen. Ad12 and fluoresceinisothiocianate-labeled antibody to Ad12 T-antigen were kindly supplied by Dr. N. Yamaguchi and Dr. H. Shimojo, Institute of Medical Science, Tokyo University.

Induction of Primary Murine SR-RSV Sarcoma. The procedure for the induction of primary murine SR-RSV sarcoma in adult mice was given in detail previously (18). In brief, mice were inoculated s.c. in the right groin area with a mixture of 5×10^6 SR-CTC and 2×10^6 MEC in 0.25 to 0.3 ml of MEM soon after mixing. The mice were observed twice a week during the period of maximum tumor development and then once a week for about 1 year.

Wing Web Test for Detection of SR-RSV Genome in the Tumor. We usually used the wing web test to detect the existence of SR-RSV genome in the tumor. Details of the wing web test were given previously (22). In this paper, 2×10^6 cells derived from, or chopped pieces of, primary murine SR-RSV sarcoma were injected bilaterally into the wing web of 1- to 2-day-old chickens.

RESULTS

Incidence of Tumors in Adult Mice Inoculated with a Mixture of MEC and SR-CTC. In order to test possible differences in sensitivity among mouse strains to tumor induction by SR-CTC plus MEC, adult mice of 7 different inbred strains were given injections of a mixture of 5×10^6 fresh SR-CTC and 2×10^6 syngeneic MEC. The incidence of murine sarcoma in the 326 mice tested is presented in Table 1. There is no significant difference in susceptibility among the strains. Tumors developed at the sites of inoculation only when MEC and the inoculated mouse were in syngeneic relation. These mouse tumors were strain specific as shown by transplantation tests. The SR-RSV genome was confirmed to exist in the tumor by the wing web test. Inoculation with SR-CTC alone or MEC alone did not induce any tumor during the observation period of about 1 year as reported previously (18).

Latent Period and Survival Time. The latent period of tumor growth as well as the survival time of the adult mice which had been given injections of a mixture of SR-CTC and MEC differed considerably. Among the strains tested, as shown in Table 2, there was a wide range in the latent period and the survival time in comparison with those of SR-RSV ascites tumors established from SR-RSV murine sarcoma in newborn mice. A possible difference between male and female mice with respect to these responses was compared in C3H/He, CBA, and DDD mice. The latent period was significantly longer in females than in males (Table 3).

Table 1

Incidence of Rous sarcoma in adult mice given injections of a mixture of SR-CTC and syngeneic embryo cells

Each mouse received a mixture of 5×10^6 SR-CTC and 2×10^6 syngeneic MEC. CBAT6/T6 and CBA mice are pooled under the designation CBA.

Mouse strain	H-2 haplotype	No. of mice with tumors/no. inoculated	% of tumor
C57BL/6	*b*	26/26	100
DBA/2	*d*	20/20	100
BALB/c	*d*	24/24	100
C3H/He	*k*	111/112	99.1
CBA/J	*k*	33/33	100
AKR	*k*	10/10	100
DDD	*s*	100/101	99.0

Table 2

Latent period and survival time of adult mice given injections of a mixture of SR-CTC and MEC

Each mouse received a mixture of 5×10^6 SR-CTC and 2×10^6 syngeneic MEC.

Mouse strain	No. of mice	Latent period (days) Mean	Latent period (days) Range	No. of mice[a]	Survival time (days) Mean	Survival time (days) Range
C57BL/6	26	20.6	12–42	24	66.5	27–190
DBA/2	20	9.5	7–21	18	105.9	78–151
BALB/c	24	22.8	14–35	5	105.4	103–110
C3H/He	111	19.3	13–31	25	127.6	80–244
CBA	33	28.7	18–73	18	126.1	72–184
AKR	10	16.9	7–34	8	75.3	41–92
DDD	92	15.2	7–28	49	52.6	37–97

[a] Fewer mice were used for the survival time study because the observation of some was terminated before death in order that these mice might be used for wing web test, transplantation test, chromosome analysis, and specimens, *etc.*

Effect of Age and Inoculation Site. The incidence of murine SR-RSV sarcoma in newborn, suckling, and adult mice was compared in C57BL/6, C3H/He, and DDD mice. As shown in Table 4, the tumor incidence and latent period in newborn mice (within 24 hr) inoculated with SR-CTC were the same as in adult mice which were inoculated with SR-CTC plus MEC. The incidence in suckling mice decreased with increasing age at the time of injection. In the case of intracranial injection of SR-CTC alone in adult mice, there were a prolongation of the latent period and difficulty in determination of the time of appearance of tumor.

Influence of the Inoculum Size and of the Recipient Mouse Strain on the Incidence of Tumor Induction. Three concentrations of C3H/He MEC and SR-CTC, varying 10-fold, were tested for tumor inducibility in C3H/He and B6C3F$_1$ mice. With the large inoculum (5×10^6 SR-CTC and 2×10^6 C3H/He MEC) and also with the medium inoculum (5×10^5 SR-CTC and 2×10^5 C3H/He MEC), 100% tumor induction was observed in the syngeneic C3H/He mice.

The incidence of tumors was lower in B6C3F$_1$ mice; the large and medium inocula induced tumors in 50 and 12.5% of the mice inoculated, respectively. No tumors were produced with a mixture of 2×10^4 C3H/He MEC and 5×10^4 SR-CTC in either strain of mice (Table 5).

Target Cells for SR-RSV. The possibility of the existence of target cells for SR-RSV in MEC was reported previously (18). To ascertain which cells, either the embryo cells or the host cells, became transformed into SR-RSV murine tumors by SR-CTC, adult B6C3F$_1$ mice were inoculated with C3H/He or C57BL/6 MEC together with SR-CTC. The cells that were derived from a tumor that appeared in an F$_1$ mouse were transplanted to adult C3H/He, C57BL/6, and B6C3F$_1$ mice. Transplantation of the primary tumor produced in the F$_1$ mouse succeeded only when there was a syngeneic relationship between the embryo cells used and the mice which received transplantation, as shown in Table 6. It is known that CBAT6/T6 mice are identical to CBA mice except that they carry the T6 marker chromosome. Therefore, adult CBA mice were given injections of a mixture of CBAT6/T6 MEC and SR-CTC. The T6 marker chromosome of the tumor cells, thus produced in the CBA mice, was analyzed mainly by following the method of Kissoglou *et al.* (10) as reported previously (16). The T6 marker

Table 3

Differences between adult male and female mice in incidence of primary Rous sarcoma

Each mouse received a mixture of 5 × 10^6 SR-CTC and 2 × 10^6 MEC.

	No. of mice		% with tumors		Latent period (days)		p
Mouse strain	M	F	M	F	M	F	M *vs.* F[a]
C3H/He	66	44	100	93.1	20.5 ± 10.4[b]	34.9 ± 18.5	<0.001
CBA	20	12	100	100	18.5 ± 1.5	68.0 ± 47.5	<0.001
DDD	92	9	100	88.8	15.2 ± 5.8	29.1 ± 6.5	<0.001

[a] Determined by the Kolmogorov-Sminov test. χ^2 comparison of these 3 fractions gives: χ^2 = 13.816; p = 0.001.
[b] Mean ± S.D.

Table 4

Tumor induction in mice with Rous sarcoma virus (SR-RSV)

Newborn mice were given s.c. or i.p. injections of 2 × 10^6 SR-CTC. Adult mice were given s.c. injections of a mixture of 5 × 10^6 SR-CTC and 2 × 10^6 MEC or i.c.[a] injections of 2 × 10^6 SR-CTC.

		Mouse strain inoculated					
		C57BL/6		C3H/He		DDD	
Age of mice	Site of injection	No. of mice with tumors/ no. of mice inoculated	Range of latent period (days)	No. of mice with tumors/no. of mice inoculated	Range of latent period (days)	No. of mice with tumors/no. of mice inoculated	Range of latent period (days)
Adult	i.c.	16/18	16–59	6/6	14–98	2/6	120–153
	s.c. with MEC	26/26	12–42	111/112	13–31	100/101	7–41
Newborn							
Within 24 hr	s.c., i.p.	24/24	13–17	29/29	8–56		
1–3 days	s.c., i.p.			33/54	19–210	12/24	22–49

[a] i.c., intracerebral.

Table 5

Tumor induction in C3H/He mice and B6C3F$_1$ mice by a mixture of SR-CTC and C3H/He MEC

		C3H/He			B6C3F$_1$		
No. of SR-CTC	No. of C3H/He MEC	No. of mice with tumors/ no. inoculated	Latent period (days)	Survival (days)	No. of mice with tumors/ no. inoculated	Latent period (days)	Survival (days)
5 × 10^6	2 × 10^6	6/6	21.6 ± 0.82[a]	67.6 ± 15.5	4/8	79.0 ± 12.5	145.5 ± 0.7
5 × 10^5	2 × 10^5	9/9	32.0 ± 11.9	112.8 ± 14.1	1/8	45	145
5 × 10^4	2 × 10^4	0/6			0/8		

[a] Mean ± S.D.

Table 6

Transplantability of adult B6C3F$_1$ tumors induced by injection of a mixture of SR-CTC and C3H/He or a mixture of SR-CTC and C57BL/6 MEC

Tumor cells or tumor mass was injected i.p.

	B6C3F$_1$ tumor induced by SR-CTC and C3H/He MEC				Tumor mass induced by SR-CTC and C57BL/6 MEC	
	5 × 10^6		1 × 10^6			
Mouse strain	No. of mice with tumors/ no. inoculated	Survival (days)	No. of mice with tumors/ no. inoculated	Survival (days)	No. of mice with tumors/ no. inoculated	Survival (days)
B6C3F$_1$	5/5	23.2 ± 8.0[a]	5/5	24.6 ± 4.4	ND[b]	
C57BL/6	0/5		0/5		5/5	50.0 ± 7.1
C3H/He	5/5	16.9 ± 6.9	5/5	23.8 ± 6.8	0/6	

[a] Mean ± S.D.
[b] ND, not determined.

chromosomes were found in all cells of the CBA SR-RSV tumors in the metaphase.

To test whether or not the target cells for SR-RSV exist in brain and kidney, the brain of a C3H/He mouse embryo and a tissue culture line of C3H/He newborn kidney were used. Brain cells were obtained from the brain of a 9- to 12-day-old C3H/He embryo by trypsinization. The tissue culture line of C3H-2K cells originating from the kidney tissue of a newborn C3H/He mouse (23) was kindly supplied by Dr. Y. Hirokawa, National Institute of Health, Japan, and was cultured in MEM containing 10% calf serum, 10% tryptose phosphate broth, 100 μg of streptomycin, and 100 units of penicillin per ml. Brain cells (4×10^6) or 2×10^6 C3H-2K cells were mixed with 5×10^6 SR-CTC and inoculated s.c. in the right groin area of adult C3H/He mice. Tumors developed at the site of injection in both cases (Table 7), but the latent periods were longer than in the case of MEC SR-CTC injection.

Ad12 Tumor Induction In Adult Mice by Injection of Ad12 with Syngeneic Embryo Cells. Induction of tumor by Ad12 is known to be difficult even in thymectomized newborn mice (9, 14) compared with newborn hamsters (21). However, the results described above prompted us to try to induce Ad12 tumor in adult mice by the same procedure. Adult CBA, C3H/He, and DDD mice were inoculated s.c. with a mixture of various concentrations of Ad12 and syngeneic embryo cells as described in "Materials and Methods." Murine Ad12 tumors developed at the sites of inoculation in a syngeneic relation between MEC and the mouse inoculated. The results are summarized in Table 8. In the case of the CBA mouse, the incidence of Ad12 tumors was 100% by doses of 2×10^6 $TCID_{50}$ (on human embryo kidney cells) of Ad12 with 2×10^7 MEC and 4×10^5 $TCID_{50}$ of Ad12 with 4×10^6 MEC. But the incidences of tumor by 4×10^5 $TCID_{50}$ of Ad12 with 4×10^6 MEC in C3H/He and DDD were 25.9 and 47.6%, respectively. No tumors appeared by the above concentrations of Ad12 without MEC in adult mice for 1 year of observation. The tumor incidence in newborn hamsters as control was 8 of 11 with 4×10^6 $TCID_{50}$ Ad12 and 3 of 5 with 4×10^5 $TCID_{50}$ of Ad12. The tumor did not develop in newborn mice of C3H/He and DDD with 4×10^5 $TCID_{50}$ of Ad12 in comparison with newborn hamsters.

Histologically, these Ad12 tumors in adult mice were neuroblastoma-like tumors composed of blastomatous polymorphic cells and contained crescent-shaped T-antigen-positive bodies in tumor cells by the immunofluorescein T-antigen test.

Table 7

Tumor induction in adult C3H/He mice by a mixture of SR-CTC and syngeneic cells

Each type of cells was inoculated s.c. along with 5×10^6 SR-CTC into mice.

	Tumor induction			
	Incidence		Latent period (days)	
Syngeneic cells injected with SR-CTC	No. of mice with tumors/no. inoculated	% of mice with tumors	Mean ± S.D.	Range
MEC (2×10^6)	111/112	99.1	19.3 ± 4.8	13–31
MBC[a] (4×10^6)	5/8	62.5	92.0 ± 28.5	62–135
C3H-2k (2×10^6)	8/9	88.8	190.8 ± 57.6	109–255

[a] MBC, mouse embryonic brain cells; C3H-2k, a tissue culture line from kidney of newborn C3H/He mice.

DISCUSSION

We proposed a system developing primary solid tumors induced by SR-RSV in adult mice for experimental studies of cancer. Rous sarcoma in mice, which is analogous to human tumors, never regresses or elaborates infectious virus (4). Target cells for Rous sarcoma virus are not part of the lymphohematopoietic system (12), and this fact is important for analysis of the immunological status in the development of the tumor.

Binger *et al.* (3) proposed a model of primary rat brain tumors for therapeutic screening. In the model, the tumors were induced in Fischer 344 rats after intracerebral inoculation of B77 ASV in 1-day-old neonates. However, the model needed a large quantity of ASV, and diagnosis was difficult. Primary Rous tumors were induced in the brain of adult mice and adult hamsters by Kumanishi (11) and Nakazato and Ishida (13). Previously, Schmidt-Ruppin succeeded in inducing tumors in adult mice and adult rats by repeated by inoculations of freshly minced chicken sarcoma or of freeze-dried extracts of chicken sarcoma. He obtained transplantable tumor strains from these primary tumors and applied the strains to chemotherapeutic experiments (15). With chicken embryo cells transformed by a mutant of Rous sarcoma virus, Kahn *et al.* (8) attempted tumor induction in 4- to 8-week-old nude mice. The tumors that developed in the mice were found to consist of chicken cells but not mouse cells. Practically, primary Rous sarcoma in the adult mouse seems to be an attractive model for chemotherapy and immunochemotherapy of experimental solid tumors. We reported earlier the induction of primary SR-RSV tumors in a few strains of adult mice (18). We report in the present paper the induction of murine tumors caused by SR-RSV and Ad12

Table 8

Various conditions of Ad12 tumor induction in mice and hamsters

Each mouse and hamster received s.c. Ad12 alone or a mixture of Ad12 and syngeneic embryo cells.

Injected materials		Animals					
Ad12 $TCID_{50}$	Syngeneic embryo cells	Strain	Age	No. inoculated	No. with tumor	Range of latent period	Incidence
4×10^6	4×10^6	C3H/He	≧5 wk	10	0		0
4×10^6		Golden hamster	≧24 hr	6	5	30–49	83.3
2×10^6	2×10^7	CBA	>25 wk	11	11	24–70	100.0
4×10^5	4×10^6	CBA	9.5 wk	18	18	47–175	100.0
4×10^5	4×10^6	C3H/He	≧5 wk	27	7	18–73	25.9
4×10^5	4×10^6	DDD	6 wk	21	10	32–67	47.6
4×10^5		C3H/He, DDD	≦24 hr	24	0		0
4×10^5		Golden hamster	≦24 hr	5	3	43–64	60.0
4×10^5	4×10^5	C3H/He	≧5 wk	10	2	115–157	20.0

in adult mice, without any immunological suppression. All of these strains of mice showed nearly 100% tumor incidence and short latency periods for tumor induction. The tumors appeared on inoculation of a mixture of more than 5×10^4 SR-CTC and 2×10^4 MEC. The latent period for tumor appearance and the survival time of the tumor-bearing mice could be varied by alteration of the inoculum size. To induce an SR-RSV tumor in an adult mouse, more than 2×10^4 MEC infected with SR-RSV were required. These results indicate the existence of target cells for SR-RSV in embryo cells of the mouse strains tested. The target cells may be transformed by virus derived from SR-CTC or by fusion with SR-CTC in the injected site.

The existence of target cells for SR-RSV in mouse embryos was confirmed by the transplantation of a B6C3F$_1$ mouse tumor which was induced by embryo cells of the parental strain of these F$_1$ mice plus SR-CTC. Another confirmation was the presence of the T6 marker chromosome in SR-RSV tumors of CBA mice induced by CBAT6/T6 embryo cells plus SR-CTC. The fibroblast-like cells from 9- to 14-day-old mouse embryos were competent as target cells. The cells of the cell line C3H/He-2K and mouse embryo brain cells were also competent, with longer latent periods. The target cells for SR-RSV may be distributed in embryos and in newborn mice in high density, in contrast to the possible low density in local areas in adult mice.

Whitmore *et al.* (19) and Whitmore and Haughton (20) studied the genetic control of susceptibility to Rous sarcoma virus tumorigenesis of 10 inbred mouse strains by neonatal injection of SR-CTC. They showed that tumor incidence was significantly associated with the H-2 type by segregation analysis of F$_2$ hybrids between the resistant strains and the susceptible strains. Mice of resistant strains immunized with allogenic Rous sarcoma developed stronger cellular anti-Rous tumor immunity than did those of susceptible strains (2). In our studies, when adult C3H/He and DDD mice were immunized with SR-RSV or SR-CTC before injection of an SR-CTC-MEC mixture, the tumor incidence was not reduced (data not shown). It is thought that the mouse strains tested in our studies are susceptible and that the difficulty in tumor induction in these adult mice with SR-RSV alone may be caused by limited distribution of the target cells in the local area and by the function of the immune system in mice. Immune responses of these mice during the process of tumor development will be reported in a separate paper.

It was known that the Ad12 tumor induced in mice does not produce infectious virus, as murine SR-RSV tumor does not. Therefore, the tumor induction by a mixture of Ad12 and syngeneic embryo cells was tested for a model of primary tumor in adult mice. Tumors were induced in CBA, C3H/He, and DDD mice without any immunosuppressive treatment when a suitable dose of virus and MEC was used. More than 10 of these tumors were found to be transplantable. As to the dose ratio of virus and MEC in the case of C3H/He, tumors were not induced by the inoculation of a mixture of 4×10^6 TCID$_{50}$ of Ad12 and 4×10^6 MEC, while some induction of tumors (7 of 27) was observed with a mixture of 4×10^5 TCID$_{50}$ of Ad12 and 4×10^6 MEC (Table 8). Therefore, the dose ratio between virus and MEC is important for the induction. In a preliminary study with simian virus 40, tumors did not appear in adult C3H/He mice by injection with a mixture of $4 \times 10^{6 \text{ to } 7}$ TCID$_{50}$ of simian virus 40 and 4×10^6 C3H/He MEC (details not shown).

Our model of the induction of primary tumors in adult mice may be a useful tool of therapy of the host during the development of tumors.

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