Comparative Studies of SV_{40} and Adenovirus Oncogenesis in Random Bred and Inbred Hamsters[1] (35631)

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Laboratory rodents, such as hamsters and mice, have played a major role in the field of cancer research for many years. It was not possible, however, to demonstrate conclusively the presence of specific tumor antigens (1–3) until the development of inbred strains of mice. The golden Syrian hamster (4) has been the animal of choice for much of the recent work concerned with tumor induction by viruses. The advantages of this species include: (a) a paucity of histocompatibility antigens which permits the use of random bred animals as though they were, for practical purpose, syngeneic, *i.e.*, ready acceptance of tumor tissue transplants from another hamster (5, 6); (b) a high level of susceptibility to tumor induction by a variety of viruses such as SV_{40}, polyoma, and certain types of adenoviruses when the virus is inoculated into the newborn animal (7); (c) a relatively low incidence of spontaneous tumors which occur late in life (8, 9); (d) a relatively low incidence of indigenous rodent viruses (10); (e) a high level of resistance to laboratory animal diseases in general (11); and (f) a relative ease with which the animals can be propagated and maintained from the point of view of animal husbandry.

Recent reports (12–14) of the preparation of vaccines from disrupted hamster tumor cells which protect against tumor development in hamsters after homologous tumor cell challenge have emphasized the need for inbred hamster model systems to rule out the possibility that normal isoantigens might play a role in the protection afforded by such cellular vaccines. Inbred hamsters have become available commercially during recent years and this made possible the development of truly syngeneic hamster model systems for cancer immunology work. The present report summarizes the comparative findings in studies of inbred strains LSH and MHA (5, 6) and random bred strain LVG hamsters after inoculation with SV_{40} or adenovirus types 7 and 12 virus, or with homologous tumor cells.

Materials and Methods. Hamsters. Pregnant random bred (LVG/LAK) and inbred (MHA/SsLAK and LSH/SsLAK) golden Syrian hamsters were purchased from Lakeview Hamster Colony, Newfield, New Jersey.

Viruses. The following virus preparations were used for inoculation of animals: (a) SV_{40} virus (strain VA45-54) which had been passed five times in primary grivet monkey kidney (GMK) cell cultures and had an infectivity titer of $10^{7.6}$ $TCID_{50}$/0.1 ml when tested in GMK, (b) adenovirus type 7 (strain Pinckney) which had been passed five times in primary human embryonic kidney (HEK) cell cultures and had an infectivity titer of $10^{7.5}$ $TCID_{50}$/0.1 ml when in HEK and (c) adenovirus type 12 (Huie strain) which had been passed 7 times in HEK and had an infectivity titer of $10^{8.7}$ $TCID_{50}$/0.1 ml when titrated in HEK.

Experimental design. Groups of newborn ($<$24 hr) hamsters were inoculated subcutaneously in the scapular region with 0.2 ml of undiluted SV_{40} virus or adenovirus type 7 (Adv. 7) or a 1:2 dilution of adenovirus type 12 (Adv. 12). Virus-inoculated and uninoculated control animals were housed in filter cages for the first 6–8 weeks after inoculation and were then transferred to conventional open-topped cages. The suckling ham-

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sters were weaned and sex-segregated at 3 weeks of age in order to reduce fighting. All surviving animals were palpated at weekly intervals for tumors. Animals which died spontaneously or were sacrificed were autopsied. Tumors were confirmed by histologic and/or gross examination. The incidence of tumors were expressed as percentage of hamsters with tumors, *i.e.*, 100 times the ratio of the number of animals with tumor divided by the number of animals with or without tumors minus the number of animals which died nonspecifically. The percentage figure was corrected for nonspecific deaths according to standard life-table procedures where indicated in the text.

Transplantation. Tumor-bearing hamsters were bled, the sera collected and stored for future use, and the tumors harvested for further study as indicated in the text. A portion of the tumor was minced in Hanks' balanced salt solution (HBSS) or disrupted in a tumor press and inoculated subcutaneously via syringe and needle or by trocar into the dorsal area of newborn, suckling, or adult hamsters of the strain in which the tumor originated. Hamsters which received transplant tissue were housed in conventional cages and observed for tumor development as described above.

Tumor cell culture lines. Tumor cell culture lines were initiated from primary tumors, either by finely mincing a portion of the tumor tissue in culture medium or by trypsinization of the minced tissue and planting in milk-dilution bottles containing 20 ml of medium. The media used for each type of tumor were as follows: (a) all SV_{40} lines were grown in medium 199 containing 10% heat-inactivated calf serum, (b) Adv. 7 lines were grown in Eagle's Basal Medium (BME) containing either 10% whole or agamma calf serum, and (c) Adv. 12 lines were grown either in Eagle's Minimal Essential Medium (EMEM) for suspension cultures containing 0.1 m*M* $CaCl_2$ and 10% heat-inactivated calf serum, or in medium 199 containing 10% heat-inactivated calf serum. Cell lines were maintained routinely by serial passage at a time when the cultures were confluent; this was usually at weekly intervals.

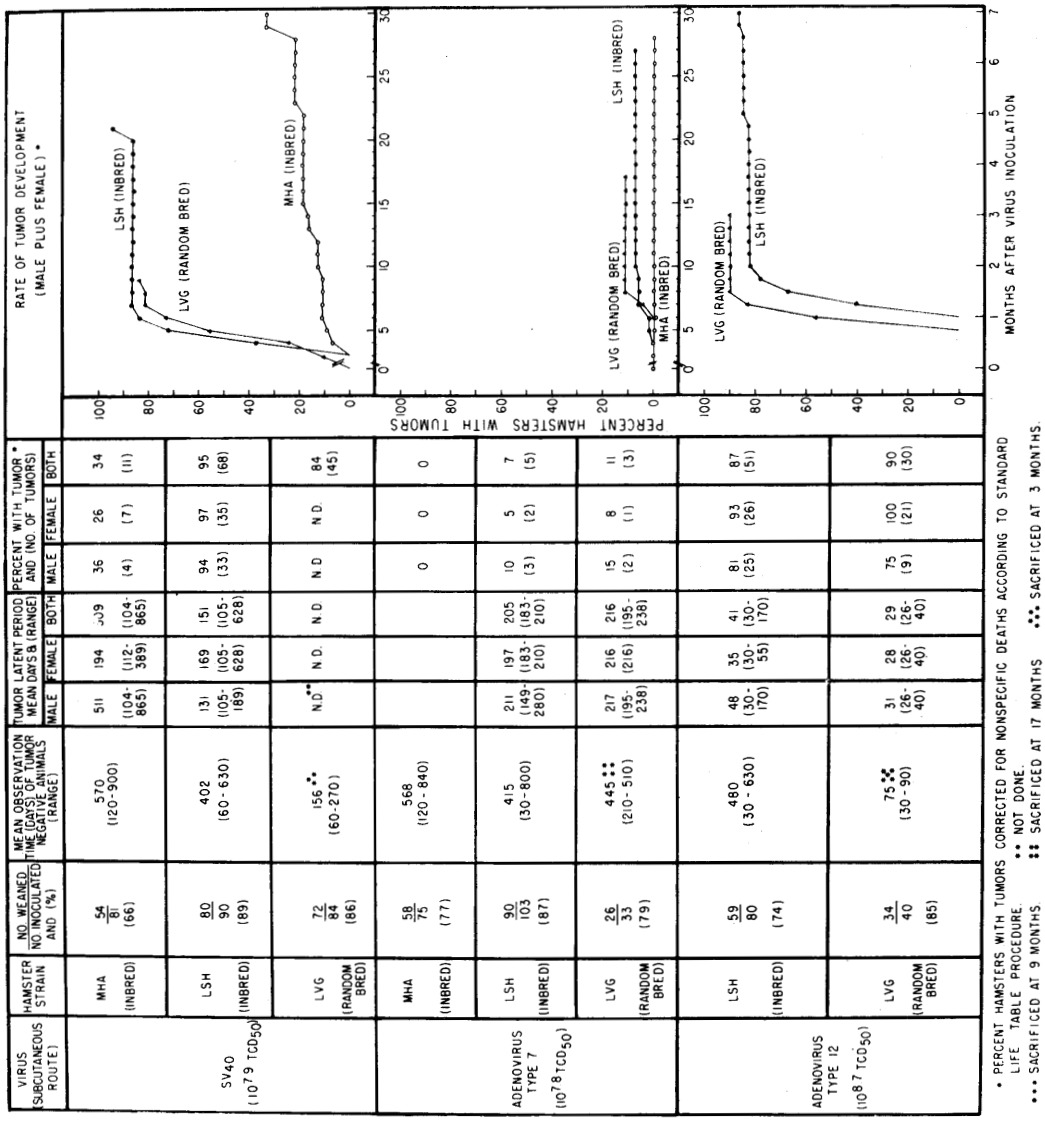
Virus isolation attempts. Supernatant fluids or cells plus fluid were collected at frequent intervals during routine *in vitro* passage of the tumor cell lines. The materials from SV_{40} tumor lines were inoculated into GMK cell cultures and the materials from the adenovirus tumor lines were inoculated into HEK cell cultures. The assay cultures were observed for specific cytopathology until they began to degenerate spontaneously. At that time, they were frozen and thawed three times, inoculated into fresh cultures, and observation continued for a total period of 50–60 days.

Oncogenicity of tumor cell lines. Cell suspensions were prepared by trypsinization, serially diluted in HBSS to the desired viable cell number, and inoculated subcutaneously in the scapular region of 8- to 9-week-old hamsters of the strain in which the tumor originated. These animals were housed and observed in the same manner as described above for transplant animals.

Results. Tumor induction by oncogenic viruses. Figure 1 presents the findings in the random bred and inbred hamsters inoculated with SV_{40} virus or with adenovirus 7 or 12. Comparative data were obtained in observations of 96 MHA, 161 LSH, and 142 LVG uninoculated control animals which were observed for 120–930 (mean 579), 30–810 (mean 533), and 60–900 (mean 378) days, respectively.

The overall rates for survival to weaning of the virus-inoculated animals (Fig. 1) was the same for the inbred LSH hamsters (229/273, 84%) and the random bred LVG strain (132/157, 84%); the inbred MHA strain showed a slightly lower survival rate (112/156, 72%). Survival among the uninoculated animals was approximately in the same proportion (78%, 80%, and 67%, respectively). None of the hamster strains showed excessive cannibalism or neglect of the young after handling of the pups during the inoculation procedure.

The findings in the tests to measure the relative susceptibility of the inbred and random bred hamsters to induction of tumor by virus is shown in Fig. 1. The inbred LSH and the random-bred LVG animals showed greater incidence of tumor than did the in-



VIRUS (SUBCUTANEOUS ROUTE)	HAMSTER STRAIN	NO. WEANED / NO. INOCULATED AND (%)	MEAN OBSERVATION TIME (DAYS) OF TUMOR NEGATIVE ANIMALS (RANGE)	TUMOR LATENT PERIOD MEAN DAYS & (RANGE) MALE	FEMALE	BOTH	PERCENT WITH TUMOR * AND (NO. OF TUMORS) MALE	FEMALE	BOTH
SV_{40} ($10^{7.9}$ TCD_{50})	MHA (INBRED)	54/61 (66)	570 (120-900)	511 (104-865)	194	309	36 (4)	26 (7)	34 (11)
	LSH (INBRED)	80/90 (89)	402 (60-630)	131 (105-189)	169 (105-628)	151 (104-865)	94 (33)	97 (35)	95 (68)
	LVG (RANDOM BRED)	72/84 (86)	156 ** (60-270)	N.D.**	N.D.	N.D	N.D	N.D	84 (45)
ADENOVIRUS TYPE 7 ($10^{7.8}$ TCD_{50})	MHA (INBRED)	58/75 (77)	568 (120-840)				0	0	0
	LSH (INBRED)	90/103 (87)	415 (30-800)	211 (149-280)	197 (183-210)	205 (183-210)	10 (3)	5 (2)	7 (5)
	LVG (RANDOM BRED)	26/33 (79)	445 ** (210-510)	217 (195-238)	216 (216)	216 (195-238)	15 (2)	8 (1)	11 (3)
ADENOVIRUS TYPE 12 ($10^{8.7}$ TCD_{50})	LSH (INBRED)	59/80 (74)	480 (30-630)	48 (30-170)	35 (30-55)	41 (30-170)	81 (25)	93 (26)	87 (51)
	LVG (RANDOM BRED)	34/40 (85)	75 ** (30-90)	31 (26-40)	28 (26-40)	29 (26-40)	75 (9)	100 (21)	90 (30)

* PERCENT HAMSTERS WITH TUMORS CORRECTED FOR NONSPECIFIC DEATHS ACCORDING TO STANDARD LIFE TABLE PROCEDURE. ** NOT DONE.
*** SACRIFICED AT 9 MONTHS ** SACRIFICED AT 17 MONTHS *** SACRIFICED AT 3 MONTHS.

FIG. 1. Susceptibility of newborn inbred (LSH and MHA) and random bred (LVG) hamsters to viral oncogenesis.

bred MHA strain of animals. Only 34% of the MHA inbred hamsters developed tumor after inoculation with the highly oncogenic SV_{40} virus in contrast to 95% and 84%, respectively, in the LSH and LVG strain hamsters. The weakly oncogenic adenovirus 7 failed completely to induce tumors in MHA animals even though the same virus preparation induced tumor in 7% of the LSH and in 11% of the LVG animals. Adenovirus 12 was tested only in LSH and LVG hamsters and induced tumors in 87% and 90%, respectively. Interestingly, 5 of 51 tumor-positive LSH animals and 6 of 30 tumor-positive LVG animals displayed liver tumor in addition to tumor occurrence at the subcutaneous site. There was no marked difference in tumor incidence or in tumor latent period, according to sex, within the numbers of animals tested and the quantities of viruses which were administered.

Only three spontaneous tumors (two MHA and one LSH strain hamsters) were noted among the uninoculated control animals and those occurred very late, *i.e.*, 541 days or more. The histopathologic type and location

TABLE I. Transplantability of SV_{40}, Adenovirus 7, and Adenovirus 12 Tumors in the Homologous Hamster Host.

Tumor-inducing virus	Hamster strain	Serial transplant no.	Age of recipient host	Results			
				No. with tumor/total inoc.	(%)	Latent period (days)	
						Range	Mean
SV_{40}	MHA	1	4 weeks	5/5	(100)	42–70	(64)
	(inbred)	2	3 weeks	5/5	(100)	41–105	(66)
		3	1 week	9/13	(69)	21–82	(47)
		4	4 weeks	5/5	(100)	20–34	(23)
SV_{40}	LSH	1	3 weeks	4/5	(80)	20	(20)
	(inbred)	2	5 weeks	5/5	(100)	19–26	(20)
		3	3 weeks	5/5	(100)	18	(18)
		3	2 days	14/14	(100)	18–24	(18)
		4	4 weeks	5/5	(100)	12–19	(15)
		4	2 days	6/6	(100)	19	(19)
SV_{40}	LVG	1	8–12 weeks	5/5	(100)	10	(10)
	(random	2	8–12 weeks	4/5	(80)	7–11	(9)
	bred)	3	12–16 weeks	5/5	(100)	5–11	(7)
		4	8–12 weeks	4/4	(100)	11–20	(16)
Adenovirus	LSH	1	3 weeks	4/5	(80)	28–37	(33)
7	(inbred)	1	2 days	8/8	(100)	27	(27)
		2	2 weeks	13/13	(100)	15–36	(19)
		3	9 weeks	5/5	(100)	15–22	(19)
		3	2 days	5/5	(100)	22	(22)
Adenovirus	LVG	1	1 day	13/13	(100)	12	(12)
7	(random	2	8–12 weeks	6/6	(100)	8–22	(15)
	bred)	3	8–12 weeks	5/5	(100)	21	(21)
Adenovirus	LSH	1	3 weeks	1/5	(20)	64	(64)
12	(inbred)	1	1 day	22/22	(100)	15–21	(16)
		2	4 weeks	3/5	(60)	15	(15)
		3	4 weeks	2/5	(40)	6	(6)
Adenovirus	LVG	1	4–12 weeks	5/5	(100)	5	(5)
12	(random	2	4–12 weeks	5/5	(100)	11	(11)
	bred)	3	4–12 weeks	4/5	(80)	19	(19)

of the three spontaneous tumors were as follows: one MHA hamster had adenocarcinoma of the intestine, liver, and skeletal muscle; another MHA animal had undifferentiated sarcoma of intestine and liver; and the LSH hamster had a subcutaneous fibrosarcoma invading the skeletal muscle. The group of control LVG animals did not develop spontaneous tumors within the timespan of the experiments though occurrence of tumors has been noted infrequently in late life of these animals during more than a decade of experience.

Transplantation of virus-induced tumors. Several hamster tumor transplant lines (5 SV_{40} MHA, 2 SV_{40} LSH, 5 adenovirus 7 LSH, and 3 adenovirus 12 LSH) were established for each hamster strain and for each viral type which induced tumors. Representative results of transplantation of tumor in the corresponding hamster strain are given in Table I. Readily transplantable lines were evolved for each hamster-virus tumor system and the percentages of successful tumor transplants were roughly the same, irrespective of age of hamster recipient. There were differences in the mean latent period; the SV_{40} MHA lines generally showed longer tumor latent periods than did lines developed in other hamster strains.

TABLE II. Establishment of Cell Culture Lines and Findings in Tests for Infectious Virus.

Tumor-inducing virus	Hamster strain	No. cell lines established/ no. attempts	Results of virus-isolation attempts: No. of culture fluids tested	Passage levels tested: Line	Passage levels tested: Levels	Results
SV_{40}	MHA (inbred)	4/5	218	1	0–60	Negative
				2	0–53	Negative
				3	0–51	Negative
				4	0–50	Negative
SV_{40}	LSH (inbred)	3/4	86	1	3–31	Negative
				2	0–35	Negative
				3	2–19	Negative
Adenovirus 7	LSH (inbred)	2/5	40	1	0–30	Negative
				2	10–26	Negative
Adenovirus 12	LSH (inbred)	2/3	9	1	0–20	Negative
				2	1–5	Negative

Histopathologic diagnosis of tumor types. The tumor types seen in the inoculated inbred hamsters were the same as found previously (15, 16) in the random-bred LVG animals inoculated with these viruses. Thus, all the primary SV_{40} virus tumors in inbred hamsters which were examined (7 MHA and 16 LSH) were fibrosarcomas. Five primary adenovirus 7 tumors and three primary adenovirus 12 tumors in LSH hamsters were either undifferentiated sarcomas or undifferentiated sarcomas resembling malignant lymphomas. All adenovirus 7 and 12 tumor transplant lines in the homologous LSH hamsters were examined at each passage level and were found to have the same histopathology as the primary tumors. All SV_{40} transplant lines in homologous MHA hamsters were fibrosarcomas at all passage levels. One SV_{40} tumor line carried in LSH hamsters was a fibrosarcoma at all passage levels; another was a fibrosarcoma in passages 1 and 2 and an undifferentiated sarcoma in two additional passages.

Tumor cell culture lines. Table II shows that several cell culture lines were established for each kind of virus-induced tumor in MHA and LSH inbred hamsters. None of these cell lines showed infectious virus when tested by the isolation procedures described. However, none of the cell lines has been tested to date for virus employing the coropagation or cell-fusion techniques which are considered to be more sensitive for detecting virus.

One inbred hamster tumor cell culture line, *i.e.*, adenovirus 12 LSH 1, was tested for tumorigenicity in the homologous host species and compared with an adenovirus 12 tumor cell line (LVG 37-4) which originated in random bred LVG hamsters (Fig. 2). Tumor line 37-4 derived in random bred LVG animals appeared to be somewhat more tumorigenic than tumor line 1 derived in the inbred LSH hamsters. For example, 5×10^4 LVG 37-4 cells produced tumor in 63% of the animals while 1×10^6 LSH 1 cells produced tumor in only 50% of the animals. The occurrence of fewer tumors (38%) in LVG animals given a large dose (1×10^6) of LVG 37-4 cells than a smaller dose (5×10^4, 63%) of the same cells, was noted earlier by us in tests of other tumor cell lines of random bred hamster origin and by other workers as well (17).

Discussion. Tests for protective efficacy of tumor cell vaccines require the use of host animals which are sufficiently syngeneic to rule out possible responses to isoantigens. The availability of inbred hamsters in commercial quantity made possible the development of syngeneic tumor–host systems for such studies.

The present report summarizes the findings

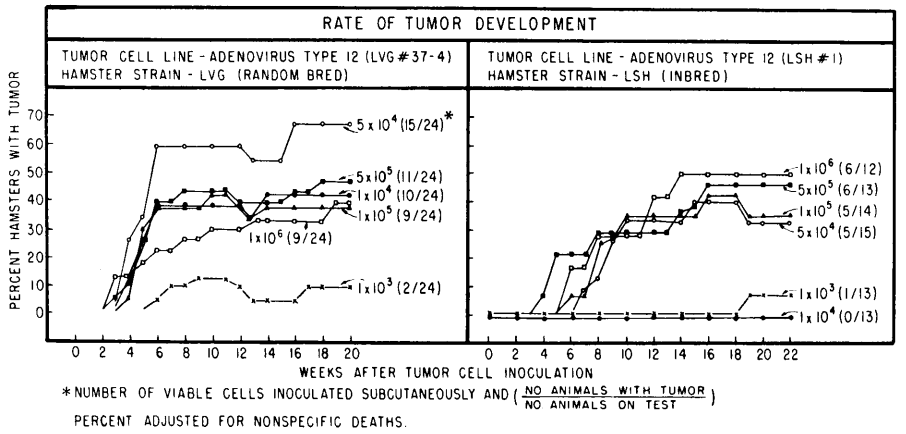


FIG. 2. Oncogenicity of adenovirus type 12 random bred (LVG) and inbred (LSH) hamster tumor cell lines in the homologous hamster strain.

in tests with the LSH and MHA lines of inbred hamsters compared with the random bred LVG hamster line from the standpoint of usefulness as experimental models in the virus-newborn hamster (12) and the tumor transplant (12) systems for immunologic investigations. The findings show the equal performance of the inbred LSH and random bred LVG animals and their superiority to the MHA inbred line.

The inbred LSH hamster line was just as susceptible to tumor induction by SV_{40} adenovirus 7, and adenovirus 12 viruses as was the random bred LVG line used heretofore. The MHA inbred line, by contrast, proved very insusceptible to tumor induction by SV_{40} and by adenovirus 7 virus. Inbred LSH hamsters were just as susceptible to homologous SV_{40} or adenovirus 7 and 12 transplant tumor development as were the random bred LVG hamsters. The MHA inbred line was tested only with homologous SV_{40} transplant tumor and showed a longer time period for tumor appearance than did the inbred LSH or random-bred LVG animals. Van Hoosier *et al.* (18) recently reported that noninbred hamsters were significantly more susceptible to adenovirus 12 than were inbred LSH animals. This difference in findings might be related to the use of a larger dose of adenovirus 12 in the present study. The incidence of SA-7 adenovirus tumors was slightly lower in noninbred than in LSH hamsters.

The inbred LSH hamsters appeared more desirable than the MHA line from the viewpoint of animal husbandry. Thus, the average survival to weaning was greater for LSH (84%) than for MHA (72%) animals. The LSH were more docile than MHA hamsters, making general care and palpation less difficult. The average lifespan and occurrence of spontaneous tumor late in life were approximately the same for the two strains and resembled those for the random bred LVG animals.

The choice of inbred LSH or random bred LVG hamsters depends upon the objectives of the investigator. Both strains are equally susceptible to tumor induction by viruses and for development of tumor after transplantation. The LSH inbred line presents the advantage of providing a truly syngeneic system for investigations in transplant tumor immunology.

Summary. Inbred LSH and MHA hamsters were compared with random bred LVG hamsters for susceptibility to induction of tumor by SV_{40}, adenovirus 7, or adenovirus 12 viruses and for appearance of tumor after homologous transplantation. The LSH inbred strain proved superior to the MHA inbred line in trems of susceptibility to induction of tumor by SV_{40} and adenovirus 7 virus and showed a shorter latent period after transplant with homologous SV_{40} virus tumor. The LSH animals were also somewhat better than MHA hamsters from the point of view

of husbandry. The susceptibilities of the inbred LSH and random bred LVG animals to induction of tumor by viruses were similar and the transplantation characteristics of tumors evolved in the two breeds of animals were approximately the same. The inbred LSH hamster provides a satisfactory model for induction of tumor by virus and for investigations of transplant tumor immunology employing a truly syngeneic system.

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