Oncogenic Evaluation in Hamsters of Human Picorna-, Paramyxo-, and Herpesviruses[1] (34335)

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A screening program initiated several years ago to test the oncogenic potential of a variety of human viruses in other species resulted in the recognition that some human adenoviruses produce tumors when inoculated into newborn hamsters (1, 2). Human adenovirus type 12 is oncogenic in species other than the hamster including newborn mice (3), rats (4), and mastomys (5).

Tests in this laboratory for the oncogenicity of other human viruses in hamsters have included serotypes from most groups of human viruses. This report summarizes the results of long-term testing for oncogenicity in newborn hamsters of picornaviruses, including 27 echoviruses, 5 rhinoviruses, and 1 coxsackie virus; paramyxoviruses, including measles, mumps, respiratory syncytial virus, and 4 parainfluenza viruses; 3 reoviruses and 1 herpesvirus.

Materials and Methods. Human virus strains were obtained from American Type Culture Collection (ATCC). One-tenth ml of each virus or serum–virus mixture was inoculated intraperitoneally (ip) into newborn hamsters (*Mesocricetus auratus*). Rhinoviruses were used undiluted. Measles, mumps, and respiratory syncytial viruses were diluted 1:10 with Eagle's BME. Before animal inoculation with echoviruses, 4 parainfluenzas, and a coxsackie virus, equal volumes of the undiluted viruses and a 1:10 dilution of SV-40 antiserum were mixed and incubated at room temperature for 1 hr. These mixtures were then diluted with Eagle's BME to obtain final virus concentrations of 1:10. Echovirus types 12, 14, and a portion of type 15 were diluted 1:10 with Eagle's BME for animal inoculations without the addition of SV-40 antiserum. Animals, facilities, observations, and treatment of neoplastic tissues have been previously described (2).

Results. As shown in Table I, 28 picornaviruses, 2 reoviruses, 6 paramyxoviruses, and 1 herpesvirus were inoculated into a total of 1268 newborn hamsters. Unusually high mortality in inoculated animals prior to weaning was observed in some groups, presumably because of the pathogenicity of the inoculum. Particularly noteworthy were reovirus types 1 and 2 in which excessive mortality was found even with virus diluted to contain only 5–500 $TCID_{50}$. Passive protection with human serum (0.1 ml ip), 1 hr following virus inoculation, was used in some groups to reduce the mortality with herpesvirus inoculated animals. Mean survival times for all groups ranged from 49–760 days. No neoplastic tissues were observed in any of these groups of animals.

Tumors were observed in hamsters inoculated with echovirus types 12, 17, 22, 23, and 29, respiratory syncytial virus, and reovirus type 3 (Table II). The one tumor observed following inoculation of respiratory syncytial virus was an undifferentiated sarcoma of the thoracic cavity with a latent period of 333 days. In the reo-3 inoculated animals, an intraperitoneal tumor of the malignant lymphoma group was observed in one male hamster necropsied at 663 days after inoculation.

The echovirus tumor incidence varied from 4.8 to 17.0%, and the latent periods were relatively long, *i.e.*, 560–1026 days. Tumors in animals inoculated with types 12 and 17 were located in the prefemoral lymph nodes while those produced by types 22 and 23

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TABLE I. Human Picornaviruses, Reoviruses, Paramyxoviruses, and Herpesviruses that Yielded no Tumors among Hamsters Inoculated at Birth.

Subgroup	Type and strain	No. weaned /no. inoc.	No. necropsied	Mean age (days) at necropsy (range)
Echoviruses	Type 1 (Farouk)	12/74	7	560 (146– 895)
	2 (Cornelis)	15/20	6	760 (593–1132)
	3 (Morrisey)	20/36	15	603 (170– 894)
	4 (Pesascek)	10/18	7	540 (249– 667)
	5 (Noyce)	15/30	11	692 (281– 876)
	6 (D'Amori)	19/24	11	486 (206– 624)
	7 (Wallace)	18/32	13	620 (147– 875)
	8 (Bryson)	12/23	6	561 (473– 590)
	9 (Hill)	10/22	6	512 (242– 658)
	11 (Gregory)	22/59	17	583 (469– 834)
	13 (Del Carmen) (11-4-D)	18/43	11	687 (498– 885)
	14 (Tow)	19/24	12	530 (294– 699)
	15 (Ch 96-51)	19/32	8	683 (392– 785)
	16 (Harrington)	21/29	12	609 (56– 819)
	18 (Metcalf)	19/38	12	490 (259– 788)
	19 (Burke)	12/19	9	628 (286– 867)
	20 (JV-1)	25/29	5	630 (603– 689)
	21 (Farina) (E26D)	15/21	7	666 (331– 909)
	24 (DeCamp)	19/32	11	549 (325– 966)
	25 (JV-4)	15/38	7	560 (24– 734)
	26 (Coronel) (11-3-6)	18/22	6	382 (32– 602)
	27 (Bacon) (1-36-4)	20/26	14	747 (526–1039)
Coxsackie-B	Type 6 (Schmitt)	12/73	11	530 (43– 839)
Rhinoviruses	Type 1A (2060)	14/21	9	556 (292– 814)
	14 (1059)	5/17	4	49 (26– 58)
	15 (1734)	19/28	11	210 (27– 425)
	16 (11757)	10/20	8	186 (41– 425)
	17 (33342)	7/17	5	197 (45– 397)
Reoviruses	Type 1 (Lang)	11/68	NA	NA
	2 (D5-Jones)	14/51	NA	NA
Measles	Edmonston	19/70	16	477 (47– 976)
Mumps	Enders	28/33	19	623 (282– 825)
Parainfluenza 1	C35	15/34	12	627 (253– 932)
2	Greer	13/40	7	536 (120– 973)
3	C 243	16/53	12	453 (155– 873)
5	DA	14/36	8	342 (63– 936)
Herpes simplex	HF	16/34	11	571 (74–1061)

were found in both the peritoneal cavity and submandibular region. The type 29 tumor was located in the subcutis of the thoracic region.

Discussion. Possible interpretations of the results obtained are summarized as follows: (a) The observed tumors are spontaneous and unrelated to the inoculum. (b) The inoculated virus(es), *per se*, are weakly oncogenic; alternatively, the virus(es) enhance the incidence of spontaneous tumors or activate a latent enzootic tumor virus. (c) A second contaminating virus is responsible for the tumors.

The overall incidence of spontaneous tumors in hamsters reported in the literature is 4.4%; the most frequently occurring types are lymphomas, adenocarcinomas of the intest-

TABLE II. Human Viruses Associated with Tumors in Hamsters Inoculated at Birth.

Serotype	Strain	No. weaned /no. inoc.	Percentage tumors	Mean days of all necropsies	Tumor type (mean days of tumor necropsies)
Echovirus type 12	Travis 2-85	24/26	9.1 (1/11)[a]	690	Malignant lymphoma (719)
17	CHHE-29	17/29	17 (1/6)	359	Sarcoma (560)
22	Harris	23/29	8.3 (1/12)	408	Unclassified[b] (681)
23	Williamson	29/36	4.8 (1/21)	570	Unclassified[b] (663)
29	JV-10	22/24	6.7 (1/15)	601	Unclassified[b] (1026)
Respiratory syncytial virus	Long	57/106	2.2 (1/46)	553	Undifferentiated sarcoma (333)
Reovirus type 3	Abney	11/43	16.6 (1/7)	545	Malignant lymphoma (663)

[a] Number with tumors per number necropsied.

[b] Possibly sarcomas, but too much autolysis to permit classification.

ines, and melanomas (6). In our closed colony, 8 malignant neoplasms have been observed in 1671 animals necropsied at a mean age of 347 days for an incidence of 0.5%. The observed groups of hamsters included breeders, uninoculated mothers of inoculated newborn, and newborn inoculated with tissue culture media. The 8 tumors observed included 2 cheek pouch tumors, 2 carcinomas, 3 malignant lymphomas, and 1 subcutaneous sarcoma. Tumors of the cheek pouch or carcinomas were not observed in inoculated animals. The incidence of neoplasia observed in which only one animal of the group developed tumors may not be significantly different than control groups either because of the absolute value as compared with reports in the literature or because of the small numbers of animals involved. However, when one also considers the predominant histological type (sarcomas) and sites of tumors in inoculated animals, the results appear different than those observed in control groups with the possible exception of the malignant lymphoma in the reovirus-3 and echo-12 experimental series.

Regarding the second possible interpretation, it is noteworthy that adenovirus type 7 produces tumors in a very low percentage of animals. Only one inoculated animal in 23 (4%) developed tumors in one series in our laboratory (2), although the incidence was significantly enhanced by thymectomy (7) and specific serological reactivity can be demonstrated between type 7 antigens produced *in vitro* or present in tumor homogenates, and antisera from tumor bearing animals (8). Therefore, a low incidence of tumors, *per se*, does not preclude specific induction by the inoculated virus.

Also worthy of consideration is whether the inoculation of some viruses, *per se*, may enhance the incidence of tumors without the inoculated virus being the direct etiologic factor. A possible mechanism would be activation of enzootic agents, *e.g.*, the hamster type A or C viruses (9, 10, 11). The type C virus particle from tumor-bearing hamsters from our colony was found to contain an antigen (MuLV-gs 3) in common with murine and feline leukemia viruses (L. J. Old, personal communication). The malignant lymphomas occurring spontaneously and in some virus-inoculated hamsters (Table II) (2) may thus relate etiologically to the enzootic hamster type C virus particle. Indeed, Graffi has recently induced lymphomas in hamsters by neonatal injection of a similar type C virus particle found in his hamster colony (12). Studies are in progress to determine if the type C virus particle in our colony, which has been found in spontaneous and transplanted tumors (9, 13), can be activated by X-irradiation to induce lymphomas, as in radiation-induced murine leukemia.

Regarding the etiologic involvement of contaminating oncogenic viruses, SV-40 deserves special consideration in the case of viruses passed in rhesus monkey kidney cell cultures. The role of this agent in the echovi-

rus-induced neoplasms is presented separately (14).

Summary. Groups of newborn hamsters were inoculated with one of each of 33 picornaviruses, 7 paramyxoviruses, 3 reoviruses, and 1 herpesvirus, of human origin, obtained from the American Type Culture Collection. Low tumor incidences were observed following inoculation of echovirus types 12, 17, 22, 23, and 29, respiratory syncytial virus, and reovirus type 3. Tumors observed in inoculated animals are compared with spontaneous tumors. The possibility of etiologic involvement of contaminating oncogenic viruses, or of enhancement or activation of enzootic latent tumor agents by non-oncogenic virus inoculation is discussed.

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