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Enhancement by Thymectomy of Tumor Formation by Oncogenic Adenoviruses* (33039)

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The role of the thymus in the development of immunological competence has been reviewed by Miller (1). Briefly, the removal of the thymus from perinatal mammals frequently results in an impairment of the production of specific immunoglobulins (antibodies), of the development of delayed hypersensitivity, and of the rejection of tissues from histoincompatible donors. The specific effects of thymectomy on the Golden hamster (*Mesocricetus auratus*) have been described by Sherman *et al.* (2).

A variety of animal neoplasms possess new antigens, functionally similar to minor histocompatibility antigens, that are capable of eliciting immune responses (3). It has been demonstrated that neonatal thymectomy reduces these immunological responses, frequently resulting in an enhanced susceptibility to tumor induction (4).

Yohn *et al.* (5) reported that thymectomy at 3 weeks following injection of adenovirus type 12 (A-12) into newborn Syrian hamsters enhanced susceptibility to tumor induction in male animals. Kirschstein *et al.* (6) found that A-12 produced tumors in BALB/c and C3H/HeN mice only if they had been thymectomized at birth. Allison *et al.* (7) observed a greater incidence of tumors in CBA mice inoculated with low doses of A-12 and either thymectomized or injected with antilymphocyte sera.

Previous reports describe the results of inoculation of 30 human adenoviruses into neonatal hamsters (8,9). Adenovirus type 12 (A-12) induced tumors in a high incidence and short latent period (8). Tumors were also observed with types 1, 7, 8, 14, 18, 21, and 24, but the incidence was generally low and the incubation period long (9). The present report gives the results of the inoculation of most of these latter viruses of suspected oncogenicity into thymectomized hamsters.

Methods. Syrian hamsters, of both sexes, less than 24 hours old were inoculated either subcutaneously (s.c.) or intraperitoneally (i.p.) with 0.1 ml of undiluted adenovirus types 1, 7, 8, 14, 21, or 24. The viruses were grown and titered in human embryonic kidney cells.[1]

For thymectomy, newborn or 1-week-old animals were secured with masking tape. Using a dissecting microscope for visualizing the surgical field, the skin and sternum were opened down to the second or third rib and the width of the incision was extended with forceps. A cotton tipped applicator stick was gently rotated about the thymus until both lobes became detached. The sternum and skin were then closed by the use of collodion. All inoculated animals were thymectomized on day 7 except those for adenovirus 21 which were done on day 0 and for adenovirus 7 which were done either on day 0 or day 7.

Results. The results of inoculation of adenovirus types 1, 7, 8, 14, 21, and 24 into thymectomized and nonthymectomized hamsters are shown in Table I. To date, tumors have been observed in thymectomized groups inoculated with types 7 and 21 (Table I). With adenovirus 7, 17% of the 76 (s.c. and i.p. routes combined) thymectomized animals

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TABLE I. Tumor Incidence in Thymectomized and Intact Adenovirus—Inoculated Animals.

Inoculum	Titer/1.0 ml	Intact or thy-x	Mean time of autopsy (days)	Percent with tumors by route of inoculation (s.c.)	(i.p.)	Mean tumor latent period	No. alive and survival time (days)
Adeno-1	$10^{6.5}$ HEK[a]	Thy-x	517	(0/4)[b]	(0/3)[b]		1 (765)
		Intact	384	(0/11)	(0/9)		4 (776)
Adeno-7	$10^{7.0}$ HEK	Thy-x	491	28 (13/46)[c]	(0/30)	421 (240–607)[d]	3 (887)
		Intact	570	8 (1/12)[c]	(0/11)	466	0
Adeno-8	$10^{4.6}$ HEK	Thy-x	413	(0/2)	(0/8)		5 (631)
		Intact	342	—	(0/6)		12 (511)
Adeno-14	$10^{7.6}$ HEK	Thy-x	489	(0/24)	(0/18)		
		Intact	354	—	(0/2)		4 (538)
Adeno-21	$10^{7.5}$ HEK	Thy-x	463	50 (3/6)[c]	4.5 (1/22)	431 (401–455)	3 (979)
		Intact	563	—	(0/25)		3 (893)
Adeno-24	$10^{6.3}$ KB	Thy-x	554	(0/9)	(0/12)		
		Intact	562	—	(0/18)		1 (910)
				Not inoculated			
Controls		Thy-x	518	0/48			11

[a] Cell type used for titration.

[b] No. of animals with tumor/no. of animals autopsied.

[c] Histological types of tumors in the animals examined were as follows: type 7, thymectomized, two malignant lymphomas and three malignant tumors, undifferentiated or unclassified; type 7, intact, one malignant lymphoma; type 21, thymectomized, three malignant lymphomas.

[d] Range.

that have been autopsied, developed tumors compared to an incidence of 4% with intact animals; 14% of the thymectomized hamsters inoculated with adenovirus 21 had tumors, as compared to no tumors with this virus in intact animals of this series. Figure 1 graphically illustrates the effects of the age of thymectomy, route of inoculation and sex on tumor induction by type 7 in thymectomized animals.

Discussion. The spontaneous tumor incidence among 1557 control animals autopsied at 188 days or older (mean of 377 days) in our hamster colony over a 5-year period was 0.45% (Van Hoosier, G. L., Jr., Trentin, J. J., Spjut, H. J., in preparation). A possible way to resolve questions of spontaneous versus induced tumors with the viral agents under investigation would be to induce tumors in a larger percentage of animals with a shorter latent period.

Adenovirus serotypes 7, 14, and 21 have been reported by others to be weakly oncogenic (10, and Green *et al.*, personal communication). In our original screening inoculations with adenovirus types 1, 7, 8, 14, 21, and 24 the tumor incidence was low, ranging from 4 to 15% and the latent period was long, i.e., 394–684 days postinoculation. Following thymectomy there was an increase in tumor incidence with adenovirus types 7 and 21. Although the number of animals thymectomized on day 0 and inoculated with type 7 is small, thymectomy at day 7 appears as effective (cf. Fig. 1), and survival at weaning age is much better. The i.p. route is less sensitive than the s.c. route for adenovirus type 7 and 21 which is in contrast to results obtained with type 12. The slightly greater incidence in thymectomized females than males is of doubtful significance, although type 12 gives a significantly higher incidence in intact females as compared to intact males (5).

There appear to be at least two limitations to the use of thymectomized animals to enhance the susceptibility to tumor induction. There is often a decrease in survival time of

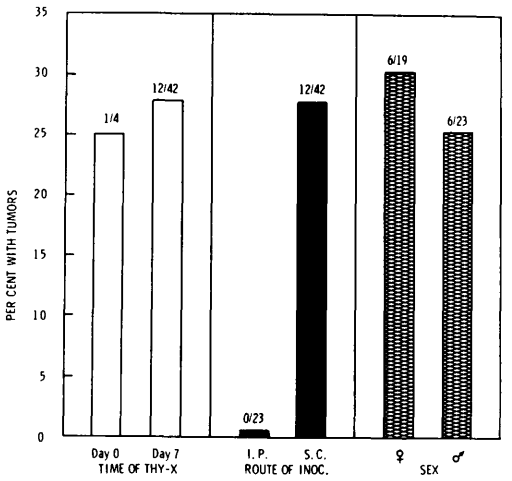

FIG. 1. Influence of time of thymectomy, route of inoculation and sex on incidence of tumors in hamsters inoculated with adenovirus 7. For time of thymectomy only animals inoculated by the s.c. route are shown. For route of inoculation only animals thymectomized on day 7 are shown. For the effects of sex, only animals inoculated by the s.c. route and thymectomized on day 7 are shown.

thymectomized animals. Thus any theoretical reduction in latent period may be nullified by earlier deaths. In addition, thymectomy has been shown to have an inhibitory effect on lymphocytic neoplasms of the mouse (11) but observations are cited by Law (4) that neonatal thymectomy in Syrian hamsters did not influence tumor induction by Rous sarcoma virus. The inhibitory effect of thymectomy on lymphocytic neoplasms of the mouse is especially relevant since, with the exception of the undifferentiated tumors induced by adenovirus types 12 and 18, the predominant histological pattern of the hamster tumors induced by the other types of adenoviruses is, in our experience, malignant lymphoma (9). For these reasons and because some inoculated animals are still alive, greater significance attaches to the increased incidence of tumors with types 7 and 21 than to the absence of tumors with other serotypes in thymectomized animals.

Summary. Adenoviral serotypes 1, 7, 8, 14, 21, and 24 were inoculated into thymectomized and nonthymectomized newborn Syrian hamsters because of their suspect oncogenicity in an initial screening program. Enhanced oncogenicity attributable to thymectomy at 1 week of age was observed when type 7 was inoculated subcutaneously. Tumor incidence increased from 4 to 17%. About 14% of the thymectomized hamsters inoculated with adenovirus 21 had tumors although this virus produced no tumors in intact animals of this series. All surviving animals remain under observation.

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