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Transplantation Resistance to Adenovirus-Induced Hepatocellular Carcinomas[1] (38954)

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Chicken embryo lethal orphan (CELO) virus, an avian adenovirus (1), is oncogenic in hamsters. Subcutaneous sarcomas (2), ependymomas (3), and adenocarcinomas or hepatocellular carcinomas of the liver (4–6) have been produced in hamsters inoculated at birth. Although viral-induced tumor-specific transplantation antigen (TSTA) and T antigen have been identified in this system (7–10), the detection of T antibodies in primary tumor-bearing animals has not been uniformly successful for all CELO-induced tumors (2, 10, 11). Utilizing the indirect immunofluorescence technique, we found that 60% of hamsters bearing primary CELO-induced fibrosarcomas produced antibodies to the viral T antigen (10).

Even though hamster cells transformed *in vitro* by CELO virus may actively synthesize T antigen (12, 13), cell lines derived from hamster tumors can require cultivation *in vitro* for many generations in order to express its synthesis (7, 11, 14). In addition, one cell culture derived from an hepatocellular carcinoma has lost the ability to synthesize this antigen (5, 14). Therefore, the presence or absence of T antigen or T antibodies may not be an adequate immunologic test for the presence of viral genetic material in CELO-transformed cells.

Primary CELO-induced carcinomas arising in the livers of hamsters are remote from the site of sc injection and may contain type C viruses, as well as abundant intracytoplasmic type A virus particles (4, 5). We have been attempting to determine if a cooperative relationship between CELO virus and these apparently indigenous hamster viruses exists during induction of hepatocellular carcinomas. Although T antibodies were present in the sera of each animal bearing a primary hepatocellular carcinoma (5, 10), similar antibodies were not found in the sera of hamsters bearing transplantable tumors derived from the carcinomas (10). The presence of antibodies in these primary tumor-bearing animals could result from the ability of high doses of DNA tumor viruses to induce T antibodies without induction of tumor (15, 16). In this report, the presence of at least a part of the CELO genome in two transplantable carcinomas is demonstrated by *in vivo* assays for CELO-specific TSTA.

Materials and Methods. The Phelps strain of CELO virus (1) was produced (10) in specific pathogen free chicken eggs (SPAFAS, Inc., Norwich, Conn.) and assayed as described (17). The human adenoviruses were grown in HEp 2 cells and assayed in either HEp 2 or secondary cultures of human embryonic kidney cells. Simian adenovirus type 7 (SA7) was grown and assayed in BSC-1 cells; canine hepatitis virus, in MDCK cells.

The transplantable tumors employed, CILT and CILT/2, were derived from hepatocellular carcinomas occurring in inbred LSH hamsters following sc injection of CELO virus into newborn animals. After transplantation, the tumors grew as anaplastic carcinomas. Although T antibodies were produced in primary tumor bearing animals, sera from hamsters bearing transplantable tumors were generally negative for antibodies. Adult caesarian-derived inbred LSH/Lak hamsters (18) were immunized three times by weekly ip injection of approximately 10^8 PFU of virus. One week following the final injection, animals were challenged with graded sc doses of viable tumor cells (usually 8 hamsters/group) obtained by trypsinization of transplantable tumors. The hamsters were examined weekly for 3 mo and the 50% tumor producing dose (TPD_{50}) was calculated by the method of Reed and

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TABLE I. TRANSPLANTATION OF CELO-INDUCED HEPATIC TUMORS IN CELO IMMUNE AND CONTROL INBRED HAMSTERS

No. of tumor cells	CELO immune[a] No. tumors/No. inoculated		Allantoic fluid immune No. tumors/No. inoculated	
	CILT Tumor	CILT/2 Tumor	CILT Tumor	CILT/2 Tumor
1×10^7	—	4/4	—	4/4
1×10^6	1/4	2/8	8/8	7/7
1×10^5	0/8	0/6	1/8	2/7
1×10^4	0/8	0/7	0/8	0/8
1×10^3	0/8	0/4	0/8	0/8

[a] Tumor incidence significantly decreased in both tumor systems by immunization with CELO virus ($P < 0.03$).

TABLE II. TRANSPLANTATION RESISTANCE INDUCED BY VARIOUS AMOUNTS OF CELO VIRUS

Immunizing virus titer (PFU)	Log_{10} TPD_{50} of CILT/2 tumor cells		
	Virus group	Allantoic fluid group	Log_{10} difference
1.9×10^8	6.6[a]	5.4	1.2
5×10^7	6.1[a]	4.4	1.7
1×10^7	5.4[b]	4.6	0.8
5×10^6	4.3	4.7	−0.4
5×10^5	4.4	4.4	0

[a] Significantly greater than allantoic fluid control ($P < 0.01$).
[b] $P \sim 0.06$.

TABLE III. INDUCTION OF TRANSPLANTATION RESISTANCE TO CILT/2 TUMOR BY CELO VIRUS BUT NOT BY MAMMALIAN ADENOVIRUSES

Virus	Titer (PFU)	Log_{10} TPD_{50} of CILT/2 tumor cells		
		Virus group	Control group	Log_{10} difference
CELO	10^8	6.4[a]	5.0	1.4
Human adeno, type 1	10^8	5.3	5.6	−0.3
Human adeno, type 7	10^8	6.2[b]	5.5	0.7
Human adeno, type 12	10^8	6.2	5.7	0.5
Simian adeno SA7	10^8	<4.4	4.4	<0
Canine hepatitis	$10^{7.8}$	4.6	4.8	−0.2

[a] Significantly greater than control group ($P < 0.01$).
[b] Not significantly greater than control group ($P \sim 0.12$).

Muench (19). Controls were inoculated with normal cell culture or allantoic fluids, as required. A tenfold or greater increase in the TPD_{50} represents a positive tumor resistance test. This assumption was confirmed statistically by calculating exact probabilities comparing the total positive and negative responses in those control and treated groups in which tumors developed.

Results and Discussion. Several injections of CELO virus specifically immunize adult hamsters to transplantation of CELO-induced tumors, but not to hamster tumors induced by SV40 (20), polyoma (21), or Rous sarcoma virus (21). Table I demonstrates that animals previously immunized with CELO virus reject tumor cells from two different hepatic carcinomas when compared to groups of animals immunized with normal allantoic fluid ($P < 0.03$). Approximately $1–5 \times 10^7$ PFU of virus were necessary to elicit rejection of the carcinomas (Table II). Although the immunization obtained at 10^7 PFU approaches statistical significance ($P \sim 0.06$), 10^7 PFU represent a marginal dosage of virus. In further studies, 10^8 PFU were chosen as optimal immunogen.

The specificity of the immunizing virus required to produce rejection of the tumor cells is shown in Table III. Statistically significant tumor resistance ($P < 0.05$) was induced only by CELO virus, and not by human, simian, or canine adenoviruses which transform cells *in vitro* or *in vivo*. In other studies, human adenovirus type 12 (22) and SA7 (23) have been shown to induce resistance to transplantation of homologous tumor cells. CELO virus itself does not prevent development of primary hamster tumors induced by bovine adenovirus type 3 (24). Our present tests indicate that at least part of the genetic information of the avian adenovirus is present in the transplantable carcinomas.

The avian adenoviruses lack the group specific complement-fixing antigen common to the mammalian adenoviruses (25). Although the T antigen of some human and simian adenoviruses cross-react immunologically (26), serological tests have failed to establish a relationship between the T antigens of CELO virus and oncogenic human adenoviruses (7, 9). Evidence has also suggested that the TSTA induced by SA7 may cross react with that induced by human adenovirus type 12 (27, 28). In our experiments (Table III), no resistance could be induced to the TSTA of CELO virus by representative oncogenic human, simian, or canine adenoviruses. A similar lack of cross reactivity between CELO virus and the TSTA of the bovine group of adenoviruses was recently demonstrated (24). Therefore, this avian adenovirus apparently produces no common TSTA, no common adenovirus group antigen, and no common hemagglutinating components (29, and El Mishad, McCormick, and Trentin, in preparation) with the mammalian adenoviruses. The avian adenoviruses are structurally comparable to the mammalian group of viruses, but appear to have no antigenic cross reactivity with them.

In addition, it is likely that, except for possible cross reactivity of the simian and human groups (27, 28), each species of adenovirus may induce its own TSTA(s). Ankerst and Sjogren have found two group-specific TSTAs in human adenovirus induced-tumors (30). The bovine adenoviruses induce TSTA cross reactive within the bovine viral species but unrelated immunologically to the human, avian, simian, or canine antigens (24). Similarly, in the present study, the avian virus-induced TSTA is specific for the avian adenovirus. Further studies on cross-reactions between TSTA of other adenoviruses will clarify the position of adenoviral TSTA as viral species antigens.

Summary. Two transplantable hepatic carcinomas of the hamster contained CELO virus-induced TSTA, confirming the etiological role of this avian adenovirus in the induction of hepatic tumors. Transplantation resistance could not be induced with oncogenic adenoviruses of human, simian, or canine origin.

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