Occurrence of Antibodies to the T Antigen of Chicken Embryo Lethal Orphan Virus

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SUMMARY

The occurrence of viral and T antibodies in hamsters bearing chicken embryo lethal orphan virus (CELO)-induced tumors was determined. By complement fixation tests, approximately 11% (5/44) of primary tumor-bearing animals had viral antibodies in either serum or ascitic fluids. Although both complement fixation tests and indirect immunofluorescence tests detected antibodies to CELO T antigen induced in chick kidney cell cultures, the immunofluorescence test was more sensitive. About 62% (29/47) of primary tumor-bearing hamsters (fibrosarcomas, hepatomas, liver adenocarcinomas, and sarcomas) produced T antibodies detectable by the immunofluorescence reaction of sera or ascitic fluid against CELO-infected chick kidney cell cultures. Fewer positive sera were detected in animals bearing transplanted tumors. In contrast to other reports, the T antigen of CELO-induced tumors does appear to be expressed in the primary tumor, although it may not be as easily detected as with other DNA-tumor virus systems.

INTRODUCTION

The oncogenicity of CELO, an avian adenovirus, was first reported by Sarma et al. (18). The virus produced well-differentiated fibrosarcomas at the site of injection. Recently, the oncogenic activity of CELO has been confirmed (10, 12). In addition to fibrosarcomas, the virus induces various other histological types of sarcomas (10), ependymomas (13), hepatomas (2, 4), and adenocarcinomas. The in vitro transformation of hamster and human cells has also been demonstrated (5–7).

A T antibody response could not be shown in the sera of primary tumor-bearing hamsters (10, 18). However, experiments with either hamster cells transformed by CELO (5) or CELO-induced hamster tumor cells grown in vitro (17) indicated that an antigen detectable in these cells was possibly the equivalent of a CELO-induced T antigen. In both of these studies, cells had been cultured for relatively long periods in vitro. Therefore, it was suggested (10) that cells containing the CELO T antigen may require “long-term cultivation in a favorable environment” to allow the phenotypic expression of this antigen. It was subsequently demonstrated (3, 4, 16) that this avian adenovirus does induce a specific T antigen in lytically infected chick kidney cells. The purpose of this report is to present data on the prevalence of antibodies to CELO T antigen in sera of hamsters bearing primary CELO tumors.

MATERIALS AND METHODS

Preparation of Virus

The Phelps strain of CELO was used (21). Virus pools were prepared in CELO-free chicken eggs. Approximately 10⁶ PFU's of virus were inoculated into the allantoic cavity of 10-day embryonated eggs. The eggs were incubated at 37° for 3 days and chilled overnight at 4°. The allantoic fluids were collected, pooled, and frozen at −20°.

Assay of Virus

The virus was assayed by the plaque technique (1). Plaques were counted on Day 11.

Inoculation of Hamsters

Newborn hamsters were inoculated either i.p. or s.c. with 0.1 to 0.2 ml of CELO. Random-bred (19) and inbred LSH/LAK (8) hamsters from our colony were used. The inoculated animals were weaned and sex segregated at 3 weeks of age. The hamsters were observed until death. Animals were sacrificed by cardiac puncture. Ascitic fluids were collected, pooled, and frozen at −20°.

Transplantation of Tumors

Viable tumor tissue was selected and transplanted to weanling hamsters by trocar. Tumors were transplanted to either inbred or random-bred animals, depending on the origin of the primary tumor.

Cell Cultures

Chick kidney cells were prepared by trypsinization of kidneys from 21-day-old chick embryos (1). The cells were grown in Eagle's minimum essential medium containing 10% inactivated fetal calf serum.
Preparation of CF Antigens

**T Antigen.** Monolayers of chick kidney cells were infected with approximately 50 to 100 PFUs/cell and kept at 37°C for 1 hr. The cells were then maintained in minimum essential medium containing 2% fetal calf serum and 10 μg of CA per ml. At 24 hr, the cells were washed with Veronal-buffered saline and scraped off the glass. After centrifugation, the cells were resuspended to 20% (v/v) concentration and frozen at −70°C. The antigens were thawed at room temperature and allowed to settle before use.

**Viral Antigens.** Infected allantoic fluids were used as the source of viral antigen for the CF test. Uninfected allantoic fluids served as control. Eight to 16 units of antigen were used in the CF test.

**Tumor Antigens.** The method of extraction of hamster tumors and the technique of the CF test have been reported.

Preparation of T Antigen for Indirect IF

Monolayers of chick kidney cells grown in Leighton tubes were infected with 30 PFUs/cell and treated as described in Ref. 3. Maintenance medium was used either with or without the addition of 10 μg of CA per ml. Coverslips were harvested at 18 to 20 hr postinfection, at which time approximately 85% of the nuclei stained for T antigen and 0 to 2% stained for V antigen (3). The latter staining was completely eliminated if CA was added to the medium. The slides were washed with Hanks' balanced salt solution, fixed in acetone for 10 min at room temperature, and frozen at −70°C. Hamster sera were tested at a 1:4 dilution. The IF technique has been described.

RESULTS

**Induction of Tumors.** The detailed incidence of tumors will be reported in another communication. However, fibrosarcomas, hepatocellular carcinomas (hepatomas), adenocarcinomas of the liver, and sarcomas of the liver were found. Hepatomas (2) and liver sarcomas occurred at a low incidence in the LSH/LAK inbred strain of hamsters. Adenocarcinomas were found only in random-bred animals. No tumors appeared in control animals given allantoic fluid when newborn.

**Anti-CELO Antibodies in Tumor-bearing Hamsters.** The CF test was used to detect antibodies to CELO structural antigens. Antiviral antibodies were detected in the sera of 3 animals (2 inbred, 1 random bred) out of 37 bearing primary fibrosarcomas and in the sera and/or ascitic fluids from 2 animals out of 3 bearing primary adenocarcinomas of the liver (Table 1). The titers ranged from 1:20 to 1:80. No antiviral antibodies appeared in 26 animals carrying transplanted fibrosarcomas or in 20 animals bearing transplanted hepatomas.

**CF Tests for T Antigen in Infected Cells.** The CF antigens prepared in lytically infected chick kidney cells did not react with 8 to 16 units of hamster sera (3) prepared against CELO. However, sera from 2 of 34 inbred animals with primary fibrosarcomas and the serum and ascitic fluid from the 1 hamster tested with a primary adenocarcinoma fixed complement with the T antigen preparation (Table 1).

**CF Tests for T Antigens in Primary Tumors.** Only 2 hepatomas and 1 fibrosarcoma were tested by CF. Although no cross-reactions were found with sera from hamsters bearing tumors induced by human adenovirus types 12 and 21, polyoma, Rous sarcoma, or SV40, we also found no specific reactions of the CELO tumors and sera in these limited tests. However, because most fibrosarcomas were difficult to homogenize and the tumors appeared to be highly anticomplementary, this approach to detection of antigen and antibody was abandoned.

**IF Tests for CELO T Antigen.** This technique was superior to the CF test for detecting the CELO T antigen in lytically infected chick kidney cells. The antigen was localized to the nucleus of the infected cells (Fig. 1) and appeared early in the infectious cycle (3). Table 2 summarizes the results of these experiments. Anti-T antibodies were detected in approximately 57% of hamsters bearing primary fibrosarcomas. Antibodies were also found in sera from animals carrying primary hepatomas or primary adenocarcinomas of the liver. Ascites obtained from the latter animals were also positive for T antibody. The percentage of hamsters developing T antibodies on transplantation of tumors was considerably lower than in primary tumor-bearing hamsters: 11% for fibrosarcomas and 0% for hepatomas. Antibodies to CELO T antigen were not found in 2 hamsters tested bearing primary sarcomas of the liver.

Although the IF test may be used to detect viral antibodies in tumor-bearing hamster sera, appreciable amounts of viral antigen do not appear in lytically infected cells until 24 to 36 hr after infection (3). Therefore, the cells studied in this report contained little or no detectable viral antigens.

**Heat Stability of T Antigen.** Control and infected cultures were heated at 56°C for 30 min by the method of Lewis et al. (11). The intranuclear antigen could not be detected after heating when used in the indirect IF test with T-positive hamster sera.

DISCUSSION

Our results indicate that the CF test for the detection of CELO T antigen is apparently less sensitive than the indirect

### Table 1

| CF antibodies in the sera of tumor-bearing hamsters |
|---|---|---|
| Type of tumor | Viral | T |
| Fibrosarcoma, primary (at site of injection) | 3/37 | 2/34 |
| Fibrosarcoma, transplanted | 0/26 | 0/16 |
| Hepatoma, primary | 0/2 | NT |
| Hepatoma, transplanted | 0/20 | 0/9 |
| Liver adenocarcinoma, primary | 2/3 | 1/1 |
| Liver sarcoma, primary | 0/2 | NT |

* a Eight to 16 units of viral antigen were used.
* b Two to 4 units of T antigen prepared in chick kidney cells were used.
* c Values are the no. of sera positive/no. of sera tested; NT, not tested.
* d Either serum or ascitic fluid was used as source of antibodies.
Occurrence of Antibodies to T Antigen of CELO

Fig. 1. Indirect IF staining of CELO T antigen in lytically infected chick kidney cells 17 hr after infection. Note the absence of fluorescence in the nucleolar areas. × 160.

Table 2
Detection of T antibodies by indirect IF

<table>
<thead>
<tr>
<th>Serum</th>
<th>No positive/no. tested</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosarcoma, primary (at site of injection)</td>
<td>21/37</td>
<td>57</td>
</tr>
<tr>
<td>Fibrosarcoma, transplanted</td>
<td>4/37</td>
<td>11</td>
</tr>
<tr>
<td>Hepatoma, primary</td>
<td>4/4</td>
<td>100</td>
</tr>
<tr>
<td>Hepatoma, transplanted</td>
<td>0/33</td>
<td>0</td>
</tr>
<tr>
<td>Liver adenocarcinoma, primary</td>
<td>4/4b</td>
<td>100</td>
</tr>
<tr>
<td>Liver sarcoma, primary</td>
<td>0/2b</td>
<td>0</td>
</tr>
</tbody>
</table>

a Three ascitic fluids and 2 sera were tested.
b Two sera and 1 ascitic fluid were tested.

Although Jones et al. (10) reported no T antigen activity in CELO-infected chicken cells, our results show that this system can be used to produce T antigen. The difficulties encountered by these authors were probably due to their choice of chick embryo fibroblasts as host cells. The chick kidney cell is the preferred host cell (15), and our use of infected chick kidney cells resulted in the synthesis of large amounts of T antigen. The antigen was heat labile, and its development was independent of DNA synthesis and followed the time sequence expected for the formation of T antigens (3).

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REFERENCES

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