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Specific Tumour Antigen Induced by Chick Embryo Lethal Orphan (CELO) Virus

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The cells of tumours induced in laboratory animals by the inoculation of DNA viruses contain antigens, termed tumour antigens, not present in normal cells (Huebner, 1967; Macpherson, 1967). These antigens are specific for the tumour-inducing virus, and have been described in tumour cells induced by polyoma virus (Habel, 1965), simian virus SV40 (Black *et al.* 1963) and the oncogenic adenoviruses of human, simian, bovine, dog and mouse origin (Huebner, 1967). Sarma, Huebner & Lane (1965) reported the induction of tumours in newborn hamsters by chick embryo lethal orphan (CELO) virus. This preliminary report describes a tumour antigen specific for CELO virus.

The PHELPS strain of CELO virus (Petek, Felloga & Zolletto, 1963) was used to inoculate the allantoic cavity of 7-day chick embryos. After 5 days' incubation at 36° the allantoic fluids were harvested, pooled and stored at −80°. This stock virus pool had a titre of $10^{8.5}$ TCD50/ml. in tissue cultures of chick kidney cells (Chomiak, Luginbuhl & Helmboldt, 1961).

Newborn hamsters (*Mesocricetus auratus*) were inoculated subcutaneously with 0·2 ml. of stock CELO virus; nine of 17 inoculated hamsters developed tumours during the following 12 months. From one tumour a tissue-culture cell line was established, and these cells have been serially passed in the laboratory. Cells of the 51st *in vitro* passage were transplanted subcutaneously in hamsters aged 3 to 4 weeks. Palpable tumours developed 4 weeks later and were excised, minced and the fragments inoculated subcutaneously into newborn hamsters (Gilden, Beddow & Huebner, 1967). Tumours approximately 15 mm. in diameter were removed 18 days after inoculation. The tumours were freed from adhering tissues, minced and briefly homogenized in a Waring blender as a 20% suspension in phosphate-buffered saline, pH 7·4. After freezing at −80° and thawing, the suspension was centrifuged at 3000 rev./min. for 30 min. and the supernatant fluid, which constituted the 'tumour antigen', stored at −80° in 1·0 ml. volumes. No infective CELO virus was recovered from the tumour antigen preparation after two serial allantoic passages in chick embryos followed by inoculation to cultures of chick kidney cells. A single serum pool obtained from hamsters bearing transplanted CELO tumours > 50 mm. in diameter was used for all complement-fixation and immunofluorescence studies. Similar procedures were used to obtain SV40 and adenovirus 12 tumour antigens and sera from tumour-bearing hamsters.

Tables 1 and 2 show the results of complement-fixation tests (Sever, 1962) with CELO-virus-induced tumour antigen and the homologous serum from tumour-bearing hamsters using overnight fixation at *c.* 4° with two units of complement. The specificity was controlled in parallel tests with SV40 and adenovirus 12 tumour antigens and sera from tumour-bearing hamsters. The CELO-tumour antigen had a titre of 1/32 against four complement-fixing units of antiserum from hamsters bearing CELO-

induced tumours but did not react at a 1/4 dilution with normal hamster sera or with sera from hamsters bearing adenovirus 12 or SV40 tumours. The serum pool from hamsters with CELO tumours had a titre of 1/80 against four units of homologous tumour antigen but failed to react (titre < 1/10) with the other antigens. No fixation of complement was detected when a 1/5 dilution of a fowl serum with CELO-virus-neutralizing antibody at a dilution of 1/640 was reacted with four units of CELO-tumour antigen. The CELO-tumour antigen did not react with COFAL hamster serum at dilution 1/10 and the sera from hamsters bearing CELO-induced tumours

Table 1. *Titres of complement-fixing antigen in virus-induced hamster tumours*

Serum from hamsters bearing tumours induced by	Complement-fixing titre of tumour antigen induced by		
	CELO	SV40	Adenovirus 12
CELO	32*	< 4	< 4
SV40	< 4	32	< 4
Adenovirus 12	< 4	< 4	128
Normal hamster serum	< 4	< 4	< 4

* Reciprocal of dilution of tumour antigen tested against 4 units of serum from tumour bearing hamsters.

Table 2. *Titres of complement-fixing antibody in sera of hamsters bearing virus-induced tumours*

Extract of hamster tumour induced by	Complement-fixing titre of serum from hamsters bearing tumours induced by		
	CELO	SV40	Adenovirus 12
CELO	40*	< 10	< 10
SV40	< 10	80	< 10
Adenovirus 12	< 10	< 10	80
Normal hamster tissue	< 10	< 10	< 10

* Reciprocal of serum dilution tested against 4 complement-fixing units of tumour antigen.

did not react with COFAL antigen at 1/4 (Sarma, Turner & Huebner, 1964). The COFAL antigen and hamster serum had homologous complement-fixing titres of 1/40 and 1/640 respectively. Thus, the fixation of complement by CELO-tumour antigen and homologous serum from tumour-bearing hamsters was not due to contaminating avian leukosis virus.

In further experiments the localization and specificity of the CELO-tumour antigen was examined in tissue cultures derived from virus-induced hamster tumours. All the tumour cells studied had been sub-cultivated at least 50 times *in vitro*. Coverslip monolayers of the hamster tumour cells induced by adenovirus 12, SV40 and CELO viruses were each examined for fluorescence using the indirect technique with sera from tumour-bearing hamsters (Pope & Rowe, 1964). Specific granular fluorescence, similar to that described for SV40 tumour antigen (Pope & Rowe, 1964), was detected in the nuclei of 100% of CELO tumour cells treated with 1/8 dilution of serum from hamsters bearing transplanted CELO tumours. In control preparations no fluorescence was detected when serum from hamsters bearing CELO-induced tumours was used to treat SV40 or adenovirus 12 tumour cells. Similarly, sera from hamsters bearing trans-

planted adenovirus 12 or SV40 tumours failed to induce fluorescence in CELO tumour cells.

These results indicate the presence of a specific tumour antigen in hamster tumours induced by CELO virus. This antigen is demonstrable by complement-fixation and immunofluorescence and is apparently immunologically distinct from tumour antigens induced by adenovirus 12 and SV40 virus. Serological studies with early *in vitro* and *in vivo* passages of CELO-virus-induced tumour cells failed to detect a specific tumour antigen. In the present study tumour antigen was present in tumour cells which had been repeatedly sub-cultivated *in vitro*. This may explain the absence of a tumour antigen detectable by complement-fixation tests in the first report of hamster tumours induced by CELO virus (Sarma *et al.* 1965).

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