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Highly efficient induction of type C retroviruses by a human tumor in athymic mice

(murine leukemia/peptide maps/structural markers)

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ABSTRACT **We have found that 1 of 20 human tumors tranplanted and passaged in *nude* mice was associated with a massive induction of endogenous murine leukemia virus (MuLV). Separation and growth of these viruses on various substrates indicated that both ecotropic and xenotropic MuLV were present in the induced mixture. Tryptic peptide fingerprints of the p30 and gp70 structural elements of the viruses indicated that all of the known endogenous MuLVs of BALB/c mice were present in the mixture. In addition, a new xenotropic MuLV was identified. The human tumor that induced the viruses was an oat cell carcinoma. The oat cell carcinoma possibly produced a specific hormone or factor that acts as a potent inducer of endogenous type C retroviruses.**

The use of the athymic *nude* mouse as a vehicle for the maintenance of heterotransplanted tumor tissue is an established procedure. It has been recognized that xenotropic and ecotropic murine leukemia viruses (MuLV), endogenous to *nude* mice, can productively infect transplanted tumor cells (1–4). The resulting addition of mouse viral proteins to the tumor cell's antigenic repertoire can complicate immunological and biochemical studies of the tumor, not to mention efforts to identify possible retroviruses endogenous to the tumor cell. In this report we describe a differential effect of various human tumors transplanted into *nude* mice to induce endogenous MuLV.

In contrast to most human tumors established in our mouse colony, it was found that passage of an oat cell carcinoma in *nude* mice caused the induction of a large variety and quantity of MuLV. The viruses were grown on mouse and heterologous cells *in vitro* to separate host range types. Peptide mapping of viral gene products gp70 and p30 indicated that all of the known MuLVs endogenous to BALB/c mice were present in the original mixed viral population. This was surprising because some of these viruses had not been inducible by previously reported means. The superior viral inductive qualities of the oat cell carcinoma, relative to other human tumors, suggests that it possessed, induced, or produced and secreted a potent inducer of endogenous MuLV.

MATERIALS AND METHODS

Tumor Transplantation. Oat cell carcinoma and other human tumors were serially transplanted subcutaneously in BALB/c *nu/nu* mice bred in our laboratory, as described (5).

Electron Microscopy. Tissues for electron microscopy were fixed in 2% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon. Thin sections were counterstained in uranyl acetate and lead citrate.

Cocultivations. Tumor tissue was excised after subcutaneous growth in BALB/c *nu/nu* mice, portions were minced and trypsinized, and cultures were prepared by seeding 3×10^6 cells per 75-cm^2 tissue culture flask. SC-1 (murine) or CCL 64 mink lung cells were cocultivated with primary tumor cell suspensions in a 1:1 ratio (1×10^6 mouse or mink cells with 1×10^6 tumor cells). Cultures were re-fed and subcultured at weekly intervals.

Virus Isolation and Detection. Viruses were grown on SC-1 mouse cells, mink lung cells (CCL-64), rabbit endothelial cells, and W138-VA13 [simian virus 40-transformed human fibroblasts (CCL-75)] as described (6, 12). Filtered supernatant fluids were concentrated and assayed for reverse transcriptase activity, as described (6). Cultures that were positive for reverse transcriptase activity were further purified according to host range by passage of cell-free virus from mouse to nonmouse cells and conversely for at least two passages.

Ecotropic MuLV infectivities were tested on secondary NIH Swiss and BALB/c embryo fibroblasts by the XC or SXC assays (7, 8).

Virus Purification. Virus was concentrated by centrifugation of filtered supernates through 15% (wt/vol) sucrose onto a cushion of 50% sucrose. Centrifugation was for 60 min at 25,000 rpm ($105,000 \times g$) in a Beckman SW 27 rotor in the Beckman LS-50 centrifuge. The concentration step was repeated once and the virus was finally purified by isopycnic sedimentation through a 15–50% (wt/vol) sucrose gradient in STE buffer at pH 7.5 (0.1 M NaCl/0.01 M Tris·HCl/0.1 mM EDTA). Centrifugation was for 16 hr at 35,000 rpm ($155,000 \times g$ av.) in the Beckman SW 41 rotor. At the end of the run, fractions were collected from the gradient and the refractive index was determined on each fraction with a Bausch and Lomb refractometer.

Peptide Maps. Proteins from sucrose gradient-purified viruses were separated by $NaDodSO_4$/polyacrylamide slab gel electrophoresis according to Laemmli (9). Viral p30 bands were excised from the stained gels, labeled with ^{125}I, and digested with trypsin; the peptides were separated in two dimensions on cellulose-coated plates as described (10, 11). The isolation and peptide mapping of MuLV gp70 were as described (6).

RESULTS

Human tumors that were transplanted directly to *nude* mice without prior *in vitro* cultivation were periodically examined

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Abbreviation: MuLV, murine leukemia virus.

Table 1. Virus particles in human tumors passaged in *nude* mice

Tumors	Passage	Virus particles*
Astrocytoma (T-24)	22nd, 34th, 37th	−
Oat cell carcinoma (T-293)	10th, 11th, 15th	++++
Melanoma (T242)	21st	−
Colon (T84)	27th	−
Teratocarcinoma (T-259)	10th	−
Epidermal carcinoma of lung (T-222)	19th	−
Colon adenocarcinoma (T219)	20th	−
Colon adenocarcinoma (T183)	22nd	−
Colon adenocarcinoma (T347)	2nd	−
Lung adenocarcinoma (T291)	11th	−
Rectal adenocarcinoma (T348)	4th	−
Colon adenocarcinoma (metastatic to lymph nodes)(T245)	10th	−

* −, No virus particles seen in fields encompassing at least 50 cells.

by electron microscopy. In striking contrast to all other tumors thus examined, T293, an oat cell carcinoma, showed the presence of a large number of type C particles. Table 1 summarizes these findings with T293 and 11 other tumors. Fig. 1 is a typical electron micrograph of a cross section through T293 cells after 10 passages in *nude* mice; an unusually large number of intercellular mature type C particles were evident.

After type C retroviral particles were seen by electron microscopy, T293 cells were cocultivated *in vitro* with mink lung and SC-1 cells. Viruses produced by these cell lines were purified, and the viral proteins were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis. Fig. 2 shows tryptic peptide maps of the viral core p30s from the viruses isolated from the T293 cocultivations with mink lung and SC-1 cells. The distinguishing structural markers on MuLV p30 are indicated in the peptide profiles. As reported (11, 12), MuLV p30 tryptic peptide no. 12 (p30-T12) will migrate to different positions depending upon the functional subclass to which the virus belongs. The positions of T12 have been given letter designations. Most wild mouse virus p30s have T12A; T12B is a characteristic of B-ecotropic and X^b xenotropic MuLV (MuLV-X^b); T12C distinguishes N-tropic MuLV of inbred mice; and T12D is a marker of X^a xenotropic viruses (MuLV-X^a). An additional marker in MuLV p30, relevant to these studies, has been noted in the B-ecotropic virus WN1802B. This structural marker (1802B) had not been seen on any other viral p30 until the present study (11).

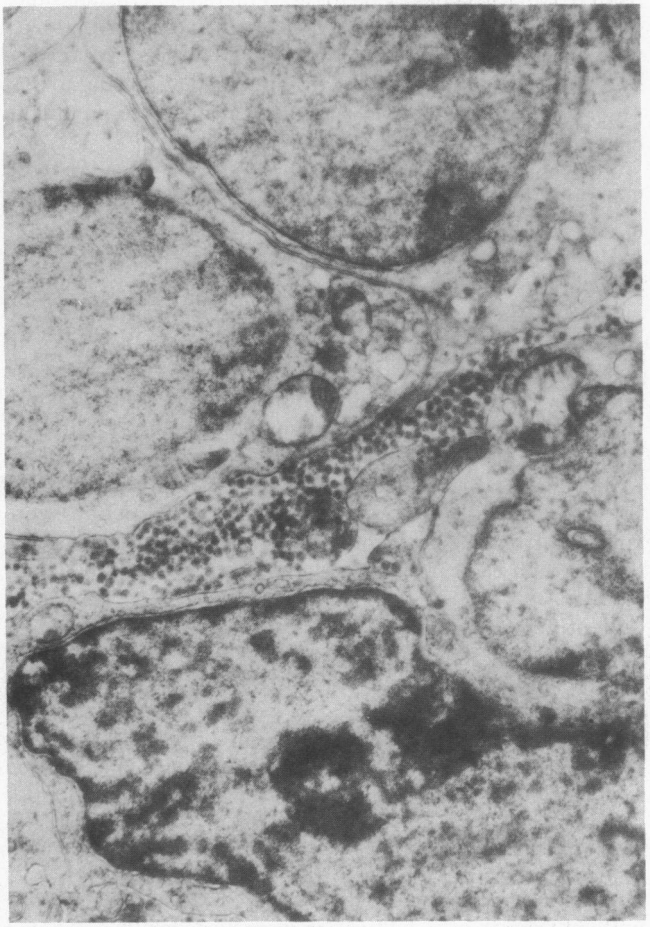

FIG. 1. Electron micrograph of a human oat cell carcinoma after 10 passages in the *nude* mouse. There are more than 200 type C particles visible in this photograph. (×8900.)

Table 2. Isolation protocol for type C retroviruses associated with T293 oat cell carcinoma after 10 passages in BALB/c *nu/nu* mice

	Viral core p30 marker	Virus
Cocultivation:		
A. T293 and mink lung cells	T12D	MuLV-X^a
B. T293 and SC-1 cells	T12C	N-Ecotropic
	T12B	B-Ecotropic
	(T12D)	MuLV-X^a
	(1802B)	MuLV-X^b or B-ecotropic
Cell-free viruses from cocultivation B used to infect:		
Mink lung cells	T12B	MuLV-X^b-1
	(T12D)	MuLV-X^a
Rabbit endothelial cells	T12B	MuLV-X^b-1
	T12D	MuLV-X^a
SC-1 mouse cells	T12B	B-Ecotropic
	T12C	N-Ecotropic
Transformed human fibroblast, WI-38–VA13	T12B	MuLV-X^b-1
	1802B	MuLV-X^b-2

Summary of distinct viruses of viral proteins associated with T293

Xenotropic:
- MuLV-X^a
- MuLV-X^b-1
- MuLV-X^b-2 (1802B marker)

Ecotropic:
- N-ecotropic
- B-ecotropic

As shown in Fig. 2*a*, the virus isolated from cocultivation of T293 with mink lung cells showed a p30-T12D marker only. This indicated that MuLV-X^a was either the only virus that grew in this culture or that it was in excess (greater than 10-fold) to the point such that other viruses could not be detected by this method. However, peptide analysis of the p30s from the viruses isolated from T293 and SC-1 cocultivation (Fig. 2*b*) showed that the T-12B and C markers are present, signalling a mixture of at least two distinct MuLV. A cell-free supernatant containing this mixture of viruses was then used to infect mink, human, rabbit, and mouse cells. Two to 3 weeks after infection, when the four cultures were positive for particle-associated reverse transcriptase, the viruses were purified and peptide maps were obtained as described above. The peptide profile (Fig. 3*a*) showed the presence of p30 marker T12B and a trace of T12D, indicating that MuLV-X^b-1 and MuLV-X^a were produced by rabbit cells. Fig. 3*b* shows, by the presence of the T12B and

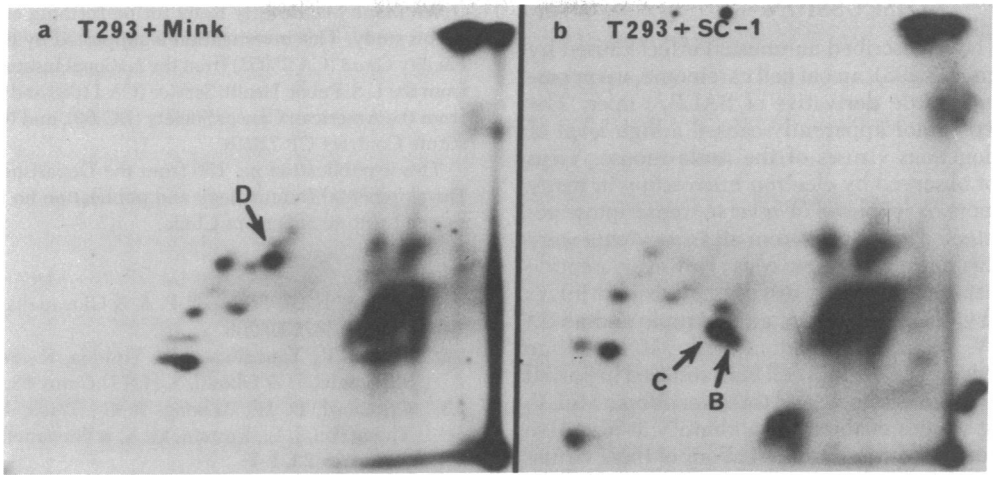

FIG. 2. Two-dimensional ^{125}I-labeled tryptic peptide maps of p30s isolated from virus produced by cocultivation of T293 and mink cells (*a*) and of T293 and SC-1 cells (*b*). The indicated lettered peptides are structural markers of specific MuLV p30 subtypes, as described in the text.

T12D markers, that MuLV-X^b-1 and MuLV-X^a also grew in mink cells. The presence of p30-T12B and T12C markers (Fig. 3*c*) suggests that SC-1 mouse cells are producing a mixture of N- and B-ecotropic MuLV in approximately equal numbers. In confirmation of this, viruses isolated from individual SXC plaques (8) displayed either N- or B-tropism when assayed on homozygous *Fv-1^n* or *Fv-1^b* mouse embryo fibroblasts (data not shown). Fig. 3*d* shows a tryptic peptide map of the p30s from the virus mixture produced by the transformed human fibroblast cells. The presence of both the prototype p30 peptides (small arrows) and the 1802B marker indicated that two xenotropic viruses were present in the mixture. Both virus p30s displayed T12B, so each was of the class III or MuLV-X^b category of xenotropic MuLV, but the distinguishing feature was that one had the 1802B marker whereas the other did not. These were designated MuLV-X^b-2 and MuLV-X^b-1, respectively.

Table 2 summarizes the above-described isolation and partial purification scheme of the viruses from passage-10 T293 cells. MuLV-X^a and ecotropic MuLV were isolated from passage-4 T293 cells, suggesting that extended passage of T293 in *nude* mice is required to induce the full repertoire of BALB/c viruses. Peptide maps of viral gp70 (not shown) indicated that both ecotropic and xenotropic viruses were associated with T293, in agreement with the p30 structural studies.

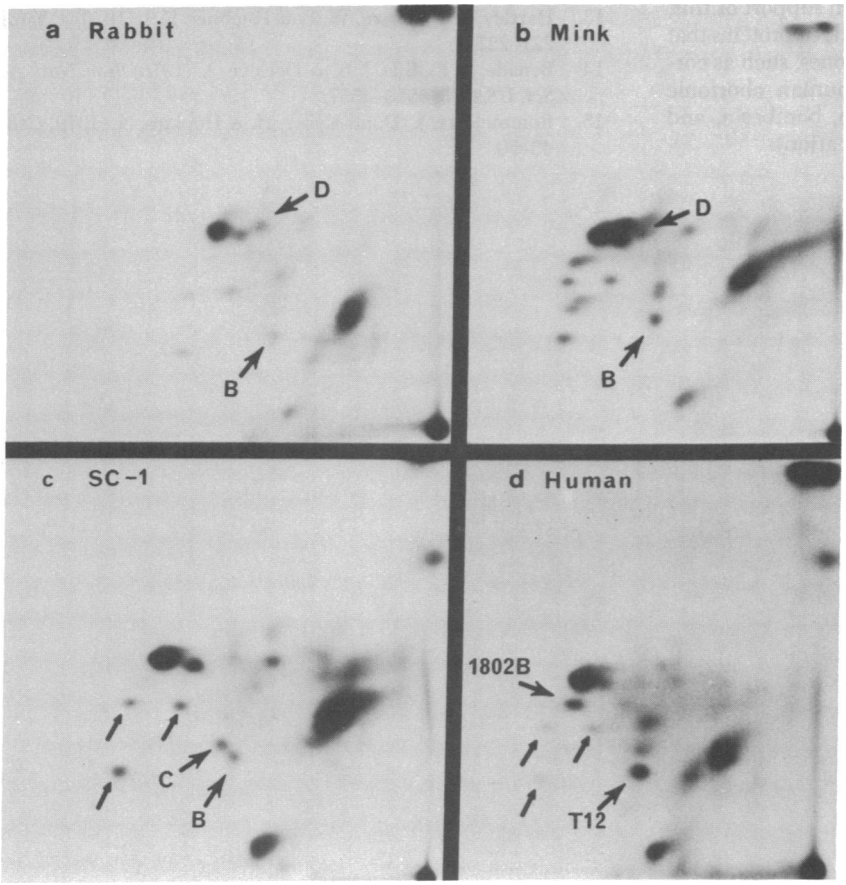

FIG. 3. Two-dimensional tryptic peptide fingerprints of MuLV p30s isolated from rabbit (*a*), mink (*b*), SC-1 mouse (*c*), and WI-38-VA13, simian virus 40-transformed human fibroblast (*d*). Cell cultures had been infected with cell-free culture harvest from the T293 + SC-1 cocultivation described in Fig. 2*b*. The lettered peptides are various positional markers of tryptic peptide 12 (p30-T12) that are indicative of MuLV p30 subtypes. The small arrows in *c* and *d* indicate peptides that are present in prototype p30 fingerprints. The 1802B marker in *d*, in addition to the prototype spots, indicates a mixture of viruses.

DISCUSSION

In this report we have described an unusual effect caused by a human lung tumor (T293), an oat cell carcinoma, upon passage in the athymic *nude* derivative of BALB/c mice. The heterotransplanted tumor apparently caused a high level of induction of endogenous viruses of the *nude* mouse. Virus particles were not observed by electron microscopy in many other human tumors. A low level of reverse transcriptase activity could be detected when cells from an astrocytoma were cocultivated with mouse and mink cells. However, peptide maps indicated that there were only two distinguishable MuLVs being produced by the astrocytoma, an ecotropic and an X^a xenotropic MuLV. *In vitro* cultivation of the 10th passage T293-associated viruses on various cell lines resulted in partial purification of N- and B-ecotropic and three xenotropic MuLV. Peptide mapping studies enabled us to identify at least five distinct viruses from the original mixture. Four of these viruses (MuLV-X^b-1, MuLV-X^a, and N- and B-ecotropic MuLV) were MuLV that have been previously isolated from BALB or *nude* mice. One xenotropic MuLV, MuLV-X^b-2, that displayed the distinct 1802B p30 structural marker, had not previously been isolated from mice. The p30 1802B structural marker had previously been associated only with a B-ecotropic MuLV (WN1802B, formerly BALB/c-S2B) originally isolated from a BALB/c mouse (13). B-Ecotropic viruses cannot be induced from BALB/c or C57BL mice directly and, in general, are thought to arise by recombination between endogenous MuLV-X^b and N-ecotropic viruses (11, 12, 14, 15). The association of the 1802B marker with the p30 from an endogenous xenotropic MuLV of BALB/c *nu/nu* suggests the likelihood that this virus was the progenitor of WN1802B.

The ability of the T293 oat cell carcinoma to elicit a strong inductive response of endogenous MuLV in *nude* mice, in contrast to most other human tumor cells tested, suggests that T293 cells may produce a factor or hormone that acts as a potent inducer of endogenous type C retroviruses. In support of this, it has recently been shown that T293 cells produce proteins that react with specific antisera to peptide hormones, such as corticotropin (adenocorticotropic hormone), human chorionic gonadotropin-releasing hormone, calcitonin, bombesin, and gastrin (R. DiAugustine, personal communication).

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