

**EXPRESSION OF ENDOGENOUS XENOTROPIC RETROVIRUS
BY METHYLCHOLANTHRENE-INDUCED SQUAMOUS CELL
CARCINOMA OF THE MOUSE RESPIRATORY TRACT***

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Physical and chemical agents as well as immunological stimulation are known both to induce tumors and to be associated with the expression of endogenous murine leukemia viruses (MuLV) (1-3). In at least two systems that have been well studied, the MuLV activated by an inciting oncogenic stimulus appears to be the agent responsible for subsequent tumor development (1, 2, 4, 5). The resulting tumors were composed of lymphoid cells. We wanted to know if retroviruses are expressed in chemically induced epithelial cell tumors because the large majority of known chemically induced cancers in man are derived from and are composed of epithelial cells. Here we report the expression of a complete MuLV in 3-methylcholanthrene (3-mc)-induced respiratory tract carcinomas of BALB/c mice.

Materials and Methods and Results

Our system adapted a tumor induction technique which had first been worked out in rats (6, 7) to mice. 8-wk-old BALB/cJ or (C57BL/6J × C3H/HeJ)_{F1} donor mice were killed with ether. Their tracheas were cut at the first cartilage ring and at the carina. The tracheas were removed and placed in Hanks' balanced salt solution. 3-mc was finely ground with mortar and pestle and 100 mg/ml was suspended at 100°C in an aqueous solution of 9 mg/ml sodium chloride and 120 mg/ml gelatin. The suspension was drawn into a glass syringe and allowed to harden at 4°C. One end of the removed trachea was closed with a 3-mm tantalum hemostatic clip (Edward Weck & Co., E. R. Squibb & Sons, Research Triangle Park, N. C.); a 23-gauge needle was inserted into the open end and the gel was forced through the needle into the trachea; the needle was withdrawn and the open end of the trachea was closed with a second clip. The same procedure was followed for control tracheas except that 3-mc was omitted from the gel. 8-wk-old recipient syngeneic mice were anesthetized with ether; the dorsal skin was shaved and washed with 70% ethanol and a 1-cm incision was made. One tracheal graft per animal was inserted under the skin and the wound was closed with a 9-mm stainless steel wound clip.

By 32 wks of age (24 wk after the graft was inserted), all experimental animals had developed gross tumors involving the graft. None of the twelve control animals had developed tumors and at 35 wk of age when autopsied, they each had normal-appearing pseudostratified ciliated tracheal epithelium in the graft. As seen in Table I, of the first 10 tumors examined, 3 were squamous cell carcinomas, 4 were sarcomas, and 3 were mixed tumors with elements of both sarcoma and carcinoma. Fig. 1 is a photomicrograph of a well-differentiated squamous cell

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TABLE I
Individual Histological and Virological Results of the First 10 Experimental Animals Killed

Tumor number	Time (from graft insertion to animal sacrifice)	Tumor histology	Mouse strain, sex	Virus titer (ecotropic)		Virus estimate (xenotropic)
				B tropic	N tropic	
	<i>mo</i>					
1	6	Mixed (carcinoma plus sarcoma)	BALB/c female	4×10^3	8×10^2	++§
2	6	Sarcoma	BALB/c female	3.5×10^3	3×10^2	Negative
3	6	Carcinoma	BALB/c female	Negative	Negative	++
4	7	Sarcoma	BALB/c female	Negative	Negative	Negative
5	7	Carcinoma	BALB/c female	Negative	Negative	++
6*	3	Sarcoma	C57BL × C3H male	Negative	Negative	Negative
7‡	4	Mixed (carcinoma plus sarcoma)	C57BL × C3H male	Negative	Negative	++
8	4	Mixed (carcinoma plus sarcoma)	C57BL × C3H male	Negative	Negative	+
9‡	4	Carcinoma	C57BL × C3H male	Negative	Negative	++
10*	4	Sarcoma	C57BL × C3H male	Negative	Negative	Negative

For each tumor, 2×10^6 tumor cells were applied to each of two 35-mm dishes of mouse embryo cells (one BALB and one NIH Swiss) and to a 60-mm dish of CCL64 cells, with the following exceptions:

* In tumors No. 6 and 10, a 0.1-ml vol of a 20% (wt/vol) cell-free extract was applied to each dish (because tumor No. 6 produced few free cells and tumor number 10 appeared to contain pus cells and possible bacterial contamination).

‡ In tumors No. 7 and 9, a 0.1-ml vol of a 2% (wt/vol) cell suspension was applied to each dish (because cells from these two tumors clumped and were impossible to count) and;

§ (+) designates up to 10 foci counted on the 22- × 11-mm cover slip at passage two; (++) designates 10-100 foci on the cover slip.

|| C57BL × C3H mice are Fv1^{nb} and would not be permissive for N or B tropic virus.

carcinoma that arose in and involved one of the grafts (animal No. 9).

To screen tumors for ecotropic MuLV, tumor cells were cocultivated with both BALB and NIH Swiss mouse embryo cells and assayed for MuLV using the UV-XC (8) assay modified as previously described (2, 4). To screen tumors for xenotropic MuLV, a fluorescent antibody focus assay (9) was used as follows. Tumor cells were cocultivated with CCL64 (mink) cells (American Type Cell Culture Collection, Rockville, Md.). The mink cells were subsequently passaged twice and at the second passage duplicate dishes, each containing a glass coverslip, were also seeded with the inoculated mink cells. The cell sheet that grew on the coverslip was fixed with acetone and incubated with fluorescein isothiocyanate-conjugated goat antiserum prepared against Tween ether-disrupted Moloney MuLV (Dr. Roger Wilsnack, Huntington Research Center, Brooklandville, Md.). Foci of cells infected with MuLV and expressing group-specific MuLV antigens fluoresce when viewed through a fluorescence microscope and these foci were counted.

The results are shown in Table I. Ecotropic MuLV was found in two of the three BALB/c tumors containing sarcoma and was absent from the pure BALB/c carcinomas and from the (C57BL/6J × C3H/HeJ)_{F1} tumors. Xenotropic MuLV was found in six out of six tumors containing carcinoma and was absent from the four pure sarcomas.

Discussion

Chemically induced respiratory tract tumors of the alveolar cell type have previously been found to contain retrovirus markers. Alveolar cell tumors, however, do not resemble any chemically induced tumors of humans, arise spontaneously in mice and merely increase in incidence in the presence of agents applied remotely such as intraperitoneal urethane, and usually do not invade and metastasize. In alveolar cell tumors, C-type particles have been seen by electron microscopy (10) and, when transplanted, these tumors have been positive for gs-1 and gs-3 antigens (11). From our model we have isolated complete infectious virus. The tumors in our model are

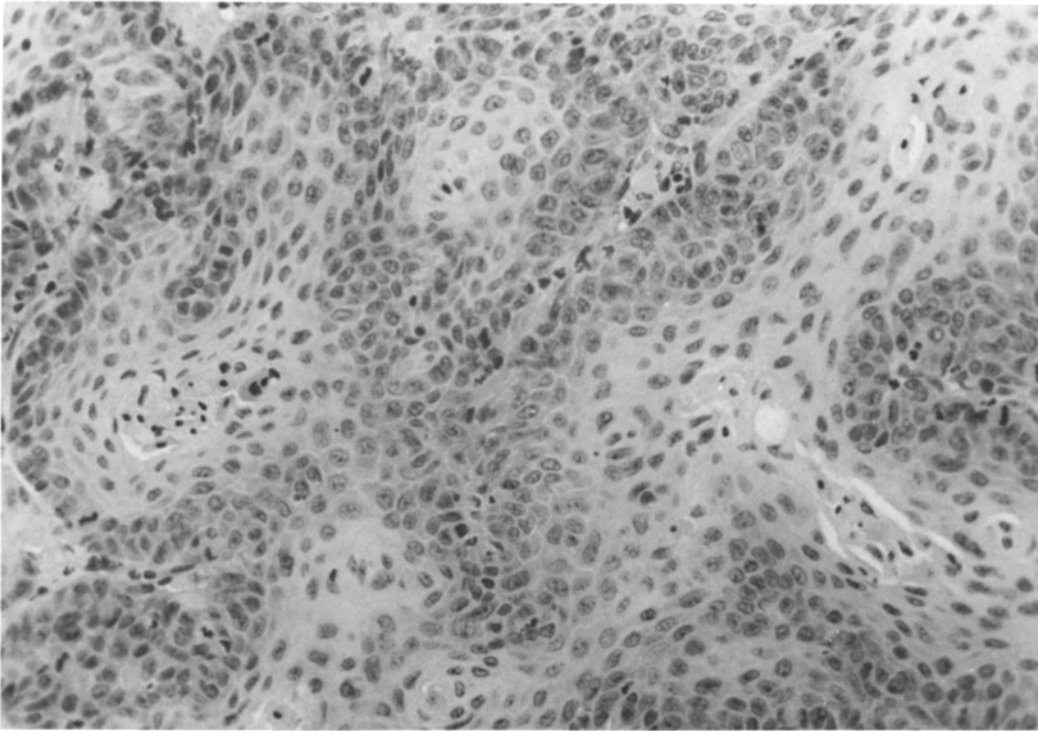


FIG. 1. Photomicrograph of a well-differentiated squamous carcinoma that arose in and involved the tracheal explant graft of animal No. 9. Typical epithelial pearls—swirling epithelial cells around a center—are seen. From the periphery of a pearl the cells flatten toward the center and at the center there is necrosis with pyknotic nuclei.

induced by exposing respiratory tract epithelium to 3-mc, a polycyclic hydrocarbon found in cigarette smoke. The grafts go through a sequence of morphological steps—basal cell hyperplasia, squamous metaplasia, and carcinoma *in situ*—that are indistinguishable from the changes found in the respiratory tract of human smokers. The final tumor, squamous cell carcinoma, is histologically identical to the most commonly found human lung cancer.

Summary

As a model for human lung cancer, squamous cell carcinomas were induced by 3-methylcholanthrene in mouse tracheas which had been explanted to a subcutaneous site. The tumors that developed were examined for both ecotropic and xenotropic infectious murine leukemia virus (MuLV). From all squamous carcinomas—six out of six—a xenotropic MuLV was isolated. From some of the fibrosarcomas that occurred incidentally in our induction system, ecotropic MuLV was isolated. However, in the fibrosarcomas, no xenotropic MuLV at all was found.

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