Lung Tumor-bearing Strain A Mice with Coincident Leukemia: An Electron Microscopic Study

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SUMMARY

Two instances of coincident occurrence of lung adenomas and leukemia in older strain A mice have been encountered. One mouse had received urethan 21 months prior to sacrificing and the other mouse was an untreated control. In both animals, thymuses, spleens, and livers were greatly swollen. Microscopically, these organs showed extensive infiltration by plasma cells. Electron microscopic examination of the tissues revealed type A-2 mouse leukemia virus particles within plasma cells and histiocytes in thymus and spleen and within plasma cells located in liver sinusoids. Type C mouse leukemia virus particles were found extracellularly in thymus and spleen. Virus particles were not observed in other cell types of thymus, spleen, or liver. Plasma cells were rarely seen in lung. Some lung tumor cells from the urethan-treated mouse contained type A-2 virus particles, and type C particles were observed extracellularly. Virus particles were not found in lung tumor cells from the untreated mouse. An etiological relation between the leukemia-associated virus particles and the lung adenomas is considered unlikely. A lung tumor cell susceptibility toward infection by these viruses would, however, appear to exist.

INTRODUCTION

Administration of urethan to mice of certain strains produces coincident lung adenomas and leukemia in a small proportion of animals (5, 13). In normal mice, coincidence of occurrence of these 2 neoplastic conditions is rare.

In the course of electron microscopic studies on mouse lung tumors, 2 instances of coincident lung adenomas and leukemia have been encountered in strain A mice. One of the mice had been treated with urethan; the other was not. This report describes and illustrates the ultrastructure of neoplastic cells from these mice.

MATERIALS AND METHODS

Strain A mice were purchased from Jackson Laboratory, Bar Harbor, Maine. One of the 2 mice with coincident lung adenomas and leukemia had received drinking water containing 0.1% urethan for a period of 24 days starting at the age of 3 months. This mouse was sacrificed at 24 months. The 2nd mouse, an untreated control, was 16 months old when sacrificed.

Lung, thymus, liver, and spleen tissues were removed from the urethan-treated mouse, and lung, thymus, and spleen tissues were taken from the untreated mouse. Tissue blocks, about 1 cu mm in volume, were cut from these organs and fixed in an aldehyde-osmium tetroxide combined fixative solution. Tissue blocks from the untreated mouse were immersed in a fixative made by combining, immediately prior to fixation, ice-cold solutions of 1.5% glutaraldehyde and 1% sucrose in 0.067 M cacodylate buffer, pH 7.4, and 1% OsO₄ in 0.067 M cacodylate buffer, pH 7.4. Tissue blocks from the urethan-treated mouse were immersed in a fixative made as above but containing 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, and 2% OsO₄. Tissues from the 1st animal were fixed for 3 hr at 5°, rinsed for 2 hr in cold 0.1 M cacodylate buffer (pH 7.4), and finally postfixed in 1% OsO₄ for 2 hr. Tissues from the 2nd animal were fixed for 2 hr at 5° and then immersed in 0.5% uranyl acetate for 30 min. A few lung tumor blocks from the urethan-treated mouse were fixed sequentially in the aldehyde and osmium fixatives—15 min in aldehyde fixative and 2 hr in OsO₄ fixative. Following fixation, tissues were dehydrated with ice-cold 50, 70, 95, and 100% ethanol and then with 100% ethanol and propylene oxide at room temperature. Blocks were infiltrated and embedded with Araldite according to the method of Luft (8). Sections were cut with glass or diamond knives using a Servali Porter-Blum II microtome, stained with 5% uranyl acetate and lead citrate (11), and examined with a Philips EM 200 electron microscope.

RESULTS

Thymus, spleen, and liver were greatly enlarged in both mice, but lymph nodes were not enlarged. The urethan-treated mouse had numerous lung tumors throughout the lungs. The untreated mouse had microscopic tumors only.

On electron microscopic examination, the thymuses of both mice were seen to contain large numbers of plasma cells in addition to thymocytes and histiocytes (Figs. 1 to 6). Virus particles were found in plasma cells and histio-
cytes but not in thymocytes. In plasma cells, virus appeared to form at the granular endoplasmic reticulum membrane (Fig. 3) and were also found free within the cisternae (Fig. 4). Viruses were noted, in addition, at the plasma membrane as well as outside but immediately adjacent to plasma cells. In histiocytes, virus particles occurred usually in groups (Figs. 5 and 6) unassociated with cell organelles, although virus could be found occasionally in relation to the endoplasmic reticulum as in plasma cells. Groups of virus particles occurring together with short rod forms (Fig. 7) were also seen in plasma cells. Cylindrical forms associated with leukemia viruses were described previously by Dalton et al. (2) in giant cells. Cylindrical forms associated with leukemia viruses were described previously by Dalton et al. (2) in giant cells from bone marrow of a C57BL/Kalw strain mouse with radiation-induced leukemia. Intracellular virus in both plasma cells and histiocytes in the present study had the appearance of type A-2 particles and varied in diameter from 650 to 750 Å, with some being as large as 800 Å (Fig. 9). Extracellular virus had the appearance of type C particles and measured about 850 Å in diameter.

The spleens of the 2 mice were similar in appearance to the thymuses in that both were characterized by massive plasma cell infiltration. Virus particles were found predominantly in plasma cells in the spleen.

In liver from the urethan-treated mouse, hepatocytes were uninvolved by virus. Numerous virus-containing plasma cells were found within the sinusoids. In many areas of liver, plasma cells virtually filled the sinusoids. A small fraction of hepatocytes contained long, fibrillar, membrane-enclosed bundles up to 1500 Å wide and greater than 2 μ long. These structures were similar to those observed and described by Essner (4) in hepatocytes from an apparently normal 18-month-old C3H strain mouse.

Plasma cells were rare in the lungs of either mouse and were not noted in the lung tumors. Lung tumor cells of the untreated mouse appeared unaffected by the coincident plasma cell leukaemia. The majority of tumor cells from the urethan-treated mouse were also unaffected. Ultrastructural differences noted between tumor cells from the 2 animals could be attributed to effects of fixation, as previously shown (1). A minority of lung tumor cells from the urethan-treated mouse contained virus particles (Figs. 9 to 12). The virus were the same size and had the same structure as those within plasma cells previously illustrated. Virus particles were also observed within vacuoles of alveolar macrophages (Fig. 12). The extent of viral involvement varied from cell to cell. Fig. 9 illustrates a tumor cell showing slight virus involvement. Here, only a few particles are present within the cytoplasm or budding from the plasma membrane. Other lung tumor cells (Fig. 10) contained masses of virus particles. At higher magnification (Fig. 11), particles are noted budding from the plasma membrane as well as lying free in a lumen formed by the tumor cell apices. Viruses particles outside the tumor cells appear as type C particles. Viruses were not observed in normal lung cells, with the exception of alveolar macrophages associated with tumors.

DISCUSSION

The mouse lung adenoma is not considered generally to be caused by viruses. In recent years, however, the issue of viral involvement was introduced by Klärner and Gieseking (7). In an electron microscopic study of mouse lung tumors, these investigators described certain intracellular particles as "elementary particles," and suggested that they were possibly virus. These particles, about 200 Å in diameter, were not membrane coated but were within a matrix which, from comparison with light microscopic sections, stained positively by the periodic acid-Schiff method. Svoboda (12), in a later electron microscopic study of mouse lung tumors, observed similar particles but considered them to be nonviral. Hattori et al. (6) also noted 200-Å particles in lung tumor cells and pointed out that they were similar to glycogen in size and shape, but discounted the possibility that the particles were glycogen because they were observed within nuclei also. The morphological appearance of glycogen in hepatocytes and other cells is now well known. On the basis of this knowledge and previous observations of lung tumor cells (1), I conclude that the "elementary particles" described and illustrated by Klärner and Gieseking are very likely to be glycogen.

Rabotti (9) described myxo-like virus particles in the connective tissue of a spontaneous lung tumor in a BALB/c mouse. These particles measured 1200 to 1400 Å in diameter and consisted of a nucleoid surrounded by a single membrane. No particles within tumor or other lung cells were illustrated.

Raine et al. (10) illustrated virus particles in an old BSVA strain mouse lung tumor. The particles, 800 Å in diameter and of the A-2 type, budded off the plasma membrane of tumor cells. The lung tumor occurred in a mouse that had received, 18 months previously, an intracerebral inoculation from the brain of a mouse similarly inoculated with a multiple sclerotic brain suspension. No abnormality other than a single lung tumor was noted in the mouse. A coincident leukaemia condition was not reported.

Yumoto and Dmochowski (14) reported on spontaneous reticulum cell neoplasms in SJL/L strain mice. These investigators noted that, in 1 form of neoplasm, type A virus particles, 750 to 800 Å in diameter, were present within reticulum cells and plasma cells. The observations in the present study appear to parallel this finding of Yumoto and Dmochowski. However, as these investigators point out, the significance of this type of virus particle is still unknown. Previously, Dalton et al. (3), in discussing particles with the same structure, suggested that, "Evidence indicates that the particles do not represent an agent responsible for the development of neoplastic plasma cells...."

The occurrence of virus particles in some lung tumor cells in this study is related most likely to the coincident plasma cell virus involvement and is unlikely to have any etiological relation to the lung tumor.
A tentative conclusion may be reached on the basis of the observations reported here, namely: some lung tumor cells appear to share, with some plasma cell and histiocytes, a common feature relating to susceptibility to a specific type of viral infection. It is of interest, in regard to this tentative conclusion, that Yumoto and Dmochowski (14) found murine leukemia type C particles in a spontaneous mammary tumor of a SJL/J mouse with coincident reticulum cell neoplasm.

The presence of virus particles in some lung tumor cells from a urethan-treated mouse and the apparent absence in similar cells from an untreated mouse does not, in itself, indicate an effect of urethan. An insufficient amount of tissue was examined to warrant such a conclusion.

REFERENCES


Fig. 1. Thymus tissue from untreated mouse. Portions of a plasma cell (PC) and thymocyte (TH) are shown. The plasma cell has a large nucleolus, several nuclear bodies (NB), peripherally oriented nuclear chromatin, abundant granular endoplasmic reticulum (GER), and 2 viral particles (arrows) within the cisternae of the granular endoplasmic reticulum. X 29,200.

Fig. 2. Thymus tissue from untreated mouse. Portions of several plasma cells (PC) and intervening histiocytes (H) are shown. Several of the plasma cells have widely dilated granular endoplasmic reticulum cisternae. A few virus particles appear within the plasma cells (arrows). X 22,900.

Fig. 3. Thymus histiocyte from untreated mouse. A virus particle appears to be budding from the membrane of granular endoplasmic reticulum. X 79,000.

Fig. 4. Thymus plasma cell from untreated mouse. Two type A-2 virus particles are within the cisternae of the granular endoplasmic reticulum. X 137,000.

Fig. 5. Thymus from urethan-treated mouse. A plasma cell (PC) and part of a histiocyte are shown. A group of viral particles (arrow) are seen in the histiocyte (H). X 13,700.

Fig. 6. Thymus from urethan-treated mouse. Enlargement of a portion of the previous figure showing virus particles in a histiocyte and a single particle (arrow) budding from the granular endoplasmic reticulum membrane of a plasma cell. X 49,600.

Fig. 7. Thymus plasma cell from urethan-treated mouse. Virus particles and rod-like structures occur together in this cell. X 71,000.

Fig. 8. Thymus histiocyte from urethan-treated mouse. A group of virus particles are located in close spatial relation to membrane-enclosed dense bodies (DB). X 79,000.

Fig. 9. Lung tumor from urethan-treated mouse. Portions of several tumor cells are shown. Virus particles are noted within the cytoplasm and budding from the plasma membrane (arrows). X 29,200.

Fig. 10. Lung tumor from urethan-treated mouse. Portions of several tumor cells are shown. Groups of type A-2 virus particles are present in the apical region of 1 tumor cell. X 29,200.

Fig. 11. Lung tumor cells from urethan-treated mouse. At higher magnification, virus particles are noted budding from the cell and free outside the cells within a luminal space. Extracellular particles are type C. X 86,000.

Fig. 12. Lung tumor from urethan-treated mouse. Portions of 2 tumor cells (T) and an alveolar macrophage (MAC) are shown. Virus particles are within a tumor cell, between 2 tumor cells, and within a vacuole of the macrophage (arrow). X 32,000.
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