Fine Structure of a Transplanted Chemically Induced Nonlymphoid Thymoma

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SUMMARY

A chemically induced, serially transplanted nonlymphoid thymoma capable of immunologic restoration of neonatally thymectomized mice was examined by electron microscopy. The cytoplasm of most of the cells in this tumor is filled with single ribosomes and polysomes. A moderate amount of rough endoplasmic reticulum is present. Cells with the identifying characteristics of normal thymic epithelial cells are absent. Dense granules similar to those in thymic epithelial cells are observed in some tumor cells. Type A virus particles are present in association with rough endoplasmic reticulum.

INTRODUCTION

The immunologic deficiencies caused by neonatal thymectomy of mice may be corrected by host reconstitution after thymus grafting (6, 14). Immunologic restoration occurs when thymus fragments are within cell-impermeable diffusion chambers (18). It was demonstrated that a chemically induced nonlymphoid thymoma was effective in the restoration of immunologic functions when grafted subcutaneously into neonatally thymectomized mice (21, 22). These studies suggest that a humoral or inductive factor is produced by normal and neoplastic reticuloepithelial cells of the thymus.

In the present study the fine structure of a nonlymphoid thymoma capable of immunologic restoration was investigated. Observations have indicated an absence, in the transplanted tumor, of features characteristic of thymic reticuloepithelial cells. Type A virus particles were consistently present, usually in association with rough endoplasmic reticulum.

MATERIALS AND METHODS

The details of the technic for tumor induction have been presented in previous papers (21, 22). Briefly, 0.1 mg 7,12-dimethylbenzanthracene in 0.01 ml peanut oil is injected directly into the thymus of newborn inbred A mice. The strain A mice used in the experiments are highly inbred and belong to the colony of the late Dr. J. J. Bittner. They are presently at F12 in our colonies and are comparable to the description in the Fourth Listing of the Standardized Nomenclature for Inbred Strains of Mice (4). Mammary tumor incidence is approximately 3% in virgins and 88% in female breeders.

Cell suspensions containing 1 X 10^6 nucleated tumor cells were implanted subcutaneously into syngeneic 5-week-old mice of both sexes. The tissue examined in the present study is from the twelfth generation of such successive subcutaneous transplants. In previous work it was observed that the functional capacity of the strain A thymoma to restore neonatally thymectomized mice decreased after serial transplantation. Restoration of neonatally thymectomized strain A mice subcutaneously grafted at 15 days of age with the functional thymoma was 66% (8 of 12) when 3rd generation transplants were used, 20% (2 of 10) when treated with 9th generation transplantation thymomas, 8% (1 of 12) when treated with 12th generation transplants, and negative (0 of 25) when treated with 19th and 22nd generation transplants. Lactic dehydrogenase activity in plasma was measured according to the method of Wroblewski and LaDue (26). The procedure is based on the oxidation of reduced diphosphopyridine nucleotide by sodium pyruvate and is expressed as units (1 unit = decrease in optical density of 0.001 per minute per ml). Normal values ranged from 80 to 400 units.

For electron microscopy, small fragments of tissue were fixed for 2 hours in a modification of Karnovsky's fixative (12) containing 1% paraformaldehyde and 1% glutaraldehyde in phosphate buffer, pH 7.2-7.3. Postfixation in phosphate-buffered 1% osmium tetroxide (19) for 90 minutes was preceded by a 5-minute rinse in cold buffer (15). The tissue was dehydrated through graded ethanols and embedded in Dow epoxy resin 334 (25) or Epon 812 (5).

Thin sections were cut with glass knives on a Porter-Blum microtome, placed on uncoated 300- or 400-mesh grids, and stained with aqueous uranyl acetate and lead citrate (24). Specimens were examined with the Hitachi HU-11E-1 electron microscope at 50 kv. Images were recorded on DuPont Cronar film at original magnifications of 3,600 to 18,000.

Thick sections of plastic-embedded tissue were mounted on glass slides and stained with toluidine blue for examination with the light microscope.
RESULTS

The transplanted tumor (Fig. 1) is pleomorphic and composed of elongate and rounded cells with frequently irregular nuclei. Nucleoli are prominent. Mitotic figures (arrows, Fig. 1) are present in moderate quantity. The architecture typical of a normal thymus is not observed.

Electron microscopic examination reveals a large number of cells with a dense cytoplasmic matrix filled with single ribosomes and polysomes (Figs. 2–4). In addition, many cells contain a moderate amount of rough endoplasmic reticulum (Figs. 2, 4, 5). Lipid droplets (Fig. 2) and lysosomes (Figs. 3, 4) frequently are observed in the cytoplasm. Chromatin is clumped both centrally and peripherally in rounded and irregular nuclei.

Long cellular extensions frequently are sectioned transversely or obliquely and appear as unattached areas of cytoplasm in the intercellular space (open arrows, Fig. 2). Small, microvillous-like extensions from the cells also project into the extensive intercellular spaces (Figs. 2, 3; mw, Fig. 4).

Some cells, like the one in Fig. 3, bear some resemblance to thymic epithelial cells. They have an electron-lucent cytoplasm which contains many small vesicles, a moderate number of single ribosomes and polysomes, and scattered endoplasmic reticulum. However, no cells containing bundles of tonofilaments within the cytoplasm are observed. Desmosomal connections with adjoining cells are not observed. Large cytoplasmic vacuoles are absent. Thus, there seem to be no cells in the tissue examined which are identical in morphology with thymic epithelial cells. Dense granules (G, Fig. 2), which resemble similar structures in normal thymic epithelial cells, are present in a few cells.

The presence of virus particles (Figs. 2, 4, 5) is a fairly constant feature in all samples examined. They are called "virus" on the basis of their morphology. No biologic activity has been established. The number of particles in any particular cell profile is never very large. Although virus is not evident in all cell profiles, its presence in a number of different cells in all areas of thin sections leads to the impression that most cells have particles in some part of the cytoplasm, and, therefore, that the total number of particles is large.

Virus particles are located only intracisternally; none were observed in intercellular spaces. They occur in association with rough endoplasmic reticulum as shown in Figs. 2, 4, and 5. Most particles appear circular in profile. Their diameter is approximately 70 nm or larger. An interval of approximately 10 nm separates the outer membrane from the inner membrane. The central core usually is electron lucent but occasionally exhibits a moderate density. The appearance and dimensions of these particles correspond to the type A particles of Bernhard (1), and more specifically to intracisternal A particles (23).

Monthly determinations of lactic dehydrogenase levels in the plasma of normal, thymectomized, or thymoma-bearing strain A animals have always been within normal limits. Monthly or bimonthly control experiments to determine lactic dehydrogenase values are performed routinely in our mouse colony, and normal values have been found for the last year in all instances. The tested animals are selected at random.

DISCUSSION

The 12th generation transplanted thymoma examined in the present study was functional, i.e., it could restore the deficiencies produced by neonatal thymectomy although less efficiently than the original thymoma or earlier transplant generations and normal thymus. Thymic reticuloeipithelial cells have seemed a likely source of the humoral or inductive factor responsible for immunologic restoration. However, the identifying ultrastructural characteristics typical of thymic epithelial cells (tonofilaments, desmosomes, and large vacuoles) were not observed in the tumor.

Clark (3) constructed a hypothetical natural history of thymic secretion in the mouse, based on an extensive series of histochemical and ultrastructural studies. In this scheme, dense granules were suggested as the first evidence of thymic secretion while the larger vacuoles which accumulated later represented a shift from rapid release to storage of the secretory product. It is possible that the storage phase is not present in tumor cells observed in the present study. Large vacuoles with amorphous materials would not be evident, but scattered dense granules (Fig. 2) might represent a stage in the rapid secretory process. Tonofilaments and desmosomes have no known relationship to secretion, so that their absence only results in a loose structural configuration.

Virus particles have been observed in the thymus under a variety of conditions. de Harven (8) demonstrated virus in thymic lymphocytes and epithelial cells of conventional and germ-free mice. The particles which he described in lymphocytes appear somewhat similar to those observed in the present investigation, except they were not associated with rough endoplasmic reticulum. Similar particles were observed in a transplanted thymic lymphoma (8).

Gross and Feldman (11) examined thymus and other lymphoid tissue of C3Hf mice a few days after total-body X-irradiation and after the radiation had induced leukemia. In both groups, virus particles were observed budding from the cell membranes, with type C particles located in the intracellular spaces. The appearance of virus particles in relatively large numbers in lymph nodes, spleen, and bone marrow a few days after irradiation led these authors to suggest that irradiation may activate a latent leukemogenic virus. Similar particles were described by the same workers (10) in thymus and other lymphoid tissues of mice with virus-induced leukemia. In addition, they found smaller viruses, similar to those in the present investigation, in leukemic and normal C3Hf mice. Feldman and Gross also thought that the type A particles were probably unrelated to the mouse leukemia virus since they were not found in either leukemic or normal rats.

A comparative electron microscopic study by Dalton et al. (7) of murine lymphoid neoplasms induced by a variety of agents clearly indicated the involvement of type C particles in a majority of cases. Occasionally, type A particles were observed in cells considered to be malignant lymphoblasts in tumors induced with the Moloney agent; no relationship with type C particles was noted.

Stephens et al. (20) described type A intracytoplasmic viral particles in one cell type of a murine testicular interstitial cell tumor. These workers thought this virus was directly involved...
in the establishment of the tumor because B and C-type particles were not present. Cell types having a large amount of rough endoplasmic reticulum, however, were devoid of virus particles. Type A virus particles were encountered in relation to the endoplasmic reticulum in studies of melanoma by Nathaniel et al. (16) and Channing, et al. (2). Kuff et al. (13) correlated chemical and fine structural alterations of intracisternal A particles liberated from mouse plasma cell tumors.

The particles observed in the present study bear some morphologic resemblance to the lactic dehydrogenase virus that superinfects many spontaneous and induced tumors in mice (9, 17). The normal enzyme values in the plasma of our mice, whether normal or tumor-bearing, are against this possibility since high lactic dehydrogenase values in plasma are characteristic of this viral infection (17).

Thus, it seems that although the type C particle is usually associated with virus-containing neoplasms, the possibility that type A particles, including intracisternal A particles, may be directly associated with some tumors should not be ruled out. The possibility that, in the present study, direct application of dimethylbenzanthracene activated latent virus within the neonatal thymus should receive further consideration.

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REFERENCES

Fig. 1. Epoxy thick section stained with toluidine blue. The pleomorphic nature of the tumor is evident. Mitotic figures are indicated by arrows. × 670.

Fig. 2. An abundance of ribosomes in a dense cytoplasmic matrix may be seen in these tumor cells. Moderate amounts of rough endoplasmic reticulum and mitochondria (m) are evident in the middle two cells. Sections of large cell processes (open arrows) are in an extensive intercellular space. Intracisternal virus particles are indicated by arrows. Dense granules (G) occur in one cell. × 15,700.

Fig. 3. The cell in the center of this electron micrograph bears some resemblance to a thymic epithelial cell but has no tonofilaments, desmosomes, or large vacuoles. A lysosome is located directly below the nucleus. Extenuated cell processes are in the intercellular space. Smaller, microvillus-like projections from the lower tumor cells are evident. × 15,700.

Fig. 4. Virus particles (arrows) are associated with rough endoplasmic reticulum. Individual and aggregated ribosomes are numerous in the dense cytoplasmic matrix. Microvillus-like projections (mv) extend into the intercellular space. Cytoplasm of cells in lower part of micrograph is relatively less dense and contains fewer free ribosomes. A lysosome is seen in lower right corner. × 44,600.

Fig. 5. Virus particles (solid arrows) associated with rough endoplasmic reticulum. Open arrow indicates site of probable budding. × 117,000.
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