Transplantable Plasma Cell Tumor of Mice in Later Generations

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SUMMARY

An Adj-PC-5 variety of experimental mouse plasma cell tumor, obtained at its 40th generation, was studied by light and electron microscopy through 16 transplant generations. Tumor cells were poorly differentiated and showed loss of plasma cell characteristics when compared to earlier generations of the same variety of tumor. However, the tumors continued to produce a high level of the IgG type of immunoprotein, as did the earlier generations. Considerable morphologic differences exist between the cells of human multiple myelomas and those of later generations of experimental plasma cell tumors.

INTRODUCTION

Experimentally produced plasma cell tumors in laboratory mice elaborate immunoproteins which are physicochemically similar to those seen in human multiple myelomas and other plasma cell proliferative disorders (13). The initiation of the tumors is achieved by intraperitoneal introduction of a variety of substances, including mineral oils such as Bayol F (14), plastics (9), and incomplete Freund’s adjuvant (16). Each of these substances is capable of acting as a chronic irritant to the reticuloendothelial system, because they are not readily removed by the cellular response that they elicit. The initial reaction resembles an inflammatory granuloma consisting of lymphocytes, histiocytes, and plasma cells. From this reaction there gradually evolves a well-differentiated plasma cell tumor which subsequently metastasizes to different organs (15). The host animal develops multiple tumor nodules during transformation from a granulomatous to a neoplastic process. All of the nodules have a similar morphologic appearance; however, each nodule has the ability to produce a physicochemically distinct immunoprotein. Therefore, several different plasma-cell tumor lines can be produced in transplantation from one primary animal. Once the nature of the immunoprotein is established in a clone, it persists in successive transplants (12). Although neither the production of the immunoprotein nor its nature alter with successive transplants, the morphology of the tumor cells appears to change from a well-differentiated plasma cell type to an undifferentiated cell type.

The histologic and ultrastructural characteristics of the early generations of experimental plasma cell tumors have been previously described (4, 5, 11). We have studied 16 successive transplants of an Adj-PC-5 variety of experimental plasma cell tumor, obtained by us at the 40th generation, and we present a light and ultramicroscopic description of later generations of this tumor.

MATERIALS AND METHODS

Six BALB/c female mice (BALB/cAn), carrying the transplantable plasma cell tumor, were obtained from the National Institutes of Health, Bethesda, Maryland. The tumor type was Adj-PC-5, which produces a high level of the IgG type of immunoprotein (13). It was supplied at its 40th generation. In our laboratory, the tumor was transplanted to male and female BALB/cJ mice, an inbred mice strain of BALB/c mice, obtained from the Jackson Laboratories, Bar Harbor, Maine. The mice were 8—14 weeks old at the time of transplantation. Tumor tissue from each donor mouse was transplanted to recipients, numbering 2—4 in each group, and this procedure was carried out in 16 tumor generations. Female mice made up most of the experimental animals; no sex difference in growth pattern was noted.

The tumor tissue was minced in normal saline or Medium 199 obtained from the Grand Island Biological Company, Grand Island, New York, and 0.2 ml of the fluid medium, containing a few tiny particles of tumor tissue, was injected subcutaneously into the right axillary region with a 20-gauge needle. The tumor grew in all recipients, and the tumor-bearing mouse, selected as the donor to the next batch of mice, was sacrificed at the 9th to 12th day by severance of the spinal cord. Necropsies of all animals in each group were done. For light microscopy, tissues were fixed in 10% formalin and absolute alcohol. Sections of paraffin-embedded tissues were stained with hematoxylin and eosin, hematoxylin-phloxin-saffronin, Giemsa, Masson’s trichrome, periodic acid-Schiff (PAS), methyl green pyronin, and with stains for reticulin fibers and inclusion bodies. Representative sections from the transplanted tumor mass, heart, lungs, liver, spleen, thymus, mediastinal lymph nodes, kidneys, and ribs were examined in all mice. Decalcified portions of the limb bone adjacent to the
main tumor mass, and sections from the brain, gastrointestinal tract, and other sites were examined in selected cases.

For electron microscopy, a sample was obtained from the subcutaneous tumor, growing at the site of inoculation, from one mouse chosen at random in each transplant generation. On reflection of the overlying skin, the tumor surface was flooded immediately with buffered glutaraldehyde. Selected pieces were taken from the viable, actively growing peripheral region of the tumor. The pieces were kept in phosphate-buffered, 5% glutaraldehyde at 4°C for 1.5–2 hours. They were then washed with sucrose buffer and postfixed in phosphate-buffered 1% osmium tetroxide for 1½ hours. After serial dehydration in increasing concentrations of ethyl alcohol, the pieces were embedded in epoxy resin. One-half micron sections were cut on a Servali Porter-Blum microtome and stained with toluidine blue for preliminary light microscopy assessment. Sections for electron microscopy were made, using a Reichert “Om U2” ultramicrotome, and double stained with uranyl acetate and lead citrate. Sections were examined and photographed with the Philips EM 75B and 100B instruments.

For immunoelecrophoresis, pooled sera of 3 tumor-bearing animals and of 3 control mice of the same strain, sex, and age were obtained. Analysis was done with LKB immunophor instrument (LKB Producter, Stockholm) using agar gel as the supporting medium with a current of 20 milliamperes and 200 volts over a 1-hour period. Antiserum to mouse serum for preliminary light microscopy assessment. Sections for electron microscopy were made, using a Reichert “Om U2” ultramicrotome, and double stained with uranyl acetate and lead citrate. Sections were examined and photographed with the Philips EM 75B and 100B instruments.

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RESULTS

Results in the 16 transplants did not vary with respect to manner of tumor growth and cell morphology. The following descriptions pertain to all of the transplant generations studied.

In animals with minced tissue implantation, the tumor became apparent at about the 5th day as a diffuse swelling at the base of the right forelimb. The swelling rapidly increased in size and by the 9th or 10th day usually measured about 3.0 cm in principal dimension. The host animals became lethargic, moved with difficulty, and the fur became coarse; the animals died between the 11th and 13th day. On palpation the tumor was firm in the early stage of growth but became progressively softer. Occasionally there was ulceration of the skin over the tumor.

**Gross Findings**

At autopsy the tumor was very soft and difficult to grasp with forceps. Centrally, there were chalky white necrotic areas and foci of both recent and old hemorrhage. Peripherally the tissue was grayish-tan, moist, and glistening. The mass at the site of implantation was firmly adherent to the overlying skin and had invaded extensively the muscles at the base of the right forelimb and of the chest wall, with firm fixation about the ribs. In some animals the tumor had penetrated into the right pleural cavity causing hemorrhagic effusion. Except for slight splenic enlargement, no other significant abnormalities were noted.

**Light Microscopy**

The tumors growing at the implantation site were composed of compact cellular lobules and islands. They were surrounded by delicate connective tissue strands, composed of reticulin fibers containing capillary blood vessels, and were separated from each other by an empty space or by homogenous eosinophilic material and red blood cells. The tumor cells measured from 35 to 50 microns in diameter and had scanty, often vacuolated, basophilic cytoplasm and distinct cell borders. Their vesicular nuclei occupied two-thirds or more of the cell and were indented or lobulated; the majority had one or more nucleoli (Fig. 1).

The peripheral chromatin strands were of irregular thickness but did not reproduce the clock-face pattern of mature plasma cells. Mitoses were frequent and included abnormal tripolar forms. Most of the viable tumor cells exhibited pyroninophilia, and a few contained tiny PAS-positive cytoplasmic droplets. Cytoplasmic inclusions were not demonstrated. Invasion of the upper corium and underlying musculature was evident. Cells in the center of the tumor showed degeneration and necrosis.

The mediastinal lymph nodes showed metastatic involvement. In all nodes the marginal sinusoids contained tumor cells, and in some the medulla was partially replaced by the tumor. Despite its proximity to involved mediastinal nodes, the thymus was not involved. Tumor cells were commonly seen in the sinusoids of the liver (Fig. 2), spleen, and pulmonary septal vessels, and were infrequently identified in glomerular capillaries (Fig. 3). Metastases were not found in the other organs or in the bone marrow.

**Electron Microscopy**

The plasma membrane was generally stretched out, with slight undulations, but without deep infolding. No surface microvilli were present. The plasma membranes of adjacent cells were in close apposition, but intercellular junctions were not seen; a narrow space was present between cells, and frequently triangular spaces between cells contained fine granular material (Fig. 4).

The cytoplasmic volume was considerably less than the nuclear volume. In the normal plasma cell and in the well-differentiated plasma cell tumors, the quantity of cytoplasm far exceeds the nuclear volume, and most of the cytoplasm is occupied by stacks of cisternae of rough endoplasmic reticulum. In the series of tumors that we examined, the endoplasmic reticulum was neither abundant nor arranged in typical lamellar fashion. Although present in every cell, its amount was markedly decreased, and the cisternae were scattered irregularly in the hyaloplasm. Many cisternae were dilated and electron-lucent, but occasionally contained amorphous electron-dense material. Most of the surface of the individual cisternae was granular, alternating with smaller agranular areas of variable length. Numerous aggregates of free ribosomal granules filled the hyaloplasm. Osmiophilic lamellated structures, of irregular size and shape
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Characteristic cytoplasmic components of the experimental plasma cell tumors were virus-like particles, resembling the type-A particles of Bernhard (1), measuring about 75 millimicrons in diameter (4). They were uniform in size and had a concentric double membrane, with the inner membrane slightly more electron-dense than the outer. Their number and distribution in individual cells varied considerably. Almost all were found free within the dilated cisternal spaces of the endoplasmic reticulum (Fig. 5). Infrequently, isolated particles were attached to the inner aspect of the cisterna, appearing incomplete at the site of attachment.

The nucleus had a bizarre shape and configuration with at least one indentation, which sometimes was deep enough to simulate a binucleated appearance under the light microscope. The hyaloplasm extended into the indented region. The outer nuclear membrane was separated from the inner by an electron-lucent space of variable width. The nuclei had an irregular chromatin pattern. Usually clumps of peripheral chromatin protruded into the depth of the nucleus giving the appearance of a nucleolus under the light microscope. Nucleoli were rarely seen.

Immunoelectrophoresis of the pooled sera of the tumor-bearing animals showed an increased level of IgG immuno- protein compared to the sera of the control animals (Fig. 8).

DISCUSSION

Light microscopic and ultrastructural characteristics of the cells of the earlier generations of experimental plasma cell tumors resemble well-differentiated plasma cells (4, 5, 11). It appears that tumor cells in later generations have developed considerable morphologic changes without loss of their distinct protein-producing ability. Larger cell size, increased nuclear-cytoplasmic ratio, and frequent mitoses of abnormal pattern denote that the tumor has become less differentiated and has acquired an increased growth potential. The development of the new characteristics falls within the general concept of tumor progression as described by Foulds (6). Dissemination of tumor cells in visceral sinusoids and capillaries indicates rapid hematogenous spread of the tumor. Antibody-producing plasma cells under antigenic stimulation display abundant rough endoplasmic reticulum (2), which also accounts for their marked basophilia. The tumor cells of the earlier generations of experimental plasma cell tumors contain similar amounts of rough endoplasmic reticulum (4), producing high levels of immunoprotein. In comparison, tumor cells of later generations exhibit a considerable reduction in rough endoplasmic reticulum. We could detect no evidence of proliferation of nonneoplastic plasma cells in the bone marrow, the lymph nodes, the tissues around the tumor, or elsewhere in these animals; therefore, we attribute the continued elevated level of immunoprotein in their sera to the activity of the tumor cells, despite the relative decrease in the rough endoplasmic reticulum. Sustained IgG production due to increase in cell numbers, although with decreased output per cell, may be the explanation of this apparent paradox.

Mitochondrial swelling and cisternal dilatation have been described in a great many experimental situations. Both of these changes may be seen in rapidly growing cancer (10), and similar changes in our tumors are probably due to their rapidly growing nature.

Significant morphologic differences exist between the later generations of this experimental plasma cell tumor and human multiple myeloma. The ultrastructure of which has been described in detail (3, 7, 18). In contrast to the larger cells with indented nuclei of our experimental tumors, the typical myeloma cell varies from 15 to 30 microns in diameter (20), with usually one eccentric round nucleus occupying only one-third of the cellular volume. The cytoplasm of the myeloma cell is deeply basophilic and frequently contains Russell bodies and exhibits PAS positivity. Large, round, dense intracisternal bodies, which account for the light microscopic appearance of Russell bodies (8), are absent in the experimental tumor cells. PAS positivity of the myeloma cell is due to the presence of dense amorphous intracisternal substance (19), whereas dilated cisternal spaces in experimental tumors are usually empty, correlating with the lack of PAS staining. Experimental tumor cells are devoid of surface microvilli, which are characteristically seen in myeloma cells. Virus-like particles and a few multivesiculated bodies are invariably present in these tumor cells, while they are generally absent in myeloma cells. However, both single- and double-membraned particles, measuring 30—50 millimicrons, have been described in myeloma cells on rare occasions (17).

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REFERENCES


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