An Electron Microscopic Examination of Murine Plasma Cell Neoplasms with and without Paraproteinemia

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SUMMARY

We have examined seven generalized neoplasias which developed in BALB/c mice subsequent to inoculation of subcellular leukemic material from mice with plasma cell leukemias. Based on light microscopic criteria the malignancies were classified as immature plasma cell neoplasms. Four were associated with paraproteinemia of the gamma-globulin G type and 3 had normal electrophoretic patterns. An electron microscopic examination of malignant infiltrates from spleens and peripheral lymph nodes from both groups of animals showed the dominant malignant cell to be a rather primitive mesenchymal cell with some plasmacellular differentiation. Cells from leukemias with paraproteinemia usually exhibited more nucleoli, ergastoplasmic channels, and free ribosomes than cells from leukemias with normal serum electrophoresis. Amyloid fibrils were present both extra- and intracellularly in leukemic infiltrates from both groups of leukemias.

INTRODUCTION

Plasma cell neoplasms may occur in several strains of mice (4, 18, 20, 22) and are often associated with paraproteinemia (17, 21). The paraproteins are specific for each individual tumor, are usually preserved in transplantation (16), and are products of the malignant cells (12, 25).

By inoculation of a subcellular leukemic extract from mice with plasma cell leukemia, an induction of leukemias in BALB/c mice was accomplished (6). On the basis of morphologic, light microscopic criteria (22), the majority were classified as plasma cell neoplasias. One-third of these neoplasias were associated with paraproteinemia, mostly of the gamma-globulin G or H type (23). The present publication deals with the ultrastructure of some of the neoplasias, in an attempt to determine if any correlation can be established between production of paraprotein and maintenance of plasma cell morphology.

MATERIALS AND METHODS

Mice. Seven of the BALB/c females which developed neoplasia subsequent to inoculation with subcellular leukemic material (6) were used in the present investigation. Four of the neoplasias were associated with paraproteinemia of the gamma-globulin G type (23), whereas the serum electrophoretic findings were normal with the remaining 3 neoplasias. The animals were killed shortly before expected death at an age varying from 17 to 24 months. Total necropsy was performed, and sections were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), Unna Pappenheim, and alkaline Congo red (11, 19).

Electron Microscopic Technic. Fragments of spleen and peripheral lymph node tissue were prefixed in 3 percent glutaraldehyde for 1 hour and postfixed in 2 percent osmium tetroxide. After embedding in Vestopal, one-micron sections were cut and stained with toluidine blue. Ultrathin sections were made on the LKB ultratom and stained with 5 percent aqueous uranyl acetate and lead hydroxyde according to the method of Reynolds (24). A Siemens electron microscope was used. Electron micrographs were examined under code.

RESULTS

Gross Pathology and Light Microscopy

All mice investigated revealed peripheral lymph nodes measuring about 10 mm in diameter, mesenteric lymph nodes about 35 x 8 x 8 mm, and moderately enlarged spleens. Other organs appeared normal. The malignant infiltrates consisted of densely packed cells with round or slightly irregular nuclei and moderate amounts of slightly basophilic cytoplasm (Fig. 1). Many irregular chromatin clumps were scattered adjacent to the nuclear membrane. In some cases the cytoplasm stained weakly positive with Unna Pappenheim, but PAS positivity was never observed. The characteristic malignant cells were interspersed with some reticulum cells and lymphocytes (Fig. 2). Using the criteria of Rask-Nielsen and Gormsen (22), all neoplasias were classified as plasma cell neoplasias of differentiation grade III or II-III (Table 1). In lymph nodes the...
infiltrating cells erased the normal architecture except for the subcapsular sinus. In spleens the malignant cells were mostly located in the follicles. Smaller infiltrates were found perivascularly in lungs, livers, and kidneys, whereas the thymus and thyroid glands were only slightly involved. Amyloid was not observed, and both dichroism and birefringence were lacking in sections stained with alkaline Congo red.

**DISCUSSION**

The findings of some of the characteristics of plasma cells in the neoplastic cells justifies the term immature plasma cell neoplasia for these neoplasms (6). However, the numerous similarities with reticulum cells indicate a close relationship with reticulosarcomas, especially of type B (3, 10, 26).

From previous work by several investigators (2, 8, 13), the ultrastructure of well-differentiated murine plasma cell neoplasms, many of which are paraprotein producers, is fairly well known. Furthermore, three cases of undifferentiated murine plasma cell neoplasms have been investigated by Dalton et al. (2). Paraprotein was looked for in one of these leukemias and found not to be present. In our group of undifferentiated plasma cell neoplasias, the protein-secreting apparatus seemed to be more highly developed than in the cases reported by Dalton et al. As paraproteinemia is less frequent among undifferentiated than among well-differentiated plasma cell neoplasias (6), the ultrastructure described here may exemplify the minimal requirements for paraprotein production to occur.

Both the neoplasias with paraprotein production and those without it showed the usual morphologic signs of protein production. The fact that the morphology could be correlated with paraprotein production only to a very limited extent, might indicate that the structures observed were seldom deficient to such a degree as to prevent paraprotein production. It might, however, also be that striking differences between paraprotein producing and nonproducing neoplasias are not detected because the paraprotein-producing cells only comprise a minority of the total cell population. Several investigations, however, point out the monoclonal nature of advanced cases of murine plasma cell neoplasms (9, 14, 15, 23), and immunofluorescent examinations by Solomon et al. (25) have produced direct evidence for a participation of most malignant cells when paraprotein production occurs.

In spite of negative reactions for amyloid by polarization optical examinations of alkaline Congo red-stained sections, the electron microscopic findings of amyloid fibrils in malignant infiltrates suggest that amyloidosis may occur more frequently with plasma cell neoplasms than previously recorded (1). Furthermore, the presence of amyloid both intra- and extracellularly indicates that the fibrils are produced by the malignant cells in analogy with the amyloid production

### Table 1

<table>
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<tr>
<th>Treatment</th>
<th>Mouse number</th>
<th>Age in months</th>
<th>Differentiation</th>
<th>Paraprotein in serum</th>
<th>N/C</th>
<th>Nucleoli</th>
<th>Ribosomes</th>
<th>Ergastoplasma</th>
<th>Golgi complex</th>
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<td>806</td>
<td>23</td>
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<td>Gamma G</td>
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<td>III</td>
<td>Gamma G</td>
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<td>++</td>
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<td>17</td>
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Plasma cell neoplasias in BALB/c female mice. Light microscopic, serum electrophoretic, and electron microscopic findings are present. N/C, nucleus to cytoplasm ratio.

*Graded according to the light microscopic criteria of Rask-Nielsen and Gormsen (22).*
REFERENCES

Fig. 1. Imprint of peripheral lymph node from 17-month-old BALB/c female mouse with plasma cell leukemia of Differentiation Grade II-III (mouse number 20603). Most nuclei are located eccentrically and surrounded by moderate amounts of cytoplasm. Serum contained paraprotein of gamma-globulin type G. Giemsa stain, X 525.

Fig. 2. Section of peripheral lymph node from 21-month-old BALB/c female mouse (mouse number 20027). The normal architecture is nearly erased by infiltrating malignant plasma cells of Differentiation Grade II-III. Among the malignant plasma cells there are a few reticulum cells and lymphocytes. Electrophoresis of serum showed nothing abnormal. Periodic acid-Schiff, X 840.

Fig. 3. Lymph node of mouse number 20603 (see Fig. 1). Leukemic cells with indistinct cell borders are closely packed. The nuclei with prominent nucleoli are somewhat irregular in shape, large, and rich in chromatin which is located mainly in clumps along the nuclear membrane. Some mitochondria and a few dense bodies are seen in addition to numerous ergastoplasmic dilations containing a finely granular material. The perinuclear space appears dilated. X 5000.

Fig. 4. Peripheral lymph node from mouse number 902, age 22 months. A region where 3 tumor cells are separated by a thin brim of possible cellular material from a branching reticular cell. The cytoplasm of the 3 tumor cells is extremely rich in ribosomes, mostly as rosette formations. X 38,000.

Fig. 5. Lymph node from mouse number 20603 (also depicted in Figs. 1 and 3). Most malignant cells from this mouse showed dilated ergastoplasm containing secretion products. The cell in the middle of this picture is, however, of a more primitive type with many ribosomes mostly free in the cytoplasm or as rosettes, rather few ergastoplasmic channels, some vesicles, and some mitochondria. X 26,000.

Fig. 6. Low-power view of leukemic lymph node cells from mouse number 20327, age 24 months. The malignant cells have an immature appearance with a rather high nucleocytoplasmatic ratio. The amount of cell organelles is low, and the nuclei exhibit less chromatin in the peripheral parts than the nuclei in Fig. 3. X 8000.

Fig. 7. Malignant lymph node cells from mouse number 20027 (see Fig. 2). The cells have large nuclei of immature appearance. The one to the left has a large nucleolus. In the cytoplasm there are a few ergastoplasmic channels, a moderate amount of free ribosomes, and two dense bodies (right). X 26,000.

Fig. 8. Lymph node of mouse number 1109 (paraproteinemic), age 21 months. In an edematous area between cell protusions, a fibrillar material is seen. These fibrils cannot be distinguished from the fibrils of amyloid. X 52,000.

Fig. 9. Lymph node of mouse number 870 (nonparaproteinemic), age 22 months. A rim of finely granular or homogeneous material (hyaline) is present between the cells, which have the lowest degree of differentiation observed in the present leukemias. The cell to the right contains an area in which an amyloid-like substance is located, X 37,500.
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