AxC Rat Lymphoma in Tissue Culture; Electron Microscopic Observations(*)

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SUMÁRIO

Neste trabalho foram estudados os aspectos ultra-estruturais de um linfoma obtido espontaneamente em ratos da cepa AxC. As células derivadas desse tumor, crescendo em cultura de tecido, bem como o sarcoma obtido da inoculação dessas células em animais da mesma cepa, foram também examinadas ao microscópio eletrônico.

As células do tumor original mostraram características descritas para linfoblastos. Na cultura, as células que mostraram aspecto morfológico diferente do das células do tumor original apresentaram pontos de junção entre as membranas celulares semelhantes ao que se vê entre as células epiteliais. O significado deste achado é discutido. A estrutura das células do sarcoma mostrou características de células fagocitárias, reforçando a natureza histiocítica do tumor, o que já havia sido sugerido no trabalho anterior. A pesquisa de vírus neste estudo foi negativa.

INTRODUCTION

A previous publication documented a sarcomatous transformation observed in a cell line derived from a rat lymphoma growing in monolayer culture. (9) The morphological and cytogenetic changes found, suggested the presence of virus and the differentiation of the malignant lymphoblasts toward malignant histiocytes in culture. The present paper describes, under the electron microscope the structural features of the original tumor, of the cells growing in culture and of the derived sarcoma. This study will serve to complement the prior observations.

(*) Este trabalho foi realizado no Departamento de Patologia de Tulane University Medical School. New Orleans, Louisiana, USA.

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MATERIAL AND METHODS

Original tumor — The tumor was a transplantable lymphoblastic lymphosarcoma that arose spontaneously in an AxC (August-Copenhagen) strain of rats. For electron microscopy, fragments of tumor were fixed in 2% glutaraldehyde and post fixed in 1% osmium tetroxide, dehydrated through a series of graded ethanol solutions, treated with propylene oxide and embedded in maraglas epoxy resin. (13)

Cells in culture — The cells were obtained at passage 22nd in monolayer culture and prepared for the electron microscope study using the technique that has ben carried out at the Department of Pathology of the Tulane Medical School (New Orleans, Louisiana — USA).

The culture bottle is washed with Hank's solution without serum, fixed in situ with cold 2% glutaraldehyde/0,05M cacodylate for 15 minutes. After fixation the cells are removed from the bottle wall with a rubber in a gentle maneuver and the fixative changed and left for 45 minutes. The cells are washed in buffer overnight, changed to 1% osmium tetroxide and then to 70% alcohol, centrifuged quickly and the supernatant poured off. 0,5ml of melted agar is added to the centrifuge tube containing the cells and left until it hardens. The block is removed gently, cut into small blocks containing the cells and put in 70% alcohol. The subsequent steps of the technique follow the same procedure for maraglas embedding.

Derived sarcoma — The tumor was obtained by intraperitoneal inoculation of cells from the 28th passage in culture. It was classified histologically as a highly undifferentiated sarcoma. The fragments taken for electron microscopy were processed following the same procedure used for the original tumor.

All the sections were obtained in a Poter Blum MT-2 ultramicromtome, stained with 2% uranyl-acetate and lead citrate and examined in a Philips EM 300 or a Siemens Elmiskop I electron microscope.

RESULTS

Light microscopy — The morphology of the original tumor was that of a lymphoblastic lymphosarcoma, showing the typical starry sky appearence. The details of the morphology of the cells growing in culture and of the derived sarcoma have been described elsewhere. (9)

Electron microscopy — Under the electron microscope, the cells of the original tumor, presented with the same structural organization, resembling the general morphology of lymphoblasts (fig. 1). The cytoplasm appeared packed with free ribonucleoprotein particles and few mitochondria grouped at one pole of the cell (fig. 2). The endoplasmic reticulum was represented by few scattered rough surfaced cisternae. The round nuclei was surrounded by a double membrane, had marginated chromatin clumps and showed a large nucleoli which appeared with evidente granular type nucelolonema.

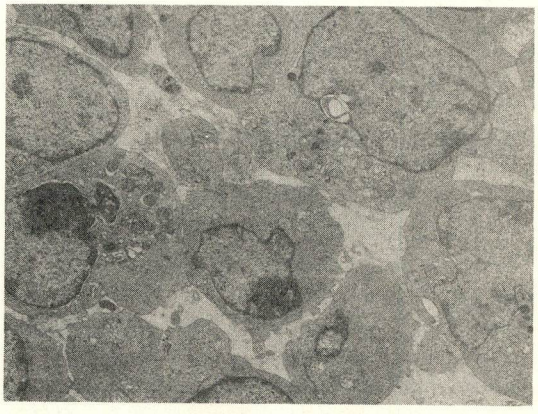

FIGURE 1 — Low magnification of section from the original tumor. The cells have large nuclei with prominent nucleoli. The cytoplasm shows abundant free ribosomes and few organelles. X 12000.

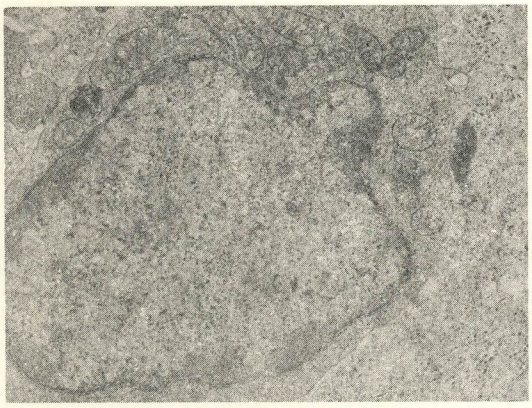

FIGURE 2 — Higher magnification of a lymphoblast. The large nucleus is limited by double membrane. The cytoplasm contains groups of mitochondria, abundant free ribosomes and scanty endoplasmic reticulum. X 30000.

The cells in culture were elongated and some of them showed few fingerlike projections extending from the cell surface. (Fig. 3). The cytoplasm of the cells had few dilated smooth surfaced endoplasmic reticulum, few well preserved mitochondria

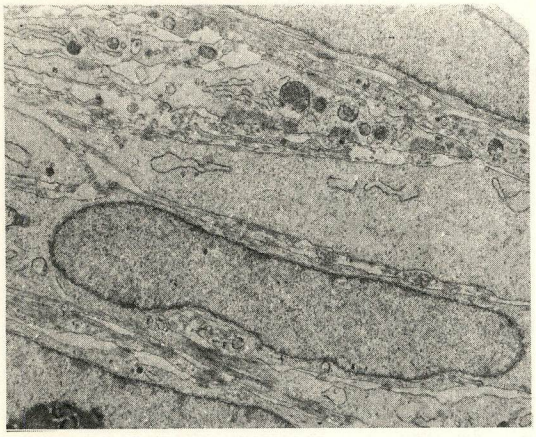

FIGURE 3 — Elongated cells from the culture. Bundles of fibrills are seen in the cytoplasm with condensation in points of cellular contacts, on the left lower quadrant of the field. X 14000.

and few clear vacuoles. A characteristic change in most of the cells was the hypertrophy of the Golgi complex that showed a large number of small, smooth surfaced vesicles and few lamellae (fig. 5). Some cells presented in the cytoplasm bundles of fibrils with areas of condensation in points of contact of the cellular membranes, resembling the specialized junctions of the epithelial tissues (fig. 4). The derived sarcoma presented, under the electron microscope, cells with the same nuclear structure, but with different organization of the cytoplasmic organelles. The cells had irregular surface, with large number of fingerlike projections extending from the cytoplasm. The nuclei with a double membrane, showed marginated dense clumps of chromatin. The nucleoli were large with a

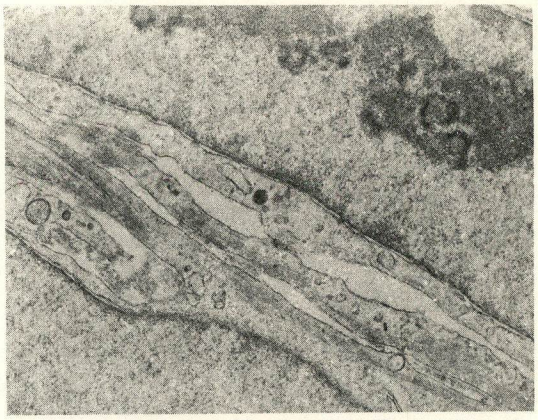

FIGURE 4 — Higher magnification of the figure 3. Note the cytoplasm fibrils condensed in points of cellular junctions. The nucleolus shows prominent nucleolonema. X 31000.

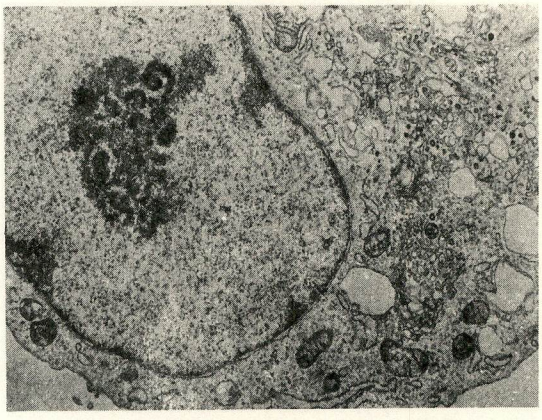

FIGURE 5 — High magnification of a cells from the culture. There is hypertrophy of the Golgi complex, characterized by an increase number of small vesicles and few lamellae. Note the large nucleolus with prominent nucleolonema. X 24000.

very prominent granular type nucleolonema.

Some of the tumor cells had the cytoplasm packed with rough surfaced endoplasmic reticulum and few well preserved mitochondria (fig. 6). Other cells had few dilated endoplasmic reticulum cisternae but the prominent feature was the presence of large number of well preserved mitochondria, numerous membrane bound lipid droplets, dense cytoplasmic inclusions and myelin figures, suggesting phagocytic activity (fig. 7). A third type of cells presented in the cytoplasm, besides the large number of endoplasmic reticulum, and mitochondria, numerous membrane bound lipid droplets. This type seems to represent an intermediate cell between the other two above mentioned.

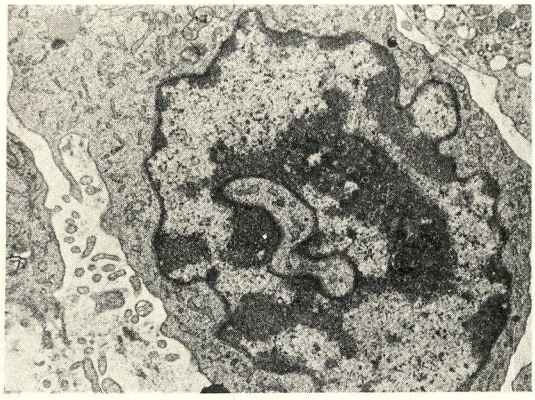

FIGURE 6 — Cell from the derived sarcoma showing irregular surface with fingerlike projections and large number of rough surfaced endoplasmic reticulum in the cytoplasm. The large nucleolus has prominent granular nucleolonema. X 24000.

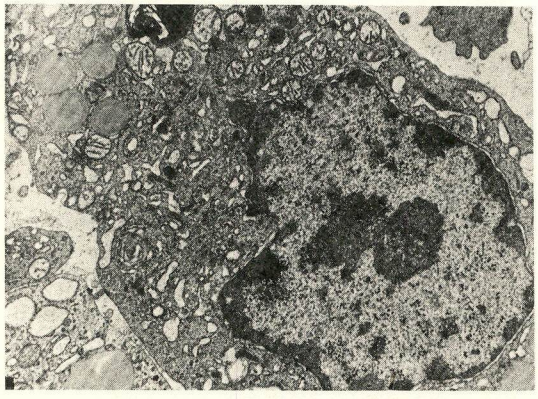

FIGURE 7 — Cell from the derived sarcoma. The cytoplasm shows, besides the dilated endoplasmic reticulum cisternae, numerous mitochondria, lipid droplets and dense laminated bodies (myelin figures). X 24000.

Exhaustive search for virus particle in the present material resulted negative.

COMMENTS

Under the electron microscope the cells of the original tumor showed the same structure of lymphoblasts as described by other investigators (6,7), with an outstanding feature represented by an evident granular type nucleolonema. The ultrastructure of the tumor confirms the morphology observed under the light microscope.

The morphology of the cells in culture was quite different from that of the original tumor. They showed severe degree of dedifferentiation, assuming the characteristics common to cells in long-term culture derived from different tissues (10, 12). The cells showed evidence of low cytoplasmic activity with few endoplasmic reticulum and few mitochondria but hypertrophy of the Golgi complex was prominent. The latter morphological feature resembles that of the phagocytes growing in culture (2)

An interesting finding was the presence of condensed fibrillary matter in the cytoplasm, in points of cellular contacts that resembled the specialized epithelial junctions. These points of attachments between cells have been described in tissues of mesenchymal origin (11, 3) and referred as specialized, intercellular junctions. In tissue culture studies, intercellular attachments were demonstrated between guinea-pig fibroblast and referred as "close associations of the plasma membranes". (5) Such attachments, like the epithelial specialized junctions, appear to function

maintaining the intercellular relationship and the organization of the tissues, but in tissue culture the significance of such system is not clear.

The cells of the derived sarcoma, contrary to the other cells studied, showed a great cytoplasmic activity, with abundant endoplasmic reticulum and great phagocytic activity. The latter feature suggests strongly the histiocytic nature of the derived tumor and supports the hypothesis of the differentiation of the original tumor cells toward malignant histiocytes during the culture, as stated previously. (9) The lack of activity in the culture cells can be explained based on the fact that in long-term culture, the cells stop their functions. (4)

Another interesting finding in the present study was the prominence of the granular type nucleolonema observed in the culture cells and in the derived tumor. This kind of variation of the nucleolar components appears more pronounced under pathological conditions, particularly in the malignant or virus-infected cells, **in vivo or in vitro** (1,8). Since no virus-infected cells could be detected, the desbribed nucleolar structure can only be related to the malignant nature of the cells studied.

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