

EPITHELIOID SARCOMA: ULTRASTRUCTURAL OBSERVATION OF LYMPHOID CELL-INDUCED LYSIS OF TUMOR CELLS

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Abstract. Epithelioid sarcoma of the palm of 7 months' duration was observed in a 30-year-old man. Six months after wide surgical excision there was no evidence of recurrence or metastasis. By light microscopic examination the tumor showed typical nodular arrangement of malignant cells, with necrosis of these cells in the centers of the nodules. Patchy lymphocytic infiltrates were observed at the peripheries of the nodules and also extended in places between the tumor cells. Other types of inflammatory cells were practically absent. By electron microscopic examination it was noted that numerous neoplastic cells formed firm close contacts with lymphocytes. Considerable numbers of neoplastic cells so contacted were damaged or even disintegrated. The damaged tumor cells contained abundant lysosomes. The release of enzymes from these lysosomes in the disintegrating tumor cells might be an important factor underlying the extracellular tissue injury and necrosis so conspicuous in epithelioid sarcoma. The very slow growth of this neoplasm and its slow tendency to metastasize might be related to the high efficacy of lymphocyte-mediated defenses against this tumor.

Epithelioid sarcoma was first described by Enzinger in 1970 (4). Since then there have been several case reports (2, 5, 6, 8, 11, 14, 16) of this tumor including light and ultrastructural descriptions and in 1977 it was reported in the dermatologic literature by Saxe & Botha (15). We recently had the opportunity to see a patient with a single primary lesion of epithelioid sarcoma and to study this lesion by means of light and electron microscopy.

CASE REPORT

A 36-year-old white man first noticed a firm, non-tender, skin-colored nodule on the palmar aspect of his right hand in October 1976. Six months later the lesion spontaneously fell off, leaving an ulcerated base which failed to heal.

In early May 1977 he presented with a single, very indurated, ulcerated nodule (Fig. 1) covered by a black crust and having an annular raised border with surrounding erythema and induration. There was no regional adenopathy. The lesion had been diagnosed previously

and treated elsewhere as an infected verruca vulgaris. A biopsy specimen was taken and a diagnosis of epithelioid sarcoma was made. Subsequently, the lesion was widely excised and a split thickness skin graft applied. On examination 6 months after surgery there was no evidence of recurrence or of metastases and there was good function of the hand.

MATERIALS AND METHODS

The surgically excised specimen was divided into two pieces. The larger piece was fixed in formalin, embedded



Fig. 1. Clinical appearance of the tumor.

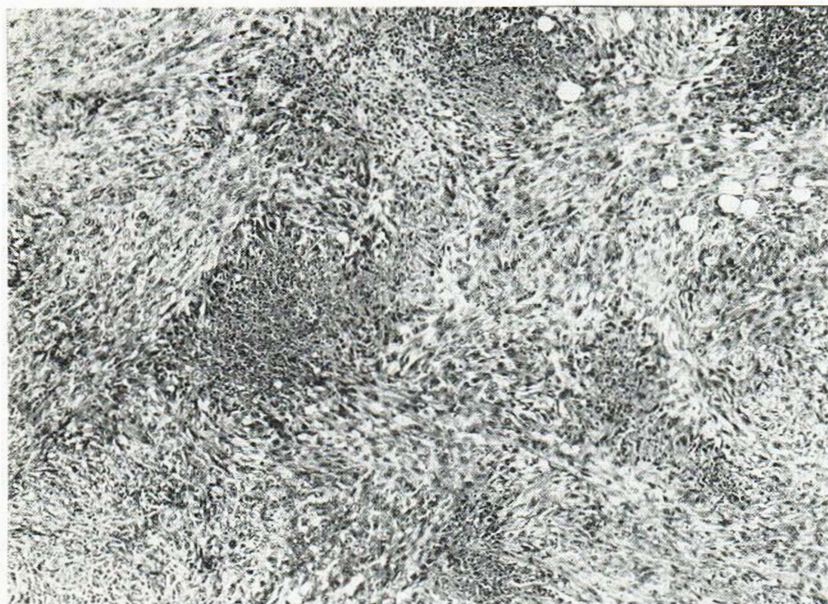


Fig. 2. Histologic section of the tumor, showing its nodular pattern and central necrosis (Hematoxylin-eosin, original magnification $\times 55$).

in paraffin, routinely processed and stained with hematoxylin-eosin, reticulin and colloidal iron stains. The smaller piece was cut into small fragments and fixed in 3% glutaraldehyde with Millonig's buffer. These fragments were post-fixed for 1 1/2 hours in collidine-buffered osmic acid, dehydrated in ethanol, embedded in Epon and sectioned on a Porter-Blum ultramicrotome. Sections 600–800 Å thick were stained with uranyl-acetate and lead citrate and examined with a Siemens' Elmiskop I at 80 kV. Sec-

tions 1 to 2 μm thick were stained for light microscope examination with a mixture of 1% pyronine and 1% toluidine blue in a ratio of 1:4.

RESULTS

Sections stained with hematoxylin-eosin revealed numerous confluent nodules (Fig. 2) composed of

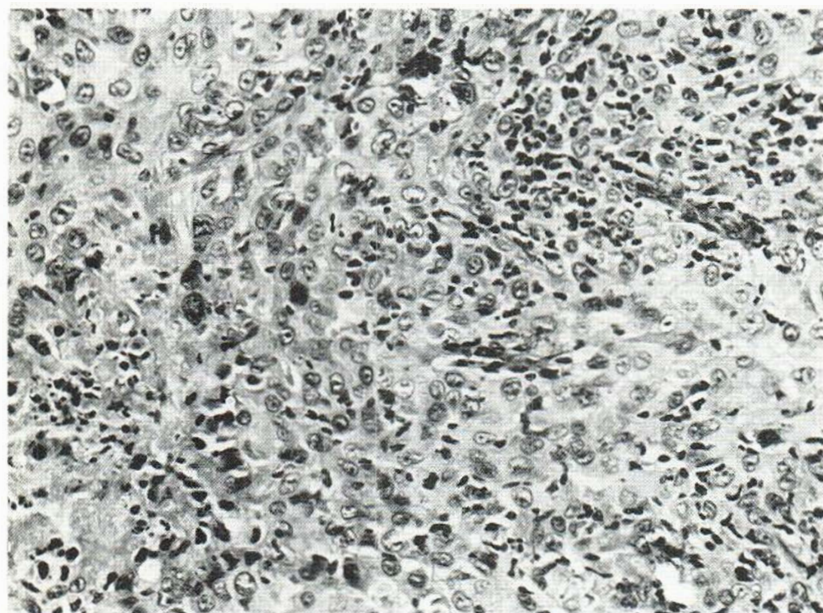


Fig. 3. Higher magnification, showing admixture of tumor cells and lymphocytes (Hematoxylin-eosin, original magnification $\times 175$).

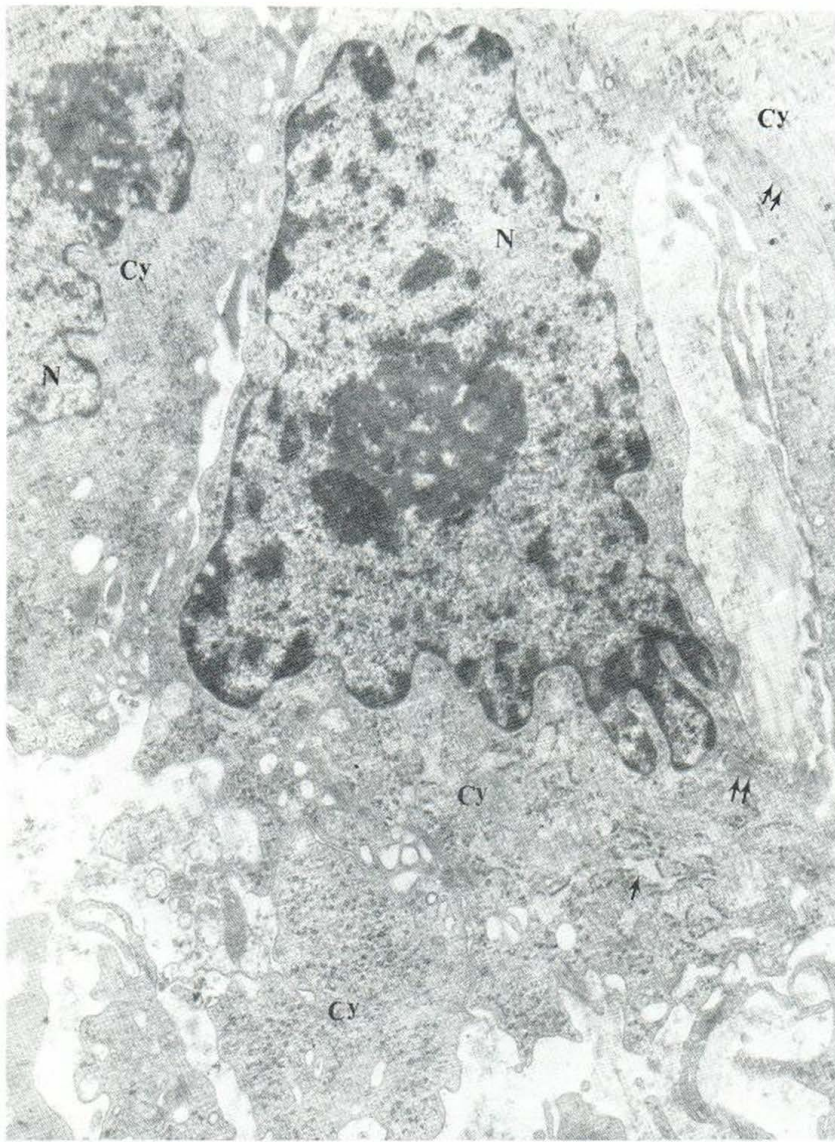


Fig. 4. Electron micrograph, showing one tumor cell closely apposed to three other tumor cells. Nucleus (N). Cytoplasm (CY). Rough endoplasmic reticulum (Single arrow). Filaments (Double arrow). (Original magnification $\times 3400$.)

closely packed large polygonal atypical cells with abundant acidophilic cytoplasm and large irregular nuclei. Patchy infiltration with lymphocytes (Fig. 3) was present especially in peripheral areas but also extended in places between the tumor cells. Many of the nodular areas showed central necrosis (Fig. 2). Reticulin stain showed increased reticulin fibers. Colloidal iron stain revealed an increased amount of acid mucopolysaccharides in the stroma and in a few neoplastic cells. A Mason-Fontana stain did not demonstrate melanin pigment.

By electron microscopic examination the tumor

cells were closely packed (Fig. 4). Their nuclear membranes disclosed narrow invaginating folds and condensed heterochromatin was distributed as a narrow peripheral band. The cytoplasm was always abundant and had moderate numbers of mitochondria and numerous free ribosomes. Varying amounts of rough-surfaced endoplasmic reticulum and filaments were in inverse proportions. A few electron-dense lysosomes and some modestly developed Golgi structures were observed. Occasional cells contained a few lipid droplets. The cell membrane formed numerous filopodia. Interdigita-

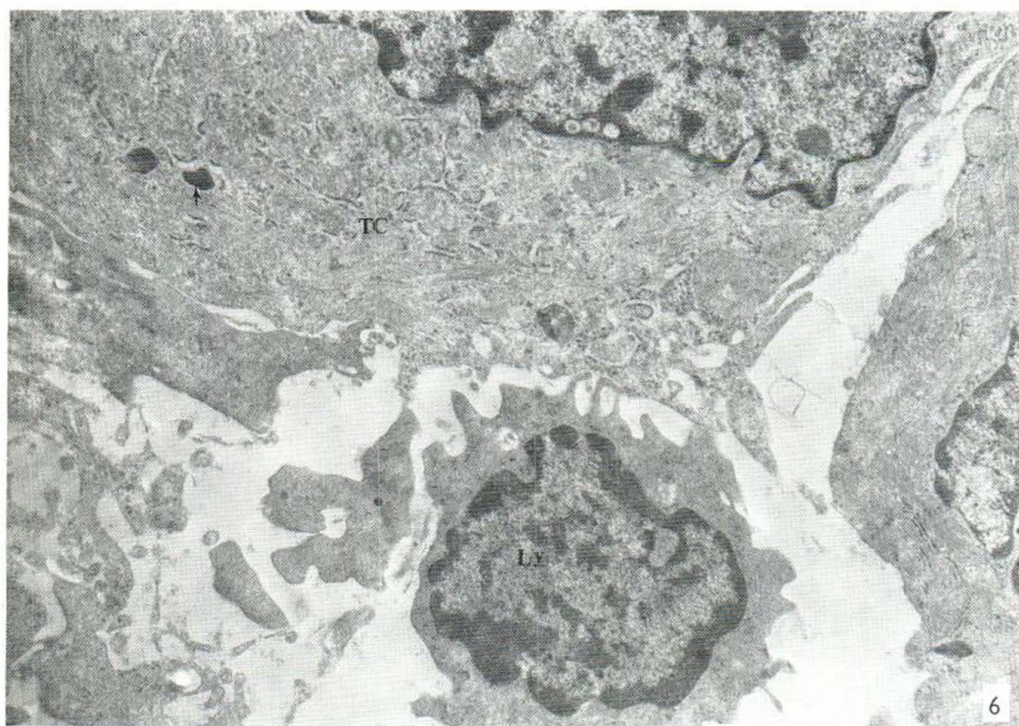
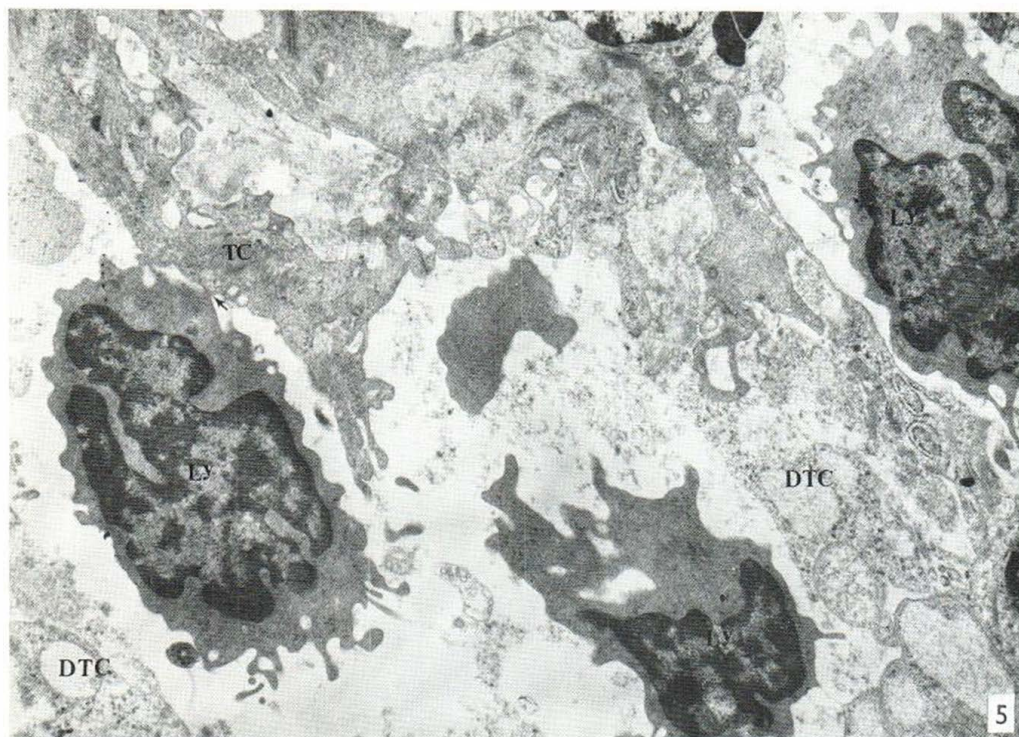


Fig. 5. Three lymphocytes (*Ly*). One is in contact (single arrow) with a tumor cell (*TC*). Damaged tumor cells (*DTC*). (Original magnification $\times 1500$.)

Fig. 6. A lymphocyte (*Ly*) in close contact with a tumor cell (*TC*) showing focal discontinuities of the cell membrane and a few lysosomes (single arrow). (Original magnification $\times 4500$.)

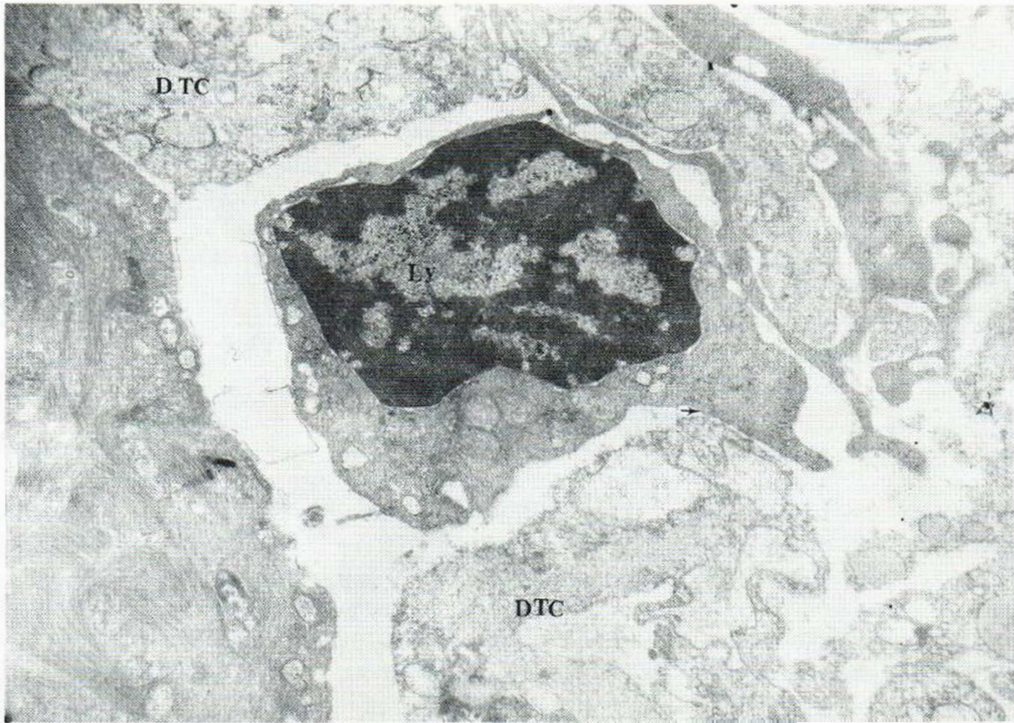


Fig. 7. Several damaged tumor cells (DTC) showing vacuolization of cytoplasm and discontinuities of the cell membrane. One lymphocyte (Ly) is in close contact (sing-

le arrow) with damaged tumor cell (DTC). (Original magnification $\times 4500$.)

tions with neighboring cells were frequent and at the lines of attachment the cell surfaces formed maculae adherentes and true desmosomes. The cells near the necrotic foci showed a decrease in ribosomes, dilatation of the rough endoplasmic reticulum, and granular nuclei. Some of the cells contained moderate numbers of lysosomes and lipid droplets. The spaces between the cells were filled with amorphous ground substance of variable density, microfibrils, and cellular debris. At the peripheral regions of the tumor nodules, extracellular spaces contained collagen bundles, and there were scattered fibrocytes and normal-appearing capillaries. In the necrotic foci the few scattered tumor cells which were present had granular nuclei and their cell membranes showed multiple discontinuities. Some of the cells were filled with lysosomes and lipid droplets. Numerous small cellular fragments, floating lysosomes, and mitochondria were also seen in the necrotic areas. Capillary endothelial cells were degenerated. Many lymphocytes were seen among tumor cells and some dem-

onstrated markedly folded nuclei and moderate amounts of cytoplasm filled with ribosomes (Fig. 5). A few mitochondria, rough endoplasmic reticulum and numerous microvilli were also present. In every section several lymphocytes were seen in close contact with tumor cells (Figs. 5, 6, 7). Many of the neoplastic cells so contacted showed vacuolization of cytoplasm, a decreased number of filaments, and discontinuities of the cell membrane. Some of the cells were disintegrated into small fragments. In the necrotic foci among tumor cells intact lymphocytes were seen virtually touching the damaged cells and cell fragments with their microvilli. Plasma cells were not seen. Polymorphonuclear leukocytes were rarely noted.

DISCUSSION

Histologically the most characteristic features of this neoplasm (Figs. 2 and 3) are its nodular arrangement, acidophilia of the cytoplasm of the malignant cells, and central necrosis of many of the

nodules. The deep location of some of the tumors and their close association with tendons and fascial structures indicate the mesenchymal nature of this neoplasm. The exact cell type has remained debatable. Fibrocytic (4), histiocytic (5), and synovial (8) origins of these cells have been proposed. We agree with Gabbiani et al (8) that the tumor cells show close similarity to the stromal component of synovial sarcoma (7) on the basis of ultrastructural observations, the main features of which are: 1) narrow invaginating folds of the nuclear membrane and prominent marginal heterochromatin; 2) the presence of varying amounts of microfilaments and endoplasmic reticulum in inverse proportions; 3) the presence of lipid droplets and lysosomes; 4) interdigitations of cell membranes and formation of maculae adherentes and true desmosomes between neighboring cells; 5) formation of filopodia. The presence of acid mucopolysaccharides in the stroma and in a few neoplastic cells as revealed by colloidal iron stain and by light microscopy further supports the synovial origin of this tumor. By light microscopic examination, Seemeyer et al. (16) and Saxe & Botha (15) noted the presence of many lymphocytes in the tumor. Seemeyer et al. (16) observed in one of their cases that so many lymphocytes were present in the initial biopsy specimen that a diagnosis of lymphocytoma cutis was considered. Five years later, however, sarcomatous features became predominant and the patient died with metastases 4 years later.

The features not described by the previous authors are the close contacts between neoplastic cells and lymphocytes and the virtual absence of other types of inflammatory cells. Considerable numbers of infiltrating lymphocytes showed irregular nuclei (Fig. 5) dispersed aggregates of heterochromatin and moderate amounts of cytoplasm, which are morphologic features of T-cells (12). Many neoplastic cells which were in close contact with the lymphocytes demonstrated changes indicating injury to the cell membrane, cytoplasm and nucleus which may lead to the eventual complete disintegration of these cells. Some of the lymphocytes appeared as though they had just dissociated from damaged tumor cells.

Many tumors in man and laboratory animals stimulate immune responses involving antibody formation, T-lymphocyte mediated tumor cell toxicity, antibody dependent cytotoxicity by K-cells, and activated macrophages (3). The cytolytic effect

of lymphocyte mediated tumor toxicity has been studied by many investigators in recent years (1, 3, 9, 10, 17). This effect is antigen specific and is a product of T-cell response to surface membrane antigen of tumor cells. Such cytotoxicity can occur in the absence of detectable antibody or complement. It involves close, firm contact between sensitized (9) lymphocytes and target tumor cells which leads to tumor cell membrane damage and, finally, to disintegration of the injured cell. Similar cytolytic target cell destruction can be brought about by K-cells (13) which attach via FC receptor to immunoglobulin-coated antigen on tumor cells. Our electron microscopic observation in epithelioid sarcoma of activated lymphocyte to tumor cell firm contacts with associated tumor cell damage cannot distinguish between these two mechanisms, although possibly the absence of plasma cells in the tumor might suggest less likely participation of the antibody dependent K-cell mechanism. Among factors which might help account for the characteristic, quite extensive foci of necrosis in the tumor nodules is the presence of fairly abundant lysosomes in the tumor cells. With disintegration of tumor cells under lymphocytic attack, enzymes released from tumor cell lysosomes could amplify extracellular tissue injury. The very slow growth of epithelioid sarcoma and its slowness to metastasize might possibly be related to the apparent high efficacy of lymphocyte mediated defenses against it.

Dermatologists should be aware of epithelioid sarcoma because early recognition and wide excision of this slowly evolving, potentially lethal tumor offers the greatest chance for cure.

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