Vascular Invasion by Glioma Cells in Man: An Electron Microscopic Study*

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One of the unique characteristics of primary brain tumors is that they rarely metastasize outside the central nervous system, irrespective of their grade of malignancy. Although at present cases of extracranial metastasis are being reported with increasing frequency, they still remain relatively uncommon.\textsuperscript{1,8,10-21,24} Such metastases are supposedly based on hematogenous dissemination of the tumor cells since there is no lymphatic system in the central nervous system although the role of propagation along the sheaths of cranial or spinal nerves must also be taken into consideration. It has been assumed that the vascular invasion by neoplastic cells takes place through a physically damaged wall of a blood vessel within the tumor or in its vicinity, but meningeal vasculature and venous sinuses are rarely involved.\textsuperscript{25}

The hematogenous metastases develop according to a certain sequence:\textsuperscript{9,16} 1) direct intravasation of the tumor cells into the blood stream across the wall of a blood vessel in the brain or spinal cord; 2) embolization of a group of tumor cells in the small vessels of a distal organ; and 3) implantation of tumor cells in a new location where they survive to produce a secondary neoplasm. The stages of embolization and implantation have been studied in detail, both experimentally and clinically,\textsuperscript{6} but the first step, that of the invasion of cerebral vasculature, has not been adequately investigated. Our observations are centered on the ultrastructural sequence of vascular invasion by gliomatous cells with particular reference to glioblastoma multiforme.

Material and Methods

Biopsy specimens were obtained during craniotomy from five patients with histologically verified glioblastoma multiforme and two with astrocytoma. The specimens consisted of tumor and peritumoral tissues. The biopsies were carried out with special care; there was no previous interference by coagulation with the blood supply of the tissue, and any manipulation of the core of the specimen was avoided. The specimens were immediately immersed in phosphate-buffered 4% glutaraldehyde. After trimming, they were postfixed in 1% osmium tetroxide, followed by Maraglas embedding. The detailed procedure of tissue preparation for electron microscopy has been previously reported.\textsuperscript{12} The sections stained with lead citrate were studied under a JEM-T6S electron microscope.

Results

Vascular invasion by tumor cells at the ultrastructural level was observed in both glioblastoma multiforme and astrocytoma. The manner of invasion was the same in all cases but the incidence was higher in malignant gliomas.

The tumor cells were extremely pleomorphic. Their neoplastic character was clearly revealed by the presence of bizarre nuclei, the occurrence of dense bodies and vacuoles in the cytoplasm, a lack of cohesiveness between adjacent cells, and a considerable variation in the extent of cytoplasmic processes (Fig. 1). A few of the tumor cells had fine fibrils in their cytoplasm, reminiscent of astrocytes.

In the core and periphery of tumors, the neoplastic cells were often concentrated in the perivascular region. They might be found in different parts of the vascular wall. Many were present in the basement membrane,
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Fro. 1. Junctional area between glioblastomatous tissue and cerebral cortex. In the tumor tissue there is enlarged extracellular space (Es) filled with flocculent material, while the intercellular space in the adjacent neuropile (N) appears intact. The nucleus (Nu) of tumor cell contains clumps of chromatin. The cytoplasmic processes (P) of tumor cells vary in shape and size. Vacuoles (V) and dense bodies (D) are present in the cytoplasm and its processes (P). Tc = tumor cell; Ap = astrocytic processes. × 11,880.

which was consequently widened or split (Fig. 2). The splitting gave rise to two dense zones separated by a wide, light area. One zone remained in close contact with the endothelium, and another was related to the perivascular cell processes; they have been described as the endothelial and glial basement membranes, respectively, in the peritumoral brain tissue.4,14,23 The light area or space was always wide in the tumor tissue and contained flocculent material and collagen fibrils in addition to the neoplastic cells. The presence of mitotic figures indicated that the tumor cells might multiply in the widened basement membrane.

Some neoplastic cells protruded into the endothelial basement membrane and were covered only by a thin layer of endothelial cytoplasm (Fig. 3). Others sent out cytoplasmic processes insinuating through the adjacent endothelial cells toward the vascular lumen (Fig. 3). Varying extents of the processes could be seen inside the lumen (Figs. 3 and 4). In favorable sections, whole tumor cells were observed in the same positions as the cytoplasmic processes with reference to the vascular lumen. Within the lumen, the tumor cells were either free or attached to the endothelial lining (Fig. 5). The freely floating neoplastic cells appeared very dense and their nuclei were pyknotic.

Tumor cells invaded the peritumoral tissue. They were not particularly concentrated in the perivascular region, and did not invade the blood vessels.

Discussion

The hematogenous spread of tumors in general has been known since the establishment of the cellular theory. Virchow23 con-