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.1,8,16-21,24 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.25

The hematogenous metastases develop according to a certain sequence:5,16 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,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.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,
Fig. 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.\(^4,14,22\) 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. Virchow\(^{23}\) con-
FIG. 2. Perivascular region at the periphery of a glioblastoma. A tumor cell (Tc) is inside the widened basement membrane (Bm). A red blood cell (R) is seen in the lumen (L) of a blood vessel. E = endothelial basement membrane; G = glial basement membrane; P = cytoplasmic processes of tumor cell; V = vacuoles. x 11,880.

considered metastasis as the result of the circulation of tumor juice in the blood. Improved hematological techniques revealed that tumor cells are frequently found in the circulating blood.⁵,¹⁵ Although most malignant tumors spread through the lymphatic channels, hematogenous dissemination does exist and it is perhaps instrumental in many rapidly advancing malignant neoplasias.³

The lack of lymphatic drainage from the brain and the anatomical peculiarity of the cranial cavity have been held responsible for the fact that primary intracranial tumors usually do not metastasize to tissues outside the skull.²⁶⁻²⁸ It is still a matter of conjecture whether extracranial metastases are promoted by surgical intervention or radiation therapy of the primary tumor.⁵,⁷,⁹,¹¹

Winkelmann, et al.,²⁷ suggest that vascular channels opened at the time of surgery may be the reason of such dissemination. It has to be pointed out, however, that extracranial dissemination has been reported in patients without previous surgery or irradiation.¹⁹ Potter, et al.,¹⁸ stated that tumors with differing characteristics and degrees of invasiveness have a similar chance of developing metastases when introduced directly into the blood stream. According to Ley, et al.,¹³ access of glioblastoma cells to the vascular system determines whether or not they will form distant metastases.

It seems reasonable to assume that the penetration of cells of malignant brain tumors into the blood stream through either intact or damaged vascular walls is a necessary first step to the formation of distant metastases. As far as the mechanism of such penetration is concerned our findings indicate that neoplastic cells are present in the vascular basement membrane, between the endothelial cells and even inside the lumen of blood vessels. This seems to describe the sequence of migration of these cells across
the interface between tumor tissue and blood. In other words, a tumor cell situated in the perivascular space may first indent the endothelial basement membrane and then, having ruptured it by mechanical or enzymatic action, separates two adjacent endothelial cells by its cytoplasmic processes. Eventually, the gap is large enough for the whole cell to squeeze through into the lumen of the vessel.

It is perhaps surprising how frequently this sequence occurs in all gliomas studied, in view of the fact that the formation of distant metastases still remains a very remote possibility. It is obvious that although the presence of glioma cells in the blood stream is probably a common phenomenon, implantation and viability of the dislodged tumor cells in a new site is most uncommon. One can only speculate on the reason for this. A metabolically hostile environment or immune rejection by the prospective host tissue might be responsible. It is equally possible that glioblastoma cells shed individually into the blood stream do not stand a chance of implantation, a development that is reserved only for larger cell colonies. Potter, et al., 17,18 established the following conditions necessary for neoplastic cells to gain access to the blood stream: 1) decrease of cohesiveness between adjacent tumor cells to facilitate the detachment of the migrating cells from the main body of the neoplasm; 2) increase in cell motility thought to be proportional to metastatic potential; 19 and 3) capability of transendothelial migration. This latter aspect has not been adequately studied in the past.

As far as our own investigations are concerned, loss of cohesiveness between tumor cells was a common finding; it manifested itself by the presence of large intercellular spaces in glioblastoma multiforme. The presence of numerous cytoplasmic processes on
tumor cells might be an indication of increased motility. There was a definite difference between the ultrastructure of benign and malignant gliomas. The cells of astrocytomas had shorter cytoplasmic processes and their body was less irregular in shape than those of glioblastomas. It may be due to this difference that intravasation of cells occurred in astrocytomas less frequently than in glioblastoma multiforme.

The occurrence of tumor cells in the basement membrane and their migration through endothelial gaps into the vascular lumen is confined to the tumor tissue proper. Therefore, this phenomenon should not be considered as a breach of the blood-brain barrier because vascular invasion by neoplastic cells has not been observed in the surrounding edematous brain tissue. A blood-brain barrier effect, in its proper sense, does not exist within the tumor tissue as evidenced by its ultrastructural morphology and the presence of flocculent, plasma-like material in the widened perivascular space.

**Summary**

Surgical specimens of glioblastoma multiforme and astrocytoma were studied by electron microscopy with special regard to the entrance of tumor cells into the bloodstream. Vascular invasion by neoplastic cells was observed in all cases but with a higher rate of frequency in glioblastomas. The sequence of penetration by the cells from the perivascular space to the vascular lumen has been described, and the different factors necessary for this passage through the neoplastic blood vessels have been discussed.

**References**

Fig. 5. Tumor tissue of a glioblastoma. A tumor cell (Tc) is inside the vascular lumen (L) but still in close contact with the endothelial cell (Ec). Another tumor cell with a small, dense nucleus (Nu) is free in the lumen. Tumor cells are also present in the basement membrane (Bm). $\times 10,800$.

16. Potter, J. F. Concepts of the mechanisms
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