GLIAL SCAR INSTABILITY AFTER BRAIN INJURY

Glial scar is formed following surgical damage to the cerebral cortex. In the present study we examined the ultrastructural status of the cerebral cortex 14 to 180 days following surgical damage to cerebral parenchyma. The results showed a contribution of astrocytes, but also mesodermal cells, to the process of scar formation. Furthermore, our study showed that the process initiated by trauma did not terminate with the formation of a glial scar. Late phases of repair following tissue damage were associated with lytic processes and a disassembly of the cerebral parenchyma. These findings indicate a changing and unstable nature of the glial scar and its components.

Key words: astrocytes, glia scar, macrophages, ultrastructure

INTRODUCTION

Studying models of cerebral damage may contribute significantly to our knowledge regarding pathology of the central nervous system. Human brain trauma occurs during numerous neurosurgical procedures associated with disrupted continuity of the meninges followed by interventions within the cerebral parenchyma. Such interventions result in damage to morphological components of the blood-brain barrier, disruption of the continuity of nerve fibers, and damage to various cells forming the cerebral parenchyma (1-3).

Studies show that plasma proteins present in the perivascular zone form scaffolding that supports newly formed blood vessels (4). Cellular aggregates seen in these areas show the ultrastructural features of endothelial cells but also some unusual features, such as the presence of cytoplasmic filaments. Using electron microscopy, we defined stages of new blood vessel formation.
Morphological characteristics of endothelial cells, components of the basal membrane material and, other structures of cerebral parenchyma adjacent to new blood vessels suggest the contribution of stem cells to vasculogenesis in the areas of the cerebral cortex that are in direct contact with the posttraumatic lesion (5). Immunocytochemistry using the Flk-1 antibody, which interacts with receptors for the vascular endothelial growth factor (VEGF), a marker of immature endothelial cells, confirmed these suggestions and showed that the development and growth of new blood vessels in the brain is not limited to angiogenesis but may also involve other processes, with a likely contribution of immature endothelial cells. These ultrastructural findings show that surgical trauma of the cerebral cortex may induce new vessel formation at the site of surgical intervention and in the areas adjacent to damaged cerebral parenchyma. Thus, evaluation of the later phases of cerebral parenchyma response to surgical brain trauma is of interest. The objective of our study was to assess the ultrastructural characteristics of the early and late phases of damaged cerebral parenchyma in rats, 14, 30, 90 and 180 days following surgical trauma.

MATERIAL AND METHODS

The study was approved by a local Ethics Committee. Male adult Wistar rats weighing 200-250g were anesthetized with ketamine hydrochloride - 20 mg/kg, i.m. Traumatic brain injury was induced in the frontotemporal region of the cerebral cortex after skull exposure as described earlier (6). After recovery from anesthesia, the rats remained under standard laboratory conditions for 14 to 180 days. Then, the animals were euthanized and the material for microscopic studies was dissected and processed for transmission electron microscopy. Each experimental group consisted of 3 operated on and 3 non-operated animals; the latter were used as controls.

RESULTS

The results showed that 14 days following surgical brain trauma in rats, fibroblast-like cells filled with ribosome-rich endoplasmic reticulum and containing elongated nuclei were present at the site of the trauma (Fig. 1A). Cytoplasmic processes of these cells were connected and formed a network around bundles of collagen fibers. Both these cells and striated collagen fibers were present in this area until 6 months following trauma.

In the zone immediately adjacent to the traumatic lesion, capillaries showing ultrastructural features of young vessels were seen after 14 days, surrounded by astrocyte processes. These vessels lacked a fully developed basal membrane and were surrounded only by basal membrane-like material. The vessel walls contained pericytes. These vessels were in direct contact with the astrocyte plasmatic processes (Fig. 1A). In addition to capillaries, larger precapillary vessels were seen. Both capillary and precapillary vessel formation also occurred
in more remote areas until 3 months following trauma. Collagen fibers were found within the basal membrane of precapillary vessels in the remote areas.

A characteristic feature of the zone surrounding the traumatic lesion was the presence of basal membrane-like material defining lesion borders, with adjacent astrocyte plasmatic processes containing numerous glial filaments (Fig. 1B). Subsequently, the number of glial filaments in astrocytes increased, and the filaments filled morphologically differentiated processes of varying morphology. From 30 days following trauma, nerve fibers and synaptic endings were also seen in the zone adjacent to the traumatic lesion (Fig. 2A). At 6 months, the layer of basal membrane-like material and plasmatic processes of fibroblast-like cells formed a distinct border separating cerebral parenchyma from the posttraumatic lesion. Astrocytes and blood vessels were present adjacent to the layer of basal membrane-like material. In addition to glial filaments, irregular fibers of 7–9 nm in diameter were seen in astrocytes. A basal membrane that often formed a network with collagen fibers surrounded capillaries.

From 30 days following trauma, macrophages filled with phagolysosomes were seen in the traumatic lesion and adjacent areas. After 3 and 6 months,
phagocytic cells were localized both in the borderline layer of the scar and in the remote areas (Fig. 2B). Microscopic pictures of the brain at 6 months following trauma showed large loss of cerebral parenchyma (Fig. 3A). In addition to macrophages, the glial scar contained astrocytes with rich lysosomal apparatus, suggesting these cells contributed to phagocytosis. Using electron microscopy, populations of perivascular macrophages were also seen.

Neuronal cell death occurred in cerebral parenchyma at some distance from the lesion border from 14 to 180 days following trauma (Fig. 3B). These areas showed an increased number of microglial cells and loss of cerebral parenchyma, which was likely associated with neuronal cell death.

DISCUSSION

Damage to the central nervous system initiates a series of cellular and molecular processes leading to the formation of a glial scar. Cells involved in these processes include not only astrocytes but also microglial cells, oligodendrocyte precursors, meningeal cells, and stem cells (7-9).
The initial phenomenon following cerebral parenchymal damage is cellular death within neurovascular units and other components of the neuropile (10, 11). During initial phases, we noticed capillary vessel formation supported by plasma protein scaffolding. Macrophages migrated to the lesion site, followed by phagocytic cells originating from the surrounding parenchyma (5, 12). Four days following surgical trauma, we could see fibroblast-like cells that were involved in the repair processes and collagen protein synthesis. Close interaction between these cells and astrocytes suggests their contribution to the formation of the glial scar (13, 14). The latter is eventually composed mainly of tightly connected astrocyte processes that form a network surrounding nearby fibroblasts.

Our study also showed that the process initiated in the cerebral parenchyma by surgical trauma does not terminate with the formation of a glial scar. Initial mechanisms of repair and reconstruction become disturbed after approximately 3 months following trauma of the cerebral parenchyma. At that time the number of macrophages is increased, with an associated marked microglial response. Astrocytes forming the glial scar show features of edema. The solid glial scar surrounded by basal membrane-like material becomes loosened. Macroscopic
pictures of the brain 6 months after trauma show large loss of cerebral parenchyma suggesting that the glial scar is not a stable structure, and its remodeling involves various cells contributing to formation of the glial scar during different phases of this process.

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REFERENCES


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