Epileptogenic Role of Astrocyte Dysfunction

Astrocytes Are a Specific Immunological Target in Rasmussen's Encephalitis. Bauer J, Elger CE, Hans VH, Schramm J, Urbach H, Lassmann H, Bien CH. *Ann Neurol* 2007;62(1):67–80. OBJECTIVE: The current histopathological criteria of Rasmussen's encephalitis (RE) include the presence of T-cell-dominated inflammation, microglial activation, neuronal loss, and astrocytic activation. An in vitro study, however, suggested glutamate receptor 3 (GluR3) antibody-mediated astrocytic loss. Therefore, we investigated astrocytic apoptosis and loss in situ. METHODS: Histochemical, immunohistochemical, terminal deoxynucleotidyltransferase-mediated biotin-dUTP nick end labeling, and in situ hybridization techniques were applied to paraffin sections of 20 RE cases, 6 healthy control subjects, and 6 paraneoplastic encephalomyelitis, 10 Ammon's horn sclerosis, and 5 focal cortical dysplasia cases. RESULTS: Astrocytic apoptosis and subsequent loss of these cells is a specific feature of RE. Such lesions are not found in the control groups. In RE, astrocytic apoptosis and loss was present both in cortical and in white matter areas. Astrocytes in these tissues showed major histocompatibility complex class I expression. Furthermore, granzyme-B^+ lymphocytes were found in close apposition to astrocytes bordering astrocyte-deficient lesions. Granzyme-B^+ granules in these lymphocytes were polarized and faced the astrocytic membranes. No evidence was found for an antibody-mediated destruction. INTERPRETATION: We suggest a specific attack by cytotoxic T lymphocytes as a possible mechanism responsible for astrocytic degeneration in RE. The loss of astrocytes might play a role in neuronal dysfunction, seizure induction, and enhancement of neuronal cell death.

COMMENTARY

It is increasingly recognized that astrocytes may play an active role in brain pathology occurring in various CNS disorders (1,2). Alterations in astrocyte function, often associated with reactive astrogliosis, are described in epilepsy using both experimental models and human tissue resected at surgery. The repertoire of gliotransmitters that mediate the functional communication between astrocytes and neurons as well as the ability of astrocytes to affect neuronal excitability (for example, by regulating the clearance of extracellular K^+ and glutamate) are the basis for studying whether and to what extent specific defects in astrocyte function and signaling may contribute to neuronal network hyperexcitability underlying seizures (1). In this context, recent evidence has shown that reactive astrocytes represent a chronic source of inflammatory mediators in human and experimental epileptic brain tissue and that some of these mediators, such as IL-1β, have a proconvulsant action and can contribute to neuronal cell loss (3,4).

In this article, the authors report the interesting observation of astrocytic cell loss in Rasmussen's encephalitis (RE), predominantly observed in brain areas of prominent neuronal degeneration. The study was performed in both surgically resected tissue and diagnostic brain biopsies from RE patients who were diagnosed according to the criteria described by Bien et al. (5). Brain tissue from epilepsy surgery cases of Ammon's horn sclerosis, focal cortical dysplasia, and paraneoplastic encephalitis was used for comparison. Immunocytochemical analysis of astrocytes positive for two specific markers, glial fibrillary acidic protein (GFAP) and S100β protein, showed reactive gliosis in large parts of the RE cortex. A qualitative assessment of astrocyte and neuron density, using immunocytochemistry, demonstrated that sizeable portions of RE cortex indeed were devoid of immunopositive cells; in particular, lesions differing in extent were detected, and often the lesional areas were surrounded by hypertrophic activated astrocytes. Since lack of GFAP staining does not unequivocally demonstrate cell loss, these authors also evaluated GFAP mRNA levels, thus confirming the loss of astrocytes. Double-labeling studies with apoptosis markers (caspase-3 immunoreactivity, nuclear condensation, and DNA fragmentation) showed that a portion of the GFAP-positive astrocytes was undergoing apoptosis. The differences found in the degree of apoptosis and in the loss of GFAP-positive cells among the RE patients may reflect different stages of astrocytic cell loss. Areas with loss of astrocytes, as seen in RE, were found neither in focal cortical dysplasia nor in Ammon's horn sclerosis patients, although a considerable degree of neuronal cell loss and astrocytes with hypertrophic morphology were found in these tissue specimens. In RE patients, astrocytic and neuronal cell losses were positively correlated, although the extent of neuronal loss was larger than that of astrocytes. Astrocytic cell loss and apoptosis in RE were not restricted to cortical areas but also were observed in subcortical white matter. Oligodendrocytes and myelin in these areas were unaffected.

In order to understand the mechanisms of astrocytic cell loss, the authors investigated a few possible mechanisms, including complement deposition and immunoglobulin-mediated cell death. Both phenomena have been shown to induce astrocyte death in in vitro preparations, but they were not detectable on astrocytes in RE tissue. Instead, T-lymphocytes secreting granzyme B (GrB) were present around blood vessels and in the

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lesion areas where astrocyte loss was detected. Moreover, numerous lymphocytes were identified, demonstrating that GrB-positive granules polarize toward astrocyte processes. Expression of MHC I also was detected in astrocytes; these molecules are able to present antigens to T cells, in particular they can be recognized by cytotoxic CD8 T cells.

Studies in rodent models of seizures and epilepsy have reported astrocyte loss in the hippocampus following status epilepticus and subsequent newly generated astrocytes with altered functional properties (6,7). Astrocyte loss preceded and was strictly associated with neuronal cell degeneration in the hilus of the hippocampus. These studies suggest that astrocytic cell loss is a consequence of epileptic activity, raising the possibility that the same phenomenon explains the findings in RE. However, the authors of this paper did not detect astrocyte loss in Ammon's horn sclerosis or focal cortical dysplasia tissue in patients experiencing drug-refractory seizures. The option that cytotoxic T cells indeed are responsible for astrocytic cell loss in RE, therefore, appears the most plausible. The T-cell attack may be mediated by autoimmunity or, as an attractive alternative, by viral infection of astrocytes. Various viruses can cause cytoskeleton alterations (8); for example, in vitro infection of astrocytoma cell lines with measles virus may disrupt the cytoskeleton (9), thus also offering a process for direct cell damage. Interestingly, HHV6-mediated infection of astrocytes has been demonstrated in a population of patients with mesial TLE. The astrocytes infected with this type of virus show a reduced ability to reuptake glutamate, which is due to down-regulation of excitatory amino acid transporter 2 (EAAT2) (10). Consequently, it is possible that an as yet unknown virus may contribute to astrocytic dysfunction and death in RE.

Astrocytes could play an important pathophysiological role in RE since their dysfunction (presumably occurring in reactive astrocytes) or loss in the lesional areas may compromise energy metabolisms, K^+ homeostasis, or GABA and glutamate catabolism and reuptake (2). Moreover, activated astrocytes may release excitotoxic amounts of glutamate (1,2), proinflammatory cytokines (3,4), and reduce the availability of adenosine (a brain metabolite endowed with anticonvulsant activity) via endogenous upregulation of adenosine kinase (11). These changes in astrocytic cell function, stemming from their pathological activation or apoptotic loss, potentially could contribute to the acquisition of the properties of the epileptic tissue in RE patients. Further elucidation of these novel aspects may offer a new perspective into the mechanisms of epileptogenesis in RE and possibly also in other types of epilepsies.

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