

INNATE IMMUNITY AND INFLAMMATION IN TEMPORAL LOBE EPILEPSY: NEW EMPHASIS ON THE ROLE OF COMPLEMENT ACTIVATION

Complement Activation in Experimental and Human Temporal Lobe Epilepsy. Aronica E, Boer K, van Vliet EA, Re-deker S, Baayen JC, Spliet WG, van Rijen PC, Troost D, da Silva FH, Wadman WJ, Gorter JA. *Neurobiol Dis* 2007;26(3):497–511. We investigated the involvement of the complement cascade during epileptogenesis in a rat model of temporal lobe epilepsy (TLE), and in the chronic epileptic phase in both experimental as well as human TLE. Previous rat gene expression analysis using microarrays indicated prominent activation of the classical complement pathway which peaked at 1 week after SE in CA3 and entorhinal cortex. Increased expression of C1q, C3 and C4 was confirmed in CA3 tissue using quantitative PCR at 1 day, 1 week and 3–4 months after status epilepticus (SE). Upregulation of C1q and C3d protein expression was confirmed mainly to be present in microglia and in a few hippocampal neurons. In human TLE with hippocampal sclerosis, astroglial, microglial and neuronal (5/8 cases) expression of C1q, C3c and C3d was observed particularly within regions where neuronal cell loss occurs. The membrane attack protein complex (C5b-C9) was predominantly detected in activated microglial cells. The persistence of complement activation could contribute to a sustained inflammatory response and could destabilize neuronal networks involved.

COMMENTARY

The complement system includes a proteolytic cascade of events representing an important component of the human immune response and an essential effector of both humoral and cellular immunity (1). The complement system consists of several fluid-phase and cell-membrane proteins that are divided into three activation pathways (i.e., classical, alternative, and lectin) and the membrane attack complex (MAC), a cytolytic or terminal pathway that results in the formation of a lytic pore-forming complex (1,2). Complement participates in the host defense against pathogens by triggering the formation of the MAC, which damages the phospholipid bilayer to lyse the target cell. In addition to their role in pathogen clearance, complement factors, such as C1q, play an important role in the clearance of apoptotic cells (2). However, activation of the complement system either at inappropriate sites and/or to an inappropriate extent can lead to host tissue damage. To protect against self-damage, host cells express a battery of regulatory proteins (i.e., complement inhibitors) that interfere with complement activation at several steps of the proteolytic cascade. These inhibitors can be associated with the cell membrane or

can be soluble and secreted by the cells (e.g., C1q inhibitor and clusterin) (1–3).

In mammals, the liver is the major source of most complement proteins, but many cell types, including monocytes, fibroblasts, and epithelial and endothelial cells, can also synthesize most of the complement components. Since 1987, it has been known that brain cells, including astrocytes, microglia, neurons, and oligodendrocytes (2,4), also synthesize complement components (5). In particular, human astrocytes express and secrete all the components of the three complement pathways. Synthesis of all components is constitutively low but can be enhanced by interferon- γ and inflammatory cytokines, such as IL-1 β and TNF- α . Indeed, astrocytes themselves can synthesize cytokines in an inflammatory context, raising the possibility that they may switch on complement biosynthesis in an autocrine manner. Given the high level of expression of membrane regulators in the complement system, human astrocytes appear to be resistant to complement lysis, while oligodendrocytes and neurons are much less resistant in vitro, suggesting that these cells are constantly at risk of complement-mediated damage (6).

Complement has long been thought of as a double-edged sword, with the capacity to harm as well as to heal. Indications of a general role for complement in neurodegenerative processes comes from evidence of chronic complement activation and synthesis in various neuropathological conditions, such as

multiple sclerosis, stroke, chronic neurodegenerative disorders (e.g., Alzheimer's and Parkinson's disease), as well as in Rasmussen's encephalities (6,7). Cytokines produced in diseased brain tissue may constitute a driving force in stimulating local complement synthesis by resident cells. Furthermore, complement receptors and regulatory proteins allow viruses to enter cells in CNS. Interestingly, HHV6-mediated infection of astrocytes has been demonstrated in a population of patients with mesial TLE and apparently leads to a reduced ability of cells to reuptake glutamate, highlighting the possibility that complement components may play a role in this infection (8).

Aronica and colleagues investigated the complement activation in both experimental and human TLE. Their work is a more extensive evaluation of this inflammatory pathway than previously presented by Rozowski et al. (9) and Xiong et al. (10) in seizure models. Rozowski and colleagues showed increased C1q and C4 mRNA in rat pyramidal neurons after systemic injection of convulsant doses of kainic acid in neuronal layers of limbic areas that are vulnerable to kainic acid-induced neurodegeneration (9); moreover, clusterin and C1q immunoreactivities were observed in both neurons and astrocytes, while increased immunoreactivities (as observed in vivo after seizures) were demonstrated following prolonged exposure of primary cultures of hippocampal neurons to glutamate. One important question is whether activation of the complement cascade could be responsible for increased susceptibility to seizures and neuronal injury. Preliminary, but compelling, evidence, in favor of a detrimental role of complement activation, is provided by Xiong et al., who showed that the sequential infusion into the rat hippocampus of individual proteins of MAC induced both behavioral and electrographic seizures as well as cytotoxicity (10).

Aronica and colleagues reported the increased transcript expression of C1q, C3, and C4 in the CA3 hippocampal layer in rats from day 1 to 4 months after status epilepticus (SE) induced by stimulation of the angular bundle. Their findings clearly show that complement activation occurs chronically after the first damaging event (i.e., SE) but before epilepsy develops, thus during the epileptogenesis phase when no epileptic-like activity is present. Immunohistochemical analysis showed that protein expression was mainly present in microglia, parenchymal and perivascular astrocytes, as well as hippocampal neurons. This pattern of activation persists in chronic epileptic tissue in rats with spontaneous seizures, although immunoreactivity in neurons is scarce compared to day 1 and week 1 following SE, suggesting that the complement-positive neurons in the early phases after SE are damaged and may be destined to die.

Using human sclerotic hippocampi from TLE patients, Aronica et al. show that there are abundant C1q and C3 immunopositive astroglial cells in areas of prominent gliosis and that immunoreactivity in microglia/macrophage lineage cells

occurs in regions where cell death predominates (i.e., pyramidal cell layers and hilus). Activation of components of the MAC was mainly observed in microglia/macrophages, rarely in neurons, and not in astrocytes. These components were only barely detectable in control tissue specimens (i.e., in autaptic tissue from patients without history of seizures or other neurological diseases and in nonsclerotic tissue from patients with a focal lesion from a ganglioglioma not involving the hippocampus).

The main finding by Aronica et al. is that there is a prominent activation of the complement cascade during the epileptogenesis phase in the experimental model and in sclerotic hippocampi from rats and human TLE. Interestingly, the expression of CD59, a complement inhibitor of MAC, was increased in microglia/macrophages but only modestly in neurons, suggesting that in this cell population complement activation may be poorly controlled.

The parallel validation of experimental and human tissue findings confirms and expands previous evidence indicating the occurrence of a complex, chronic inflammation involving the innate immune system in TLE and in other epilepsies or epileptic syndromes of different etiology (11,12). Increasingly, evidence in experimental models of seizures shows that inflammatory processes may contribute to lower seizure threshold and possibly play a role in epileptogenesis and cell death (13). Further mechanistic insights into these processes and the development of strategies to control their overactivation in diseased conditions may highlight potential new targets for therapeutic intervention.

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