

A Role for Leukocyte-Endothelial Adhesion Mechanisms in Epilepsy. Fabene PF, Navarro Mora G, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, Zanetti L, Schio F, Osculati A, Marzola P, Nicolato E, Homeister JW, Xia L, Lowe JB, McEver RP, Osculati F, Sbarbati A, Butcher EC, Constantin G. *Nat Med* 2008;14(12):1377–1383. The mechanisms involved in the pathogenesis of epilepsy, a chronic neurological disorder that affects approximately one percent of the world population, are not well understood.^{1–3} Using a mouse model of epilepsy, we show that seizures induce elevated expression of vascular cell adhesion molecules and enhanced leukocyte rolling and arrest in brain vessels mediated by the leukocyte mucin P-selectin glycoprotein ligand-1 (PSGL-1, encoded by *Selplg*) and leukocyte integrins $\alpha_4\beta_1$ and $\alpha_L\beta_2$. Inhibition of leukocyte-vascular interactions, either with blocking antibodies or by genetically interfering with PSGL-1 function in mice, markedly reduced seizures. Treatment with blocking antibodies after acute seizures prevented the development of epilepsy. Neutrophil depletion also inhibited acute seizure induction and chronic spontaneous recurrent seizures. Blood-brain barrier (BBB) leakage, which is known to enhance neuronal excitability, was induced by acute seizure activity but was prevented by blockade of leukocyte-vascular adhesion, suggesting a pathogenetic link between leukocyte-vascular interactions, BBB damage and seizure generation. Consistent with the potential leukocyte involvement in epilepsy in humans, leukocytes were more abundant in brains of individuals with epilepsy than in controls. Our results suggest leukocyte-endothelial interaction as a potential target for the prevention and treatment of epilepsy.

Myelomonocytic Cell Recruitment Causes Fatal CNS Vascular Injury during Acute Viral Meningitis. Kim JV, Kang SS, Dustin ML, McGavern DB. *Nature* 2009;457(7226):191–195. Lymphocytic choriomeningitis virus¹ infection of the mouse central nervous system (CNS) elicits fatal immunopathology through blood-brain barrier breakdown² and convulsive seizures³. Although lymphocytic-choriomeningitis-virus-specific cytotoxic T lymphocytes (CTLs) are essential for disease⁴, their mechanism of action is not known. To gain insights into disease pathogenesis, we observed the dynamics of immune cells in the meninges by two-photon microscopy. Here we report visualization of motile CTLs and massive secondary recruitment of pathogenic monocytes and neutrophils that were required for vascular leakage and acute lethality. CTLs expressed multiple chemoattractants capable of recruiting myelomonocytic cells. We conclude that a CD8⁺ T-cell-dependent disorder can proceed in the absence of direct T-cell effector mechanisms and rely instead on CTL-recruited myelomonocytic cells.

COMMENTARY

Epileptogenesis has traditionally been regarded as a purely neurological phenomenon, with little or no input from outside the central nervous system (CNS). This perception of exclusivity, to some extent, has been reinforced because of the known functions of the blood–brain barrier (BBB) as a physical and metabolic barrier that maintains local brain homeostasis and affords protection from xenobiotics and circulating cells, while allowing passage of essential molecules, like glucose (1,2). As such, the brain always has been considered both chemically and immunologically privileged. The principal defense at the BBB is provided by cerebrovascular capillary endothelial cells that are characterized by: 1) tight intercellular junctions, 2) a relative lack of fenestrations and pinocytotic vesicles, and 3) the expression of efflux transport proteins and metabolic enzymes (e.g., P-glycoprotein and cytochrome P450 enzymes). Endothelial cells are supported in this role by the end-foot processes of perivascular astrocytes that express specialized proteins involved in volume and ionic regulation (1,2).

Seizures are known to interfere with the integrity of the BBB. For instance, the escape of serum albumin into the interstitial fluid of the brain (extravasation), indicted by penetration of Evans blue dye into brain parenchyma, is routinely observed in experimental seizure models and human epileptic tissue. However, it is now apparent that the reverse may be true—that disruption of the BBB can promote seizures or exacerbate a preexisting epileptic state. Topical application of bile salts to the cerebral cortex of naive animals results in the extravasation of albumin, activation of glial cells, and the development of localized epileptiform discharges as a result of impaired potassium buffering by astrocytes (3,4). Leakage of serum proteins into brain parenchyma is also observed following intravenous mannitol administration and leads to a concurrent and often progressive increase in seizure frequency in chronic epileptic rats (5). Mannitol also has been shown to elicit immediate seizures in patients undergoing deliberate osmotic disruption of the BBB prior to chemotherapy for the treatment of primary brain lymphomas (6). This evidence puts a new complexion on the interplay between seizures and the BBB and challenges the traditional view that epilepsy develops entirely from within the confines of the barrier.

Two recent studies, one by Kim et al. and the other by Fabene and colleagues, have furthered the notion that external contributors to the pathogenesis of seizures may involve BBB disruption. In addition to the extravasation of serum albumin and impaired potassium buffering, these studies imply that compromising the integrity of the BBB triggers an immune response characterized by rapid influx of white blood cells into the cerebrovasculature and their escape into the extravascular compartment, which results in the precipitation of seizures.

Fabene et al. induced a short period of status epilepticus in mice by systemic injection of pilocarpine, which when terminated by diazepam, was followed by a latent period of 1–2 weeks and then the subsequent development of chronic, recurrent seizures. The investigators showed an elevated cerebrovascular endothelial cell expression of various vascular cell adhesion proteins, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), P-selectin, and E-selectin, in response to the pilocarpine challenge. This upregulation, in concert with the accompanying seizure activity, caused an increased adherence of both polymorphonuclear leukocytes (i.e., neutrophils) and polarized T-helper cells to the blood vessel wall. Adherence was mediated by interactions between VCAM-1 and P-selectin and their respective leukocyte surface proteins, α_4 -integrin and P-selectin glycoprotein ligand 1 (PSGL-1), and was diminished by selective antibody blockade. Fabene and colleagues then demonstrated that the initial status epilepticus could be effectively prevented by pretreatment with α_4 -integrin antibody and that after status epilepticus, administration of both α_4 -integrin and VCAM-1 antibodies reduced the frequency of spontaneous seizures and protected against the neuronal cell loss typically associated with pilocarpine-induced epileptogenesis. These intriguing findings were validated in α_4 -integrin and PSGL-1 knockout mice, which proved to be resistant to status epilepticus and exhibited a marked reduction of late-onset chronic seizures. Prepilocarpine depletion of neutrophils, induced by using a granulocyte-specific antibody, had a similar effect. Finally, Fabene et al. showed that BBB leakage associated with pilocarpine could be prevented by pretreatment with α_4 -integrin antibody and was absent in the α_4 -integrin and PSGL-1 knockouts. This elegant study clearly demonstrates the contribution of BBB permeability and the vascular adherence of leukocytes to acute seizure initiation and longer-term epileptogenesis in the pilocarpine model and supports previous evidence suggesting that BBB disruption is a prerequisite for pilocarpine-induced seizures (7).

A subsequent paper by Kim et al. demonstrated that infection of the CNS by direct injection of lymphocytic choriomeningitis virus could result in fatal immunopathology, mediated by breakdown of the BBB and convulsive seizures. They showed that activation of CD8⁺ T-lymphocytes by transient contact with cells infected by the lymphocytic choriomeningitis virus in the subarachnoid space was sufficient to precipitate a chain of events involving the initial release of various chemokines, most notably CCL3, CCL4, and CCL5, which in turn, resulted in the localized influx of neutrophils and monocytes at 6 days postinfection. A release of cytokines from these inflammatory cells and their diffusion across the vascular endothelial cell layer compromised the BBB, permitting extravasation of leukocytes and the onset of fatal seizures. Depletion of myeloid cells prior to lymphocytic choriomeningitis virus

infection minimized BBB disruption (as measured by the escape of serum proteins) and delayed seizure onset but did not avert seizure-associated lethality. In contrast, treatment with anti-CD8 antibody prevented seizures, suggesting that interventions targeting early mediators of the immune cascade may be more effective at halting an otherwise insidious process. These findings suggest that enhanced permeability of the BBB, resulting from an immune-mediated inflammatory response, is not simply a feature of exposure to seizure-inducing chemicals, such as pilocarpine, but may be an inherent and required characteristic of epileptogenesis in general.

It is widely acknowledged that prolonged clinical seizures are associated with a mildly elevated white cell count in the cerebrospinal fluid, but until now, this inflammatory response was assumed to be a consequence of the seizures rather than a possible cause. It now appears that leukocyte infiltration of the brain is allied to the onset of seizures and may even be a prerequisite for epileptogenesis. This observation has potential therapeutic applicability, although not necessarily for established epilepsy in which the damage arguably already has taken place and theoretically is irreversible, but rather in the area of disease modification (8). It offers promise for the use of immunomodulatory agents in the aftermath of epileptogenic insults, such as traumatic brain injury and stroke. However, a more obvious and immediate application might be the opportunity to arrest acute status epilepticus as well as prevent the long-term sequelae (including epilepsy) associated with CNS infections.

Numerous bacterial, parasitic, and viral etiologies of epilepsy are recognized, most notably those affecting patients in the developing world. A recent study from rural Kenya reported that 18% of 5,000 acute pediatric admissions were seizure-related and over 80% of those were associated with infections, such as malaria and pyrogenic meningitis (9). Even in developed countries, one-third of patients with herpes simplex virus encephalitis experience acute symptomatic seizures, and one-in-four survivors are subsequently diagnosed with epilepsy (10). Immunosuppressive therapy may be counterintuitive in cases of epilepsy with infectious etiology; however, the work by Fabene et al. and Kim et al. suggests that control of the inflammation associated with CNS infections might alleviate acute seizures and reduce the subsequent risk of epilepsy. Their data

substantiate the contribution of BBB disruption and immune-mediated mechanisms in the development and perpetuation of seizures and open a novel avenue of research that challenges the conventional wisdom of epileptogenesis as an exclusively neurological phenomenon, offering new opportunities for therapeutic intervention.

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