

Current Review

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A Swell in the Armamentarium of Antiepileptic Drug Targets

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Although long granted the status of immune privilege, the central nervous system is not completely isolated from the immune system. Increasing evidence suggests that this system contributes to seizures and epilepsy and that the molecular mediators of inflammation and immunity may serve as appropriate targets in the quest to develop novel antiepileptic drugs that are more efficacious and potentially disease modifying.

The year 2012 will mark the 100-year anniversary of the publication by Dr. Alfred Hauptmann that described his serendipitous observation of the utility of the soporific phenobarbital (PB)—St. Barbara's compound—as a treatment for epilepsy in a chronically institutionalized population (1). Compared with bromide, the standard medical therapy of the time, Hauptmann observed that PB was better tolerated, safer, easier to use, and potentially more efficacious, as it permitted several patients to be discharged and return to work. However, he cautioned that his discovery was not a cure: "Because, naturally, [PB] is not a cure for epilepsy, it does not specifically influence the epileptiform brain process, it is merely capable of reducing the sensitivity of the cerebral cortex and by so doing to stall the attacks" (as translated by D.F. Scott [2]).

In the intervening years, approximately 35 new medications have been marketed as antiepileptic medications (AEDs), and several more are in the pipeline (3). As was the case with PB in 1912, many of these AEDs are better tolerated, safer, and easier to use compared to their predecessors. Yet, despite these important advances, a substantial proportion of patients remain pharmacoresistant (4); and, no AED has been proven to be disease modifying or truly antiepileptogenic (5).

Therefore, there is a continued need to develop new AEDs that act on novel therapeutic targets. It is possible that, like PB, such a drug will result from a serendipitous observation. The most recent example is the discovery of the antiepileptic efficacy of the potassium channel opener flupirtine, the forerunner of ezogabine, which was recently approved by the FDA (6). Originally synthesized with the goal of developing a centrally acting nonopioid analgesic, flupirtine had unknown antiepileptic efficacy and mechanism of action at the time of its submission to the National Institutes of Health–sponsored Antiepileptic Screening Program (7).

Yet, because serendipity cannot be completely relied upon, drug discovery and development has moved toward a greater dependence on rational drug design, in which drugs are developed to target specific proteins/molecules or selective signaling pathways that are based on the current understanding of pathogenesis. Among the new targets garnering increasing attention are the molecular mediators of neuroimmunity (8–10).

The CNS and the Immune System: A Pseudo-Privilege

The immune system provides for the detection of pathogens and the efficient elimination of these pathogens while minimizing damage to the body that can potentially be caused by both the pathogen and the immune system itself. To complete this task, two complementary and interconnected subsystems have evolved: innate and adaptive immunity. Innate immunity is an evolutionarily primitive process that controls inflammation and provides the host a rapid but nonspecific response to invading pathogens or injury. This response is mediated via epithelial barriers, macrophages (e.g., microglia, the resident macrophage of the brain), leukocytes, natural killer cells, circulating proteins (e.g., pro-inflammatory and anti-inflammatory cytokines, complement), and pattern recognition receptors (e.g., Toll-like receptors [TLRs]). Antigen presentation by professional (e.g., dendritic cells) or nonprofessional (e.g., thymic epithelial cells, glia) antigen-presenting cells triggers the evolutionarily, newer, adaptive immune system. This system, which provides for long-lasting pathogen-specific immunologic memory, is mediated by T and B cells, specialized lymphocytes that contribute to cell-mediated and humoral, antibody-mediated, immunity.

Although the CNS has long been granted the status of immune privilege, the system is not completely isolated from the immune system (11); both innate and adaptive immunity can be triggered in response to CNS infection or injury. Experimental studies demonstrate that seizures are also capable of activating a response that includes an increase in the expression of cytokines and their receptors as well as the activation of complement and prostaglandin pathways (as reviewed by



Vezzani and Granata [12]). Clinically, the activation of innate and adaptive immunity has been demonstrated in a number of epileptic conditions, including the epilepsies associated with hippocampal sclerosis, focal cortical dysplasia, tuberous sclerosis complex, Rasmussen encephalitis, and an expanding list of autoantibody and paraneoplastic conditions (13).

Neuroimmunity and Seizures: Consequence and Cause

Interleukin (IL) 1 β (IL-1 β) is a 17-kDA pro-inflammatory cytokine that is activated by the proteolytic processing of its 31-kDA proprotein (pro-IL-1 β) by IL-1 β converting enzyme (ICE, caspase 1). The effects of IL-1 β occur in response to IL-1 β binding to the IL-1 type 1 receptor (IL-1R1).

IL-1R1 and the TLRs share a common intracellular domain (toll/IL-1 receptor domain [TIR]). The TLRs are constitutively expressed by microglial cells, astrocytes, ependymal cells lining the cerebral ventricles, and neurons. Like IL-1 β , TLR signaling is an essential component in the rapid activation of innate immunity. The TLR receptors can be activated by pathogens, such as lipopolysaccharide (14), and by protein and nonprotein damage-associated molecular patterns (DAMPs), such as heat shock protein, S100 proteins, and high mobility group box-1 protein (HMGB1), which are released by degenerating neurons and activated glia as a homeostatic response to pathologic perturbations including trauma (15) and seizures (16, 17).

IL-1R1/TLR receptor activation can precipitate changes in excitability. In addition to inhibiting the uptake of glutamate via astrocytes (18, 19), IL-1 β has been shown to reduce GABA-mediated inhibition (20) and to facilitate NMDA-receptor mediated calcium influx (21). Activation of TLR4 by lipopolysaccharide (LPS), the major component of the outer membrane of gram-negative bacteria, has been shown to result in a concentration-dependent enhancement in the frequency and amplitude of spontaneous and evoked field potentials in the cortex (16).

Whole-animal studies have demonstrated that the changes in neuronal and network excitability induced by both IL-1 β and activation of the TLRs have the potential to influence seizure generation. For example, Vezzani et al. (22) demonstrated that the intrahippocampal injection of IL-1 β was capable of prolonging the duration of kainic acid (KA) – induced seizures in adult animals. This effect of IL-1 β could be blocked when it was coadministered with the IL-1 receptor antagonist, IL-1ra, a naturally occurring competitive inhibitor of IL-1R1.

Additional support for IL-1 β as a modulator of ictogenesis is provided by studies investigating the pathogenesis of febrile seizures. In an elegant study that employed the use of transgenic IL-1R1 deficient (IL-1R1^{-/-}) mice, Baram and co-workers (23) found that postnatal day (P) 14 and P15 IL-1R1^{-/-} mice were relatively resistant to the induction of febrile seizures induced via the use of a regulated stream of hot air and that the intracerebroventricular (ICV) infusion of a low dose of IL-1 β reduced the temperature threshold for hyperthermic seizures in the wild type but not in the IL-1R1^{-/-} mice. In addition, the ICV infusion of a high dose of IL-1 β , in the absence of another provoking factor, could induce seizures in normothermic wild-type controls but not in the IL-1R1^{-/-} mice. In a separate study, febrile seizures were induced by using a combination of LPS followed by a subconvulsant dose of KA (LPS/KA; 24). In this

study, the ICV infusion of IL-1 β resulted in a dose-dependent increase in the percentage of animals that convulsed in response to the intraperitoneal injection of LPS/KA, and the ICV infusion of IL-1ra resulted in a reduction in the percentage of animals that convulsed in response to LPS/KA.

The DAMP HMGB1 can bind to TLR4 and TLR2 as well as to the Receptor for Advanced Glycan End products. Vezzani and colleagues (17) recently demonstrated that the intrahippocampal injection of HMGB1 in the adult C57B7/6 mouse could decrease the time to seizure onset as well as increase the total number of seizures and the time spent in seizure after KA induction. A specific role for TLR4 as the key mediator of this proictogenic response was demonstrated by the finding that the effect of HMGB1 was not observed with the coadministration of two TLR4 antagonists and was absent in the C3H/HeJ mouse in which the TIR is functionally inactive. This same group of investigators provided additional support for the HMGB1-TLR4 axis as a potential new target for AED development by demonstrating that antagonism of the HMGB1-TLR4 axis reduced the frequency of spontaneous seizures in a mouse model of chronic epilepsy.

With respect to epileptogenesis, the recent demonstration by Dube et al. (25) that the level of IL-1 β in hippocampal homogenates from animals that develop spontaneous seizures after febrile seizures induced on P11 was higher than the level in the hippocampal homogenates from animals that did not develop spontaneous seizures and controls provides support that the IL-1 β may also contribute to epileptogenesis. Although this study is limited by the absence of additional experiments that demonstrate that the blockade of IL-1 β production during the latent period prevents the occurrence of spontaneous seizures, other studies provide additional evidence that activation of inflammatory mediators may contribute to epileptogenesis. For example, Galic et al. (26) mimicked a viral infection in animals on P14 via the ICV injection of polyinosinic:polycytidylic acid. As young adults, the mRNA expression of NMDA and AMPA receptor subunits was altered, and the animals were more susceptible to chemically induced seizures. A separate study by this group (27) raised the question of whether the effects of neuroinflammation on epileptogenesis may be age dependent, because they found that only rats in which a neuroinflammatory response was triggered by the injection of LPS on P7 and P14, but not P1 or older than P20, were more susceptible than controls to chemically induced seizures as adults.

Evidence linking neuroinflammation to ictogenesis and epileptogenesis is not limited to only IL-1 β and TLRs. Other examples include tumor necrosis factor α (TNF α), which has been shown to have proictogenic action (28) when upregulated in neurons (29) but not when overexpressed only in the astrocytes (30). The proictogenic action of TNF α perhaps is mediated by an increase in excitatory synaptic strength and decrease in GABA-mediated inhibition via an influence on the trafficking of both glutamate receptor 2–lacking AMPA receptors and GABA_A receptors (31). The role of IL-6 is less clear; though there is some evidence that enhanced IL-6 levels could predispose to seizures (32). In addition, disruption of the blood-brain barrier (BBB) in response to cytokines or other mechanisms results in the admission of proteins (e.g., albumin) and other normally



nonpermeant molecules (e.g., immunoglobulin G) that potentially can contribute to ictogenesis and epileptogenesis (as reviewed by Oby and Janigro (33)) as demonstrated by Seiffert et al. (34), who found that there was a focus of epileptiform activity, which occurred within 4 to 7 days of a focal disruption of the BBB and persisted for up to 1.5 months, in the region of the cortex underlying the focal disruption.

Lastly, as noted in this paper, there is an expanding list of autoantibodies and paraneoplastic conditions associated with seizures and epilepsy. In an early study, Delzenne (35) reported that the administration of duck anti-dog forebrain antiserum into the forebrain of the dog was capable of inducing seizures (“clear epileptic signs: loud crying, salivation, tonic and clonic convulsions, which were repeated several times before being followed by clonic convulsions that intensified with time”). The mechanisms that link antibodies to changes in network excitability and seizures likely depend on the target. Whereas serum obtained from patients with systemic lupus erythematosus and seizures was found to inhibit chloride conductance (36), the anti-NMDA receptor antibody associated with anti-NMDA receptor encephalitis has been demonstrated to alter the surface availability of NMDA receptors and their synaptic localization (37).

Take Two Anti-inflammatories and Call Me in the Morning?

The foregoing experimental studies suggest the hypothesis that activation of immunoregulators in response to infection, trauma, or autoimmune conditions can be a maladaptive, self-perpetuating, homeostatic mechanism that contributes to the maintenance of the hyperexcitable state required for the generation of seizures and epilepsy. Targeting selective neuroimmune pathways, therefore, may offer a potentially viable therapeutic strategy in the treatment of epilepsy. Currently, at least two putative AEDs that target inflammatory pathways are in various phases of clinical trials.

VX-765

VX-765 prevents the production of IL-1 β by inhibiting ICE (38). The potential utility of this agent in treating epilepsy has been demonstrated in several experimental studies. The ICV infusion of VX-765 has been shown to delay the onset to seizures, the number of seizures, and the total time spent in seizures after the intrahippocampal injection of KA in naïve adult rats and mice (39). In a mouse model of spontaneous limbic seizures, the repeated systemic administration of VX-765 reduced the frequency of seizures in a dose-dependent manner (40). Of import, VX-765 has also been shown to block the kindling in adult rats (41). This agent has recently completed a phase II clinical trial for the adjunctive treatment of medically refractory partial epilepsy (<http://clinicaltrials.gov/ct2/show/NCT01048255>).

Minozac (MCZ)

MCZ is an orally bioavailable aminopyridazine that blocks pro-inflammatory cytokine production by activated glia (42, 43) and that is now in a phase I clinical study (TT-301/TT-302). MCZ administered 3 and 9 hours after a KA-induced seizure at P15 has been shown to reduce the severity of seizures induced by a second hit of KA at P45 (44). In addition, in adult mice, MCZ

administered 3 and 6 hours after closed head injury (i.e., traumatic brain injury (TBI) induced via a pneumatic compression prevented the increased seizure susceptibility observed with a 30-mA electroconvulsive shock applied 7 days after the TBI (45).

But Will it Be Better Than PB?

The current clinical and experimental evidence as reviewed here and elsewhere (8–10, 12, 46, 47) support the hypothesis that activation of neuroimmunity is a contributing factor in seizures and epilepsy. Therefore, the rational design and development of drugs that target these pathways appears warranted.

It is suspected that not all pathways in this complex homeostatic process will be effective targets, and it is likely that targeting of certain pathways will also potentially exacerbate seizures. For example, studies investigating the role of cyclooxygenase (COX, e.g., COX-2) and prostaglandin pathways have produced varying results that depend on model, timing, and duration (48–50). Caution is also warranted, because our current immunosuppressive therapies (e.g., adrenocorticotropic hormone, corticosteroids, intravenous immunoglobulin) do not always meet with success. Even in Rasmussen encephalitis, in which biopsy specimens demonstrate evidence for an inflammatory process, chronic immunotherapy may decrease the atrophy and cognitive decline but may not decrease the frequency of seizures, and hemispherectomy still is often required (51). One potential explanation for this observation is that that targeting of these pathways may have to occur within a critical time period (27).

The chronic targeting of inflammation and immunity has been successful in modifying several human diseases (e.g., inflammatory bowel disease) and is currently a mainstay in the treatment of multiple sclerosis. Whether drugs targeting neuroimmunity will prove more efficacious than the current armamentarium of AEDs or have disease-modifying effects for epilepsy is not known. Although it is recognized that rational drug design has not always proved successful in the development of AEDs (5), the continued attempts to develop AEDs that target novel mechanisms that influence excitability and a reduced reliance on serendipity is a step in the right direction.

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7. Other	<input checked="" type="checkbox"/>				

* This means money that your institution received for your efforts on this study.

** Use this section to provide any needed explanation.

Section #3 Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the “Add” box. You should report relationships that were present during the 36 months prior to submission.

Complete each row by checking “No” or providing the requested information. If you have more than one relationship just add rows to this table.

Type of relationship (in alphabetical order)	No	Money Paid to You	Money to Your Institution*	Name of Entity	Comments**
1. Board membership	<input checked="" type="checkbox"/>				
2. Consultancy	<input checked="" type="checkbox"/>				
3. Employment	<input checked="" type="checkbox"/>				
4. Expert testimony	<input checked="" type="checkbox"/>				
5. Grants/grants pending	<input checked="" type="checkbox"/>				
6. Payment for lectures including service on speakers bureaus	<input type="checkbox"/>				
7. Payment for manuscript preparation.	<input checked="" type="checkbox"/>				
8. Patents (planned, pending or issued)	<input checked="" type="checkbox"/>				
9. Royalties	<input checked="" type="checkbox"/>				
10. Payment for development of educational presentations	<input checked="" type="checkbox"/>				
11. Stock/stock options	<input checked="" type="checkbox"/>				
12. Travel/accommodations/meeting expenses unrelated to activities listed.**	<input checked="" type="checkbox"/>				
13. Other (err on the side of full disclosure)	<input checked="" type="checkbox"/>				

* This means money that your institution received for your efforts.

** For example, if you report a consultancy above there is no need to report travel related to that consultancy on this line.

Section #4 Other relationships

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- No other relationships/conditions/circumstances that present a potential conflict of interest.
 Yes, the following relationships/conditions/circumstances are present:

Thank you for your assistance.
Epilepsy Currents Editorial Board