

Current Review

In Basic Science



Inflammation and Epilepsy: The Foundations for a New Therapeutic Approach in Epilepsy?

Lauren Walker¹ and Graeme J. Sills^{2,*}¹Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, U.K.²Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, U.K.

*Address correspondence to Graeme J. Sills, PhD, Wolfson Centre for Personalised Medicine, Block A: Waterhouse Buildings, 1–5 Brownlow Street, Liverpool L69 3GL, U.K. E-mail: g.sills@liv.ac.uk

Emerging data from experimental epilepsy models and resected human brain tissue support the proposed involvement of innate immune system activation and consequent inflammation in epilepsy. Key mediators of this process include interleukin-1 β , high-mobility group box protein 1 (HMGB1), and Toll-like receptor (TLR) signaling. These recent findings constitute the basis for a novel avenue of drug development in epilepsy, one that is not only distinct from previous approaches but uniquely based on sound neurobiological evidence.

Inflammatory pathways are widely acknowledged to contribute to the pathogenesis of several neurodegenerative disorders, including multiple sclerosis and Alzheimer disease, and are known to be activated following neurologic infection, ischemic stroke, and traumatic brain injury (1). Increasing evidence also supports a link between inflammation and epilepsy, both in terms of epileptogenesis and the long-term consequences of seizures (2). Complex febrile seizures in childhood have long been associated with the later development of temporal lobe epilepsy; febrile illnesses in people with otherwise well-controlled epilepsy can trigger seizures; and immunomodulatory agents such as steroids and adrenocorticotrophic hormone (ACTH) have shown efficacy in some epileptic encephalopathies and occasionally in refractory status epilepticus (3, 4).

More recently, it has been reported that surgically resected brain tissue from individuals with refractory focal epilepsy displays all of the hallmarks of a chronic inflammatory state, with infiltration of leukocytes, reactive gliosis, and overexpression of cytokines and their target proteins (2). This finding is backed up by data from studies of animal models that confirm the intimate involvement of inflammatory mechanisms in the generation of epileptic discharges and in the cellular damage associated with focal-onset seizures (2). Targeting brain inflammation may accordingly represent a novel therapeutic strategy for epilepsy, consistent with efforts to shift focus away from the symptomatic control of seizures to disease-modifying treatments that better target the underlying pathological mechanisms.

Brain Inflammation: Cellular Mechanisms

Inflammation is a natural physiological response to insult, infection, or biological stress and is mediated by the innate im-

mune system. It can be activated by invading pathogens or by cellular damage elicited under otherwise sterile conditions (5). In the brain, innate immunity is predominantly conferred by microglial cells, which act as the resident macrophages of the nervous system and represent the first line of defense against injury (6), but emerging evidence suggests that both neurons and astrocytes also play an important role (7). Chemical mediators and endogenous danger signals (also known as alarmins) released by pathogens and damaged neuronal cells, respectively, attract microglia and cause them to become activated. This, in turn, elicits further, extensive microglial proliferation and the release of cytokines and chemokines. Activated microglial cells perform phagocytic functions by digesting foreign materials and cellular debris and, together with astrocytes, release cytotoxic substances such as hydrogen peroxide, nitric oxide, and proteases to destroy infectious organisms (8). Cytokines and chemokines released from activated microglia initiate a pro-inflammatory signaling cascade that ultimately leads to localized vasodilation, the extravasation and recruitment of leukocytes, and activation of the adaptive immune response, in which microglia also play a role by acting as antigen-presenting cells (9).

Ordinarily, this process is halted by removal or elimination of the injurious stimulus, at which stage the immune response is scaled back, and astrocytes and microglia turn their attention to repair through the release of anti-inflammatory cytokines, the pruning of damaged synapses, and the promotion of neuronal regrowth (10). However, under circumstances that remain poorly understood, the resolution of inflammation is compromised, the proliferation of activated microglia is perpetuated and their attendant cytotoxic functions exaggerated. In chronic neuro-inflammation, astrocytes and microglial cells appear to act in a deleterious manner, contributing to rather than reversing the neuronal damage, by the sustained release of pro-inflammatory cytokines and chemokines and proteases such as cathepsins and metalloproteinases (11).

Epilepsy Currents, Vol. 12, No. 1 (January/February) 2012 pp. 8–12
© American Epilepsy Society

OPEN ACCESS Freely available online



Molecular Mechanisms: The Role of Interleukin-1 β

Much of the early evidence to support a role for inflammation in epilepsy arose from studies of the cytokine interleukin-1 β (IL-1 β), its target, interleukin-1 receptor type 1 (IL-1R1), and its naturally occurring competitive antagonist, interleukin-1 β receptor antagonist (IL-1RA) (2). All three are upregulated in rodent brain following chemically and electrically induced seizures (12), with IL-1 β expression in glial cells remaining elevated for up to 60 days after experimental status epilepticus (13). They are similarly overexpressed in human epileptogenic brain tissue in association with a variety of pathologies including hippocampal sclerosis, focal cortical dysplasia, and tuberous sclerosis (14–17). In addition to their overexpression arising as a result of seizures, IL-1 β and IL-1RA can also modulate susceptibility to seizure-inducing stimuli. When injected directly into the CNS, IL-1 β exacerbates seizures induced by kainic acid and bicuculline (18) and lowers the seizure threshold in models of febrile convulsions (19, 20). In contrast, IL-1RA has anticonvulsant activity following intracerebral administration and transgenic mice that overexpress this protein in astrocytes have reduced seizure susceptibility (21, 22). Similarly, IL-1R1 knock-out mice are less sensitive to experimentally induced febrile seizures (19) and the convulsant effects of bicuculline (21).

The proconvulsant effects of IL-1 β are believed to be mediated via IL-1R1-dependent activation of neuronal sphingomyelinase and Src kinases, resulting in phosphorylation of the NR2B subunit of the NMDA receptor, stabilization of the receptor at the cell surface, enhanced NMDA-mediated calcium conductance, and an increase in glutamatergic neurotransmission and the propensity for excitotoxicity (23, 24). Other putative effects of IL-1 β include a reduction in astrocytic glutamate uptake (25), an enhanced release of glutamate from glial cells, possibly via enhanced tumor necrosis factor- α (TNF- α) production (26), and the generation of acquired channelopathies (27).

Novel Molecular Mechanisms

Recent work has identified the possible role of TLRs in inflammatory pathways associated with epilepsy. Ordinarily, these proteins play a key role in pathogen recognition by binding molecules of microbial origin and triggering localized inflammation by increasing the transcription of various cytokines, including IL-1 β (28). However, TLRs are also activated by HMGB1, a chromatin component and DNA-binding motif molecule that is released by necrotic cells and secreted in a hyper-acetylated form by activated immune cells and other cells following immune challenge or biological stress (5, 29). HMGB1 acts as a “danger signal” and alerts the immune system to damaged or dying cells. The hyperacetylated form of HMGB1 regulates transcription of various pro-inflammatory cytokines, including IL-1 β , through binding to TLR2 and TLR4 and also to the receptor for advanced glycation end-products (RAGE) (5, 29). Nontranscriptional mechanisms have also been described, similar to those reported for IL-1 β (30). Thus, HMGB1-TLR-RAGE may represent a novel pro-inflammatory axis, acting in concert with the traditional IL-1 β pathway following sterile brain injury and perhaps without the requirement for overt microglial activation.

Numerous other molecules, pathways, and mechanisms have also been proposed to contribute to inflammatory events associated with seizures and epileptogenesis. These include TNF- α , transforming growth factor- β , cyclo-oxygenase 2, and blood-brain barrier (BBB) disruption (31–34). It is not possible to address each of these associations within a short review, but the BBB merits a brief comment. While it is widely accepted that the BBB is transiently compromised in the aftermath of seizures (35), recent evidence suggests that BBB disruption may also contribute to the generation of seizures and to the process of epileptogenesis following neurological infection or proconvulsant challenge (36, 37). The resulting extravasation of serum albumin leads to hyperexcitability via an alteration in glutamate and potassium ion homeostasis (38), and the coincident infiltration of blood-borne leukocytes into the brain, aided and abetted by vascular cell adhesion proteins, can initiate a localized inflammatory reaction (36).

An important footnote is that the above mechanisms are not mutually exclusive. HMGB1 enhances the expression of IL-1 β via an effect on TLRs (29) and increases the expression of vascular cell adhesion proteins in the cerebrovascular endothelium (39); IL-1 β promotes the nuclear to cytoplasmic transfer of HMGB1 (40); several inflammatory mediators influence BBB permeability (41) and, on the basis of current evidence, most ultimately impact on excitatory transmission and excitotoxicity mediated by glutamate (5). Any intervention strategy designed to alleviate the unresolved inflammation associated with ongoing seizure activity needs to consider this interplay between mechanisms and the apparent redundancy in the cascade (42) and should, perhaps, aim to target a common upstream initiator (i.e., IL-1/TLR signaling) rather than the more disparate downstream pathways (i.e., BBB disruption, enhanced TNF- α production, etc.).

Recent Developments: Animal Models

The potential contribution of the HMGB1-TLR-RAGE axis to seizures and epileptogenesis has been extensively investigated in two models of acute seizures involving unilateral intrahippocampal administration of kainate and bicuculline, respectively, and a model of chronic epilepsy in which spontaneous seizures arise 1 week after kainate-induced status epilepticus (30). This latter model is considered representative of human temporal lobe epilepsy in terms of its histopathological profile and relative resistance to treatment with conventional AEDs (43). Progressive increases in the nuclear and perinuclear staining of HMGB1 in both ipsilateral and contralateral hippocampal astrocytes were observed in response to both acute and chronic seizures and were more pronounced in the kainate models than with bicuculline. Upregulation of HMGB1 did not appear to extend to neurons in these models, although neuronal cytoplasmic staining for HMGB1 was observed in a mixed neuronal/glial culture in response to challenge with cytotoxic concentrations of glutamate. TLR4 expression was also elevated by experimental seizures in all three models, with increases noted in both neurons and astrocytes in the acute and chronic kainate models, but in neurons alone following bicuculline (30). Differences in the expression patterns of HMGB1 and TLR4 in the kainate and bicuculline models may be explained by the



lack of evident cell death and a shorter duration of seizures in the latter.

In addition to expression studies, the authors reported a reduced latency to and increased severity of acute kainate-induced seizures in otherwise normal C57BL/6 mice pretreated with intrahippocampal HMGB1 (30). This effect was reversed with ifenprodil, a selective antagonist of NR2B-containing NMDA receptors, which showed no anticonvulsant activity alone and was absent in the C3H/HeJ mice that harbor a spontaneous mutation in the TLR4 gene. These mice also proved less susceptible to kainate-induced seizures in general, suggesting that the interaction of HMGB1 and TLR4 has a role in determining seizure threshold in this strain. Finally, selective antagonists of HMGB1 and TLR4 showed anticonvulsant activity, increasing the latency to onset and frequency and duration of seizures in acute models and the number of spontaneous seizures in the chronic kainate model (30). Taken together, these data further implicate TLR4 and, at a more downstream level, NMDA receptors in the proconvulsant action of HMGB1 and highlight the potential significance of HMGB1-TLR4 signaling in the development and perpetuation of seizures. Pharmacologic interventions targeting HMGB1 are already in development for other disorders (44) and should be considered candidates for further evaluation in the treatment of epilepsy. Recent experimental evidence suggests that they may also prove beneficial in alleviating some of the common comorbidities associated with chronic epilepsy, including cognitive dysfunction and memory deficits (45, 46).

Recent Developments: Human Tissue

The findings of preclinical studies of HMGB1 and TLRs are supported by recent analyses in human brain tissue. Surgically resected temporal lobe tissues from people with hippocampal sclerosis and refractory epilepsy display expression patterns of HMGB1 and TLR4 that are distinct from those observed in non-epileptic postmortem controls. These include perinuclear (i.e., cytoplasmic) staining for HMGB1 in astrocytes and microglial cells and expression of TLR4 in astrocytes and neurons, both of which are absent in controls (30). This is consistent with a nonspecific upregulation of TLR4 expression and nuclear to cytoplasmic transfer of HMGB1 in glial cells under pathological conditions, as suggested by the preclinical data.

A subsequent study has reported similar changes in the profiles of HMGB1, TLR2, TLR4, and RAGE in a variety of malformations of cortical development associated with partial epilepsy, including focal cortical dysplasia, tuberous sclerosis, and gangliogliomas (47). TLR2 was predominantly detected in microglial cells, whereas TLR4 and RAGE were expressed in astrocytes and dysplastic neurons. Real-time PCR confirmed mRNA expression, for all three proteins in all pathologies, that was otherwise absent in epilepsy controls (perilesional tissue from the same patients), nonepilepsy controls (peritumoral tissue from patients without seizures), and postmortem brain samples from individuals without overt neurologic disorders (47). As in temporal lobe tissues, HMGB1 displayed extranuclear staining in astrocytes and activated microglia in the pathological specimens but not in controls. Failure to detect a change in HMGB1 mRNA suggested that this again represented the release of nuclear HMGB1 into the cytoplasm in

response to cellular injury/stress. This hypothesis was confirmed by a further experiment in human astrocyte cultures that clearly demonstrated nuclear to cytoplasmic transfer of HMGB1 following exposure to IL-1 β (47).

Together, these data support the observations made initially in experiment models, that HMGB1 and its corresponding receptors are upregulated in epileptic tissue and may play a role in the development and perpetuation of seizures. The volume of available evidence remains relatively modest, at least in comparison with that for IL-1 β , and further work in this area is required. At the very least, the influence of endogenous ligands for RAGE needs to be clarified, as does the interaction of this novel pathway with other postulated mechanisms of the innate immune response (48).

Targeting Inflammation as a Therapeutic Strategy in Epilepsy

Current clinical evidence to suggest that counteracting inflammation is therapeutically beneficial in epilepsy is limited. However, with growing evidence to support its potential contribution to the generation of seizures, and possibly to epileptogenesis itself, anti-inflammatory agents can be considered as candidates in the ongoing search for novel AEDs. The compounds arguably showing greatest promise, and furthest down the development pipeline, are inhibitors of interleukin converting enzyme (ICE)/caspase-1, the protease that catalyses the conversion of the inactive precursor pro-IL-1 β to active IL-1 β (49).

Inhibition of ICE/caspase-1 reduces the release of IL-1 β in organotypic hippocampal slices following exposure to pro-inflammatory stimuli (50), decreases acute seizure activity following intrahippocampal kainate in rats (50), and restricts the generalization of seizures in a rapid kindling model (17). These effects are closely correlated with a reduction in the expression of IL-1 β in hippocampal astrocytes. They are unsurprisingly absent in mice in which the corresponding gene has been knocked out and that consequently display an inherent resistance to experimental seizures (50). These initial observations have recently been corroborated in mouse models of both acute seizures and chronic epilepsy (51). Systemic administration of VX-765, a prototypic ICE/caspase-1 inhibitor, increased the time to seizure onset and decreased cumulative duration of electrographic seizures induced by acute intrahippocampal kainate; whilst in the chronic model, VX-765 decreased the time spent in spontaneous epileptic activity by up to 75%. This anticonvulsant action was again correlated with a reduction in the expression of IL-1 β in hippocampal astrocytes and microglia (51). These data confirm the previously reported efficacy of VX-765 in preclinical models, further support its proposed mechanism of action, and suggest that this class of compounds merits further evaluation as putative AEDs.

VX-765 was originally developed for the treatment of inflammatory and autoimmune conditions (52). It is a prodrug with good oral bioavailability, whose active metabolite, VRT-043198, is known to cross the BBB following systemic administration, making it an attractive candidate for the treatment of CNS disorders with a proposed inflammatory component. It has recently undergone a phase 2a trial in drug-resistant partial epilepsy (53) and preliminary, unpublished results sug-



gest that it is safe and well tolerated when administered over a 6-week period. A phase 2b trial is planned, with efficacy and longer-term safety data eagerly anticipated. These will give an important insight into the viability of anti-inflammatory strategies in the treatment of chronic epilepsy.

Implications and Conclusions

These recent publications (30, 47, 51) are consistent with the emerging hypothesis linking activation of the innate immune system and consequent inflammation to epilepsy. They suggest that multiple pro-inflammatory mechanisms are initiated by seizures and may also contribute to the cellular damage and inherent epileptogenicity of brain lesions, pathogenic or otherwise. Activation of innate immunity and inflammatory pathways as a consequence of epilepsy is, on the basis of available evidence, almost beyond doubt. This is an important finding with therapeutic potential for the control of preexisting seizure disorders. Whether anti-inflammatory agents also possess antiepileptogenic potential is another matter. The animal model data are supportive of such a causal involvement, but the relevance of these models to the human condition may be considered questionable. Reconciling a persistent, unresolved inflammatory state with an episodic disorder that is neither progressive nor overtly neurodegenerative once established also needs to be addressed, as does the long-term safety of an intervention that targets a fundamental pathway that exists to protect rather than do harm. Despite these issues, the emerging data on inflammation and epilepsy are grounds for cautious optimism. They represent a potentially novel avenue for drug development in epilepsy and one that is not only distinct from previous approaches but also based on sound neurobiological evidence. We will soon know whether anti-inflammatory agents have genuine efficacy in the treatment of established epilepsy. Harder to establish will be their antiepileptogenic or disease-modifying potential—those trials have yet to be designed, far less undertaken.

References

- Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell* 2010;140:918–934.
- Vezzani A, French JA, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol* 2011;7:31–40.
- Hart YM, Cortez M, Andermann F, Hwang P, Fish DR, Dulac O, Silver K, Fejerman N, Cross J, Sherwin A, Caraballo R. Medical treatment of Rasmussen's syndrome (chronic encephalitis and epilepsy): Effect of high-dose steroids or immunoglobulins in 19 patients. *Neurology* 1994;44:1030–1036.
- Snead OC. How does ACTH work against infantile spasms? Bedside to bench. *Ann Neurol* 2001;49:288–289.
- Maroso M, Balosso S, Ravizza T, Liu J, Bianchi ME, Vezzani A. Interleukin-1 type 1 receptor/Toll-like receptor signalling in epilepsy: The importance of IL-1beta and high-mobility group box 1. *J Intern Med* 2011;270:319–326.
- Becher B, Prat A, Antel JP. Brain-immune connection: Immunoregulatory properties of CNS-resident cells. *Glia* 2000;29:293–304.
- Vezzani A, Maroso M, Balosso S, Sanchez M-A, Bartfai T. IL-1 receptor/Toll-like receptor signalling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures. *Brain Behav Immun* 2011;25:1281–1289.
- Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2001;2:734–744.
- Aloisi F, Ria F, Adorini L. Regulation of T-cell responses by CNS antigen-presenting cells: Different roles for microglia and astrocytes. *Immunity Today* 2000;21:141–147.
- Stoll G, Jander S, Schroeter M. Cytokines in CNS disorders: Neurotoxicity versus neuroprotection. *J Neural Transm Suppl* 2000;59:81–89.
- Wood PL. Microglia: Roles of microglia in chronic neurodegenerative diseases. In: *Neuroinflammation: Mechanisms and Management*. (Wood PL, ed.) Totowa: Humana Press, 2003:3–27.
- Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. *Brain Behav Immun* 2008;22:797–803.
- De Simoni MG, Perego C, Ravizza T, Moneta D, Conti M, Marchesi F, De Luigi A, Garattini S, Vezzani A. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. *Eur J Neurosci* 2000;12:2623–2633.
- Crespel A, Coubes P, Rousset MC, Brana C, Rougier A, Rondouin G, Bockeaert J, Baldy-Moulinier M, Lerner-Natoli M. Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Res* 2002;952:159–169.
- Ravizza T, Boer K, Redeker S, Spliet WG, van Rijen PC, Troost D, Vezzani A, Aronica E. The IL-1beta system in epilepsy-associated malformations of cortical development. *Neurobiol Dis* 2006;24:128–143.
- Boer K, Jansen F, Nellist M, Redeker S, van den Ouweland AM, Spliet WG, van Nieuwenhuizen O, Troost D, Crino PB, Aronica E. Inflammatory processes in cortical tubers, and subependymal giant cell tumors of tuberous sclerosis complex. *Epilepsy Res* 2008;78:7–21.
- Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: Evidence from experimental models and human temporal lobe epilepsy. *Neurobiol Dis* 2008;29:142–160.
- Vezzani A, Conti M, De Luigi A, Ravizza T, Moneta D, Marchesi F, De Simoni MG. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: Functional evidence for enhancement of electrographic seizures. *J Neurosci* 1999;19:5054–5065.
- Dubé C, Vezzani A, Behrens M, Bartfai T, Baram TZ. Interleukin-1beta contributes to the generation of experimental febrile seizures. *Ann Neurol* 2005;57:152–155.
- Heida JG, Pittman QJ. Causal links between brain cytokines and experimental febrile convulsions in the rat. *Epilepsia* 2005;46:1906–1913.
- Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni MG, Sperk G, Andell-Jonsson S, Lundkvist J, Iverfeldt K, Bartfai T. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Natl Acad Sci U S A* 2000;97:11534–11539.
- Auvin S, Shin D, Mazarati A, Sankar R. Inflammation induced by LPS enhances epileptogenesis in immature rat and may be partially reversed by IL1RA. *Epilepsia* 2010;51 (suppl):34–38.
- Viviani B, Bartsaghi S, Gardoni F, Vezzani A, Behrens MM, Bartfai T, Binaglia M, Corsini E, Di Luca M, Galli CL, Marinovich M. Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. *J Neurosci* 2003;23:8692–8700.
- Balosso S, Maroso M, Sanchez-Alavez M, Ravizza T, Frasca A, Bartfai T, Vezzani A. A novel non-transcriptional pathway mediates the proconvulsive effects of interleukin-1beta. *Brain* 2008;131:3256–3265.
- Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC. Cytokine effects



- on glutamate uptake by human astrocytes. *Neuroimmunomodulation* 2000;7:153–159.
26. Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A. CXCR4-activated astrocyte glutamate release via TNF α : Amplification by microglia triggers neurotoxicity. *Nat Neurosci* 2001;4:702–710.
 27. Viviani B, Gardoni F, Marinovich M. Cytokines and neuronal ion channels in health and disease. *Int Rev Neurobiol* 2007;82:247–263.
 28. Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med* 2007;13:460–469.
 29. Bianchi ME, Manfredi AA. Immunology: Dangers in and out. *Science* 2009;323:1683–1684.
 30. Maroso M, Balosso S, Ravizza T, Liu J, Aronica E, Iyer A, Rossetti C, Molteni M, Casalgrandi M, Manfredi A, Bianchi M, Vezzani A. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. *Nature Med* 2010;16:413–420.
 31. Riazi K, Galic MA, Pittman QJ. Contributions of peripheral inflammation to seizure susceptibility: Cytokines and brain excitability. *Epilepsy Res* 2010;89:34–42.
 32. Cacheaux LP, Ivens S, David Y, Lakhter AJ, Bar-Klein G, Shapira M, Heinemann U, Friedman A, Kaufer D. Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. *J Neurosci* 2009;29:8927–8935.
 33. Kulkarni SK, Dhir A. Cyclooxygenase in epilepsy: From perception to application. *Drugs Today* 2009;45:135–154.
 34. Friedman A, Kaufer D, Heinemann U. Blood-brain barrier breakdown-inducing astrocytic transformation: Novel targets for the prevention of epilepsy. *Epilepsy Res* 2009;85:142–149.
 35. Cornford EM. Epilepsy and the blood brain barrier: Endothelial cell responses to seizures. *Adv Neurol* 1999;79:845–862.
 36. Fabene PF, Navarro Mora G, Martinello M, Rossi B, Merigo F, Otoboni 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. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. *Nat Med* 2008;14:1377–1383.
 37. Kim JV, Kang SS, Dustin ML, McGavern DB. Myelomonocytic cell recruitment causes fatal CNS vascular injury during acute viral meningitis. *Nature* 2009;457:193–195.
 38. David Y, Cacheaux L, Ivens S, Lapilover E, Heinemann U, Kaufer D, Friedman A. Astrocytic dysfunction in epileptogenesis: Consequence of altered potassium and glutamate homeostasis? *J Neurosci* 2009;29:10588–10599.
 39. Fiuza C, Bustin M, Talwar S, Tropea M, Gerstenberger E, Shelhamer JH, Suffredini AF. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. *Blood* 2003;101:2652–2660.
 40. Hreggvidsdottir HS, Ostberg T, Wähämaa H, Schierbeck H, Aveberger AC, Klevenvall L, Palmblad K, Ottosson L, Andersson U, Harris HE. The alarmin HMGB1 acts in synergy with endogenous and exogenous danger signals to promote inflammation. *J Leukoc Biol* 2009;86:655–662.
 41. Friedman A, Dingledine R. Molecular cascades that mediate the influence of inflammation on epilepsy. *Epilepsia* 2011;52(suppl):33–39.
 42. Auvin S, Shin D, Mazarati A, Nakagawa J, Miyamoto J, Sankar R. Inflammation exacerbates seizure-induced injury in the immature brain. *Epilepsia* 2007;48(suppl):27–34.
 43. Bouillere V, Ridoux V, Depaulis A, Marescaux C, Nehlig A, Le Gal La Salle G. Recurrent seizures and hippocampal sclerosis following intrahippocampal kainate injection in adult mice: Electroencephalography, histopathology and synaptic reorganization similar to mesial temporal lobe epilepsy. *Neuroscience* 1999;89:717–729.
 44. Yang H, Wang H, Czura CJ, Tracey KJ. HMGB1 as a cytokine and therapeutic target. *J Endotoxin Res* 2002;8:469–472.
 45. Mazarati A, Maroso M, Iori V, Vezzani A, Carli M. High-mobility group box-1 impairs memory in mice through both toll-like receptor 4 and receptor for advanced glycation end products. *Exp Neurol* 2011;232:143–148.
 46. Costello DA, Watson MB, Cowley TR, Murphy N, Murphy Royal C, Garlanda C, Lynch MA. Interleukin-1alpha and HMGB1 mediate hippocampal dysfunction in SIGIRR-deficient mice. *J Neurosci* 2011;31:3871–3879.
 47. Zurolo E, Iyer A, Maroso M, Carbonell C, Anink J, Ravizza T, Fluiter K, Spliet WGM, van Rijen P, Vezzani A, Aronica E. Activation of toll-like receptor, RAGE and HMGB1 signalling in malformations of cortical development. *Brain* 2011;134:1015–1032.
 48. Andersson U, Rauvala H. Introduction: HMGB1 in inflammation and innate immunity. *J Intern Med* 2011;270:296–300.
 49. Kuida K, Lippke JA, Ku G, Harding MW, Livingston DJ, Su MS, Flavell RA. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. *Science* 1995;267:2000–2003.
 50. Ravizza T, Lucas SM, Balosso S, Bernardino L, Ku G, Noe F, Malva J, Randle JC, Allan S, Vezzani A. Inactivation of caspase-1 in rodent brain: A novel anticonvulsant strategy. *Epilepsia* 2006;47:1160–1168.
 51. Maroso M, Balosso S, Ravizza T, Iori V, Wright CI, French J, Vezzani A. Interleukin-1 β biosynthesis inhibition reduces acute seizures and drug resistant chronic epileptic activity in mice. *Neurotherapeutics* 2011;8:304–315.
 52. Randle JCR, Harding MW, Ku G, Schönharting M, Kurre R. ICE/Caspase-1 inhibitors as novel anti-inflammatory drugs. *Exp Opin Invest Drugs* 2001;10:1207–1209.
 53. Vertex Pharmaceuticals Incorporated. Study of VX-765 in subjects with treatment-resistant partial epilepsy. ClinicalTrials.gov database. <http://clinicaltrials.gov/ct2/show/NCT01048255>. Accessed October 18, 2011.



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.

Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships

Use this section to report 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.



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today's Date: 2/10/2012
2. First Name Lauren Last Name Walker Degree MBChB
3. Are you the Main Assigned Author? Yes No

If no, enter your name as co-author: Dr Lauren Walker (co-author)

4. Manuscript/Article Title: Inflammation and epilepsy: the foundations for a new therapeutic approach in epilepsy?
5. Journal Issue you are submitting for: Unknown

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

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 | No | Money Paid to You | Money to Your Institution* | Name of Entity | Comments** |
|---|-------------------------------------|-------------------|----------------------------|----------------|------------|
| 1. Grant | <input checked="" type="checkbox"/> | | | | |
| 2. Consulting fee or honorarium | <input checked="" type="checkbox"/> | | | | |
| 3. Support for travel to meetings for the study or other purposes | <input checked="" type="checkbox"/> | | | | |
| 4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | | | | |
| 5. Payment for writing or reviewing the manuscript | <input checked="" type="checkbox"/> | | | | |
| 6. Provision of writing assistance, medicines, equipment, or administrative support. | <input checked="" type="checkbox"/> | | | | |
| 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 checked="" 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



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.

Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work’s sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

4. Other relationships

Use this section to report 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.



American Epilepsy Society

Epilepsy Currents Journal

Disclosure of Potential Conflicts of Interest

Section #1 Identifying Information

1. Today's Date: 2/15/2012

2. First Name Graeme Last Name Sills Degree PhD

3. Are you the Main Assigned Author? Yes No

If no, enter your name as co-author:

4. Manuscript/Article Title: Inflammation and epilepsy: the foundations for a new therapeutic approach in epilepsy?

5. Journal Issue you are submitting for: Unknown

Section #2 The Work Under Consideration for Publication

Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

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 | No | Money Paid to You | Money to Your Institution* | Name of Entity | Comments** |
|---|-------------------------------------|-------------------|----------------------------|----------------|------------|
| 1. Grant | <input checked="" type="checkbox"/> | | | | |
| 2. Consulting fee or honorarium | <input checked="" type="checkbox"/> | | | | |
| 3. Support for travel to meetings for the study or other purposes | <input checked="" type="checkbox"/> | | | | |
| 4. Fees for participating in review activities such as data monitoring boards, statistical analysis, end point committees, and the like | <input checked="" type="checkbox"/> | | | | |
| 5. Payment for writing or reviewing the manuscript | <input checked="" type="checkbox"/> | | | | |
| 6. Provision of writing assistance, medicines, equipment, or administrative support. | <input checked="" type="checkbox"/> | | | | |
| 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 type="checkbox"/> | Yes | | GlaxoSmithKline | |
| 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"/> | Yes | | GlaxoSmithKline, UCB Pharma, Eisai Ltd, Alapis Pharma, Pfizer | |
| 7. Payment for manuscript preparation. | <input type="checkbox"/> | Yes | | Elsevier Ltd, STAC Consultancy LLP | |
| 8. Patents (planned, pending or issued) | <input checked="" type="checkbox"/> | | | | |
| 9. Royalties | <input checked="" type="checkbox"/> | | | | |
| 10. Payment for development of educational presentations | <input type="checkbox"/> | Yes | | GlaxoSmithKline | |
| 11. Stock/stock options | <input checked="" type="checkbox"/> | | | | |
| 12. Travel/accommodations/meeting expenses unrelated to activities listed.** | <input type="checkbox"/> | Yes | | GlaxoSmithKline, Janssen-Cilag, UCB Pharma | |
| 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.

