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 epileptiform activity 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 immune 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).
Molecular Mechanisms: The Role of Interleukin-1β

Much of the early evidence to support a role for inflammation in epilepsy arose from studies of the cytokine interleukin-1β (IL-1β), its target, interleukin-1 receptor type 1 (IL-1R1), and its naturally occurring competitive antagonist, interleukin-1β receptor antagonist (IL-1RA) (2). All three are upregulated in rodent brain following chemically and electrically induced seizures (12), with IL-1β 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-1β and IL-1RA can also modulate susceptibility to seizure-inducing stimuli. When injected directly into the CNS, IL-1β 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-1β 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-1β 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).

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 for 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-1β, 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-1β (30). Thus, HMGB1-TLR-RAGE may represent a novel pro-inflammatory axis, acting in concert with the traditional IL-1β pathway following sterile brain injury and perhaps without the requirement for overt microglial activation.
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 extra-nuclear 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-1β (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-1β, 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-1β to active IL-1β (49).

Inhibition of ICE/caspase-1 reduces the release of IL-1β 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-1β 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-1β 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.

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</tr>
<tr>
<td>8. Patents (planned, pending or issued)</td>
<td>☒</td>
<td></td>
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<tr>
<td>9. Royalties</td>
<td>☒</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10. Payment for development of educational presentations</td>
<td>☐</td>
<td>Yes</td>
<td>GlaxoSmithKline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Stock/stock options</td>
<td>☒</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>12. Travel/accommodations/meeting expenses unrelated to activities listed.**</td>
<td>☐</td>
<td>Yes</td>
<td>GlaxoSmithKline, Janssen-Cilag, UCB Pharma</td>
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</tr>
<tr>
<td>13. Other (err on the side of full disclosure)</td>
<td>☒</td>
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</tr>
</tbody>
</table>

* 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.