Inflammation and epilepsy: the contribution of astrocytes
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1.2 Astrocyte immune responses in epilepsy

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Abstract

Astrocytes, the major glial cell type of the central nervous system (CNS), are known to play a major role in the regulation of the immune/inflammatory response in several human CNS diseases. In epilepsy-associated pathologies, the presence of astrogliosis has stimulated extensive research focused on the role of reactive astrocytes in the pathophysiological processes that underlie the development of epilepsy. In brain tissue from patients with epilepsy, astrocytes undergo significant changes in their physiological properties, including the activation of inflammatory pathways. Accumulating experimental evidence suggests that proinflammatory molecules can alter glio-neuronal communications contributing to the generation of seizures and seizure-related neuronal damage. In particular, both in vitro and in vivo data point to the role of astrocytes as both major source and target of epileptogenic inflammatory signaling. In this context, understanding the astroglial inflammatory response occurring in epileptic brain tissue may provide new strategies for targeting astrocyte-mediated epileptogenesis.

This article reviews current evidence regarding the role of astrocytes in the regulation of the innate immune responses in epilepsy. Both clinical observations in drug-resistant human epilepsies and experimental findings in clinically relevant models will be discussed and elaborated, highlighting specific inflammatory pathways (such as interleukin-1β/toll-like receptor 4) that could be potential targets for antiepileptic, disease-modifying therapeutic strategies.
Astrocytes as key players in brain inflammation

The central nervous system (CNS) has commonly been considered an immune-privileged site in the presence of an intact blood brain barrier (BBB). However, this concept is gradually changing as a result of recent developments in the field of innate immunity supporting the role of CNS-resident cells acting as innate-immune-competent cells; [for reviews see (Amor et al. 2010; McNaull et al. 2010; Schwartz and Kipnis 2011)]. Both innate and adaptive inflammatory responses occur in the CNS. The innate immune response is a non-specific, acute defense against external agents or local injuries, and cell types chiefly involved in this response include monocytes/macrophages and microglia. The adaptive immune response is antigen-specific, and B and T lymphocytes are the key players in adaptive immunity. Communication between the innate and adaptive responses involves cell-cell interactions, as well as soluble factors such as cytokines and chemokines. In earlier studies, a considerable amount of attention was focused on the immune function of microglial cells, which are generally considered to be the “CNS-based resident macrophages” (Aloisi 2001; Graeber and Streit 1990). However, over the past decade there has been accumulating evidence supporting the central role of astrocytes in the CNS innate immune response induced by a variety of insults. Astrocytes have been shown to initiate, regulate and amplify immune-mediated mechanisms involved in different human CNS diseases, including epilepsy [for reviews see (Farina et al. 2007; Seifert et al. 2010)]. In this review we will largely focus on protoplasmic astrocytes that predominate in gray matter, and fibrous astrocytes that predominate in white matter. Astrocytes undergo complex morphological and functional changes. These changes may vary with the severity and the type of insult and a continuum of progressive morphological alterations can be observed in human brain tissue under different pathological conditions [for review see (Sofroniew and Vinters 2010)].

Astrocytes as innate-immune-competent cells

Astrocytes as antigen-presenting cells

In contrast to the well-established role of microglia as antigen presenting cells (APCs) the role of astrocytes in antigen presentation is still a matter of debate. Previous studies have shown the induction of major histocompatibility (MHC) class I and II molecules on interferon-γ (IFN-γ) exposure in vitro [for reviews see (Aloisi et al. 2000a; Dong and Benveniste 2001)]. However, there are discrepancies in the literature concerning the ability of astrocytes to act as fully competent APCs (Aloisi et al. 2000a; Becher et al. 2000; Cornet et al. 2000; Cross and Ku 2000; Dong and Benveniste 2001; Girvin et al. 2002; Weber et al. 1994). Moreover, the demonstration of MHC-positive astrocytes in pathological human brain remains a controversial topic, even in inflammatory neurological disorders such as multiple sclerosis (MS) (De...
Keyser et al. 2003; Gobin et al. 2001; Traugott and Raine 1985). In Rasmussen’s encephalitis (RE), a severe inflammatory epileptic encephalopathy of childhood, expression of MHC class I molecules has been reported in astrocytes (Bauer et al. 2007; Farrell et al. 1995), suggesting an MHC class I–restricted T-cell response as a possible mechanism for the occurrence of the astrocytic degeneration observed in RE (Bauer et al. 2007). However, the final effect of astrocyte-T-lymphocyte interactions is complex and depends on the type of responding T cells (Th1 or Th2 cells). Accordingly, whereas microglia may activate both Th1 and Th2 cells, astrocytes have been shown to stimulate mainly Th2 responses, providing homeostatic mechanisms which may limit brain inflammation (Aloisi et al. 1998; Aloisi et al. 2000b).

The physiological role of MHC molecules (MHC class I), as well as of other immunological molecules/receptors expressed by astrocytes during brain development, is another important area for future research. Recent studies suggest that immune molecules critically modulate the development and function of the CNS [for review see (Glynn et al. 2011)].

**Astrocytes as source and target of inflammatory molecules**

Astrocytes represent an important source of immunologically relevant cytokines and chemokines. *In vitro* studies document the ability of astrocytes (particularly reactive astrocytes) to produce cytokines such as interleukin(IL) -1β, IL-6, tumor necrosis factor (TNF)-α, transforming growth factor beta (TGF)-β and chemokines, such as monocyte chemoattractant protein-1 (MCP-1; chemokine, C-C motif, ligand 2; CCL2), which are highly expressed in both experimental and human epileptogenic brain tissue [for reviews see (Aronica and Crino 2011; Vezzani et al. 2008b) Fig. 1]. The expression pattern and the role of astrocyte-derived inflammatory molecules in seizure generation and progression will be discussed in the following sections.

Astrocytes are also the target of inflammatory molecules which, through the activation of specific receptors (including pattern-recognition receptors (PRRs) and related intracellular signaling pathways), may aggravate astrogliosis and amplify the pro-epileptogenic inflammatory signaling [for reviews see (Aronica and Crino 2011; Farina et al. 2007; Sofroniew and Vinters 2010); Fig. 1]. Particular attention has recently been focused on the role of the IL-1 receptor/toll-like receptor superfamily (IL-1R/TLR) in epilepsy (Aronica and Crino 2011; Maroso et al. 2011; Vezzani et al. 2010; Vezzani et al. 2011d; Vezzani et al. 2008b). The IL-1R/TLR superfamily comprises cell surface PRRs sharing a conserved region termed the Toll/IL-1R (TIR) domain (O’Neill 2008; O’Neill and Dinarello 2000). Several TLRs are expressed in human astrocytes *in vitro*, including TLR2, TLR3 and TLR4 (Farina et al. 2007). However, whereas TLR3 shows consistent expression in the resting state, studies examining TLR2 and TLR4 expression in
Astrocytes have produced conflicting results, which may reflect differences in cell source and culture conditions (Crack and Bray 2007; Farina et al. 2007; Kielian 2006). Expression of IL-1R1 or TLRs in astrocytes has also been demonstrated in human brain, with low levels in resting astrocytes and upregulation in reactive astrocytes under different pathological conditions, including epilepsy [(Maroso et al. 2010; Maroso et al. 2011; Ravizza et al. 2006a; Ravizza et al. 2008a; Vezzani et al. 2011d; Zurolo et al. 2011); Fig.1].

TLRs have a key role in pathogen recognition (Kawai and Akira 2007), but in the absence of pathogens, TLR can be activated by endogenous molecules, named damage-associated molecular patterns (DAMPs), released from injured or activated cells. One of these molecules is the high mobility group box 1 (HMGB1) (Bianchi and Manfredi 2009), a ubiquitous chromatin component that can be actively secreted by immuno-competent cells in response to immune challenges (Muller et al. 2004). Both in vitro and in vivo findings suggest that astrocytes are a source of extracellular HMGB1 (Maroso et al. 2010; Zurolo et al. 2011). In particular, HMGB1 release has been shown to be induced in both rat (Hayakawa et al. 2010) and human astrocytes in culture (Zurolo et al. 2011) in response to the pro-inflammatory...
cytokine IL-1β, and nuclear to cytoplasmic translocation has been observed in human and experimental epileptic tissue (Maroso et al. 2010). In addition, astrocytes have also been shown to respond to HMGB1 stimulation with induction of several inflammatory mediators (Pedrazzi et al. 2007).

Recently, a new class of regulators of the immune responses has been recognised in the form of microRNAs (miRNA), acting as post-transcriptional regulators of gene expression (Gantier 2010; Quinn and O’Neill 2011; Sonkoly et al. 2008). In particular, the miRNA-146a has been specifically associated with the regulation of TLR signaling (Cui et al. 2010; Quinn and O’Neill 2011; Sheedy and O’Neill 2008; Taganov et al. 2006). miRNA-146a is expressed in human brain, and astrocytes have been shown to be key players in the regulation of this miRNA in response to inflammatory molecules, such as IL-1β (Aronica et al. 2010; Cui et al. 2010).

Another important component of the innate immune response is the complement system; this consists of a variety of soluble and surface proteins which, when activated, result in a complex cascade of processes contributing to the amplification of the inflammatory response [for review see (Bonifati and Kishore 2007)]. Reactive astrocytes are a source of complement components and also express complement-regulatory proteins, as well as complement receptors [for review see (Farina et al. 2007); Fig. 1]. Complement activation products, such as C3, regulate cytokine synthesis, and cytokines (such as IL-1β) may also induce complement factor expression in human astrocytes (Barnum and Jones 1995; Bonifati and Kishore 2007; Veerhuis et al. 1999). Astrocytes can also contribute to regulation of this inflammatory pathway by induction of inhibitory factors, such as the complement factor H (CFH) (Aronica et al. 2007; Boon et al. 2009; Griffiths et al. 2009). In addition, recent evidence suggests an extensive and complex cross-regulation between complement and the TLRs, which deserves further investigation in astrocytes (Hajishengallis and Lambris 2010).

**Astrocyte immune-inflammatory function and neurotrasmitter receptors**

Signaling via neurotransmitter receptors provides an additional mechanism by which astrocytes can sense and respond to changes in the extracellular environment, influencing the inflammatory and immune response under pathological conditions associated with astrogliosis. An example is provided by the activation of astroglial G protein-coupled glutamate and purinergic receptors, the expression of which is deregulated in epileptogenic brain tissue (Byrnes et al. 2009; D'Antoni et al. 2008; Gomes et al. 2011; Hasko et al. 2005; Matute and Cavaliere 2011).

Both in vitro and in vivo studies suggest an up-regulation of group I and II metabotropic glutamate receptor (mGluR) subtypes (mGluR5 and mGluR3) in reactive astrocytes ([Aronica et
Activation of mGluR3 in human astrocytes in culture modulates the release of IL-6 in the presence of IL-1β, supporting the role of this receptor subtype in regulating the capacity of activated astrocytes to produce inflammatory cytokines (Aronica et al. 2005a).

Increasing evidence points towards a critical role of purinergic receptors in neuron–glia communication and neuroinflammation (Boison 2010; Gomes et al. 2011). Stimulation of the adenosine receptor (P1 receptor) A2B induces the release of IL-6 from astrocytes and the activation of the A3 receptor induces the synthesis of the chemokine MCP-1 [for reviews see (Abbracchio and Ceruti 2007; Gomes et al. 2011; Hasko et al. 2005)]. The P2 purinergic receptors modulate the cytokine-mediated signal transduction in human astrocytes in culture (Liu et al. 2000). Although the expression of both P2X (4,6,7) and P2Y(1,2) receptor-subtypes has been reported in cultured astrocytes, P2Y rather than P2X receptor-subtypes have been suggested as being involved in the modulation of intracellular Ca²⁺ (Fischer et al. 2009). In human fetal astrocytes, the blockade of P2Y receptors affects both IL-1β and TNFa signaling (Liu et al. 2000), whereas the P2X7 receptor has been implicated in the regulation of chemokine synthesis in astrocytes (Panenka et al. 2001). In addition, inflammatory molecules, such as IL-1β, may modulate the expression of adenosine kinase (ADK), providing a potential modulatory crosstalk between the astrocyte-based adenosine cycle and inflammation (Aronica et al. 2011).

Finally, it has also been suggested that cannabinoid (CB) receptors, as mediators of endocannabinoid signaling, exert an immunomodulatory function on astrocytes (Sheng et al. 2005).

ASTROGLIAL INFLAMMATORY RESPONSE IN EPILEPSY

Increasing evidence supports the concept of activation of innate immune responses in both experimental and human epilepsy and the critical involvement of inflammatory processes in the etiopathogenesis of seizures (Aronica and Crino 2011; Vezzani et al. 2010; Vezzani et al. 2011d). In particular, recent clinical-neuropathological and experimental observations support the notion that dysregulation of the astrocyte immune-inflammatory function (discussed above) is a common factor, which may predispose or directly contribute to the generation of seizures and to seizure-related neuronal damage in epilepsy of various etiologies (Fig. 1). Current knowledge concerning common inflammatory signaling pathways involving astrocytes that may alter neuronal excitability is discussed below.
ASTROCYTES AND INFLAMMATORY PROCESSES IN PATIENTS WITH MEDICALLY REFRACtORY EPILEPSY

Reactive astrogliosis is a pathological hallmark of various types of medically refractory focal epilepsy, including epilepsy that develops following ischemic, traumatic, or infectious brain injury (Sofroniew and Vinters 2010). Reactive astrogliosis is also the pathological hallmark of two major epilepsy-associated pathologies [hippocampal sclerosis and focal malformations of cortical development (MCD; FCD and cortical tubers in tuberous sclerosis complex TSC)]. Hippocampal sclerosis (HS) is the most common neuropathological finding in patients undergoing surgery for intractable temporal lobe epilepsy [TLE; (Wieser 2004)]. Although specimens obtained from patients undergoing surgery for intractable TLE often represent the end-stage of the pathological cascade that leads to HS, histopathological and molecular analysis of this tissue is essential to confirm the relevance and cellular sources of inflammatory molecules and related signaling. Large-scale analysis of gene expression profiles suggests a prominent upregulation of genes related to astroglial activation and innate immune/inflammatory response in human TLE [for review see (Aronica and Gorter 2007)] This evidence, at gene expression level, has been confirmed by histopathological studies demonstrating the association between activated astrocytes and microglial cells and the induction of major proinflammatory pathways in human TLE (Aronica and Gorter 2007; Ravizza et al. 2008a; Vezzani et al. 2010).

The transcription factor nuclear factor-kappa B (NFkB) plays a central role in regulating immune and inflammatory responses, including the IL-1R/TLR signaling pathways (Oechkinghaus et al. 2011). Activation of IL-1R1-mediated signaling in cells targeted by the released IL-1β induces, via an NFkB-dependent mechanism, the transcription of other genes encoding downstream mediators of inflammation, including IL-6, TNF-α, cyclooxygenase-2 (Cox-2) or CCL2 (i.e. monocyte chemotactic protein-1) (Andjelkovic et al. 2000; Dinarello 2004; Meeuwsen et al. 2003). Crespel et al. (Crespel et al. 2002) reported NFkB over-expression in reactive astrocytes in human HS specimens. The activation of the NFkB signaling pathway in astrocytes has been confirmed by subsequent studies demonstrating prominent expression in astrocytes of both L-1β and its functional receptor, IL-1R1, providing evidence of a persistent activation of this specific inflammatory pathway in human tissue from people with chronic epilepsy (Ravizza et al. 2008a). In addition, up-regulation of astroglial Cox-2 and CCL2 has been reported in human TLE (Desjardins et al. 2003; Holtman et al. 2009; Wu et al. 2008) suggesting the activation of a complex, highly interconnected, cytokine network. Human studies in TLE also support the involvement of the complement system. Expression of various complement components, such as C1q, C3c and C3d, has been observed in reactive astrocytes within the sclerotic hippocampus of people with TLE (Aronica et al. 2007).
Complement activation in astrocytes may regulate cytokine synthesis thus critically contributing to the propagation and persistence of the inflammatory response. The activation of the plasminogen system in astrocytes in human TLE may contribute to the regulation of the immune responses and related inflammation within the epileptic lesion (Benarroch 2007). Accordingly, in addition to neurons and microglial cells, expression of both tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) has been observed in reactive astrocytes (Iyer et al. 2010). Although PAs may contribute to the activation of astrocytes (Gravanis and Tsirka 2005), astrocytes are also involved in the neutralization of tPA toxicity via an endocytic tPA receptor (Fernandez-Monreal et al. 2004).

Induction of the pathways discussed above (IL-1β, complement and PA) in perivascular astrocytes suggests that the alterations of BBB permeability described in human TLE (Rigau et al. 2007; van Vliet et al. 2007) probably result from the convergence of the actions of different inflammatory molecules released from parenchymal brain cells, then acting on blood vessels. In this context, the vascular endothelial growth factor A (VEGFA)-signaling pathway may also affect the integrity of the BBB (Schoch et al. 2002). VEGFA is upregulated in reactive astrocytes (including perivascular astrocytes) in human epileptogenic tissue [HS and FCD; (Boer et al. 2008; Morin-Brureau et al. 2011; Rigau et al. 2007)], and increased VEGFA expression has recently been reported within cortical tubers of people with TSC (Parker et al. 2011).

As mentioned above, the activation of the TLR signaling pathway in astrocytes plays a key role in the regulation of the innate immune response. Interestingly overexpression of TLR4 and its endogenous ligand HMGB1 has been demonstrated in human TLE in reactive astrocytes, confirming the role of these cells as both source and target of HMGB1 (Maroso et al. 2010). The functional consequences of the astrocyte-mediated HMGB1-TLR signaling in ictogenesis and epileptogenesis in experimental models are discussed below (see Fig. 2). Reactive astrogliosis is also a major feature of focal MCD, such as FCD and cortical tubers in TSC (Blumcke et al. 2011; Blümcke et al. 2009; Orlova and Crino; Sosunov et al. 2008; Wong 2008).

The association between astrogliosis and activation of inflammatory pathways is supported by gene expression analysis of cortical tubers in TSC; high expression levels have been observed for genes encoding complement components, chemokines, MHC elements and components of the IL-1R/TLR signaling pathways (Boer et al. 2010). Activation of various proinflammatory molecules and related pathways has been confirmed at the cellular level in both TSC and FCD specimens, pointing to the central role of reactive astrocytes in the immune/inflammatory response of these developmental epileptogenic lesions as well [for review see (Aronica and Crino 2011)].
A recent study (Zurolo et al. 2011) demonstrates the activation of the TLR signaling pathway in reactive astrocytes both in FCD type and TSC specimens, as shown by overexpression of TLR4 and RAGE (receptor for advanced glycation end products) and cytoplasmic translocation of HMGB1 [as reported in human HS specimens; (Maroso et al. 2010)].

The transforming growth factor (TGF)-β mediated pathway is also upregulated in focal MCD (Boer et al. 2010; Kim et al. 2003). TGF-β is a multifunctional cytokine which, acting through its specific receptors, modulates the astrocyte immune responses and which has recently been suggested as playing a critical role in neocortical epileptogenesis acting through its specific receptors (Ivens et al. 2007).

A controversial issue is whether the dysregulation of astrocyte immune-inflammatory responses is related to the underlying cellular pathology in epileptic tissue or whether it occurs as a consequence of recurrent seizures. The observation of prenatal TLRs and HMGB1 expression in giant cells within the tuber in TSC (Aronica et al. 2008; Samadani et al. 2007; Zurolo et al. 2011) suggests that the induction of these signaling pathways could be intrinsic to the developmental lesion per se, and that the development of seizures later in life could contribute to perpetuation of their activation.

These observations provide evidence of the potential role of astrocytes in the chronic in-
flammatory state observed in focal MCD. Whether the dysregulation of astrocyte immune-inflammatory responses could contribute to progressive cognitive dysfunction in children deserves further investigation (Chew et al. 2006; Cohly and Panja 2005).

Finally, both gene expression and immunocytochemical studies suggest prominent activation of major proinflammatory pathways within the astroglial component of highly epileptogenic tumors (glioneuronal tumors, such as gangliogliomas) (Aronica et al. 2008; Samadani et al. 2007; Zurolo et al. 2011).

**ASTROCYTE AND BRAIN INFLAMMATION IN EXPERIMENTAL MODELS OF SEIZURES AND EPILEPSY**

Experimental studies provided the first evidence showing a significant contribution of reactive astrocytes to the inflammatory processes developing after seizures or induced by an epileptogenic brain injury. Acute seizures induced in rodents (following intracerebral application of kainate or bicuculline, or electrically induced status epilepticus) were shown rapidly to upregulate prototypical inflammatory cytokines in microglia and astrocytes in the brain areas where seizures originate and spread; as a consequence of this event, a downstream cascade of inflammatory mediators is transcriptionally upregulated in brain tissue similar to what has been shown in human epilepsy [(De Simoni et al. 2000; Gorter et al. 2006; Vezzani et al. 1999; Vezzani et al. 2000); reviewed by (Kulkarni and Dhir 2009; Vezzani et al. 2008a; Vezzani et al. 2011b)]. Activation of astrocytes in the absence of neuronal degeneration has been reported in a kindling model (Khurgel et al. 1995) and induction of GFAP has been observed even after a single electroconvulsive seizure (Steward et al. 1992). A detailed time-course analysis of these inflammatory processes occurring after status epilepticus in rats was instrumental in demonstrating that the mRNA of inflammatory mediators are induced within 30 minutes of seizure onset (De Simoni et al. 2000). The immunohistochemical analysis of IL-1β in status epilepticus models showed that the expression of this cytokine in microglia is time-locked to the occurrence of seizures and the extent of expression depends on the recurrence of seizures, while astrocytes appear to be involved in perpetuating inflammation even in the long-term after the initial injury (Ravizza et al., 2008a). Moreover, as in human epileptic tissue, astrocytes often express both the inflammatory mediator and the cognate cell signaling receptors, thus highlighting that these cells serve as sources and targets of inflammatory molecules (reviewed in (Maroso et al. 2011; Vezzani et al. 2011c). Experimental findings clearly show that both parenchymal and perivascular astrocytes are activated and express inflammatory molecules in epilepsy models with functional consequences on BBB function (Fig. 2) (Bauer et al. 2008; Friedman et al. 2009; Ravizza et al. 2008a; van Vliet et al. 2009; Vezzani et al. 2011c). Notably, specific anti-inflammatory molecules, such as the IL-1
receptor antagonist (IL-1ra) (De Simoni et al. 2000) or the C59 inhibitor of the complement system (Aronica et al. 2007), are induced to a limited extent by seizures or following brain injury, suggesting that the mechanisms involved in the resolution of brain inflammation are not very efficient, and possibly explaining why inflammation is detrimental for tissue excitability and cell survival.

Although induction of various inflammatory molecules has been demonstrated in astrocytes in seizure models [reviewed in (Friedman and Dingledine 2011; Vezzani et al. 2008a; Wetherington et al. 2008)], the IL-1/TLR is the first inflammatory signalling to be induced during innate immunity activation, either by a pathogen or a danger signal serving as endogenous ligand (Maroso et al. 2011; Vezzani et al. 2011c). IL-1/TLR signalling is rapidly induced by tissue injury or seizures in neurons, microglia and astrocytes resulting in transcriptional activation of inflammatory genes, and other genes potentially involved in synaptic and molecular changes underlying epileptogenesis [reviewed in (Vezzani et al. 2011c)]. Two endogenous ligands, IL-1β and HMGB1 (Fig. 2) are released by glial cells; IL-1β activates IL-1R1 and HMGB1 activates TLR4 in neurons with significant consequences for ictogenesis, mainly mediated by post-translational effects (see later).

FUNCTIONAL CONSEQUENCES OF ASTROCYTE-MEDIATED BRAIN INFLAMMATION ON NEURONAL EXCITABILITY

The activation of inflammatory pathways and the consequent release of inflammatory molecules by astrocytes alter neural network excitability via induction of various mechanisms, with either direct or indirect impact on neuronal functions. Here we focus on the IL-1R1/TLR4 signaling because of its prominent involvement in seizures and epileptogenesis [(Maroso et al. 2011; Vezzani et al. 2011c); Fig. 2].

IL-1β, by acting on IL-1R type 1, can inhibit the astrocytic reuptake of glutamate (Hu et al. 2000; Ye and Sontheimer 1996) and increases its glial release likely via induction of TNFa (Bezzi et al. 2001). These effects result in elevated extracellular glutamate levels, which in turn can promote tissue excitability. IL-1β can also increase neuronal glutamate release via the activation of inducible nitric oxide synthase in glial cells (Casamenti et al. 1999; Hewett et al. 1994). Astrocytic glutamate release may have a role in the genesis or strength of seizure-like events (Carmignoto and Fellin 2006; Tian et al. 2005).

In hippocampal neurons, IL-1R1 co-localizes with NMDA receptors, a subtype of glutamate receptor involved in the onset and spread of seizures. IL-1β potentiates NMDA receptor function in cultured hippocampal neurons (Lai et al. 2006; Viviani et al. 2003) by enhancing N-Methyl-D-aspartate (NMDA)-mediated Ca²⁺ influx via IL-1R1 dependent activation of Src kinases and subsequent NMDA receptor subunit 2 (NR2B) phosphorylation. This rapid
mechanism (within minutes) involves ceramide-mediated activation of Src kinases (Viviani et al. 2003), and contributes to seizure generation and recurrence (Balosso, 2008). IL-1β also down-regulates AMPA receptor expression and phosphorylation in hippocampal neurons in a Ca\(^{2+}\)- and NMDA-dependent manner (Lai et al. 2006).

Interactions of IL-1β with GABA-mediated inhibitory neurotransmission have also been reported. However, the results obtained are not consistent. Thus IL-1β can either decrease or increase GABA inhibition depending on the brain area (Alam, 2004; Wilkinson, 1993), the cytokine concentration (Wang, 2000; Zeise, 1997; Serantes, 2006) and the functional properties of the cells (Hori, 1988), highlighting a dual role of IL-1β in affecting GABAergic inhibitory system.

In the hippocampus, IL-1β affects synaptic transmission, and inhibits long-term potentiation (LTP) via activation of Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) (Bellinger et al. 1993; O’Donnell et al. 2000; Schneider et al. 1998). This cytokine can also modulate neurotransmitter release via inhibition of voltage-dependent Ca\(^{2+}\) channels, an effect that involves pertussis-sensitive G proteins and protein kinase C (PKC) (Plata-Salaman and ffrench-Mullen 1994).

TNF-α is a cytokine released from activated astrocytes and microglia, and tightly associated with IL-1β, since the two molecules reciprocally induce their respective release from glia and mutually activate their gene transcription. TNF-α has been shown to increase the mean frequency of AMPA-dependent miniature excitatory postsynaptic currents in hippocampal neurons and to decrease GABA\(_{\alpha}\)-mediated inhibitory synaptic strength. These effects are mediated by its ability to activate the recruitment of AMPA receptors lacking the GluR2 subunit at neuronal membranes (thus in a molecular conformation which favors Ca\(^{2+}\) influx into neurons) and to induce endocytosis of GABA\(_{\alpha}\) receptors (Beattie et al. 2002; Stellwagen et al. 2005).

TLR4 is the TLR subtype most extensively studied for its involvement in brain excitability. Cortical rat exposure to lipopolysaccharide (LPS), a prototypical activator of TLR4, induces rapid increases in neuronal excitability leading to seizures (Rodgers et al. 2009), which are prevented by IL-1ra, implicating a role of released IL-1β.

Regarding IL-1β, LTP and long-term depression (LTD) impairment are induced by TLR4 stimulation, compatible with cognitive deficits induced in rodents by brain inflammation (Galic et al. 2009; Harre et al. 2008; Spencer et al. 2005). These modifications in brain physiology are dependent on the release of TNF-α and IL-1β from activated glial cells (Riazi et al. 2010). Cognitive deficits are associated with specific changes in NMDA receptor subunit expression in the cortex and hippocampus, predicting modifications in CNS excitability (Galic et al. 2009; Harre et al. 2008; Spencer et al. 2005).
These fast post-translational effects of inflammatory cytokines are examples of novel pathways by which inflammatory molecules produced in diseased tissue by glia can modulate neurotransmission and contribute to hyperexcitability and associated neuropathology. In general, the amount and persistence of cytokines in brain tissue appears to be a crucial factor which determines their consequences on neuronal excitability. Another important aspect is the pattern of expression of cytokine receptors in the tissue exposed to inflammation. A typical example is represented by TNF-α, which may have inhibitory or permissive effects on seizures depending both on its brain levels and on the receptor subtypes predominantly activated (Balosso et al. 2005). Moreover, transgenic mice with low to moderate overexpression of TNF-α in astrocytes show decreased susceptibility to seizures (Balosso et al. 2005), whereas mice with high overexpression of TNF-α in astrocytes develop signs of neurologic dysfunction (Akassoglou et al. 1997; Probert et al. 1995).

FUNCTIONAL CONSEQUENCES OF ASTROCYTE-MEDIATED BRAIN INFLAMMATION ON SEIZURES AND EPILEPTOGENESIS

The role of cytokines released by astrocytes in seizures and epileptogenesis has been investigated at various levels, initially using genetically-modified mice with perturbed cytokine systems and subsequently by pharmacological intervention using receptor antagonists or cytokine synthesis inhibitors, or by injecting the cytokines themselves into rodent brain [reviewed in (Vezzani et al. 2008a; Vezzani et al. 2011b)]. IL-1β, TNF-α and HMGB1 are among the most studied cytokines for their permissive role in seizures. Mice with upregulation of IL-1ra in astrocytes or lacking IL-1R1 are intrinsically resistant to seizures (Vezzani et al. 2000) and the intracerebral injection of IL-1ra mediates powerful anticonvulsant effects (Vezzani et al. 2000; Vezzani et al. 2002). Because the only action of IL-1ra is to inhibit the effects of IL-1β, these data demonstrate that an endogenous increase in brain IL-1β contributes to seizures. Mice lacking caspase-1, the biosynthetic enzyme of IL-1β, and therefore unable to release the biologically active form of this cytokine, show decreased seizure susceptibility (Ravizza et al. 2006b). Pharmacological data support a proconvulsant role of IL-1β in several acute and chronic seizure models (Vezzani et al. 1999; Vezzani et al. 2000), as well as in the kindling model of epileptogenesis (Ravizza et al. 2008b). Moreover, recent data showed that inhibition of IL-1β biosynthesis in astrocytes reduces spike-and-wave discharges in rats with genetic absence epilepsy (GAERS) (Akin et al. 2011). Increase in astrocytic IL-1β in the hippocampus due to fever/hyperthermia is involved in decreasing the seizure threshold in the immature rodent (Dube et al. 2005; Heida and Pittman 2005).

Recently, we have shown that endogenous release of “danger signals” produced by stressed
or injured neurons (i.e. HMGB1), promotes seizures by activation of neuronal TLR. Seizures, in turn, induce an additional wave of HMGB1 release from activated astrocytes and microglia, leading to a vicious positive feedback cycle of seizures and inflammation. This novel pathway may be a crucial mechanism for recurrent seizures [(Maroso et al. 2010); reviewed in (Maroso et al. 2011; Vezzani et al. 2011d); Fig. 2].

Cytokines and other inflammatory mediators have been shown to contribute to both excitotoxic and apoptotic neuronal death (Allan et al. 2005), highlighting the possibility that they contribute to seizure-mediated neuronal damage. The deleterious effects of cytokines on neuronal survival involve the production of neurotoxic compounds via autocrine or paracrine mechanisms (Allan et al. 2005; Vezzani and Baram 2007). Importantly, although cytokines can promote neurodegeneration (Fig.2), their effects on the threshold, frequency and duration of seizures are not dependent on cell death (Vezzani et al. 2011c).

Notable examples exist of a dual role of cytokines on neuronal survival in diseased tissue (Allan et al. 2005; Bernardino et al. 2005); it has been shown the ability of cytokines to induce the synthesis of growth factors in astrocytes, to activate antioxidant pathways, manganese superoxide dismutase, or calbindin which counteracts the elevation of intracellular Ca²⁺ induced by cell injuries (Allan et al. 2005), thus promoting cell repair mechanisms. In this respect, IL-1β and TNF-α can either reduce or exacerbate glutamate receptor–mediated excitotoxicity in organotypic slice cultures, depending on their extracellular concentrations, the length of time the tissue is exposed to these cytokines, and the receptor types activated by these cytokines (Bernardino et al. 2005).

Finally, the possible involvement of inflammatory mediators in epileptogenesis has been suggested by two main lines of evidence: the induction of an inflammatory state in the brain by administering proinflammatory molecules in rodents, or the use of mice that overexpress specific cytokines in astrocytes. This leads to decreased seizure threshold and induces long-term neurological deficits, particularly if applied to immature rodents, thus suggesting long-term effects of inflammation on brain functions [reviewed by (Ravizza et al. 2011; Riazi et al. 2010)]. In this context, brain inflammation has been implicated in the pathophysiology of several neuropsychiatric conditions (such as depression, memory impairments, and autism spectrum disorder) which are comorbidities of epilepsy, (Vezzani et al. 2011a).

Pharmacological interference with specific inflammatory pathways activated during epileptogenesis may reduce the severity and frequency of spontaneous seizures [reviewed in (Ravizza et al. 2011)]. Additionally, cytokines such as IL-1β and HMGB1 released in diseased tissue by parenchymal and, in particular, by perivascular astrocytes, can play a major role in BBB breakdown associated with brain inflammation in human and experimental epileptic tissue [reviewed
by (Friedman et al. 2009)]. The opening of the BBB rapidly activates the innate immune response (Cacheaux, 2002) and the accumulation of albumin in the brain because of BBB damage. Albumin triggers long-lasting hyperexcitability in surrounding tissue by impairing astrocyte capacity to buffer extracellular K⁺ and glutamate via activation of the TGF-β pathway [reviewed by (Friedman et al. 2009)].

Finally, it is increasingly recognized that pro-inflammatory molecules released by glia contribute to some of the acquired channelopathies described in epilepsy by inducing alterations in voltage- and receptor-gated ion channels via either post-translational or transcriptional mechanisms [reviewed by (Vezzani et al. 2011b; Viviani et al. 2004)].

CONCLUDING REMARKS

During the past decade, detailed molecular characterization of astrocytes, in particular reactive astrocytes, demonstrates that these cells are active players in the development and progression of the immune/inflammatory response that takes place in epileptic brain tissue. Both human and experimental data suggest the activation of specific proinflammatory pathways in astrocytes, which may also recruit neuronal cells and, in some cases, cells of the adaptive immune system. The identification of “harmful” pro-inflammatory pathways contributing to seizure onset and recurrence, as well as to comorbidities often associated with epilepsy, highlights the possibility of developing a therapeutic strategy targeting the astrocyte-mediated inflammatory signalings. However, we need to wait for the outcome of clinical studies before we can consider whether this approach is the right strategy. If this is so, it may not only improve control of seizures, but may also act as disease-modifying therapy in patients with epilepsy resistant to conventional antiepileptic drugs.

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