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Invited Review

IL-1 receptor/Toll-like receptor signaling in infection, inflammation, stress and neurodegeneration couples hyperexcitability and seizures

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ABSTRACT

Increasing evidence supports the involvement of immune and inflammatory processes in the etiopathogenesis of seizures. In particular, activation of innate immune mechanisms and the subsequent inflammatory responses, that are induced in the brain by infection, febrile seizures, neurotrauma, stroke are well documented conditions associated with acute symptomatic seizures and with a high risk of developing epilepsy. A decade ago, pharmacological experiments showed that elevated brain levels of the anti-inflammatory molecule IL-1 receptor antagonist reduced seizures in epilepsy models. This observation, together with the evidence of in situ induction of inflammatory mediators and their receptors in experimental and human epileptogenic brain tissue, established the proof-of-concept evidence that the activation of innate immunity and inflammation in the brain are intrinsic features of the pathologic hyperexcitable tissue.

Recent breakthroughs in understanding the molecular organization of the innate immune system first in macrophages, then in the different cell types of the CNS, together with pharmacological and genetic studies in epilepsy models, showed that the activation of *IL-1 receptor/Toll-like receptor* (IL-1R/TLR) signaling significantly contributes to seizures. IL-1R/TLR mediated pro-excitatory actions are elicited in the brain either by mimicking bacterial or viral infections and inflammatory responses, or via the action of endogenous ligands. These ligands include proinflammatory cytokines, such as IL-1beta, or danger signals, such as HMGB1, released from activated or injured cells. The IL-1R/TLR signaling mediates rapid post-translational changes in voltage- and ligand-gated ion channels that increase excitability, and transcriptional changes in genes involved in neurotransmission and synaptic plasticity that contribute to lower seizure thresholds chronically.

The anticonvulsant effects of inhibitors of the IL-1R/TLR signaling in various seizures models suggest that this system could be targeted to inhibit seizures in presently pharmaco-resistant epilepsies.

1. Introduction

Seizures, the hallmarks of epilepsy, originate from synchronized aberrant firing of neuronal populations due to underlying hyperexcitability phenomena. In the last decade, studies on the mechanisms underlying seizures showed that glial cells importantly contribute to neuronal network dysfunctions. Disease-related alteration in astrocyte and microglia functions include changes in the expression of ion and water channels, glutamate receptors and transporter, intracellular Ca^{2+} signaling and activation of astrocytic gliotransmission (Hanisch and Kettenmann, 2007; Seifert et al., 2006). Recently, the crucial role played in the mechanisms of seizures by proinflammatory cytokines synthesized and released mostly, although not exclusively, by activated glial cells has gained increasing recognition (Vezzani et al., 2008, 2011).

It is well established that proinflammatory cytokines in addition to their canonical involvement in immune response activation following infection, can subserve neuromodulatory functions implicated in brain physiology and may contribute to acute and chronic neurodegeneration (Allan et al., 2005; Glass et al., 2010; Nguyen et al., 2002). The possibility that cytokines also contribute to aberrant neuronal excitability underlying seizures is supported by clinical and experimental findings.

Inflammatory mediators and their receptors are up-regulated in microglia and astrocytes in epileptogenic brain tissue from clinical cases of drug-resistant epilepsies of different etiology, similarly to what is described in brain areas recruited in experimentally induced epileptic activity, like the cerebral cortex and limbic structures (Choi et al., 2009; Vezzani et al., 2011). In these brain areas, neurons and endothelial cells of the blood–brain barrier (BBB) also express proinflammatory cytokines. The frequent concomitant over-expression of releasable inflammatory mediators and of their functional receptors in the same cell types, is evidence for the

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activation of both autocrine and paracrine inflammatory signaling in the epileptic tissue. The extent of brain inflammation positively correlates with the frequency of seizures and with the severity of neuropathology both in patients and in animal models (Iyer et al., 2010; Ravizza et al., 2006a, 2008), suggesting a causal relationship between inflammation and seizures.

The active role in seizures of brain-derived inflammatory molecules, and the activation of the related cell signaling, has been demonstrated by various experimental observations. In particular, pro-epileptogenic injuries activate specific inflammatory pathways that contribute to the precipitation and recurrence of seizures (Bartfai et al., 2007; Vezzani et al., 2011). Moreover, the experimental induction of a proinflammatory state in the CNS, mimicking bacterial or viral infections, mediates long-term changes in brain excitability, and increases the likelihood of seizure precipitation (Riazi et al., 2010). This set of experimental evidence is concordant with the clinical observations that inflammatory processes are induced in brain following infection, neurotrauma, stroke, febrile seizures, status epilepticus, which are all events associated with the occurrence of symptomatic seizures and with an increased risk of developing epilepsy (Bartfai et al., 2007; Herman, 2002). Notably, infection and fever, which are concomitant with increased levels of pro-inflammatory cytokines not only in the periphery but also in the brain, can be precipitating events of seizures; moreover, a causal link between CNS infection and epilepsy has been proposed (Singh et al., 2008). It is therefore possible that microbial pathogens and non-infectious pro-epileptogenic brain insults induce *common molecules and common signaling pathways* in the brain, that may contribute via converging cellular and molecular mechanisms to the development of the epileptic process. BBB breakdown which often accompanies major systemic or CNS insults (Carvey et al., 2009; Oby and Janigro, 2006; Shlosberg et al., 2010) or active transport systems across the BBB (Banks et al., 1989) may also contribute to the entry of activators of the IL-1R/TLR signaling from the blood stream (Bianchi, 2007; Zhang et al., 2010b).

In the context of convergence of brain injury or infection and the epileptic process, an obvious candidate mechanism is represented by the *IL-1 receptor/Toll-like receptor* (IL-1R/TLR) signaling. This signaling is pivotal for activation of innate immunity and inflammation, and it may occur either following TLR mediated recognition of pathogens, or through binding of proinflammatory molecules such as IL-1β, or *danger signals*, such as HMGB1, released from activated or injured cells (Bianchi, 2007; Nguyen et al., 2002).

In this article, we will review the recent evidence on the role of the prototypical proinflammatory IL-1R/TLR signaling in seizures, following its activation by endogenous molecules, or by ligands mimicking bacterial or viral infections. We will discuss the cellular sources and the molecular targets of the activators of this signaling pathway, the molecular mechanisms by which IL-1R/TLR signaling may alter neuronal excitability, and the opportunities for therapeutic intervention emerging from the identification of the proconvulsant role of this inflammatory pathway.

2. The IL-1R/TLR signaling

IL-1R/TLR superfamily of single transmembrane domain receptors comprises 24 members (including five adaptor proteins) which share a cytosolic domain named the Toll/IL-1 receptor (TIR) domain (O'Neill and Bowie, 2007). Agonist binding to the extracellular domain of this family of receptors, and the subsequent recruitment of MyD88 and other cytosolic adaptor proteins, activates signaling via IRAK1/4 and TRAF6 to induce the expression of many genes involved in immunity and inflammation, under the control of transcription factors such as NF-κB, Activator Protein-1 (AP-1) and Interferon Regulatory Transcription Factors (O'Neill and Bowie, 2007). The IL-1R/TLR receptors are ubiquitously expressed by leucocytes (Janeway and Medzhitov, 2002), epithelial (Yoshimoto and Nakanishi, 2006) and endothelial cells (Gibson et al., 2006), and members of this family of receptors have been found also in CNS neurons, glia and astrocytes (see *Paragraph 3*).

The IL-1R family includes nine members containing extracellular Ig-like domains and intracellular TIR domains. The IL-1R type 1 (IL-1R1) and IL-1 accessory protein (AcP) form heterodimers that mediate the biological actions of the two agonists: the secreted highly inducible proinflammatory cytokine IL-1β, and the inducible, cell bound, surface expressed IL-1 α, as well as the endogenous, and inducible, IL-1 receptor antagonist (IL-1ra). IL-1β, IL-1ra and IL-1R1 are implicated in epilepsy (Vezzani et al., 2008, 2011).

The TLRs family includes 10 members which play a key role in activating the innate immune system during pathogen recognition. They are type 1 transmembrane glycoproteins recognizing a variety of microbial products to initiate a complex immune response aimed at eliminating the invading pathogens. TLRs recognize "pathogen associated molecular patterns" (*PAMPs*) which are conserved motifs of microbial origin, and each distinct TLR has a specific pattern-recognition site (Lee and Kim, 2007). However, even in the absence of pathogens, a number of endogenous molecules released by injured tissue (Bianchi, 2007; Tsan and Gao, 2004) can activate the innate immune system via stimulation of certain TLRs. These molecules are named "damage-associated molecular pattern" (*DAMPs*) or "danger signals" (*DS*); they are released by cells undergoing various "stressful" or "deadly" events such as necrosis, to alert the microenvironment to activate homeostatic programs. DAMPs include heat shock proteins, S100 proteins, components of the extracellular matrix such as hyaluronan fragments, and high mobility group box 1 (HMGB1). Excessive production of DAMPs might lead to acute and chronic diseases (Bianchi, 2007), although the molecular mechanisms mediating these pathological effects are still largely unexplored. Recently, HMGB1 has been shown to contribute significantly to seizure activity (Maroso et al., 2010).

To initiate cell signaling upon agonist binding, the TLRs heterodimerize or homodimerize depending on the receptor subtype. These dimers recognize the ligand and initiate a complex intracellular signaling cascade which initially involves the recruitment of MyD88 to a cytosolic TIR domain. Downstream events are similarly induced by IL-1R1 and TLR4 which involve activation of IL-1 receptor associated kinases (IRAK1 and 4) and the concomitant recruitment of a series of cytosolic adaptor proteins (O'Neill and Bowie, 2007) resulting in the activation of NF-κB. The IL-1R/TLR signaling also activates MAPKs (mitogen activated protein kinases) such as p38 and JNK (c-Jun terminal kinase) resulting in AP-1 mediated transcriptional events. TLR3 and TLR4 can also signal using a MyD88-independent pathway which involves TRIF (TIR-domain-containing adapter-inducing interferon-β). The TRIF-dependent signaling cascade results in the activation of IFR-3 (Interferon regulatory factor 3), which then induces interferons α and β. TRIF-mediated pathway can also lead to NF-κB activation. Finally, induction of phosphatidylinositol 3-kinase (PI3 K) can occur in response to IL-1R1 or TLR stimulation, presumably using a MyD88-independent pathway (Davis et al., 2006a; Diem et al., 2003).

Overstimulation of IL-1R1 and/or TLR4 can cause severe pathology such as sepsis; these receptors have been involved also in autoimmune diseases and in acute and chronic neurological disorders (Dinarello, 2011; Leon et al., 2008; Okun et al., 2009; Tang et al., 2007) including epilepsy (Maroso et al., 2010; Riazi et al., 2010; Rodgers et al., 2009; Vezzani et al., 2011). Both constitutive and inducible negative regulators for TLRs and IL-1Rs have been identified that control tightly the activation of this signaling pathway by acting at different strategic points in receptor recognition