

## BRAIN INFLAMMATION AND SEIZURES

**Expression of Cytokines and Cytokine Receptors in the Rat Brain after Kainic Acid-Induced Seizures**

Lehtimäki KA, Peltola J, Koskikallio E, Keränen T, Honkaniemi J

Brain Res Mol Brain Res 2003;110:253–260

We have previously shown that interleukin-6 (IL-6) protein levels are increased in cerebrospinal fluid in humans after recent tonic-clonic seizures with unchanged levels of IL-1 $\beta$  and tumor necrosis factor- $\alpha$  (TNF $\alpha$ ). Here we studied the expression of cytokines IL-6, LIF, IL-1 $\beta$ , and TNF $\alpha$  and cytokine receptors IL-6R, LIFR, and Gp130 in the rat brain after kainic acid-induced status epilepticus by using Northern blot analysis and in situ hybridization histochemistry. After seizures, IL-6 mRNA was induced in the hippocampus, cortex, amygdala, and meninges, and IL-6R was upregulated in the hippocampus. LIF was upregulated in the hippocampus, cortex and meninges after seizures, and LIFR mRNA was induced in the hippocampus and cortex. Gp130 was constitutively expressed in the brain. After seizures, Gp130 transcription was rapidly induced in the meninges. In thalamus, cortex, amygdala, and hippocampus, Gp130 mRNA was induced in a delayed fashion. IL-1 $\beta$  transcription was induced in the temporal lobe cortex and thalamus, and TNF $\alpha$  in the hippocampus. In general, the cytokines and their receptor mRNA levels were low in intact rat brain but were induced by seizures. Because IL-6 and LIF transcripts were induced in the meninges after seizures, the protein products of these transcripts may be more readily released in cerebrospinal fluid after seizures. In addition, the activity of IL-6 and LIF signaling pathways may be influenced by increased expression of their receptors after seizures.

**Formation of a Tumour Necrosis Factor Receptor 1 Molecular Scaffolding Complex and Activation of Apoptosis Signal-regulating Kinase 1 during Seizure-induced Neuronal Death**

Shinoda S, Skradski SL, Araki T, Schindler CK, Meller R, Lan JQ, Taki W, Simon RP, Henshall DC

Eur J Neurosci 2003;17:2065–2076

The consequences of activation of tumour necrosis factor receptor 1 (TNFR1) during neuronal injury remain controversial. The apoptosis signal-regulating kinase 1 (ASK1), a mitogen-activated protein kinase, can mediate cell death downstream of TNFR1. We examined the formation of the TNFR1 signaling cascade and response of ASK1 during seizure-induced neuronal death. Brief (40 min) seizures were induced in rats by intraamygdala microinjection of kainic acid, which elicited unilateral hippocampal CA3 neuronal death. Seizures caused a rapid decline in the expression of the silencer of death-domains protein within injured CA3. Coimmunoprecipitation analysis revealed a commensurate assembly of a TNFR1 scaffold complex containing TNFR-associated death-domain protein, receptor-interacting protein, and TNFR-activating factor 2. In addition, recruitment of TNFR-activating factor 2 was likely promoted by Bcl10-mediated sequestering of cellular inhibitor of apoptosis protein 2. Apoptosis signal-regulating kinase 1 was sequestered in a complex that contained the molecular chaperone 14-3-3 and protein phosphatase 5. Seizures triggered its dissociation and the phosphorylation of the ASK1 substrates, mitogen-activated protein kinase 3/6 and 4. Subsequently, protein phosphatase 5 translocated into the nuclei of degenerating CA3 neurons, while ASK1 colocalized with the adaptor proteins Daxx and TNFR-activating factor 2 at the outer membrane of injured CA3 neurons. Neutralizing antibodies to TNF reduced the numbers of DNA-damaged cells within the injured hippocampus. These data suggest that ASK1 may be involved in the mechanism of seizure-induced neuronal death downstream of a TNFR1 death-signaling complex.

**Profound Increase in Sensitivity to Glutamatergic But Not Cholinergic Agonist-induced Seizures in Transgenic Mice with Astrocyte Production of IL-6**

Samland H, Huitron-Resendiz S, Masliah E, Criado J, Henriksen SJ, Campbell IL

J Neurosci Res 2003;73:176–187

Transgenic mice with glial fibrillary acidic protein (GFAP) promoter driven-astrocyte production of the cytokines

interleukin-6 (IL-6) and tumor necrosis factor (TNF) were used to determine whether the preexisting production of these cytokines in vivo might modulate the sensitivity of neurons to excitotoxic agents. Low doses of kainic acid (5 mg/kg) that produced little or no behavioral or electroencephalogram (EEG) alterations in wild-type or GFAP-TNF animals induced severe tonic-clonic seizures and death in GFAP-IL-6 transgenic mice of age 2 or 6 months. GFAP-IL-6 mice were also significantly more sensitive to *N*-methyl-D-aspartate (NMDA)-induced but not pilocarpine-induced seizures. Kainic acid uptake in the brain of the GFAP-IL-6 mice was higher in the cerebellum but not in other regions. Kainic acid binding in the brain of GFAP-IL-6 mice had a distribution and density similar to that of wild-type controls. In the hippocampus of GFAP-IL-6 mice that survived low-dose kainic acid, no change in the extent of either neurodegeneration or astrogliosis was observed. Immunostaining revealed degenerative changes in  $\gamma$ -aminobutyric acid (GABA)- and parvalbumin-positive neurons in the hippocampus of 2-month-old GFAP-IL-6 mice, which progressed to the loss of these cells at age 6 months. Thus GFAP-IL-6 but not GFAP-TNF mice showed markedly enhanced sensitivity to glutamatergic-induced but not cholinergic-induced seizures and lethality. This may relate, in part, to a compromise of inhibitory interneuron function. Therefore preexisting IL-6 production and inflammation in the central nervous system (CNS) not only causes spontaneous neurodegeneration but also synergizes with other neurotoxic insults to induce more severe acute functional neurologic impairment.

#### Bidirectional Concentration-dependent Effects of Tumor Necrosis Factor- $\alpha$ in *Shigella dysenteriae*-related Seizures

Yuhas Y, Weizman A, Ashkenazi S

Infect Immun 2003;71:2288–2291

We previously demonstrated that pretreatment of mice with *Shigella dysenteriae* sonicate enhanced their susceptibility to pentylentetrazole-induced seizures and that tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was proconvulsive in this respect. The present study shows that TNF- $\alpha$ , at high concentrations, also may exert a suppressive effect on *Shigella*-mediated seizures. This implies that high levels of TNF- $\alpha$  may play a protective role in neurologic complications of *S. dysenteriae* infection.

## COMMENTARY

The concept that the brain is an immunologically privileged organ has been challenged by recent evidence that inflammatory-like processes and immune reactions occur in the CNS after a large variety of peripheral or local stimuli. Cytokines are expressed at very low levels in healthy brain tissue under physiologic conditions; however, they can be rapidly induced there after a variety of injuries. In particular, production of proinflammatory cytokines is an early cellular event subsequent to ischemic, traumatic, and excitotoxic insults (1). Recently, proinflammatory cytokines and related molecules, as well as activation of inflammatory pathways, have been described in epileptic tissue—both in experimental models and in humans (2–5).

Proinflammatory cytokines, such as interleukin (IL)-1 $\beta$ , IL-6, and tumor necrosis factor (TNF)- $\alpha$ , produce both detrimental and beneficial effects on brain function. The effects are dependent on a number of factors, including local concentration of the cytokine at the site of synthesis, the type of target cells, the length of time the tissue is exposed to cytokines, and which receptor subtypes are involved (1). Microglia and astrocytes are the first cells to produce cytokines during epileptic activity, and they represent the main source of local cytokine production in brain. An additional source of production consists of blood monocytes, pervading the brain several hours after acute epileptic events.

Proinflammatory cytokines affect neuronal excitability both directly, by acting on ionic currents (6,7), or indirectly, by activating gene transcription of neuroactive substances in neurons and glia (8). Recent evidence shows that proinflammatory cytokines functionally interact with glutamatergic neurotransmission, and this action may have a functional impact on neuronal excitability during seizures. Thus exogenously administered IL-1 $\beta$  has proconvulsant effects in experimental models of seizures, whereas its endogenous inactivation affords significant protection from seizures (5). The effects of IL-1 $\beta$  depend, at least in part, on enhancement of *N*-methyl-D-aspartate (NMDA)-receptor function, which is due to cytokine-mediated tyrosine phosphorylation of the NR2A/B subunits (9). Additional evidence of interactions between cytokines and glutamate is that TNF- $\alpha$  released from activated microglia can control glutamate release from astrocytes by prostaglandin formation (6). Moreover, TNF- $\alpha$  enhances synaptic efficacy by increasing surface neuronal expression of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors (7). All these events are rapid in their onset (i.e., seconds to minutes); therefore they differ from the classic actions of cytokines, which require activation of gene transcription.

Shinoda et al. and Lehtimäki et al. reported plastic changes in cytokine receptors during seizures. This finding is relevant

to the biologic activity of cytokines, which is influenced by changes in the level of cytokine-receptor expression. Lehtimäki and colleagues showed that IL-6 receptor (IL-6R) and its signal-transducer protein, Gp130, are increased in the hippocampus, meninges, thalamus, cortex, and amygdala after kainate-induced seizures in rats. The authors previously reported increased levels of IL-6 in cerebrospinal fluid (CSF) and plasma of patients with recent tonic-clonic seizures (10). The relevance of the IL-6 system in seizure modulation is strongly supported by Samland et al., who report that transgenic mice overexpressing IL-6 in astrocytes are more susceptible to kainic acid- and NMDA-induced seizures. Interestingly,  $\gamma$ -aminobutyric acid (GABA)- and parvalbumin-positive neurons in the hippocampus undergo degenerative changes in the IL-6-overexpressing mice, indicating a compromised inhibitory function when the cytokine is overexpressed in the long term in brain.

The rapid release of proinflammatory molecules during seizures also may result in long-term effects, such as reactive gliosis, alterations in blood-brain barrier permeability, and neuronal injury. The mechanism by which seizures trigger neuronal death remains incompletely understood but may involve a regulated apoptotic cell-death program. The study of Shinoda et al. shows that seizures induced in rats by kainic acid rapidly promote the formation of the assembly of complex containing TNFR1. Death-domain protein and activating factors associated with the formation of this complex may result in activation of apoptosis signal. Moreover, neutralizing antibodies to TNF- $\alpha$  reduced the number of DNA-damaged cells within the injured hippocampus. This suggests that *in vivo* activation of TNFR1 by the released cytokine may contribute to seizure-induced neuronal death, supporting previous evidence for involvement of "death receptors" in pathologic conditions (11).

TNF- $\alpha$  also can play a *neuroprotective* role, which appears to be mediated by TNFR type 2 and associated with attenuation of injury-dependent elevation of intracellular  $Ca^{2+}$ . Other potential mechanisms of TNF-mediated neuroprotection include stimulation of antioxidant pathways and enhanced expression of manganese superoxide dismutase or calbindin. In this respect, Yuhás et al. reported concentration-dependent inhibitory effects of TNF- $\alpha$  on *Shigella dysenteriae*-related seizures, an experimental condition mimicking neurologic disturbances of acute gastroenteritis caused by the bacteria. Thus the findings suggest that TNF- $\alpha$  may exert inhibitory actions in brain when expressed at relatively high levels.

Antagonism of proinflammatory molecules, as well as their receptors and signaling pathways, may represent new targets for the development of neuroprotective approaches. It is difficult

to say exactly how neuroprotection would be produced, because too little is known about the action of proinflammatory molecules in the brain. However, one mechanism simply would be a reduction in seizures, leading to a decrease in seizure-related cell death. The low, barely detectable expression of these proinflammatory molecules in healthy tissue and their limited actions in normal brain physiology may allow these systems to be pharmacologically targeted with minor impact on ordinary brain function, resulting in limited side effects.

by Annamaria Vezzani, Ph.D.

## References

1. Allan SM, Rothwell NJ. Cytokines and acute neurodegeneration. *Nat Rev Neurosci* 2001;2:734–744.
2. Sheng JG, Boop FA, Mrak RE, Griffin WST. Increased neuronal  $\beta$ -amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 $\alpha$  immunoreactivity. *J Neurochem* 1994;63:1872–1879.
3. Crespel A, Coubes P, Rousset MC, Brana C, Rougier A, Rondouin G, Bockaert J, Baldy-Moulinier M, Lerner-Natoli M. Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. *Brain Res* 2002;952:159–169.
4. Minami M, Kuraishi Y, Satoh M. Effects of kainic acid on messenger RNA levels of IL-1 $\beta$ , IL-6, TNF $\alpha$  and LIF in the rat brain. *Biochem Biophys Res Commun* 1991;176:593–598.
5. Vezzani A, Moneta D, Richichi C, Aliprandi M, Burrows SJ, Ravizza T, Perego C, De Simoni MG. Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. *Epilepsia* 2002;43(suppl 5):30–35.
6. 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 $\alpha$ : amplification by microglia triggers neurotoxicity. *Nat Neurosci* 2001;4:702–710.
7. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. Control of synaptic strength by glial TNF $\alpha$ . *Science* 2002;295:2282–2285.
8. Rothwell NJ, Hopkins SJ. Cytokines and the nervous system, II: actions and mechanisms of action. *Trends Neurosci* 1995;18:130–136.
9. Viviani B, Vezzani A, Bartsaghi S, Binaglia M, Aliprandi M, Costa L, Galli CL, Marinovich M. Interleukin-1 beta enhances *N*-methyl-D-aspartate receptor-mediated [Ca]<sup>2+</sup> increase in primary rat hippocampal neurons and potentiates NMDA neurotoxicity. *J Neurosci* 2003;23:8692–8700.
10. Peltola J, Palmio J, Korhonen L, Suhonen J, Miettinen A, Hurme M, Lindholm D, Keranen T. Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent tonic-clonic seizures. *Epilepsy Res* 2000;41:205–211.
11. Qiu J, Whalen MJ, Lowenstein P, Fiskum G, Faby B, Darwish R, Aarabi B, Yuan J, Moskowitz MA. Upregulation of the Fas receptor death-inducing signaling complex after traumatic brain injury in mice and humans. *J Neurosci* 2002;22:3504–3511.