Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration

K.A. Lehtimäki*, T. Keränen, H. Huhtala, M. Hurme, J. Ollikainen,
J. Honkaniemi, J. Palmio, J. Peltola

Department of Neurology and Rehabilitation, University of Tampere and Tampere University Hospital, 33521 Tampere, Finland

Abstract

Experimental studies suggest that cytokine production may be triggered by seizure activity. Here we determined the levels of interleukin-6 (IL-6) and its soluble receptor components (sIL-6R and sGp130) in CSF and serum from control subjects and patients after different types of seizures. IL-6 levels were increased after seizures, whereas sIL-6R levels were decreased. Interestingly, the levels of IL-6 were strongly increased after recurrent generalized tonic-clonic seizures (GTCS), whereas after single tonic-clonic or prolonged partial seizures IL-6 levels were increased to lesser extent. These results provide further support for a hypothesis of cytokine production induced by seizure activity per se.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Cytokine; GTCS; Partial seizure; CSF; Epilepsy

1. Introduction

In the central nervous system (CNS), cytokines are produced as a response to various inflammatory stimuli. Recently, experimental studies have revealed that cytokine production may be induced also by seizure activity (Jankowsky et al., 2000; Vezzani et al., 1999). Interestingly, modulation of cytokine network has been shown to affect duration and spread of seizure activity (D'Arcangelo et al., 2000; De Simoni et al., 2000; Vezzani et al., 1999), as well as seizure susceptibility (Vezzani et al., 2000). In addition, seizure-induced production of cytokines may contribute to formation of structural changes after sustained seizure activity, such as neuronal damage and gliosis (Panegyres and Hughes, 1998; Penkowa et al., 2001).

Interleukin-6 (IL-6) is a cytokine with multiple effects on various cell types and tissues throughout the body. In the CNS, IL-6 has been revealed to have both neuroprotective (Penkowa et al., 2001, 2003) and neurotoxic effects (Campbell et al., 1993). IL-6 receptor complex has two components, interleukin-6 receptor (IL-6R) and glycoprotein 130

(Gp130), both of which are also released in soluble forms. Soluble IL-6R (sIL-6R) has mainly agonistic effects on IL-6 signalling (Peters et al., 1996), whereas soluble Gp130 (sGp130) acts as an antagonist (Muller-Newen et al., 1998; Jostock et al., 2001). Due to the modulating role of these soluble receptors, the levels of these receptor components should be evaluated in order to assess IL-6 activity.

In our previous studies (Peltola et al., 1998, 2000a), increased levels of IL-6 were measured in CSF and plasma of patients with recent generalized tonic-clonic seizures (GTCS). Only patients with single GTCS were included in these studies. However, whether this observed increase in IL-6 levels is related directly to the seizure activity or some other seizure-related phenomenon is not fully understood. In the present study, we examined patients with different seizure patterns in order to evaluate the effect of seizure activity per se on IL-6 system.

2. Patients and methods

Thirty-three consecutive patients coming to the emergency department of Tampere University Hospital with acute seizures were included in the study. Patients with any signs of inflammatory or infectious disease were excluded from the study. Three groups of patients were

---

* Corresponding author. Tel.: +358-50-4123594.
E-mail address: kai.lehtimaki@uta.fi (K.A. Lehtimäki).

Table 1
Etiology of seizures in different patient groups

	sGTCS	rGTCSs	PPSs
Spontaneous seizure			
symptomatic epilepsy			
vascular		1 (10%)	4 (57.1%)
trauma		1 (10%)	
tumor	2 (12.5%)		1 (14.3%)
cryptogenic epilepsy	11 (68.8%)	1 (10%)	2 (28.6%)
Provoked seizures			
alcohol withdrawal	3 (18.8%)	4 (40.0%)	
acute trauma		1 (10%)	
electrolyte disturbances		2 (20.0%)	
(hyponatremia)			
Total	16	10	7

examined in this study. The first patient group consisted of 16 patients with single GTCS. The second group included 10 patients with recurrent GTCS, who had experienced two or more recurrent GTCS before admission to the hospital. The third patient group consisted of seven patients with prolonged partial seizures (PPS). In this group, five patients had prolonged simple partial and two patients had prolonged complex partial seizures. The duration of seizures in this group varied from 10 min up to 2 h. Serum samples were obtained from all patients, but CSF sample was unavailable from three patients with recurrent GTCS and from five patients with PPS in whom there was no clinical indication for a CSF sample. The control samples were obtained from 17 patients who were studied to exclude neurological disease, and who yielded normal neurological examination and laboratory findings. The etiology of seizures in different patient groups is listed in Table 1. All subjects were fully informed of the risks and potential benefits of the CSF examination and they gave their written informed consent to participate. The study protocol was approved by the Ethics Committee of Tampere University Hospital.

All samples were obtained within 24 h after the seizure. Commercially available enzyme linked immunosorbent assay (ELISA) kits were used for IL-6 (Pelikine Compact, CLB, Amsterdam, The Netherlands), sIL-6R and sGp130 (R&D Systems, Abingdon, UK) measurements. The sensitivities for assays for IL-6, sIL-6R and sGp130 were 0.6 pg/ml, 6.5 pg/ml and 0.08 ng/ml, respectively. Variation of levels was minimal between different plates as well as within a plate (for details, see manufacturer's information). Samples of patient groups and controls were equally distributed under several plates to minimize potential differences between plates. The assays were performed according to manufacturers' instructions.

Statistical significance of differences between groups was assessed using Mann–Whitney test. A p value of 0.05 or less was considered statistically significant. All analyses were performed using Windows SPSS 9.0 software.

3. Results

3.1. Levels of IL-6 in CSF and serum

When compared to the control subjects, the levels of IL-6 in CSF were increased both in patients with single GTCS and in patients with recurrent GTCS ($p<0.001$) (Fig. 1). Abnormal CSF levels (± 2 SD of control levels) were detected in 11/16 patients with single GTCS and in 6/7 patients with recurrent GTCS. Furthermore, the CSF levels of IL-6 were significantly higher in patients with recurrent GTCS when compared to those with single GTCS ($p=0.05$). The serum levels of IL-6 were increased in all seizure groups when compared to control subjects ($p<0.001$).
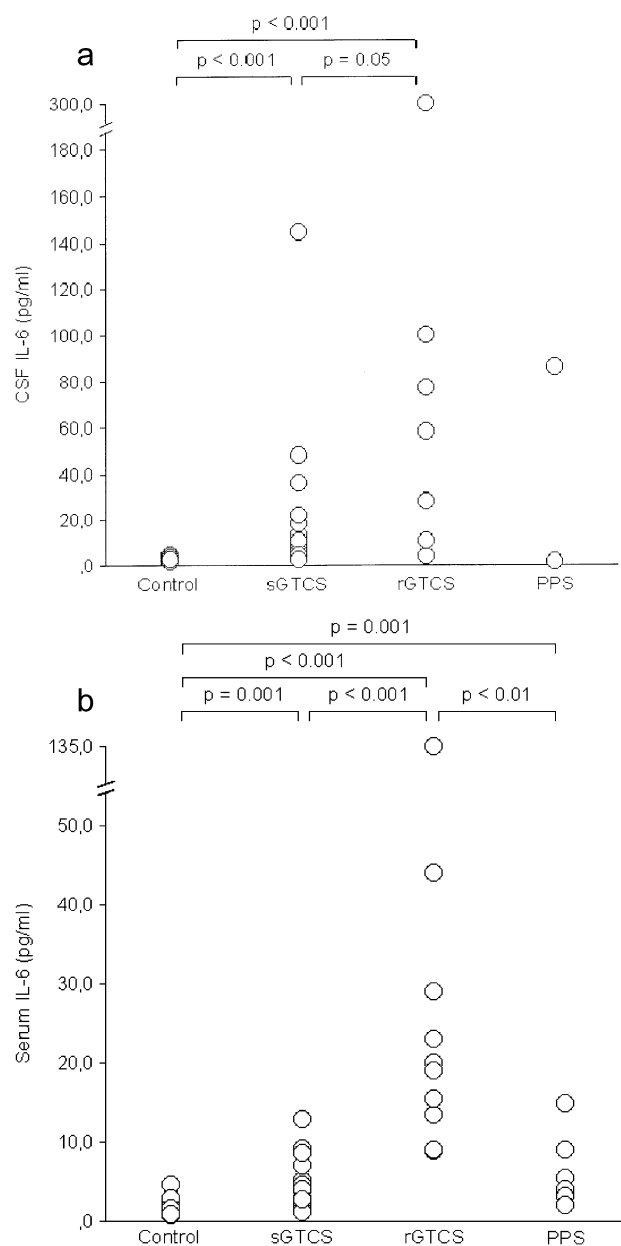

Fig. 1. (a) The levels of IL-6 in CSF. (b) The levels of IL-6 in serum.

Abnormal serum levels were found in 12/16 patients with single GTCS, in 10/10 patients with recurrent GTCS, and in 6/7 patients with PPS. Within the seizure groups, the serum levels of IL-6 were significantly higher ($p<0.001$) in patients with recurrent GTCS when compared to those with single GTCS and PPS.

3.2. Levels of sIL-6R in CSF and serum

Patients with single GTCS showed a statistically significant decrease in the CSF levels of sIL-6R when compared with control subjects ($p=0.05$) (Fig. 2). No significant differences were found from other patient groups when compared to controls, probably due to the limited number of patients in these groups. However, when all patients were pooled in a single group, the CSF levels of sIL-6R were significantly decreased ($p<0.05$). Abnormal CSF levels (±2 SD of control levels) were evident in 12/16 patients with
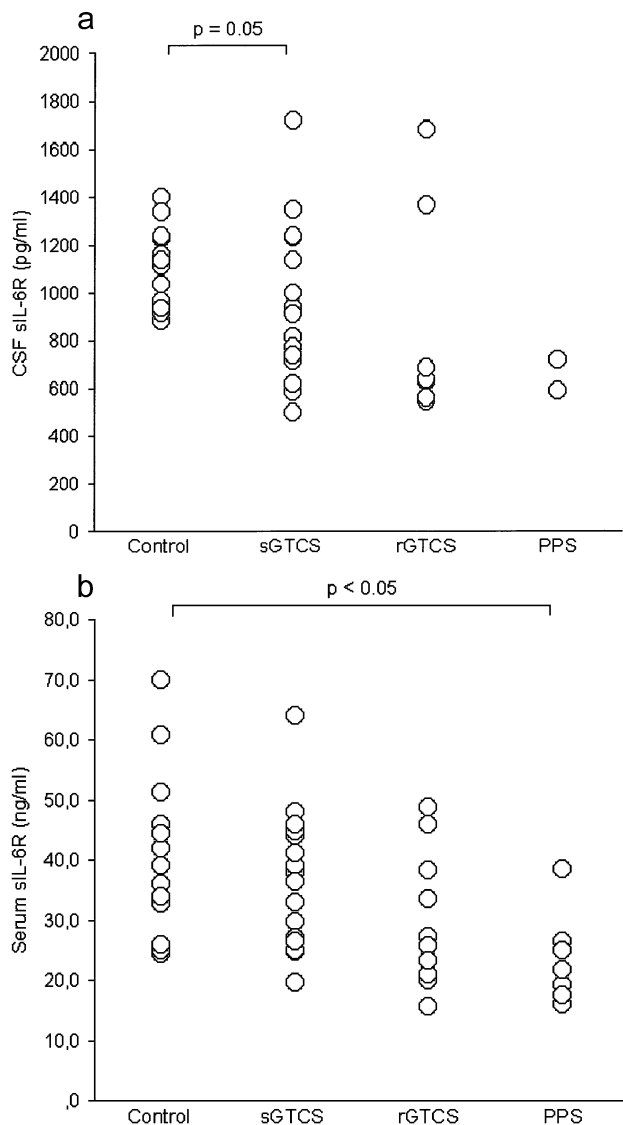
Fig. 2. (a) The levels of sIL-6R in CSF. (b) The levels of sIL-6R in serum.
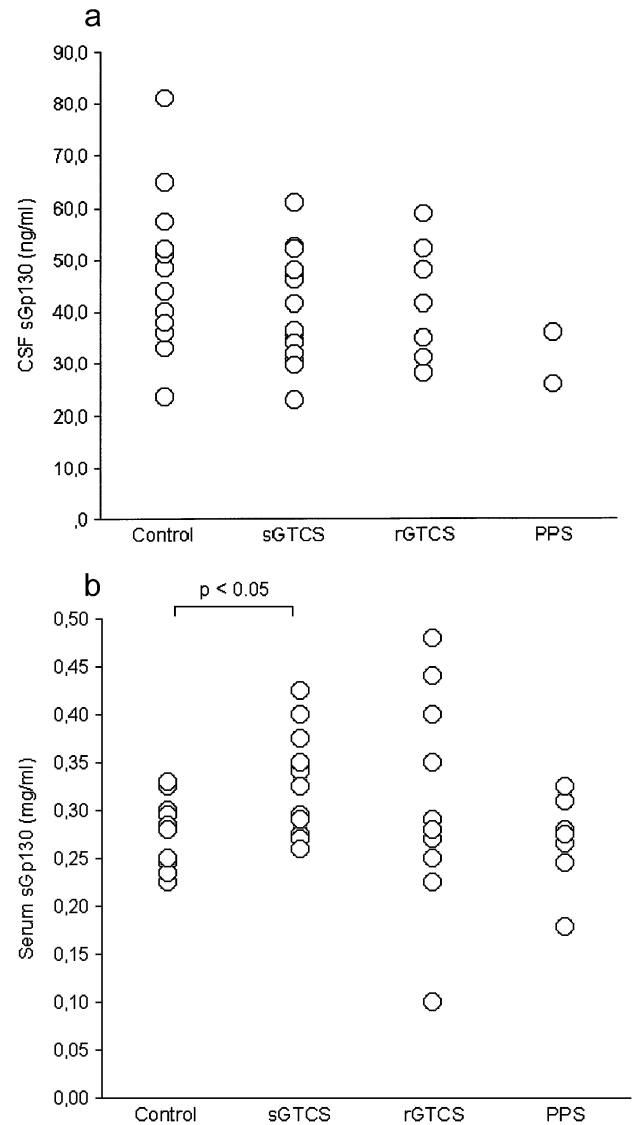
Fig. 3. (a) The levels of sGp130 in CSF. (b) The levels of sGp130 in serum.

single GTCS, in 7/7 patients with recurrent GTCS. In serum, sIL-6R levels were significantly lower in patients with PPS when compared to control subjects ($p<0.01$), whereas other patient groups showed no significant differences. When all patients were analysed as a single group, a trend to decreased levels of serum sIL-6R was found ($p=0.092$). Changed levels were found in 2/16 patients with single GTCS, in 4/10 patients with recurrent GTCS and in 4/7 patients with PPS.

3.3. Levels of sGp130 in CSF and serum

The levels of sGp130 showed no significant differences between the seizure groups and control subjects either in CSF or in serum, with the exception of an increase in serum levels of sGp130 in patients with single GTCS when compared to controls ($p<0.05$) (Fig. 3). CSF values differing from control levels were detected in 4/16 patients with

single GTCS, and in 2/7 patients with recurrent GTCS. In serum, levels were abnormal in 8/16 patients with single GTCS, in 6/10 patients with recurrent GTCS and in 3/7 patients with PPS.

3.4. The effect of time from seizure onset to sampling to IL-6 levels

The mean time from seizure onset to the sample collection was approximately 16 h in single GTCS group, 11 h in recurrent GTCS group and 10 h in PPS group. The effect of time in IL-6 concentration was studied further in the single GTCS group, since this was the largest and most homogenous group of patients. However, no correlations were found between the delay from the seizure to sample collection (data not shown).

4. Discussion

In patients with recent GTCS, we have previously found a rather selective increase in the levels of IL-6 and interleukin-1 receptor antagonist (IL-1ra) with unchanged interleukin-1β (IL-1β) and tumor necrosis factor-α (TNFα) levels (Peltola et al., 1998, 2000a). The present study confirms and further extends these findings. The levels of IL-6 both in CSF and in serum were increased in all seizure groups (except in two CSF samples in PPS group). Furthermore, the magnitude of the IL-6 activation was most pronounced in patients with recurrent GTCS, i.e. in the case of most severe cerebral epileptic activity. These human data seem to be consistent with experimental studies suggesting that the secretion of IL-6 may be induced by seizure activity per se (Jankowsky et al., 2000, Sallmann et al., 2000). A decrease in sIL-6R levels was found in the CSF when all patients were pooled in a single group, however, this decrease was most clear in patients with single GTCS. Serum levels of sIL-6R were decreased only in patients with PPS. The levels of sGp130 were increased only in serum of patients with single GTCS. These findings indicate that the levels of these soluble receptors are also affected by seizures, although no correlation to the seizure severity was found.

Experimental studies have shown that seizure activity increases expression of several cytokines in the brain parenchyma (Vezzani et al., 1999; Jankowsky et al., 2000). However, only IL-6 release into CSF seems to be clearly increased after seizures (Peltola et al., 1998, 2000a). We have found previously that after kainic acid induced status epilepticus (SE) IL-6 is up-regulated in the meninges in addition to the expression in the brain parenchyma (Lehtimaki et al., 2003). This finding indicates that meninges may also serve as a potential site of IL-6 origin in CSF. After pilocarpine-induced SE, IL-6 positive cells have been detected both in the borders and within the brain vessels, representing most likely endothelial cells and ad-

herent white blood cells (Jankowsky et al., 2000). Interleukin-6 production is also increased in the brain endothelial cells by various noxious stimuli (Reyes et al., 1999). These results suggest that seizure induced release of IL-6 into the serum compartment occurs mainly from the brain vessels. In addition, IL-6 may be partly originated from CSF compartment via venous drainage.

In assessing our results on IL-6 system, causes of seizures as well as possible seizure-related neuronal damage must be considered. Most of our patients had either remote symptomatic or cryptogenic localisation-related epilepsy, i.e. seizures of unidentifiable but probably remote symptomatic cause (Table 1). To our knowledge, there is no data suggesting that these remote symptomatic etiologies per se might be associated with permanent or long-term activation of IL-6 system. However, in the case of acute symptomatic seizures, contribution of the etiology (alcohol withdrawal, electrolyte disturbances, trauma or neoplasia) cannot be ruled out. In addition, in 7 of the 10 patients with acute symptomatic seizures the cause was alcohol withdrawal. Chronic alcoholic patients have been reported to show elevated serum levels of IL-6 due to production from monocytes (Song et al., 1999). However, whether CSF levels are affected by alcohol abuse, is currently unknown.

The potential significance of IL-6 system in epilepsy is unknown. At the systemic level, the levels of IL-6 have been shown to be associated with the parameters of systemic inflammatory response (Peltola et al., 2002). The activation of IL-6 system via stimulation of B-cells could also partly explain the increased prevalence of autoantibodies observed in patients with therapy resistant epilepsy (Peltola et al., 2000b,c). Experimental studies suggest that IL-6 may act as a protective factor against seizure induced neuronal damage (Penkowa et al., 2001). IL-6 has been also reported to inhibit spread of excitation in the rat cerebral cortex (D'Arcangelo et al., 2000). On the other hand, when chronically overproduced, IL-6 has been reported to result in seizures and accompanying histological changes such as neuronal loss and gliosis (Campbell et al., 1993). These changes, commonly referred as temporal or mesial sclerosis, are frequently seen in temporal lobe structures of patients with chronic refractory epilepsy. Therefore, it can be hypothesised that IL-6 response after acute seizures may have beneficial effects. However, when chronically produced due to recurrent seizures or some other pathological condition, IL-6 may contribute to formation of structural changes in the neuronal tissue eventually leading to refractory seizures.

In conclusion, our results demonstrate that seizures in humans cause changes in IL-6 levels in CSF as well as in peripheral blood. The magnitude of these changes is related to the severity of seizures. Seizures may also cause changes in the levels of soluble IL-6 receptor components, resulting in complex modulation of IL-6 activity in CSF and serum compartments. More generally, activation of cytokine network by seizures may serve as a link between excessive

neuronal activity and various immunological changes in patients suffering from seizure disorders.

References

Campbell, I.L., Abraham, C.R., Masliah, E., Kemper, P., Inglis, J.D., Oldstone, M.B., Mucke, L., 1993. Neurologic disease induced in transgenic mice by cerebral overexpression of interleukin 6. Proc. Natl. Acad. Sci. U. S. A. 90, 10061–10065.

D'Arcangelo, G., Tancredi, V., Onofri, F., D'Antuono, M., Giovedi, S., Benfenati, F., 2000. Interleukin-6 inhibits neurotransmitter release and the spread of excitation in the rat cerebral cortex. Eur. J. Neurosci. 12, 1241–1252.

De Simoni, M.G., Perego, C., Ravizza, T., Moneta, D., Conti, M., Marchesi, F., De Luigi, A., Garattini, S., Vezzani, A., 2000. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. Eur. J. Neurosci. 12, 2623–2633.

Jankowsky, J.L., Derrick, B.E., Patterson, P.H., 2000. Cytokine responses to LTP induction in the rat hippocampus: a comparison of in vitro and in vivo techniques. Learn. Mem. 7, 400–412.

Jostock, T., Mullberg, J., Ozbek, S., Atreya, R., Blinn, G., Voltz, N., Fischer, M., Neurath, M.F., Rose-John, S., 2001. Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. Eur. J. Biochem. 268, 160–167.

Lehtimaki, K.A., Peltola, J., Koskikallio, E., Keranen, T., Honkaniemi, J., 2003. Expression of cytokines and cytokine receptors in the rat brain after kainic acid-induced seizures. Brain. Res., Mol. Brain Res. 110, 253–260.

Muller-Newen, G., Kuster, A., Hemmann, U., Keul, R., Horsten, U., Martens, A., Graeve, L., Wijdenes, J., Heinrich, P.C., 1998. Soluble IL-6 receptor potentiates the antagonistic activity of soluble gp130 on IL-6 responses. J. Immunol. 161, 6347–6355.

Panegyres, P.K., Hughes, J., 1998. The neuroprotective effects of the recombinant interleukin-1 receptor antagonist rhIL-1ra after excitotoxic stimulation with kainic acid and its relationship to the amyloid precursor protein gene. J. Neurol. Sci. 154, 123–132.

Peltola, J., Hurme, M., Miettinen, A., Keranen, T., 1998. Elevated levels of interleukin-6 may occur in cerebrospinal fluid from patients with recent epileptic seizures. Epilepsy Res. 31, 129–133.

Peltola, J., Palmio, J., Korhonen, L., Suhonen, J., Miettinen, A., Hurme, M., Lindholm, D., Keranen, T., 2000a. Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent GTCSs. Epilepsy Res. 41, 205–211.

Peltola, J.T., Haapala, A., Isojarvi, J.I., Auvinen, A., Palmio, J., Latvala, K., Kulmala, P., Laine, S., Vaarala, O., Keranen, T., 2000b. Antiphospholipid and antinuclear antibodies in patients with epilepsy or new-onset seizure disorders. Am. J. Med. 109, 712–717.

Peltola, J., Kulmala, P., Isojarvi, J., Saiz, A., Latvala, K., Palmio, J., Savola, K., Knip, M., Keranen, T., Graus, F., 2000c. Autoantibodies to glutamic acid decarboxylase in patients with therapy-resistant epilepsy. Neurology 55, 46–50.

Peltola, J., Laaksonen, J., Haapala, A.M., Hurme, M., Rainesalo, S., Keranen, T., 2002. Indicators of inflammation after recent tonic-clonic epileptic seizures correlate with plasma interleukin-6 levels. Seizure 11, 44–46.

Penkowa, M., Molinero, A., Carrasco, J., Hidalgo, J., 2001. Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures. Neuroscience 102, 805–818.

Penkowa, M., Giralt, M., Lago, N., Camats, J., Carrasco, J., Hernandez, J., Molinero, A., Campbell, I.L., Hidalgo, J., 2003. Astrocyte-targeted expression of IL-6 protects the CNS against a focal brain injury. Exp. Neurol. 181, 130–148.

Peters, M., Jacobs, S., Ehlers, M., Vollmer, P., Mullberg, J., Wolf, E., Brem, G., Meyer zum Buschenfelde, K.H., Rose-John, S., 1996. The function of the soluble interleukin 6 (IL-6) receptor in vivo: sensitization of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. J. Exp. Med. 183, 1399–1406.

Reyes, T.M., Fabry, Z., Coe, C.L., 1999. Brain endothelial cell production of a neuroprotective cytokine, interleukin-6, in response to noxious stimuli. Brain Res. 851, 215–220.

Sallmann, S., Juttler, E., Prinz, S., Petersen, N., Knopf, U., Weiser, T., Schwaninger, M., 2000. Induction of interleukin-6 by depolarization of neurons. J. Neurosci. 20, 8637–8642.

Song, C., Lin, A., DeJong, R., Vandoolaeghe, E., Kenis, G., Bosmans, E., Whelan, A., Scharpe, S., Maes, M., 1999. Cytokines in detoxified patients with chronic alcoholism without liver disease: Increased monocytic cytokine production. Biol. Psychiatry 45, 1212–1216.

Vezzani, A., Conti, M., De Luigi, A., Ravizza, T., Moneta, D., Marchesi, F., De Simoni, M.G., 1999. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures. J. Neurosci. 19, 5054–5065.

Vezzani, A., Moneta, D., Conti, M., Richichi, C., Ravizza, T., De Luigi, A., De Simoni, M.G., Sperk, G., Andell-Jonsson, S., Lundkvist, J., Iverfeldt, K., Bartfai, T., 2000. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc. Natl. Acad. Sci. U. S. A. 97, 11534–11539.