



JUKKA PELTOLA

Cytokines and Seizures



ACADEMIC DISSERTATION

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Supervised by
Docent Tapani Keränen
University of Tampere
Professor Mikko Hurme
University of Tampere

Reviewed by
Professor Matti Iivanainen
University of Helsinki
Docent Ilkka Seppälä
University of Helsinki

Distribution
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 3 3551 6055
Fax +358 3 3551 7685
taju@uta.fi
www.uta.fi/taju
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CONTENTS

LIST OF ORIGINAL PUBLICATIONS	5
ABBREVIATIONS	6
1.INTRODUCTION	7
2.REVIEW OF THE LITERATURE	
2.1 Epileptic seizures and epileptic syndromes	8
2.2 Epileptic seizures and brain plasticity	10
2.3 The development of refractory epilepsy	
<i>2.3.1 Prognosis in epilepsy</i>	11
<i>2.3.2 Refractory epilepsy</i>	12
2.4 Cytokines as modulators of brain plasticity	
<i>2.4.1 Cytokines as regulators of immune activation</i>	13
<i>2.4.2 The effect of cytokine gene polymorphism on cytokine production</i>	16
<i>2.4.3 Cytokines and acute brain degeneration</i>	17
2.5 Cytokines and seizures	
<i>2.5.1 Experimental epilepsy and cytokine production</i>	19
<i>2.5.2 Febrile seizures and cytokines</i>	22
<i>2.5.3 Cytokines in human epilepsy</i>	23
3.PURPOSE OF THE STUDY	25
4.MATERIALS AND METHODS	26
4.1 Control subjects	26
4.2 Patients	
<i>4.2.1 Patients with acute seizures</i>	26
<i>4.2.2 Patients in the in vitro study</i>	27
<i>4.2.3 Patients in the genetic study</i>	27
4.3 Methods	
<i>4.3.1 Processing of the samples</i>	29
<i>4.3.2 Cytokine ELISAs</i>	29
<i>4.3.3 Nerve growth factor</i>	29
<i>4.3.4 Indicators of inflammation</i>	30
<i>4.3.5 Cell separation</i>	31
<i>4.3.6 Culture conditions and mitogens</i>	31

4.3.7 Intracellular IL-1 β , IL-1Ra and IL-6 detection.....	31
4.3.8 Cytokine gene polymorphism.....	32
4.3.9 Statistical methods.....	32
5.RESULTS.....	34
5.1 Patients with acute seizures.....	34
5.2 Cytokine plasma levels in the in vitro patients.....	37
5.3 In vitro cytokine production.....	38
5.4 Intracellular cytokine detection.....	39
5.5 Cytokine gene polymorphisms in refractory epilepsy.....	40
6.DISCUSSION.....	43
6.1 Which cytokines are upregulated after single seizures?	43
6.2 CSF vs. plasma: the origin of the cytokines.....	45
6.3 Time course and kinetics of cytokine production.....	46
6.4 The genetic control of the cytokine response.....	46
6.5 The implications of cytokine expression in acute seizures: CNS.....	48
6.6 The implications of cytokine activation in acute seizures: peripheral tissue.....	49
SUMMARY AND CONCLUSIONS.....	51
YHTEENVETO.....	54
ACKNOWLEDGEMENTS.....	56
REFERENCES.....	58
ORIGINAL PUBLICATIONS.....	75

LIST OF ORIGINAL PUBLICATIONS

The original publications will be referred to in the text by Roman numerals I-V:

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

II Peltola J, Palmio J, Korhonen L, Suhonen J, Hurme M, Miettinen A, Lindholm D, Keränen T. Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent tonic-clonic seizures. *Epilepsy Res* 2000;41:205-211

III Peltola J, Laaksonen J, Haapala AM, Rainesalo S, Hurme M, Keränen T. Indicators of inflammation after recent tonic-clonic epileptic seizures correlate with plasma interleukin-6 levels. *Seizure* 2002;11:44-46

IV Peltola J, Hurme M, Rainesalo S, Keränen T. Polymorphisms in IL-1 Gene Complex in Localisation-related Epilepsy. *Ann Neurol* 2001; 50:275-276

V Hulkkonen J, Koskikallio E, Rainesalo S, Keränen T, Hurme M, Peltola J. The Balance of inhibitory and excitatory cytokines is differently regulated in vivo and in vitro among therapy resistant epilepsy patients. *Epil Res* 2004;59:199-205

ABBREVIATIONS

AED, antiepileptic drug
AST, antibodies to streptolysin
BBB, blood–brain barrier
BFA, Brefeldin A
CBC, complete blood count
CI, confidence interval
CNS, central nervous system
CNTF, ciliary neurotrophic factors
CRP, C-reactive protein
CSF, cerebrospinal fluid
CT, computed tomography
EEG, electroencephalogram
EIA, enzyme immune assay
ELISA, enzyme-linked immunosorbent assay
FS, febrile seizures
IL-1 α , interleukin-1 α
IL-1 β , interleukin-1 β
IL-1R, interleukin-1 receptor
IL-1RA, interleukin-1 receptor antagonist
IL-6, interleukin-6
IL-6 receptor
IL-10, interleukin-10
JAK, Janus kinase
OKT3, anti-CD3 antibody
OR, odds ratio
OSM oncostatin M
LIF leukemia inhibitory factor
LP, lumbar puncture
LPS, lipopolysaccharide
LTP, long-term potentiation
MFI, mean fluorescence intensity
MRI, magnetic resonance imaging
mRNA, messenger ribonucleic acid
MUG, 4-methylumbelliferyl-galactoside
NF- κ B, nuclear factor kappa B
NGF, nerve growth factor
NMDA, N-methyl-D-aspartate
PBMC, peripheral blood mononuclear cells
PDBu, phorbol dibutyrate
PFS, prolonged febrile seizures
SD, standard deviation
SE, status epilepticus
STAT, signal transducers and activators of transcription
TLE, Temporal lobe epilepsy
TLE+HS, TLE with hippocampal sclerosis
TLE-HS, TLE without hippocampal sclerosis
TNF, tumor necrosis factor
VNTR, variable numbers of tandem repeats

1. INTRODUCTION

Cytokines are a heterogeneous group of polypeptide mediators that first were identified as being associated with activation of the immune system and inflammatory responses, but they also exert diverse actions on the peripheral and central nervous system. There is growing amount of experimental and clinical evidence suggesting that cytokines are also involved in epilepsy as disease modifying molecules.

Experimental studies suggest that interleukin (IL)-1 β prolongs the duration of kainic acid induced seizures and seems to promote neuronal damage (Vezzani et al, 1999), whereas its effects are blocked by IL-1 receptor antagonist (Vezzani et al, 2000) (IL-1RA). Kainic acid causes cellular damage in hippocampus, and cytokine expression may reflect this neural injury. There is also clinical evidence of activation of the cytokine network in epilepsy: IL-1 α levels are increased in brain tissue from patients with temporal lobe epilepsy (Sheng et al., 1994) and cytokine gene polymorphisms have been linked to temporal lobe epilepsy (Kanemoto et al., 2000).

The significance of cytokine production in relation to epileptic seizures is not yet fully known. Cytokines are also important for neuronal survival, and IL-1 β and IL-6 have been shown to exert neuroprotective and neurotrophic effects, on the other hand IL-1 β and IL-6 may act as mediators of astrogliosis.

The data available suggest a role for cytokines in epileptic processes and it is important to define this role further in both experimental and human studies. The search for molecular mechanism underlying progressive brain function decline in patients with seizures will reveal opportunities to prevent both the structural damage and the progression of epileptic attacks in human beings (Pitkänen and Sutula 2002).

2.REVIEW OF THE LITERATURE

2.1 Epileptic seizures and epileptic syndromes

Epilepsy is a clinically heterogeneous group of disorders; defined as spontaneous occurrence of seizures associated with electric discharges of the brain. Its prevalence is 5-10/1000, annual incidence is dependent on the age. Etiology is diverse and prognosis variable; altogether 25-30% of patients have intractable epilepsy (Brodie and Kwan, 2002).

Epileptic seizures result from excessive discharge in a population of hyper excitable neurons. Most epileptic seizures are due to discharges generated in cortical and hippocampal structures, although subcortical structures are also involved in some seizures types. The clinical expression of a seizure depends on its site of origin, time course, and discharge propagation (Avanzini and Franceschetti 2003). The seizure types can be divided into two major categories; generalized and focal seizures (Table 1). Seizure types, EEG changes, associated structural changes and age-specific factors all contribute to epileptic syndromes, which are also divided into two major groups (Table 2).

The epileptic syndrome largely determines in an individual patient the choice of antiepileptic drugs, the possibility for surgical treatment, and probability of seizure freedom and duration of drug treatment. Prognosis in localization-related (focal) epilepsy is thought to be worse than in generalized epilepsies. The impact of etiology on seizure outcome has been evaluated in some studies. In a large hospital-based survey including over 2000 patients, remission was achieved in 82 % of patients with idiopathic generalized epilepsy , in 35 % of symptomatic and in 45 % of patients with cryptogenic partial epilepsy (Semah et al, 1998). Temporal lobe epilepsy (TLE) was

TABLE 1 The International League Against Epilepsy (ILAE) classification of seizure types

I PARTIAL (FOCAL) SEIZURES

A Simple partial seizures

- i With motor signs
- ii With somatosensory or special sensory symptoms
- iii With autonomic symptoms or signs
- iv With psychic symptoms

B Complex partial seizures

- i Simple partial onset followed by impairment of consciousness
- ii With impairment of consciousness at onset

C Partial seizures evolving to secondarily generalized seizures

- i Simple partial seizures evolving to generalized seizures
- ii Complex partial seizures evolving to generalized seizures
- iii Simple partial seizures evolving to complex partial seizures evolving to generalized seizures

II GENERALIZED SEIZURES (CONVULSIVE AND NONCONVULSIVE)

A Absence seizures

- i with impairment of consciousness only, mild clonic components, atonic components, tonic components , automatisms, autonomic components

B Myoclonic seizures

C Clonic seizures

D Tonic seizures

E Tonic-clonic seizures

F Atonic seizures

- (Combinations may occur, such as myoclonic and atonic seizures or myoclonic and tonic seizures)

III UNCLASSIFIED EPILEPTIC SEIZURES

TABLE 2 The ILAE Classification of Epilepsies and Epilepsy related syndromes

I GENERALIZED

A. Idiopathic generalized epilepsies with age-related onset

- i Benign neonatal familial convulsions
- ii Benign neonatal convulsions
- iii Benign myoclonic epilepsy in infancy
- iv Childhood absence epilepsy
- v Juvenile absence epilepsy
- vi Juvenile myoclonic epilepsy
- vii Epilepsy with generalized tonic-clonic seizures on awakening

B. Cryptogenic or symptomatic generalized epilepsies

- i West syndrome
- ii Lennox-Gastaut syndrome
- iii Epilepsy with myoclonic-astatic seizures
- iv Epilepsy with myoclonic absences

C. Symptomatic generalized epilepsies

- i Non-specific aetiology
- ii Early myoclonic encephalopathies
- iii Early infantile encephalopathy with burst suppression
- iv other symptomatic epilepsies not defined above

II LOCALIZATION-RELATED

A. Localization-related epilepsies-idiopathic with age-related onset

- i Benign epilepsy with centrotemporal spikes
- ii Childhood epilepsy with occipital paroxysm
- iv Primary reading epilepsy

B. Localization-related epilepsies-symptomatic

- i Epilepsia partialis continua
- ii Syndromes characterized by specific modes of precipitation
- iii Temporal lobe epilepsies

C. Localization-related epilepsies-cryptogenic

III EPILEPSIES AND SYNDROMES UNDERTERMINED AS TO WHETHER FOCAL OR GENERALIZED

IV SPECIFIC SYNDROMES

the most common form of refractory partial epilepsy with 80 % of patients with persistent seizures. TLE with hippocampal sclerosis carries especially poor prognosis (Semah et al.,1998, Stephen et al., 2001).

2.2 Epileptic seizures and brain plasticity

Epileptic seizures can have severe and lasting effects on the architecture of the brain. They can cause neuronal cell death, enhanced neurogenesis, axonal sprouting, dendritic changes and reactive gliosis. One of the consequences of prolonged seizures is the selective neuronal loss observed at the hippocampus. The process of molecular changes leading to altered seizure susceptibility is called epileptogenesis. The first event triggering this cascade is called the initiating event, after which this process of structural and functional changes in epileptogenesis may continue for an indefinite period before the occurrence of the first seizure. Subsequent seizures also modify this molecular process, the brain plastic responses to seizures may further define whether the seizures are easily controlled or the person develops therapy resistant epilepsy. Recent experimental and human studies have shown that in some patients the development of epilepsy does not stop at the time of the diagnosis but that recurring seizures may contribute to progression of the disorder (Pitkänen and Sutula 2002).

Genetic background effects are likely to modify the brain responses to single seizures. In a genomic analysis with micro arrays genes induced by single seizures were different between two different mice strains (C57BL/6J and 129/SvEvTac) (Del Rio and Barlow 2002). Elucidation of how gene expression is altered as a function of seizure type and duration will provide important information about molecular regulation of seizure-induced plasticity of the brain (Pitkänen and Sutula 2002).

Experimental studies have convincingly shown that in animals repeated seizures induce progressive cumulative changes in neural circuits (Pitkänen and Sutula 2002). Progressive effects of seizures may depend on the previous activity in neural circuitry (Pitkänen and Sutula 2002). Kindling is a process where repetitive stimuli cause cumulative increase in seizure susceptibility, interestingly previously kindled rats are protected against damaging effects of status epilepticus (Kelly and McIntyre 1994). Also the effects of repeated seizures on neuronal damage were less pronounced in experimental models where animals had an initial episode of status epilepticus than in normal animals that had undergone kindling (Pitkänen et al, 2002). These data indicate that seizure episodes may sometimes also cause protective plastic changes in the central nervous system, even though in most cases seizure-induced molecular, cellular and network reorganization is associated with functional impairment of the brain (Pitkänen and Sutula 2002).

2.3 The development of refractory epilepsy

2.3.1 Prognosis in epilepsy

Approximately 5 % of the general population has at least one seizure during the lifetime (excluding febrile seizures). Sixty percent of the patients who have their first ever epileptic seizure do not develop epilepsy (Hauser et al., 1990), whereas 40 % need antiepileptic medication. Data from a recent study of 525 patients started on antiepileptic drug (AED) therapy at a single center suggest that patients with newly diagnosed epilepsy comprise two distinct populations. Around 60% will be controlled with a single drug (monotherapy), usually with the first or second AED chosen whereas the remaining 30 to 40% will be difficult to control from the onset (Kwan and Brodie 2000). Patients with symptomatic/cryptogenic epilepsy were more likely

to need changed treatment because of side effects compared with those with idiopathic epilepsy. This suggests lowered tolerability for drugs in these patients (Kwan and Brodie 2001).

2.3.2 Refractory epilepsy

There is no universally accepted definition of refractory epilepsy. In one recent classification refractory epilepsy was defined as a condition in which at least two AEDs have failed to control seizures because of lack of efficacy (Arroy et al 2003). Epilepsy surgery should be considered after failure of two-well tolerated treatment regimens, whether as sequential monotherapies or with one monotherapy and succeeding combination (Brodie and Kwan 2002).

At present there are some “markers” as indicators of refractory epilepsy such as age at onset, type of epilepsy, failure of the first AED, use of more than two drugs, and duration of treatment without achieving control; especially the presence of an established brain lesion is an important marker for refractory epilepsy (Arroy et al 2002). The fundamental question is whether refractory epilepsy is simply a failure to achieve seizure control or is it a distinct condition that may be characterized by progressive neuronal, cognitive and psychosocial deterioration (Kwan and Brodie 2002)? Are there identifiable clinical, molecular or genetic markers that will predict the outcome? If these processes can be monitored, there opens a possibility to develop compounds that act as anti-epileptogenic agents and not just prevent seizures. (Brodie and Leach 2003)

There are some clinical, epidemiological and experimental data suggesting that refractory epilepsy is a progressive disorder (Arroy et al 2002). Seizure activity may promote abnormal synaptic connections (axonal sprouting) (Sutula et al 1989).

The greater the number of seizures reported in patients with refractory epilepsy, the greater is the loss of volume in the hippocampus, suggesting that the loss might be induced by seizure activity (Kalviainen et al 1998). In a retrospective study with 183 patients with untreated epilepsy, the majority of patients experienced a progressive decrease in the interval between seizures, suggesting progression (Elwes et al, 1988). The new-onset seizures may be progressive in a subset of patients after initiation of drug treatment (Kwan and Brodie, 200). A progressive increase in the predicted risk of further seizures was also found with increasing count of experienced seizures in individuals with otherwise low risk of seizure recurrence; i.e. in patients with unprovoked seizures of unknown cause, with no risk factors, family history or abnormal electroencephalogram (Hauser and Lee 2002).

The hope for the future is that improved understanding of the mechanisms underlying refractoriness and advancing technology in identifying individual genetic variations might improve our ability to identify patients at risk (Arroy et al 2003). One of the newly established mechanisms leading to refractory epilepsy is over-expression of the drug transporter P-glycoprotein on blood vessel endothelial cells, encoded by the multidrug resistance gene (Tischler et al, 1995). Thus early identification of patients for risk of intractability and prompt and adequate therapeutic intervention may improve the overall outcome of the disease.

2.4 Cytokines as modulators of brain plasticity

2.4.1 Cytokines as regulators of immune activation

Cytokines are a diverse group of polypeptides that are associated with inflammation, immune activation, and cell differentiation or death. The cytokines include interleukins, interferons, tumor necrosis factors, chemokines and growth

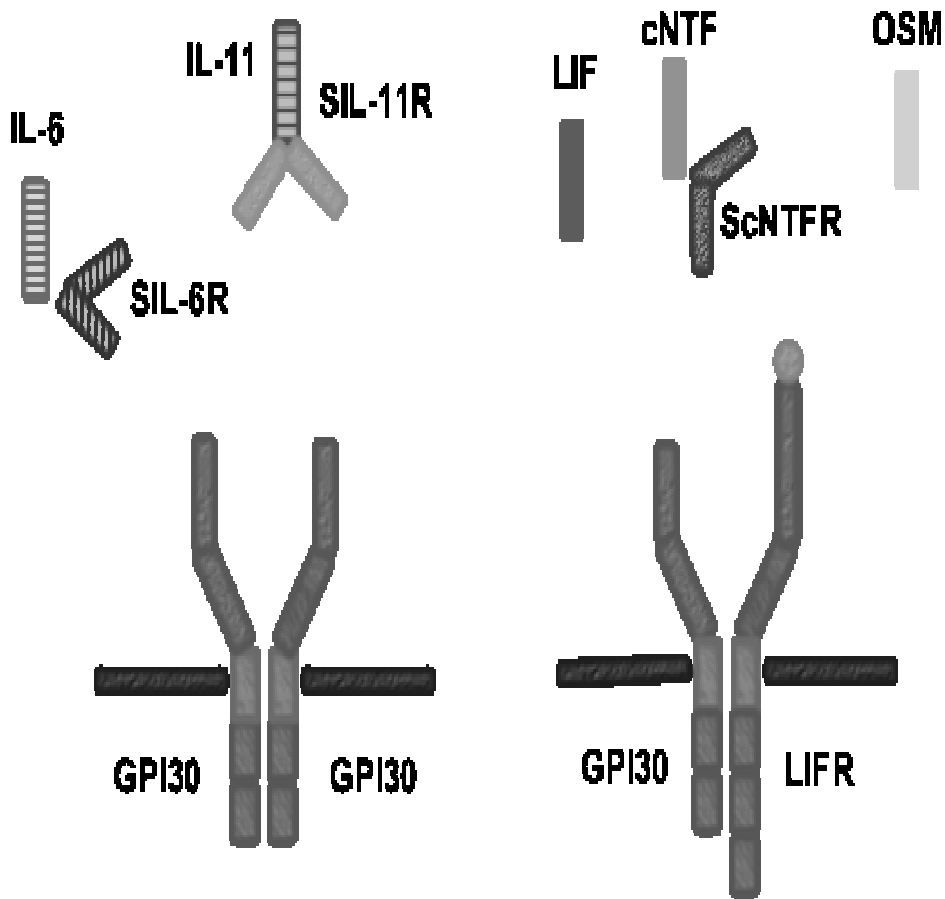
factors in rapidly expanding count. They have some function in healthy tissues, but are induced in response to tissue injury, infection or inflammation. Although first studied in association with peripheral immunity, at present their involvement in brain diseases is quickly growing area of experimental and clinical research (Allan and Rothwell 2001).

The biological response associated with immunity roughly divides into two different arms of activity: the type 1 and the type 2 responses. Type 1 response describes cellular immune response pathways that are associated with T helper cell 1 activation favoring pro-inflammation, whereas type 2 response is associated with T helper cell 2 activation favoring humoral and anti-inflammatory response. (Esch and Stefano 2002). IL-1 belongs to type 1 cytokines and IL-6 to type 2 cytokines.

IL-1 α and IL-1 β were among the first cytokines described. They are both agonists acting at the type I IL-1 receptor levels and possess overlapping biological activities. IL-1 receptor antagonist (IL-1RA) is a competitive antagonist at the level of the IL-1 receptors. There is also a type II IL-1 receptor that acts as a decoy protein. IL-1RA is an endogenously occurring antagonist with no other known effect than the blockade of interleukin-1 actions.

Interleukin-6 is part of the family of IL-6-like cytokines also known as the neuroipoietic cytokines (Figure 1). The family consists of ciliary neurotrophic factors (cNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin-1, growth promoting activity(GPA) and the interleukins IL-6 and IL-11 (Kishimoto et al, 1995).

Figure 1. The interleukin-6 family of cytokines (see text for explanation and abbreviations).



For the signal transduction IL-6 binds to its specific receptor (IL-6 receptor), furthermore a common receptor component for the whole family of cytokines is needed; gp 130. The IL-6 binds to its specific receptor forming a linkage between two molecules of gp130, leading to activation of the intracellular tyrosine kinases of the Janus kinase family (JAKs). LIF, cNTF and OSM bind to a receptor complex of gp130 and LIF-receptor (LIFR). Activated JAKs then phosphorylate transcription factors of the STAT (signal transducers and activators of transcription) family, which then translocate to the nucleus and bind to specific IL-6 response elements. (Gadient and Otten 1997).

IL-6 expression is stimulated by nuclear factor kappa B (NF- κ B); a transcription factor which is widely known for its ubiquitous roles in inflammation and immune responses, as well as in control of cell division and apoptosis (Mattson and Camandola 2001). NF- κ B in turn is activated by signals that activate inhibitory κ B kinase, including increased levels of intracellular Ca or increased glutamate release from neural cells; also stimulation of glutamate receptors and membrane depolarization stimulate NF- κ B activation, it is also activated in association with long-term potentiation (LTP) of synaptic transmission (Matson and Camandola 2001).

IL-6 is produced by a variety of cells, including fibroblasts, monocytes, T cells, B cells, microglia, endothelial cells, neurons and astrocytes (Gruol and Nelson, 1997). Major sites of IL-6 synthesis in the CNS are neurons and glial cells (Gadient and Otten 1997). The cytokines measured in CSF can be either of peripheral blood or intrathecal origin.

The possible consequences for constant pro-inflammatory stimulation of peripheral tissues include stimulation of the acute phase reaction and autoantibody production. IL-1 and tumor necrosis factor (TNF) play a major role in coordinating mechanisms which command (pro)inflammation (Esch and Stefano 2002). These immunological aspects are also interesting in patients with therapy resistant localization-related epilepsy in whom increased production of anti-nuclear and antiphospholipid antibodies have been remarkable (Peltola et al 2000).

2.4.2 The effect of cytokine gene polymorphism on cytokine production

Several of the cytokine genes have a polymorphic distribution in human populations, and most importantly, selected alleles of IL-1 gene complex are associated with either increased or decreased production of a certain cytokine (Hurme

et al, 1998). It has been shown that IL-1 β production by stimulated peripheral monocytes, stratified according to IL-1 β polymorphism, was higher in IL-1 β allele 2 carrier subjects (Pociot et al., 1992). On the other hand IL-1RA allele 2 is known to be associated with a high secretion of IL-1RA (Hurme and Santtila 1998).

2.4.3 Cytokines and acute brain degeneration

Since the studies in early 1980s reporting the production of cytokines by microglia and astrocytes, plenty of investigations have supported the notion of cytokines as mediators of development, injury, and disease states in the CNS. Cytokines are involved in controlling neuronal and glial activation, proliferation, differentiation and survival, thus influencing neuronal and glial plasticity, degeneration as well as development and regeneration of the nervous system (Munos-Fernandez and Fresno 1998). In neurological disorders such as stroke (Beamer et al., 1995) and in traumatic brain injury (McClain et al., 1991) cytokines are produced in response to cellular destruction, in bacterial meningitis by invading inflammatory cells (Waage et al., 1989) and in autoimmune disorders like multiple sclerosis by microglial or other inflammatory cells (Olsson, 1994).

Cytokines seem to play a role in normal physiology connected with neuronal activity and N-methyl-D-aspartate (NMDA) receptor activation, as illuminated with the role of IL-1 in LTP, whereas LTP is attenuated with application of IL-1RA (Schneider et al., 1998). On the other hand exogenous IL-1 β exacerbates neuronal damage induced by glutaminergic receptor activation, but external application of recombinant IL-1RA protects from kainic, acid induced neuronal damage (Lawrence et al, 1998 and Panegyres et al, 1998).

IL-6 can also play a dual role, triggering either neuronal survival after injury or causing neuronal degeneration and cell death in disorders such as Alzheimer's disease (Gadient and Otten 1997). In experimental studies IL-6 prevents NMDA receptor-mediated neurotoxicity in cultured hippocampal neurons, but it also enhances NMDA-induced neurotoxicity and cell death in cerebellar granule neurons (Yamada and Qiu). In addition, IL-6 can induce astrogliosis (Yong, 1996). Cytokines can either increase or protect from the neuronal or glial damage associated with any neuronal damage.

Injury to the brain or spinal cord induces a cascade of signaling events that stimulate NF- κ B activation in injured neurons and in injury-responsive glial cells. NF- κ B in turn influences the neurodegenerative process by directly affecting gene expression in neurons themselves and by indirectly regulating gene expression in glial cells (Mattson and Camandola 201).

Several effects of IL-1 are known to have been produced in an autocrine/paracrine fashion by very low quantities of the cytokine, and it is possible that cerebrospinal fluid (CSF) concentration reflects poorly the local production of the cytokine (Schneider et al., 1998). The effect of IL-1 may involve other co-acting inflammatory cytokines; it induces the synthesis of TNF and IL-6 in astrocytes and microglia and many actions of IL-1 in CNS are mediated by these cytokines. In CNS, IL-6 was found to be induced within hours in the rat facial nucleus following an axotomy of the facial nerve and well before other cytokines (Kiefer et al., 1993). This suggests that IL-6 may act as an activation signal for other cytokines in brain tissue and IL-6 may be elevated only transiently.

2.5 Cytokines and seizures

2.5.1 *Experimental epilepsy and cytokine production*

The significance of cytokine network in epilepsy is supported by experimental studies, where increased expression of several cytokines has been reported in brains of the animals after treatment with kainic acid. Kainic acid causes cellular damage in hippocampus, and cytokine expression may be determined by a response to neural injury.

In kainic acid and pentylenetetrazol induced seizures maximal levels of IL-1 β messenger ribonucleic acid (mRNA) have been found to be expressed in the brain 1.5–3.5 h after the seizures (Minami et al., 1990). In experimental studies, increased IL-1RA expression is detected later than increased expression of IL-1 β . Kainic acid induced expression of IL-1RA mRNA was first detected 5 h after kainic acid administration and was markedly increased at 24 h after the seizure (Eriksson et al., 1998). Increased mRNA levels of IL-1 β , IL-1RA and IL-1R have been observed by in situ, hybridization in kainic acid induced seizures (Minami et al. 1990, Nishiyori et al. 1997, and Eriksson et al., 1998).

Because kainic-acid induced status epilepticus (SE) induces relatively widespread brain damage, evaluation of local brain cytokine production during and following SE is problematic; the damage induced by status epilepticus may significantly up-regulate the cytokine network independent of seizure activity per se (Plata-Salaman et al., 2000). Bicuculline causes induction of seizures without neuronal loss, and in this model an increase both in IL-1 β and IL-1RA production was observed (Vezzani 1999). In amygdala kindling, a procedure which is known to produce minimal neuronal loss, up-regulation of IL-1 β but not IL-1RA was observed 2 hours after kindling but not 3 weeks after kindling (Plata-Salaman, 200)

In the same model application of recombinant IL-1RA produced neuroprotective effects (Panegyres and Hughes, 1998). The synthesis of IL-1 β is induced in glial cells in rodent hippocampus and limbic areas after intrahippocampal injection of kainic acid or bicuculline methiodide, producing EEG-detected and/or behavioral seizures (Vezzani et al., 1999, 2000), or after status epilepticus elicited by continuous electrical stimulation of the ventral hippocampus (De Simoni et al., 2000). IL-1 β acts as a proconvulsant. Limbic seizures are worsened by intracerebral application of picomolar amounts of recombinant IL-1 β , selective competitive antagonist of NMDA receptors prevents the proconvulsant effect of this cytokine suggesting involvement of glutamatergic neurotransmission (Vezzani et al., 1999). IL-1 β was enhanced in glia to a larger extent when seizures were associated with neuronal cell loss as compared to nonlesional models of seizures (Vezzani et al., 1999,2000; De Simoni et al., 2000).

IL-1RA is markedly induced in glia in the limbic system in models of status epileptics (Eriksson et al, 1998; De Simoni et al., 2000 and Vezzani et al., 2000) with a significant delay as compared to IL-1 β . IL-1RA act as an anticonvulsant reducing seizure activity in a variety of experimental models (Gatti et al 2002). Mice over expressing IL-1RA in astrocytes were less susceptible to bicuculline-induced seizures by inhibiting their generalization from the hippocampus to the motor cortical areas (Vezzani et al., 2000).

In an experimental study IL-6 expression was increased in astrocytes and perivascular cells in the hippocampus after LTP stimulations near the stimulation site. After pilocarpine induced status epilepticus, IL-6 was expressed within same cell types, but with more widespread distribution throughout the limbic system (Jankowsky et al., 2000). We have observed the expression of IL-6 in the brain

parenchyma and meninges after kainic acid induced status epilepticus, (Lehtimäki et al., 2003). However, IL-6 was expressed in the meninges adjacent to brain regions showing IL-1 β expression in the brain parenchyma, which is known to induce IL-6 production and release into CSF (Reyes and Coe, 1998). Therefore, meningeal expression of IL-6 may also reflect seizure induced IL-1 expression in the brain parenchyma. IL-6 was induced in neurons by membrane depolarization in cultured cortical neurons suggesting a mechanism through which IL-6 may be regulated under normal, physiological conditions in neurons (Sallmann et al., 2000).

In IL-6 knockout mice increased neuronal injury was observed after kainic-acid induced seizures simultaneously with reduced reactive astrogliosis and microgliosis (Penkowa et al., 2001). In these mice an increased susceptibility to some convulsant stimuli was observed to excitatory amino acids and β -carbolines suggesting an anticonvulsant action of IL-6 (De Sarro et al, in press). On the other hand exogenously applied IL-6 increased the severity of pentylenetetrazole-induced seizures in rats (Kalueff et al., 2004) and increased sensitivity to glutamatergic- but not cholinergic agonist-induced seizures in transgenic mice with astrocytes production of IL-6 (Samland et al., 2003).

TNF α seems to be proconvulsant. Host TNF α production plays a crucial role in enhancing ptz-induced seizures by *Shigella dysenteriae* (Yuhas et al., 2002). TNF α treatment of amygdala kindled rats increased the severity of behavioral seizures and caused changes in EEG pattern (Shandra et al., 2002)

NF- κ B activity is rapidly increased in hippocampal neurons following kainate-induced seizures, an excitoprotective role for seizure-induced neuronal NF- κ B activation is suggested based on manipulation experiments (Yu et al, 1999). NF- κ B

can also reduce the neuronal sensitivity to glutamate in an in vitro model (Furukawa and Mattson 1998)

2.5.2 Febrile seizures and cytokines

Febrile seizures (FS) occur in 2-5% of children under 5 years of age, but in certain populations (e.g., Japan, Guam) FS may be even more common (8-14%) suggesting a possibility for genetic predisposition (Stafstrom 2002). After a FS, 2-4% of children experience a subsequent unprovoked seizure, there is an association between complex FS and partial-onset unprovoked seizures, especially temporal lobe epilepsy (Hesdorffer and Hauser, 2002). Since IL-1 β is both the most potent endogenous pyrogen (Gatti et al , 2002) and lowers seizure threshold in animal models, it may be associated with FS. Also other cytokines such as IL-6 may induce fever and affect seizure parameters.

Helminen and Vesikari (1990) reported a significant increase in IL-1 β production by lipopolysaccharide (LPS)-stimulated monocytes from children with FS compared with cells from children with bacterial or viral infections without convulsions and from those with no infection suggesting that cells from children prone to seizures may produce more pro-inflammatory cytokines that may induce seizures, or on the other hand higher levels of anti-inflammatory cytokines such as IL-1RA as a defense mechanism against seizures. In another study increased production of IL-6 and IL-10 by peripheral blood mononuclear cells in response to LPS was observed in patients with previous FS in comparison with controls, whereas IL-1 β production was comparable; only after 5 hours of incubation peripheral blood mononuclear cells (PBMC) with LPS the mean concentration of IL-1 β rose two-fold compared with controls (Straussberg et al, 2001).

No significant differences were observed between mean IL-1 β levels in CSF and blood of the patients with FS group as compared with the febrile control group (Lahat 1997). CSF and plasma concentrations of IL-6 were within normal limits in children with FS (Ichiyama et al., 1998), but in that study CSF samples were taken on days 1–2 after the seizures (mean 1.1 \pm 0.2). More recently, the mean concentrations of IL-1 β were significantly increased in the CSF of the patients with febrile seizures without any changes of IL-1 β in serum levels (Haspolat et al, 2002). Moreover, in a Finnish study there were no differences in plasma levels of IL-1 β between patients with FS and febrile controls, whereas the plasma IL-1RA/IL-1 β ratio and IL-6 were significantly higher in FS patients, and also elevated CSF IL-6 concentrations were measured (Virta et al, 2002a). An increase in the frequency and the carriage of IL-1 β allele 2 was observed in patients with FS compared with healthy blood donors (Virta et al, 2002b)

2.5.3 Cytokines in human epilepsy

The data on cytokine production in human epilepsy is quite limited at present. There are some studies performed in patients operated for refractory TLE. The expression of inflammatory cytokines has been evaluated by immunohistochemistry in two studies. In the first study an increased production of IL-1 α was reported in human temporal lobe epilepsy (Sheng et al., 1994). Later another study demonstrated an over-expression of NF κ B in hippocampal neurons and glia of operated patients with temporal lobe epilepsy and hippocampal sclerosis (Crespel et al., 2002). Enhanced release of inflammatory cytokines from hippocampal slices of resected epileptic tissue has also been described (De Bock et al., 1996).

In vitro production of cytokines from stimulated mononuclear cells from epileptic patients has been investigated in one study (Pacific et al, 1995). In this study the peripheral blood mononuclear cells showed greater production of IL-1 α , IL-1 β and IL-6 in response to stimulation in patients than in control subjects (Pacifci et al, 1995).

There are several studies addressing cytokine gene polymorphism in human temporal lobe epilepsy. In a Japanese study an increased prevalence of homozygotes for IL-1 β allele 2 was reported in patients with TLE and hippocampal sclerosis compared with TLE patients without hippocampal sclerosis and controls (Kanemoto et al, 2000) (Table 3). Several other groups have studied the same polymorphism in different TLE populations without significant differences between epileptic group and the control group (Heils et al 2000, Buono et al 2001).

Table 3. IL-1 β (-511) genotypes in TLE and control populations, literature data.

	Genotypes IL-1 β alleles	TLE-HS %	Controls %
Kanemoto et al, 2000	1/1	18	28
	1/2	38	51
	2/2	44	21
Buono et al, 2001	1/1	51	37
	1/2	39	57
	2/2	10	6
Heil et al, 2000	1/1	38	42
	1/2	49	45
	2/2	13	12

3. PURPOSE OF THE STUDY

- i** To investigate the activation of the cytokine network after single tonic-clonic seizures

- ii** To search for evidence of activation of cytokine network in patients with chronic epilepsy

- iii** To evaluate the source of cytokines

- iv** To evaluate the association of cytokine activation in acute seizures with indicators of inflammation

- v** To study cytokine gene polymorphism in patients with refractory localization related epilepsy

4. MATERIALS AND METHODS

All the patients and control subjects were treated at the outpatient clinic of Tampere University Hospital. They were fully informed of the risks and potential benefits of the CSF and blood examination, and an informed consent was obtained from each subject. The study protocol was approved by the Ethics Committee of Tampere University Hospital.

4.1 Control subjects

The control samples for the acute study were obtained from 40 adult patients (mean age 40.3, range 16-67 years) on whom lumbar puncture (LP) was performed to exclude neurological disease and who yielded normal neurological examination and laboratory findings. 400 healthy blood donors served as controls for the genetic study. For the in vitro studies the control blood samples were drawn from 10 members of our laboratory staff.

4.2 Patients

4.2.1 Patients with acute seizures (Table 4)

CSF and plasma samples were collected from 37 patients with recent seizures (group I + II) previously undiagnosed and untreated and from 14 patients (group III) suffering a seizure more than 2 weeks previously. We excluded patients with seizures associated with electrolyte disturbances, metabolic causes, acute brain disease and trauma, and those with a history of major psychiatric disease or known autoimmune disease. After the first epileptic seizure the patients usually underwent EEG and

Table 4. Clinical characteristics of the patients with acute seizures.

	Group I*	Group II	Group III	Controls
	N=15	N=14	N=22	N=40
Age	33 (16-60)	30 (17-55)	37 (15-60)	38 (16-56)
Sex M/F	12/3	8/6	14/8	22/18
Symptomatic	3	0	8	
Single seizures	8	8	12	
Epilepsy	7	6	10	
Sample Collection	<72 h	>2 weeks	<24 h	

*The division of patients to groups is explained in the Text

computed tomography (CT) or magnetic resonance imaging (MRI) examinations.

There was no evidence of recent systemic or CNS infection in any of the patients.

4.2.2 Patients in the *in vitro* study (Table 5)

Blood samples from ten patients with therapy resistant localization-related epilepsy were obtained at an outpatient visit of Tampere University Hospital.

4.2.3 Patients in the genetic study (Table 6)

48 consecutive patients with therapy resistant localization-related epilepsy treated at the outpatient clinic of Tampere University Hospital were included in the study. All patients experienced at least 1 seizure/month in spite of adequate trials with at least two conventional or new antiepileptic drugs. Twenty eight patients had temporal lobe epilepsy (6 patients had hippocampal sclerosis), 12 frontal lobe epilepsy, six parieto-occipital epilepsy and two multifocal epilepsy.

Table 5. Clinical characteristics of the patients in *in vitro* study.

Patients (M/F)	Age	Duration of epilepsy	No. of seizures/month	of Medication	Epileptic syndrome
M	34	25	3	CBZ/VGB	FLE
F	65	63	2	CBZ/VGB	TLE
M	35	31	1	CBZ	TLE-HS
F	27	17	3	CBZ/LTG	TLE
F	31	17	3	OXC/LTG	TLE
M	49	42	1	CBZ/GBP	TLE
F	22	22	1	CBZ/VGB	FLE
F	53	49	2	CBZ/TPR	TLE-HS
M	62	48	3	CBZ/LTG/CLB	TLE
F	52	37	3	CBZ/TGB	TLE

CBZ= Carbamazepine, VGB= Vigabatrin, LTG= Lamotrigine, OXC=oxcarbazepine, GBP=Gabapentin, TPR= Topiramate, FLE=Frontal lobe epilepsy, TLE=Temporal lobe epilepsy, HS=Hippocampal Sclerosis

Table 6. Patient characteristics in the genetic study.

Characteristic	
Sex	
Male	26
Female	22
Age (yr.)	37 (18-64)
Age at onset of seizures (yr.)	9 (1-34)
Duration of epilepsy (yr.)	28 (3-61)
Seizure frequency (sz/month)	6 (1-30)

4.3 Methods

4.3.1 Processing of the samples

The first 2 ml of the lumbar CSF obtained was used for routine examination and further 0,2 ml for the present study. Blood was collected within 30 minutes after lumbar puncture in a Vacutainer EDTA vacuum tube and centrifuged at 3000 rpm for 10 minutes. The plasma and CSF samples were stored at – 70 °C until analysis.

Hemolyzed samples were not included for the analysis, nor CSF samples containing over 100 red cells per microliter.

4.3.2 Cytokine ELISAs

IL-1 β , IL-1Ra, TNF α and IL-6 concentrations were determined using commercially available enzyme linked immunosorbent assays. (Pelikine Compact human IL-1 β , human IL-6 and TNF- α ELISA kits, CLB, Amsterdam, Netherlands and Quantikine human IL-1Ra immunoassay, R&D systems, Mp, USA) following the manufacturer's instructions. The optical density of individual wells was determined with a "Multiscan Biochromatic 348" spectrophotometer (Labsystems, Helsinki, Finland).

4.3.3 Nerve growth factor

The concentration of nerve growth factor (NGF) in the CSF samples was determined using a sensitive two-site NGF-ELISA assay. Briefly, 0.04 g primary monoclonal anti-NGF antibody 27/21 (Roche, Bromma, Sweden) in 50 mM carbonate buffer was plated to each well in an EIA plate (Dynatech Laboratories, Virginia) and incubated overnight at 4°C. The next day, after extensive washes with washing buffer (50 mM

Tris pH 7.0, 150 mM NaCl, 5 mM MgCl₂, 0.1% Triton-X), the samples and NGF-standards (0.1–250 pg/ml) were applied onto the wells after 2 h blocking (1% BSA in carbonate buffer) at room temperature. Three wells were used for each sample and each standard concentration of NGF. Following the overnight incubation and washing, secondary -galactosidase coupled with an anti-NGF antibody (Roche, Bromma, Sweden) was added in a concentration of 0.1 U/ml to the wells and again incubated overnight. Before adding 200 M 4-methylumbelliferyl-galactoside (MUG) (Sigma, Stockholm, Sweden) to the wells, the plate was first washed with the washing buffer followed by washes with a substrate buffer (100 mM sodium phosphate buffer pH 7.3, 1 mM MgCl₂). Fluorescence signal was measured 1, 2 and 3 h after adding MUG. Typically there was a linear correlation between the fluorescence signal and NGF concentration in the range 0.1–250 pg/ml.

4.3.4 Indicators of inflammation

The complete blood count (CBC) was determined using Coulter Counter Model S- Plus IV (Coulter Group, Hialeah, FL) and C- reactive protein (CRP) by immunoturbidimetry (Hitachi Ltd, Tokyo, Japan). Fibrinogen and haptoglobin concentrations were analyzed by immunoturbidimetry (Aca, Dupoint) and nephelometric method (Dade Behring, Marburg, Germany), respectively.

Serum antibodies to streptolysin (AST) were measured by a standard nephelometric assay (Behringwerke, Hamburg, Germany). Antibodies to Salmonella, Campylobacter jejuni and Yersinia were measured by in-house enzyme immunoassay (EIA) techniques. Antibodies to Varicella zoster and Herpes simplex virus were measured by EIA (Enzygnost, Behringwerke, Germany).

4.3.5 Cell separation

The PBMCs were isolated from blood samples by centrifugation over a Ficoll-Isopaque layer (Pharmacia, Upsala, Sweden). The cells were washed twice with complete medium consisting of RPMI 1640 (Gibco, Paisley, UK), 10 mM hepes (ICN Biomedical, Costa Mesa, CA, USA), 10 % heat-inactivated fetal calf serum (Gibco), 2 mM L-glutamine and antibiotics.

4.3.6 Culture conditions and mitogens.

The PBMCs were tested for their capacity to release IL-1 β , IL-1Ra and IL-6, both spontaneously and after various stimuli. The cells were cultured in complete medium (above) at a concentration of 10^6 /mL in 96-well flat-bottomed plates (Falcon). Cells were alternatively stimulated with a pre-tested optimal dilution of plate-bound anti-CD3 antibody (OKT3; Pharmingen, San Diego, CA, USA) or phorbol dibutyrate (PDBu, 100 ng/mL; Sigma Chemicals Co., St. Louis, MO, USA) combined with calcium ionophore (A23187, 100 ng/mL; Calbiochem, La Jolla, CA, USA), or LPS (1 ug/mL; Sigma). After 24 h in culture, cell culture supernatants were collected, centrifuged and stored at -70 °C until cytokine determination.

4.3.7 Intracellular IL-1 β , IL-1Ra and IL-6 detection

Expression of intracellular IL-1 β , IL-1Ra and IL-6 was studied by flow cytometry using FacsCalibur flow cytometer (Becton Dickinson Immunocytometry systems, Palo Alto, CA, USA). Cells (10^6 /mL) were stimulated with LPS at concentration 1 ug/ml. After 4 h in culture, the cells were permeabilized and stained for intracellular cytokines with a commercially available cytostain kit (Cytofix/Cytoperm kit, Pharmingen, San Diego, CA, USA) following the

manufacturer's protocol. The protein transport inhibitor Brefeldin A (BFA, 10 µg/ml; ICN) had been added to the cell culture 4 h prior to staining. The PE-labeled antibodies for IL-1β, IL-1Ra and IL-6 (FastImmune anti-Hu IL-1β, IL-1Ra and IL-6), FITC/PE labelled CD45/14 (LeucoGate CD45/14) and PE-labeled intracellular isotype control (clone X40) were all purchased from Becton Dickinson Immunocytometry Systems (Mountain View, CA, USA). The FITC-labeled antibody for CD14 (clone Tuk4) and FITC-labeled cell surface isotype control (clone DAK-GO5) were purchased from Dako (Glostrup, Denmark). 10⁵ cells were collected for flow cytometric data analyses. The proportions of cytokine positive monocytes were analyzed by comparing the percentages of anti-cytokine labelled CD14+ cells against a control sample labeled with non-specific antibodies. The cytokine production intensities were compared using mean fluorescence intensity (MFI) values of these same cells after subtracting the background MFI from the specific MFI.

4.3.8 Cytokine gene polymorphism

The following allelisms of the IL-1 gene polymorphism were analyzed: IL-1α, base exchange polymorphism at the position -889 (McDowell et al, 1995); IL-1β, base exchange polymorphism at the position -511 (di Giovine et al., 1992); IL1-RA, variable numbers of 86-base pair tandem repeats (VNTR) in intron 2 (Tarlow et al, 1993) as previously described.

4.3.9 Statistical methods

The mean and standard deviations were calculated for continuous variables. Statistical significance of differences between groups was tested by independent two-tailed t- test for continuous variables. Associations between continuous variables were

assessed with Pearson's correlation coefficient. All analyses were performed using a microcomputer and Statistica/Win package (version 5.1; Statsoft, Inc, Tulsa, The USA) A p value less than 0.05 was considered statistically significant.

Mann-Whitney U-test was used for the comparison of group medians in in vitro studies, where correlations were calculated using the Spearman rank order correlation test. Differences were considered significant at significance level <0.05 .

The frequencies of alleles, allele 2 carrier status and genotypes of the IL-1 polymorphisms were compared between epileptic and control groups by the chi-square test. The p values <0.05 were considered statistically significant. The odds ratio (OR) and the 95% confidence intervals (CI) were calculated using the Confidence Interval Analyses program (version 1.1, MJ Gardner and the British Medical Journal, 1991).

5. RESULTS

5.1 Patients with acute seizures

The mean concentrations of both CSF and plasma IL-6 were higher in patients (groups I+III) than in controls (Table 7).

In the first set of patients measured within 72 hours from the seizure (group I) the concentrations of IL-6 were >7.4 pg/ml (mean+2 S.D. for the control group) in four out of 15 CSF samples and >5.5 pg/ml (mean+2 S.D. for the control group) in three of the plasma samples. All four patients with elevated CSF levels of IL-6 had LPs performed within 15 h of the seizures (altogether six patients in group I had LPs obtained within 15 h of the last seizure). Two of these patients were the only ones with multiple seizures during one day. Slightly elevated AST levels were measured in three, one of these patients also had elevated levels of IL-6 in CSF but not in plasma. However, these patients had normal CRP and white blood cell counts and no clinical signs of streptococcal infection. Results of other antibody studies were unremarkable in all patients.

In the second set of patients measured within 24 hours from the seizure (group III) the mean concentrations of IL-6 were elevated both in plasma and CSF, and there was also some indication of increased concentrations of IL-1RA. The concentrations of IL-6 were greater than 3.7 pg/ml (mean+2 S.D. for the control group II) in 15 out of 22 CSF samples and greater than 2.4 pg/ml (mean+2 S.D. for the control group II) in nine out of 22 plasma samples. The number of elevated values in the seizure group was statistically significant both in CSF ($P<0.0001$) and plasma ($P=0.01$). CSF levels of IL-1RA were elevated in five out of 22 patients with seizures, whereas none of the control group showed increased values ($P=0.057$). In plasma, six out of 22 patient

Table 7. Plasma cytokine levels in patients with acute seizures.

Cytokine		Patients (n=37)#	Controls (n=40)*	P
IL-1 β	CSF	0,05 \pm 0,10	0,09 \pm 0,1	0.9731
	Plasma	0,4 \pm 0,6	0,10 \pm 0.1	
IL-1Ra	CSF	60,4 \pm 56,8	33,0 \pm 21,4	0,068
	Plasma	680,1 \pm 1037,7	278,4 \pm 171,0	
IL-6	CSF	22,1 \pm 31,9	2,2 \pm 2,0	0,001
	Plasma	3,8 \pm 3,7	1,3 \pm 1,0	

Values are median [quartile range] given in pg/mL.

#n=22 for IL-1Ra

* n=18 for IL-1Ra

had increased IL-1RA levels compared with two out of 18 control patients (P=0.24), Levels of IL-6 in CSF did not correlate with any other cytokine concentrations in CSF and there was no correlation between plasma and CSF concentrations of IL-6 or IL-1RA. There was a weak correlation between plasma concentrations of IL-1 and IL-1RA (r=0.358, P=0.025). There was no correlation between CSF concentrations of IL-6 and CSF cell counts.

Indicators of inflammation in blood samples from the patients with recent seizures are shown in Table 8, The B-leukocyte and CSF leukocyte counts were higher in patients than in controls. CSF pleocytosis (defined as $> 3 \times 10^6$ white blood cells/ l) was observed in 6 out of 37 (16%) patients and in 3 out of 40 controls (7.5%). Also serum fibrinogen concentration was significantly higher in the patient group than

Table 8. The mean concentrations of indicators of inflammation.

	Patients (n=37)	Controls (n=40)	<i>p</i>
Blood-leukocytes	7,9±2,8	6,1±1,8	0.002
CSF-leukocytes	1,9±2,0	1,1±1,3	0,046
CRP	6,4±14,1	2,9±2,8	0,167
Fibrinogen	3,3±0,76	2,9±0,7	0,032
Haptoglobin	1,2±0,74	1,1±0,6	0,611

in the control group. Furthermore, we found that the CRP concentration was almost two times higher in patients than in controls (6.4 versus 3.9) but the difference was not statistically significant. In haptoglobin values no significant difference was observed.

Increased B- leukocyte count, CRP level or serum fibrinogen concentration was found in 9 out 37 patients (24 %) and in 4 out 40 (10 %) controls. We found significant correlation between the plasma IL-6 concentration and both peripheral blood white blood cell counts (correlation coefficient $r=0.51$, $p=0.001$) and plasma CRP concentration ($r= 0.42$, $p = 0.0088$). In addition, CRP had a positive correlation with time after seizure ($r=0.51$, $p=0.009$) and CSF leukocyte count had a negative correlation with time after seizure ($r = -0.40$, $p = 0.05$).

In the group of patients with measurements more than two weeks from the seizure (group II) none had concentrations of IL-6 greater than 7.4 pg/ml in CSF; in two patients in this group the plasma concentrations of IL-6 were 5.7 pg/ml and 8.7 pg/ml, respectively. In healthy controls one patient with acute headache the CSF concentration of IL-6 was 10 pg/ml and in another patient the plasma concentration of

IL-6 was 8.1 pg/ml. None of the control group showed increased values of IL-1RA in CSF. In plasma, two out of 18 control patients had increased concentrations of IL-1RA.

For the evaluation of blood–brain barrier damage, ratios of CSF albumin to serum albumin were investigated, but none of the patients or controls showed increased values (normal < 0.009). The highest TNF level measured in CSF was only 2.2 pg/ml in any group of patients. In most CSF and plasma samples IL-1 β was undetectable, the highest concentration measured in CSF being 2.18 pg/ml.

5.2 Cytokine plasma levels in the patients with refractory epilepsy; *in vitro* studies

Plasma levels of IL-1 β , IL-1Ra and IL-6 and the IL-1Ra/IL-1 β ratio are given in Table 9. Highly pro-inflammatory cytokine profile (significantly lower IL-1Ra levels, a lower IL-1Ra/IL-1 β ratio, a trend towards higher levels of IL-6) was observed in patients with epilepsy compared with controls. A positive correlation was observed between plasma IL-1 β and IL-6 in controls (R=0.50, p<0.0001). This trend was less distinct in epilepsy patients (R=0.44, p=0.21). A negative correlation between plasma IL-6 and IL-1Ra was observed in the patients (R=-0.64, p=0.047), but not in the controls (R=0.04, p=0.54).

Table 9. Plasma cytokine levels in refractory epilepsy.

Cytokine	Patients (n=10)	Controls (n=400)*	
IL-1 β	6.6 [1.3-9.9]	5.8 [2.2-13.6]	0.9731
IL-1Ra	223 [131-266]	587 [372-852]	<0.0001
IL-6	2.1 [1.22-2.49]	1.2 [0.7-2]	0.0572
IL-1Ra/IL-1 β -ratio	28.1 [10.4-109]	114 [42.3-279]	0.0207

Values are median [quartile range] given in pg/mL.

5.3 In vitro cytokine production

Spontaneous peripheral blood mononuclear cell culture cytokine release was quite similar in patients and control subjects (Table 10). This also held true in LPS stimulated cell cultures. However, when cells were stimulated with OKT3 or PDBu+A23187, the cytokine profiles in patients with epilepsy were clearly different from those observed in the control group. The IL-1 β , IL-6 and IL-1Ra productions were markedly lower and the IL-1Ra/IL-1 β ratio higher in patients than in control subjects. As was the case with plasma IL-1 β and IL-6, the production of these cytokines also correlated in vitro. Striking positive correlation was observed both in the spontaneous and OKT3 stimulated release of these factors in patients ($R= 0.88$ and 0.92 , $p<0.001$) as well as in control subjects ($R=0.87$ and 0.95 , $p<0.001$).

Table 10. In vitro cytokine responses for different stimuli among patients (n=10) and controls (n=10).

Group	No stimulus	OKT3	PDBu+A23187	LPS
IL-1β				
Patients	0 [0-11.5]	3.5 [0-8.4]	549 [442-594]	3990 [2670-10200]
Controls	5.3 [3.2-7.6]	53.1 [5.5-342]	617 [380-1420]	4410 [2570-6050]
	NS	p=0.06	NS	NS
IL-1Ra				
Patients	1075 [472-1536]	660 [467-1345]	5580 [4480-7600]	7180 [5360-13300]
Controls	1031 [504-1316]	2900 [830-5600]	5440 [4160-11080]	7800 [6800-8480]
	NS	p=0.0126*	NS	NS
IL-6				
Patients	22.3 [0-49.6]	26.4 [0-45.9]	719 [263-1420]	21200 [17200-37000]
Controls	46.5 [11.4-61.6]	593 [17.7-1290]	4310 [2720-4920]	23800 [16000-26600]
	NS	p=0.07	p=0.0156*	NS
IL-1Ra/IL-1β ratio				
Patients	107 [92.5-125]	129 [67.9-245]	9.4 [7.9-15.1]	1.8 [1.2-3.2]
Controls	156 [112-243]	31.2 [15-134]	8.2 [4-16.2]	2.3 [1.3-3]
	NS	p=0.0126*	NS	NS

Values are median [quartile range] given in pg/ml. NS= not significant. P-values <0.1 are presented

5.4 Intracellular cytokine detection

As the monocyte-macrophage lineage cells are the main cell type responsible for IL-1 β , IL-1Ra and IL-6 production in humans, we analyzed the cell-specific production of these cytokines from peripheral blood monocytes using flow cytometry. The monocyte derived cytokine production of patients and control subjects are given in Table 11. The percentages of IL-1 β , IL-1Ra and IL-6 positive monocytes were values similar in patients and control subjects. Moreover, cell specific mean cytokine production intensities were similar in these groups.

Table 11. Cytokine responses for LPS stimulation in monocytes.

Parameter	Patients	Controls	P
IL-1 β positive cells (%)	78.3 [73.0-80.9]	78.0 [67.3-88.7]	NS
IL-1Ra positive cells (%)	32.8 [29.4-41.4]	30.0 [25.5-49.0]	NS
IL-6 positive cells (%)	74.3 [72.1-78.2]	76.3 [68.8-85.4]	NS
IL-1RA/IL-1 β (%) ratio	0.452 [0.396-0.567]	0.472 [0.375-0.597]	NS
IL-1 β production MFI	489.4 [344-675]	379 [352-431]	NS
IL-1Ra production MFI	60.9 [42.0-82.9]	53.9 [49.4-78.0]	NS
IL-6 production MFI	1225.7 [694-1420]	1071.2 [903-1270]	NS
IL-Ra/IL-1 β MFI ratio	0.134 [0.078-0.234]	0.161 [0.136-0.193]	NS

Values are median [quartile range]. MFI=mean fluorescence intensity.

5.5 Cytokine gene polymorphisms in refractory epilepsy

The frequencies of the IL-1 α allele 1 and IL-1 β allele 2 were significantly higher in patients compared with controls (table 12), whereas there were no differences in IL-1RA allele frequencies (data not shown). Patients and controls were grouped according to their allele 2 status into carriers (A2+) and non-carriers (A2-), and the two-locus combinations of the allele 2 carrier status were compared. The combination of IL-1 β (-511) A 2+ /IL-1RA (VNTR)A2- was more prevalent in patients (19/47, 40%) than in control subjects (96/400, 24 %) (P=0.0148, κ^2 =5.94, OR=2.1) (Table 13).

Table 12. Allele frequencies of the genes of the IL-1 complex in epileptic patients (n=48) and healthy controls (n=400).

Allelism (position)	Patients	Controls	OR	95% CI	p
IL-1 α (-889) genotype n=47					
1.1	30 (64%)	167 (42%)	2.462	1.315-4.610	0.0039
1.2	13 (28%)	201 (50%)	0.379	0.194-0.739	0.0034
2.2	4 (8%)	32 (8%)			n.s.
IL-1 α allele frequency					
Allele 1	0.78	0.67	1.712	1.031-2.843	0.0359
Allele 2	0.22	0.33	0.584	0.352-0.970	0.0359
IL-1 β (-511) genotype n=48					
1.1	5 (10%)	146 (37%)	0.202	0.074-0.522	0.0003
1.2	31 (65%)	182 (46%)	2.184	1.171-4.074	0.0124
2.2	12 (25%)	72 (18%)			n.s.
IL-1 β (-511) allele frequency					
Allele 1	0.43	0.59	0.518	0.338-0.795	0.0023
Allele 2	0.58	0.41	1.93	1.258-2.962	0.0023

OR denotes odds ratios; 95% CI, 95% confidence interval; n, number of individuals;

n.s., not significant

Table 13. Prevalence of IL-1 β allele 2 carriage in relation to carriage of IL-1RA allele 2 in epileptic patients and healthy controls.

		IL-1β A2 carrier		OR	95% CI	p	
		yes	no				
IL-1RA A2 carrier	yes	epilepsy	24	0	NA	0.0103	
		controls	157	38			
	no	epilepsy	19	5	4.315	1.552-12.000	0.0165
		controls	96	109			

Fisher's exact test

OR denotes odds ratios; 95% CI, 95% confidence interval; n, number of individuals; A, allele; NA, not available

6. DISCUSSION

6.1 Which cytokines are upregulated after single seizures?

We have demonstrated selective upregulation of the cytokine network after a single seizure in patients with newly onset seizure disorder. Among the cytokines studied there was a robust increase in soluble IL-6 concentrations both in CSF and plasma, and some indication of increased concentrations of IL-1RA, whereas there was no increase in IL-1 β , TNF α or NGF concentrations.

IL-6 is a cytokine known to be rapidly upregulated following different kinds of tissue trauma and inflammation. IL-6 may act as an activation signal for other cytokines in brain tissue. IL-6 may be elevated only transiently in the brain, a poorly known matter. In experimental studies after kainate treatment, IL-6 mRNA was upregulated in meninges and ependymal cells (Lehtimäki et al, 2003). Depolarization causes increased expression of IL-6 in neurons (Sallman et al, 2000)

There is ample experimental evidence confirming the upregulation of the IL-1 system in seizures. In our studies there was some evidence suggesting the upregulation of IL-1RA whereas the IL-1 β levels remained unchanged. The most likely explanation is the local distribution of the expression of IL-1 β , because after kainic acid treatment the increased IL-1 β mRNA expression was limited to the hippocampus (Lehtimäki et al, 2003). Several effects of IL-1 β are shown to emerge in an autocrine/paracrine fashion by very low quantities of the cytokine, and it is possible that CSF concentration would be very insensitive to changes in local production of the cytokine (Schneider et al., 1998). It has recently been demonstrated with the use of sensitive techniques, that the expression of the IL-1 β gene is affected by physiological changes in the activity of discrete populations of hippocampal

neurons in LTP (Schneider et al., 1998). The increase in IL-1RA concentrations is also an indirect evidence of the activation of the IL-1 β , since IL-1 β is the most potent activator of IL-1RA, even though also IL-6 is an activator of IL-1RA. Furthermore increased IL-6 is one of the best markers of increased IL-1 activity (Dinarello 1997).

The upregulation of TNF α has been demonstrated in several experimental studies (Jankowsky and Patterson, 2001). After treatment with kainic acid, the timely distribution of TNF α expression was similar to IL-1 β with peak increases detected 3 h after injection of this excitotoxin (Lehtimäki et al, 2003). Immunohistochemical localization studies of TNF α following excitotoxic brain injury suggest that astrocytes and microglial cells are the primary sources of this inflammatory cytokine (Acarin et al., 2000).

Neurotrophic factors like NGF are, as the name implies, important during development but they are also supporting neurons after injury and maintaining neuronal properties. Many cytokines, especially interleukins, have direct neurotrophic effects and they can also stimulate production of certain dedicated neurotrophic factors. IL-1 and IL-6 have both been shown to stimulate the production of NGF under various conditions (Lindholm 1992; Lindholm et al, 1987). Increased neurotrophic factor production has been demonstrated in several experimental seizure models (Zafra et al, 1990 and Gall 1993). A recent study reported that a neurite stimulating effect was induced by the CSF of epileptic patients (Akoev et al., 1996). Likewise, in West syndrome, a severe epileptic syndrome of the infancy, some patients had very high levels of NGF in the CNS (Riikonen et al., 1997). In the present study on adult patients we failed to observe any differences in the NGF levels between the control and the seizure group. However it has to be kept in mind that

neither the kinetics nor the mode of production and release of NGF in the adult human brain are known and other time points need to be studied as well.

6.2 CSF vs. plasma: the origin of the cytokines

The cytokines measured in CSF can be either of peripheral blood or intrathecal origin. IL-6 is produced by a variety of cells, including fibroblasts, monocytes, T-cells, B-cells, microglia, endothelial cells, neurons and astrocytes (Gruol and Nelson, 1997). The mean concentrations of IL-6 in our study were higher in CSF than in plasma (18,6 pg/ml vs. 4,5 pg/ml) supporting the hypothesis of intrathecal production of IL-6. Seizures may increase blood–brain barrier (BBB) permeability, but none of the patients had an elevated albumin CSF/serum ratio, suggesting absence of BBB function deficit (Ruth, 1984). With IL-1RA the source of production in our patients is more difficult to assess. The IL-1RA concentrations are ten-fold in plasma compared with CSF, and IL-1RA is known to cross the BBB (Gutierrez et al., 1994). In other neurological disorders elevated levels of cytokines have been measured in different types of CNS trauma or infection (Kossman et al, 1996, Waage et al., 1989). In our patients there was no evidence of CNS trauma or infection, and the mechanism of production is most likely associated with electric brain activity whose duration is usually less than 5 minutes.

Additional evidence for CNS-origin of the cytokines is provided by the study on therapy resistant patients (V). Our study shows that during the interictal state, patients with treatment resistant epilepsy have decreased plasma levels of anti-inflammatory cytokine IL-1RA, a trend towards elevated plasma levels of soluble pro-inflammatory cytokine IL-6, but no evidence of increased production from blood mononuclear cells when both intracellular and secreted cytokines were evaluated.

These results suggest that increased cytokine responses in peripheral blood mononuclear cells are not causing the net increase in the plasma levels of these cytokines observed postictally in patients with epilepsy.

6.3 Time course and kinetics of cytokine production

In our first set of patients (I) elevated CSF levels of IL-6 were detected in 27% of patients with seizures occurring within 72 h before sampling, on the other hand all increased IL-6 levels were observed in patients sampled within 15 h from the seizure. In the second set of patients (II) the percentage of patients with increased concentrations of IL-6 was clearly higher (82%) when all patients were sampled within 24 h from the seizure. In experimental studies the expression of IL-6 mRNA was detected 2 h after the seizures and increased at 4 h after the seizure (Minami et al., 1991). It must be kept in mind, however, that experimental animal data on mRNA time courses are not directly comparable to human CSF protein levels of cytokines. The elimination of IL-6 from CSF is most likely quite rapid, since in an experimental model where radioligand bound IL-6 was applied intrathecally, the elimination half-life of IL-6 was 42 min presumably via venous drainage (Banks et al., 1994). Previously CSF and plasma concentrations of IL-6 have been studied in children with febrile seizures; results were within normal limits (Ichiyama et al., 1998). In that study CSF samples were taken on days 1–2 (mean 1.1 ± 0.2). In our previous study none of the samples studied later than 24 h from the seizure were positive.

6.4 The genetic control of the cytokine response

We observed that the distribution of IL-1 α and IL-1 β alleles were different in patients with severe localization-related epilepsy compared with control subjects. The

frequencies of the two alleles belonging to the same haplotype, IL-1 α allele 1 and IL-1 β allele 2 (Cox et al., 1998), were higher in patients than in controls. Previously it has been shown that IL-1 β production by stimulated peripheral monocytes, stratified according to IL-1 β polymorphisms, was higher in subjects carrying IL-1 β allele 2 (Pociot et al., 1992). We found no difference in IL-1RA VNTR allelic distribution between the patients and control subjects. However, the patients who were non-carriers of IL-1RA allele 2 were significantly more likely to be carriers of IL-1 β allele 2. Interestingly, a similar association has been previously observed in patients with two autoimmune disorders, ulcerative colitis (Heresbach et al., 1997) and myasthenia gravis (Huang et al., 1998). IL-1RA allele 2 is known to be associated with a high secretion of IL-1RA (Hurme and Santtila 1998). Taken together, our findings suggest that patients with severe localization-related epilepsy have a predilection for a high IL-1 β production with decreased production of IL-1RA. This in turn will lead to enhanced stimulation of type I IL-1 receptors. The patients of our study represent a highly selected population. Thus we cannot conclude whether our findings of IL-1 α/β and IL-1RA gene polymorphisms are relevant with respect to all forms of localization-related epilepsy syndromes which represent a clinically and genetically distinct group of disorders (Ottman et al. 1998).

There are several conflicting reports on the association between IL-1 β 511 allele polymorphism and temporal lobe epilepsy. An increased frequency of homozygotes for allele 2 was reported in Japanese patients with TLE with hippocampal sclerosis (TLE+HS) compared with healthy controls or TLE without hippocampal sclerosis (TLE-HS) (Kanemoto et al, 2000). In a population of patients of European ancestry, association between the IL-1 gene variation and TLE+HS could not be confirmed (Buono et al, 2001, Heils et al, 2000). There are some plausible

explanations for the contradicting results. First, the frequencies of allele 2 in different ethnic populations vary (European 0,34, Asian 0,46, African 0,6). Interestingly the frequency of allele 2 in Finland is comparable to that of Japan. On the other hand, the prevalence of prolonged febrile seizures (PFS) in TLE+HS populations vary from 25,6% (Buono, et al 2001) to that of 54% reported by Kanemoto (2000). In a subsequent study allele 2 was most frequent in patients with PFS (0,686) and TLE+HS, 0,633 with simple febrile seizures and TLE+HS, and 0,531 without febrile seizures (Kanemoto et al, 2003).

6.5 The implications of cytokine expression in acute seizures: CNS

The activation of the cytokine network in association with seizures may have consequences both for central nervous system and peripheral tissues. Exogenously applied IL-1 β and IL-6 act as proconvulsants, whereas IL-1RA is an anticonvulsant (Vezzani et al, 2000, Kalueff et al, 2004). Limbic seizures are worsened by intracerebral application of picomolar amounts of recombinant IL1- β . Furthermore, selective competitive antagonist of NMDA receptor prevents the proconvulsant effect of IL-1 β suggesting involvement of glutamatergic neurotransmission (Vezzani et al., 1999). IL-1RA acts as an anticonvulsant reducing seizure activity in a variety of experimental models (Gatti et al 2002). Mice over-expressing IL-1RA in astrocytes were less susceptible to bicuculline-induced seizures by inhibiting their generalization from the hippocampus to the motor cortical areas (Vezzani et al., 2000). IL-6 acts as a proconvulsant in pentylenetetrazol-model of epilepsy (Kalueff et al, 2004)

Cytokines seem to play a role in normal physiology connected with neuronal activity and NMDA receptor activation, as illuminated with the role of IL-1 β in LTP, whereas LTP is attenuated with application of IL-1RA (Schneider et al., 1998). On the

other hand exogenous IL-1 β exacerbates neuronal damage induced by glutamatergic receptor activation, but external application of recombinant IL-1RA protects from kainic, acid induced neuronal damage (Lawrence et al, 1998 and Panegyres et al, 1998).

IL-6 can also play a dual role, triggering either neuronal survival after injury or causing neuronal degeneration and cell death in disorders such as Alzheimer's disease (Gadient and Otten 1997). In experimental studies IL-6 prevents NMDA receptor-mediated neurotoxicity in cultured hippocampal neurons, but it also enhances NMDA-induced neurotoxicity and cell death in cerebellar granule neurons (Yamada et al., 1994, Qiu et al., 1998). In addition, IL-6 can induce astrogliosis (Yong, 1996). Cytokines can either increase or protect from the subsequent neuronal or glial damage associated with any central nervous system lesion.

6.6 The implications of cytokine activation in acute seizures: peripheral tissue

Both acute and chronic production of pro-inflammatory cytokines of peripheral tissues may mediate the acute phase reaction and autoantibody production.

The acute phase reaction is usually caused by inflammation or tissue injury. We found some evidence of an acute phase reaction provoked by an epileptic seizure in 24 % of our patients after acute seizures without any evidence of trauma or infection. Thus, the acute phase reaction is most probably caused by the seizure activity itself. Central nervous system production of IL-6 can stimulate increased production of IL-6 in peripheral blood, which subsequently activates the acute phase reaction. In our study plasma IL-6 concentrations correlated with peripheral blood leukocyte counts and CRP. Patients with single seizures have been reported to have transient CSF pleocytosis in 11 – 34 % of the cases, in line with the present study

(16%). Our study further showed a negative correlation with the time lag between the seizures and sampling.

These immunological aspects are also interesting in patients with therapy resistant localisation-related epilepsy in whom increased production of anti-nuclear and antiphospholipid antibodies was found (Peltola 2000).

SUMMARY AND CONCLUSIONS

In this dissertation I searched together with my collaborators for evidence of an association between cytokine network activation and seizures both in patients with acute seizures and chronic epilepsy. We studied the circulating levels of cytokines in cerebrospinal fluid and plasma after tonic-clonic seizures with different time-windows in order to assess the significance of recent seizures in relation to cytokine production. In these patients with acute seizures we measured a set of indicators of inflammation in order to study the potential consequences of the activation of the cytokine network. In patients with refractory chronic epilepsy we evaluated the source of cytokines with comparing the plasma levels of cytokines with the production of cytokines by blood cells. We also studied cytokine gene polymorphism in patients with refractory chronic epilepsy in order to assess the potential genetic contribution.

CSF and plasma samples were collected from 37 patients with recent seizures (<72 hours) previously undiagnosed and untreated and from 14 patients suffering a seizure more than 2 weeks previously. The control samples for this acute study were obtained from 40 adult patients on whom lumbar puncture was performed to exclude neurological disease and who yielded normal neurological examination and laboratory findings. The concentrations of IL-6 were significantly higher in the group with recent seizures both in CSF and plasma as compared with patients at more than 2 weeks from the seizure and controls. There was some indication of increased IL-1RA production both in CSF and plasma in the patients with recent seizures. There was no difference in concentrations of IL-1 β , TNF α or NGF between patients and controls. All increased concentrations of IL-6 were observed from patients sampled within 24

hours of the seizure. These studies demonstrate an increased and temporary production of IL-6 after tonic-clonic seizures with some indication of activation of the IL-1 system.

We were able to correlate the indicators of inflammation with plasma IL-6 levels after recent tonic-clonic seizures. The mean peripheral blood and CSF-leukocyte counts were significantly higher in patients compared with controls; there was some indication of increased concentration of C-reactive protein in patients with seizures but no difference in haptoglobin levels. A consequent point for patient care is that, CSF pleocytosis and increase in some indicators of inflammation should not automatically be attributed to systemic or CNS infections in patients with acute seizures.

We studied the plasma levels of IL-1 β , IL-1RA and IL-6 as well as the spontaneous and exogenously stimulated production of these cytokines from peripheral blood mononuclear cells (PBMC) from 10 patients with refractory localization-related epilepsy compared with healthy controls. Highly pro-inflammatory cytokine profile (high IL-6, low IL-1RA and low IL-1RA/IL-1 β ratio) was observed in plasma from patients with epilepsy. Spontaneous and LPS stimulated cytokine release was similar in PBMC cultures of patients and control subjects, whereas there was evidence of decreased production of these cytokines when stimulated. These results suggest that the altered plasma levels are not derived from peripheral blood, and the most likely origin for these cytokines is the brain. Formally, cytokine production by sessile cells of lymphoid organs is not excluded. This explanation would imply that there would exist a systemic disease causing proneness to seizures, that would be revolutionary to current thinking.

In the genetic study of 48 patients with refractory localization-related epilepsy compared with 400 healthy blood donors the frequencies of the IL-1 α allele 1 and IL-1 β allele 2 were significantly higher in patients compared with controls, whereas there were no differences in IL-1RA allele frequencies. The combination of IL-1 β (-511) A2+ /IL-1RA (VNTR)A2- was more prevalent in patients than in control subjects.

The significance of cytokine production in relation to epileptic seizures is not yet fully known. However, there is an increasing amount of consistent experimental data suggesting a role for cytokines as modulators of seizure activity and brain plasticity. IL-1 β is proconvulsant and neurotoxic in animal models, whereas IL-1RA antagonist acts as an anticonvulsant and neuroprotectant. The significance of TNF α and IL-6 is less well established, but IL-6 may be proconvulsant and local TNF α anticonvulsant. We have now presented clinical data on the association between the activation of the cytokine network both in acute seizures and chronic epilepsy as well evidence of genetic association. The available data suggests a role for cytokines in epileptic processes, and it is important to define this role better in the future in both experimental and human studies.

YHTEENVETO

Aikaisemmin kokeellisissa epilepsiamalleissa on todettu lisääntyntä sytokiini tuotantoa aivoissa epileptisten kohtausten yhteydessä, mutta kliinisiä tutkimuksia epilepsipotilailla ei juurikaan ole tehty. Tämän tutkimuksen tarkoituksena oli etsiä epileptisten kohtausten yhteyttä sytokiiniverkoston aktivaatioon yksittäisten kohtausten sekä kroonisen epilepsian yhteydessä. Määritimme sytokiinipitoisuuksia selkäydinnesteessä ja plasmassa toonis-kloonisten kohtausten jälkeen eri ajankohtina selvittääksemme liittyykö epilepsia kohtauksiin lisääntyntä sytokiinien tuotantoa. Lisäksi mittasimme tulehdukseen liittyviä parametrejä verestä samoilla potilailla ja etsimme korrelaatiota sytokiini tuotantoon. Vaikeahoitoista kroonista epilepsiaa sairastavilla potilailla arvioimme sytokiinien alkuperää vertaamalla sytokiinien plasmatasoja veren monosyyttien kykyyn tuottaa sytokiineja. Tutkimme myös sytokiinien geneettisen monimuotoisuuden esiintyvyyttä vaikeahoitoista epilepsiaa sairastavilla potilailla.

Keräsimme selkäydinneste- ja plasmanäytteet 37 potilaalta korkeintaan 72 tuntia toonis-kloonisen epileptisen kohtauksen jälkeen sekä 14 potilaalta yli kaksi viikkoa kohtauksen jälkeen; potilailla ei ollut aikaisempaa epilepsiadiagnoosia tai epilepsialääkitystä. Interleukiini-6:n pitoisuudet (IL-6) olivat tilastollisesti merkittävästi koholla äskettäin epileptisen kohtauksen saaneilla potilailla verrattuna kontrollipotilaisiin tai yli kaksi viikkoa aikaisemmin epileptisen kohtauksen saaneisiin potilaisiin, koholla olevat pitoisuudet oli todettavissa ainoastaan niillä potilailla joilla näytteen otosta oli vähemmän kuin 24 tuntia kohtaukseen. Interleukiini-1 reseptori antagonistin pitoisuudet olivat koholla äskettäin kohtauksen saaneilla verrattuna muihin mutta eivät tilastollisesti merkittävästi. IL-1 β , TNF α tai hermokasvutekijän (NGF) pitoisuuksissa ei ollut eroa ryhmien välillä. IL-6 pitoisuudet korreloivat

perifeerisessä veressä todettaviin tulehdukseen liittyviin parametreihin kuten veren valkosolujen määrään. Lisäksi näillä potilailla oli kohonneita valkosoluarvoja selkäydinnesteessä ilman että kysymys olisi keskushermostoinfektiosta.

Vaikeahoitoista epilepsiaa sairastavilla potilailla (N=10) todettiin selkeästi proinflammatorinen profiili plasmapitoisuuksissa (korkea IL-6 pitoisuus, matala IL-1RA pitoisuus ja matala IL-1RA/IL-1 β suhde). Kuitenkin näiden potilaiden monosytäärisissä soluissa todettiin alentunut sytokiinituotanto stimulaatiotesteissä ilman että intrasellulaarisessa värjäyksessä olisi poikkeavuutta. Näin ollen todennäköinen alkuperä proinflammatorisille sytokiineille on aivot eikä veri.

Geneettisessä tutkimuksessa vertasimme 48 vaikeahoitoista epilepsiaa sairastavien sytokiinigeenien polymorfismia 400 terveeseen verenluovuttajaan. IL-1 α alleeli 1 ja IL-1 β alleeli 2 olivat merkittävästi yleisempiä epilepsiapotilailla, lisäksi IL-1 β :n ja IL-1RA:n sellainen yhdistelmä johon liittyy voimistunut proinflammatorinen sytokiinivaste oli merkittävästi yleisempi epilepsiapotilailla.

Sytokiinituotannon merkitystä epilepsiakohtausten yhteydessä ei vielä täysin tiedetä. Kuitenkin kokeellisissa tutkimuksissa on osoitettu että tietyillä sytokiineilla voi olla joko kohtauksia estävää tai lisäävää vaikutusta, lisäksi sytokiinit voivat vaikuttaa kohtauksiin liittyvään aivojen plastisiteettiin. IL-1 β on kokeellisissa malleissa prokonvulsiivinen ja neurotoksinen, IL-1RA:n vaikutukset ovat päinvastaiset. TNF α voi olla antikonvulsiivinen ja IL-6 prokonvulsiivinen. Me olemme tutkimuksessamme osoittaneet sytokiinijärjestelmän aktivaation epileptisiin kohtauksiin liittyen sekä akuuttien kohtausten että kroonisen epilepsian yhteydessä, jatkossa on keskeistä tarkemmin kartoittaa sytokiinien merkitystä sekä kokeellisissa malleissa että kliinisissä tutkimuksissa.

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