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Refractory Focal Epilepsy

Aetiology and immunological markers in adult patients

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LIST OF ORIGINAL PUBLICATIONS

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II Liimatainen S, Peltola M, Fallah M, Kharazmi E, Haapala A-M, Peltola J. The high prevalence of antiphospholipid antibodies in refractory focal epilepsy is related to recurrent seizures. Eur J Neurol. 2009;16:134-141.

III Liimatainen S, Fallah M, Kharazmi E, Peltola M, Peltola J. Interleukin-6 levels are increased in temporal lobe epilepsy but not in extra-temporal lobe epilepsy. J Neurol. 2009;256:796-802.

IV Liimatainen S, Peltola M, Sabater L, Fallah M, Kharazmi E, Haapala A-M, Dastidar P, Knip M, Saiz A, Peltola J. Clinical significance of glutamic acid decarboxylase antibodies in chronic epilepsy. Submitted for publication.

In addition, this thesis contains unpublished data.

ABBREVIATIONS

Ab	antibody
ACL	anticardiolipin
AED	antiepileptic drug
ANA	antinuclear antibody
Apo-H	apolipoprotein H
APS	antiphospholipid syndrome
APT	antiprothrombin
AVM	arteriovenous malformation
β_2GP1	β_2 glycoprotein 1
Ca	calsium
CA	cornu ammonis
CAE	childhood absence epilepsy
CD	cortical dysplasia
CI	confidence interval
CNS	central nervous system
CPS	complex partial seizures
CSF	cerebrospinal fluid
CT	computer tomography
EEG	electroencephalogram
ELISA	enzyme-linked immunosorbent assay
FLE	frontal lobe epilepsy
FS	febrile seizure
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
GluR3	glutamate receptor 3
GM1	monosialoganglioside 1
Gp130	glycoprotein 130
GPL	IgG phospholipid unit
HLA	human leukocyte antigen
HS	hippocampal sclerosis
IEF	immunoelectrophoresis
Ig	immunoglobulin
IGE	idiopathic generalised epilepsy
IHC	immunohistochemistry
IL	interleukin
IL-1RA	interleukin 1 receptor antagonist
ILAE	International League Against Epilepsy

IS	intrathecal synthesis
IVIG	intravenous immunoglobulin
JAE	juvenile absence epilepsy
JAK	Janus kinase
JME	juvenile myoclonus epilepsy
LANGAB	Langerhans cell antibody
LE	limbic encephalitis
LOCA	late-onset cerebellar ataxia
MHC	major histocompatibility complex
MPL	IgM phospholipid unit
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
Na	sodium
NFκB	nuclear factor kappa B
NPLE	non-paraneoplastic limbic encephalitis
OLE	occipital lobe epilepsy
OR	odds ratio
PBS	phosphate-buffered saline
PGE	primary generalised epilepsy
PLE	parietal lobe epilepsy
AGPC	anti-gastric parietal cell antibody
RE	Rasmussen´s encephalitis
RIA	radioimmunoassay
SD	standard deviation
SE	status epilepticus
SGU	standard IgG β_2-glycoprotein I unit
SLE	systemic lupus erythematosus
SPS	stiff person syndrome
T	Tesla
T1D	type 1 diabetes
Th	T-helper
TLE	temporal lobe epilepsy
TNF	tumour necrosis factor
TPO	thyroperoxidase
TYGL	thyroglobulin
VGKC	voltage-gated potassium channel
VNS	vagus nerve stimulation

1 INTRODUCTION

Epilepsy is one of the most common neurological diseases. The majority of the patients with epilepsy attain seizure freedom with the use of antiepileptic drugs (AEDs). However, approximately one-third of epilepsy patients continue to have seizures despite appropriate use of AEDs. The response to the first AED seems to predict the effect of subsequent AEDs, and few patients attain remission after the first two AEDs. Certain clinical predictors, such as aetiology, have been assessed in patients with newly diagnosed epilepsy, but few studies have investigated patients with refractory focal epilepsy at the onset of the study.

The central nervous system (CNS) is regarded as an immunologically specialised site with a variety of immunological functions. In experimental models, seizures may induce the production of cytokines such as interleukin-1β (IL-1β) and IL-6. Proinflammatory cytokines may also prolong the duration of seizures. Increased production of proinflammatory mediators has been detected in brain tissue of patients surgically treated for refractory epilepsy.

Increasing clinical evidence suggests a role for immunological factors in epilepsy, and immunological mechanisms have been shown to be involved in many epilepsy syndromes. In addition, the cytokine network is demonstrated to be activated after single seizures in patients with new-onset epilepsy. Epilepsy has been found to be associated with certain autoimmune diseases, such as systemic lupus erythematosus (SLE), and patients with epilepsy have shown an increased prevalence of specific autoantibodies even when no signs of connective tissue diseases are present. Monosialoganglioside 1 (GM1) and glutamic acid decarboxylase (GAD) antibodies have been detected in patients with refractory focal epilepsy. A proportion of patients with non-paraneoplastic limbic encephalitis (NPLE), characterised by subacute amnesia, disorientation, and seizures, possess antibodies to voltage-gated potassium channel (VGKC-abs). Some patients with NPLE and VGKC-abs, as well as other forms of epilepsy, benefit from immunomodulatory drugs.

The purpose of this dissertation was to study the clinical and immunological features of refractory focal epilepsy. First, we examined whether the aetiology of refractory focal epilepsy has an influence on the possibility of seizure remission. Second, we studied immunological markers in the blood to discover patients with clinically significant immunological alteration in their epilepsy. We wanted to better characterise which factors are associated with the activation of the immune system in patients with refractory epilepsy.

2 REVIEW OF THE LITERATURE

2.1 Epilepsy

Epilepsy is one of the most common neurological diseases, affecting 50 million people worldwide. It is defined as a susceptibility to recurrent seizures without precipitating factors. Epileptic seizures result from abnormal excessive or synchronous discharge in the brain (Fisher et al. 2005). The prevalence of active epilepsy is approximately 1%, but 8%-10% of the population has at least one seizure during their lifetime. Seizure incidence depends on age, as children and the elderly exhibit a greater incidence of seizure (Banerjee and Hauser 2007).

2.1.1 Classification of epilepsies and seizure types

Epilepsy can be classified based on seizure type, patient history, age at onset, other concomitant diseases, electroencephalogram (EEG) findings, and neuroimaging studies [brain magnetic resonance imaging (MRI) or computer tomography (CT)]. The seizure types can be divided into partial seizures, which begin in a part of one hemisphere, and generalised seizures, which are bilaterally symmetrical (ILAE classification; Commission 1981) (Tables 1 and 2). The aetiology of epilepsy is classified as remote symptomatic, probably symptomatic (cryptogenic), or idiopathic based on the medical history and the results of neuroimaging studies. The current classification has been criticised, and a proposal for improving the classification has been made. This proposal includes ictal phenomenology, seizure type, syndrome (if possible to define), aetiology, and impairment (optional) (Engel 2001).

Table 1 The International League Against Epilepsy (ILAE) classification of Epilepsies and Epilepsy related syndromes

I GENERALISED	
Idiopathic generalised epilepsies with age-related onset	1. Benign neonatal familiar convulsions 2. Benign neonatal convulsions 3. Benign myoclonic epilepsy in infancy 4. Childhood absence epilepsy 5. Juvenile absence epilepsy 6. Juvenile myoclonic epilepsy 7. Epilepsy with generalised tonic-clonic seizures on awakening
Cryptogenic or symptomatic generalised epilepsies	1. West syndrome 2. Lennox-Gastaut syndrome 3. Epilepsy with myoclonic-astatic seizures 4. Epilepsy with myoclonic absences
Symptomatic generalised epilepsies	1. Non-specific aetiology 2. Early myoclonic encephalopathies 3. Early infantile encephalopathy with burst suppression 4. Other symptomatic epilepsies not defined above
II LOCALISATION-RELATED	
Localisation-related epilepsies-idiopathic with age-related onset	1. Benign epilepsy with centrotemporal spikes 2. Childhood epilepsy with occipital paroxysm 3. Primary reading epilepsy
Localisation-related epilepsies-symptomatic	1. Epilepsia partialis continua 2. Syndromes characterised by specific modes of precipitation 3. Temporal lobe epilepsies
Localisation-related epilepsies-cryptogenic	
III EPILEPSIES AND SYNDROMES UNDETERMINED AS TO WHETHER FOCAL OR GENERALISED	
IV SPECIFIC SYNDROMES	

Table 2 ILAE classification of epileptic seizures

I PARTIAL (FOCAL) SEIZURES	
A Simple partial seizures	1. With motor signs 2. With somatosensory or special sensory symptoms 3. With autonomic symptoms or signs 4. With psychic symptoms
B Complex partial seizures	1. Simple partial onset followed by impairment of consciousness 2. With impairment of consciousness at onset
C Partial seizures evolving to secondarily generalised seizures	1. Simple partial seizures evolving to generalised seizures 2. Complex partial seizures evolving to generalised seizures 3. Simple partial seizures evolving to complex partial seizures evolving to generalised seizures
II GENERALISED SEIZURES (CONVULSIVE AND NONCONVULSIVE)	
Absence seizures	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	

2.1.2 Clinical significance of epilepsy type classification

From the points of view of drug treatment selection and epilepsy surgery, the classification of epilepsy based on anatomical localisation is important. In generalised seizures, large cortical and subcortical brain areas are involved from the onset. Focal seizures arising in different anatomical locations take different forms, although clinical features between regions do overlap (Shorvon 2000). Focal onset seizures can begin at any age, depending on the aetiology and associated neurological disorders.

2.1.2.1 Temporal lobe epilepsy

Temporal lobe epilepsy (TLE) is the most common epilepsy type in adulthood. Ictal clinical manifestations depend on the ictal onset zone or sites of propagation. The first symptom is often aura (simple partial seizure), with the most common being rising epigastric aura. Other auras include autonomic, olfactory, gustatory, psychic, or somatosensory symptoms. The aura may be followed by an altered consciousness (complex partial seizure) and, secondarily, a generalisation of the seizure (Table 2). Oro-alimentary or gestural automatisms are common. Speech arrest during a seizure is common in seizures arising in the dominant temporal lobe. (Shorvon 2000)

The most important aetiology of TLE is hippocampal sclerosis (HS), which is characterised by unilateral or bilateral atrophy and a signal change of the medial hippocampal structures in the brain MRI. On an EEG, seizure onset is typically localised to the ipsilateral temporal region. Histopathological findings are comprised of neuronal loss in the CA1 region of the hippocampal sclerosis (Mathern et al. 2007).

Despite AED treatment, the majority of patients with TLE have seizures. In particular in TLE+HS, the chances of remission with drug therapy are limited. In this situation, the possibility of epilepsy surgery should be evaluated without delay, as it has been demonstrated through carefully selected patient materials that two thirds of the TLE+HS patients achieve remission with surgery, whereas the majority of patients who receive drug only treatment suffer from refractory epilepsy (Wiebe 2004).

2.1.2.2 Extra-temporal lobe epilepsy

Focal-onset epilepsies originating outside the temporal lobe are called extra-temporal lobe epilepsies. The epileptogenic focus may be located in the frontal, parietal, or occipital lobes, the central region, or several foci may be present in the brain (multifocal epilepsy). The aetiologies of extra-temporal lobe epilepsy are heterogeneous, with different congenital, acquired, and cryptogenic causes.

Seizures of frontal-lobe origin can be complex partial, simple partial, or secondarily generalised seizures. The clinical and EEG features of complex partial seizures overlap with those of TLE, partially because of the rapid spread from the frontal lobe to the mesial temporal lobe. Complex partial seizures of frontal-lobe origin are frequent, clustered, stereotyped attacks. Automatisms are usually gestural, the behaviour may be bizarre, and prominent ictal posturing or version of the head and eyes may occur (Shorvon 2000). Manifestations of epilepsies arising in the central (perirolandic) region are motor or sensory. The motor features observed may include jerking, dystonic, posturing, or paralysis, and speech arrest can occur. Seizures arising in the parietal and occipital lobe are characterised by somatosensory and visual disturbances, illusions, sexual sensations, and head and eye turning or blinking. (Shorvon 2000)

2.1.2.3 Generalised epilepsy

Generalised seizures may impair consciousness from the time of onset. Motor symptoms and EEG changes are bilateral and symmetrical over both hemispheres. Common types of generalised seizures include absence, myoclonic, tonic, clonic, tonic-clonic, and atonic seizures. Generalised seizures commonly begin in later childhood, adolescence, or young adult life.

Typical absence seizures comprise a loss of consciousness and a cessation of motor activity. An ictal EEG shows a regular, symmetrical, synchronous 3-Hz spike-wave paroxysm. Atypical absence seizures differ from typical absence seizures in clinical form, EEG, and aetiology. Generalised seizures may occur as myoclonic seizures, with brief contraction of a muscle group caused by a cortical discharge. Clonic seizures consist of clonic, often asymmetrical, jerking. Tonic seizures consist of tonic muscle contraction with altered consciousness. Tonic-clonic (grand mal) seizures initiate with a loss of consciousness, after which a tonic phase of 10-30 seconds occurs. This phase is fol-

lowed by a clonic phase of 30-60 seconds, a brief tonic phase, and sometimes incontinence. Atonic seizures are characterised by a sudden loss of postural tone. (Shorvon 2000)

2.1.3 Aetiology of epilepsy

In adulthood, the prognosis of focal-onset epilepsy is usually poorer than that of primary generalised epilepsy (Semah et al. 1998, Kwan and Brodie 2000), and epilepsy with symptomatic aetiology is often uncontrolled. The aetiology of epilepsy varies in different age groups, with a predominance of congenital and genetic causes in childhood and post-stroke epilepsy in the elderly population. In lesional epilepsy, neuroimaging often shows the underlying cause of the disorder. If neuroimaging remains negative and the patient history does not reveal any relevant cause for seizures, focal-onset epilepsy is regarded as cryptogenic.

In some MRI-negative epilepsies, the aetiology may be revealed during epilepsy surgery by a histological analysis of the resected brain tissue. The epileptogenity of the lesion varies depending on the nature of the underlying brain pathology: cortical dysplasia (CD) is hyperexcitable, whereas in epilepsy with cavernous angiomas, the epileptogenity lies in the surrounding tissue (Farrell et al. 2007).

The most common causes of symptomatic epilepsy in adulthood are HS, CD, arteriovenous malformation (AVM), brain tumour, and stroke (Semah et al., 1998; Stephen et al. 2001) (Table 3). The most common aetiology for the resected epileptic brain tissue of children is CD; this aetiology is present in over 80% of children less than 3 years of age with intractable epilepsy (Vinters et al. 2007). Certain subtypes of CD are typically encountered in epilepsy, e.g., agenesis of the corpus callosum, schizencephaly, hemimegencephaly, subedendymal heterotopias, and polymicrogyria (Shorvon 2000). Tuberous sclerosis is an autosomal-dominant, multisystem disorder associated with age-related types of epilepsy in approximately 80% of cases (Vinters et al. 2007).

AVMs are the vascular malformations most likely to cause epilepsy. Other vascular malformations associated with the possibility of epilepsy are cavernous hemangiomas and venous angiomas (Vinters et al. 2007). Epileptic seizures are common in patients with primary or metastatic brain tumours (Schaller and Rüegg 2003). Seizures occur in 80% of patients with a low-grade glioma, in almost 33% of those with a high-grade glioma, in 20% of those with a meningioma, and in 10% of patients with a central nervous system (CNS) lymphoma (Hildebrand 2004). The response to AED

therapy is often poor, and surgical tumour removal may be unpredicted (Schaller and Rüegg 2003). In patients with a traumatic brain injury, the risk of post-traumatic epilepsy depends on the severity of the brain injury (Annegers et al. 2000).

In addition to lesional causes of epilepsy, genetic aspects play an important role. In idiopathic generalised epilepsy and primary epileptic syndromes, genetic factors influence the risk of seizures. There are also single-gene disorders that demonstrate an autosomal dominant form of inheritance (Ottman and Winawer 2007).

Table 3 Aetiology of epilepsy (modified from Shorvon 2000)

INHERITED GENETIC	Epilepsy alone
	Epilepsy and other neurological manifestations
ACQUIRED	Trauma
	Neurosurgery
	Infection
	Vascular disease
	Hippocampal sclerosis
	Tumour
	Neurodegenerative disorder
	Metabolic disorder
	Toxic disorder
	Miscellaneous: celiac, Whipple´s disease, demyelinating disease, vasculitis
CONGENITAL (INHERITED OR ACQUIRED)	Cortical dysplasia or dysgenesis
	Cerebral tumour
	Vascular malformation
	Prenatal injury

2.1.4 Treatment of epilepsy

2.1.4.1 Drugs

The main goal of epilepsy treatment is cessation of seizures without intolerable side effects. AEDs protect against seizures through interactions with a variety of cellular targets, including various ion

channels, neurotransmitter transporters, neurotransmitter metabolic enzymes, and synaptic vesicle proteins. The actions of AEDs on these targets can be categorised into those that involve modulation of voltage-dependent ion channels [mainly sodium (Na) or calcium (Ca) channels], effects on γ-aminobutyric acid (GABA) systems (including alterations in the cellular disposition of GABA and enhancement of synaptic inhibition mediated by $GABA_A$ receptors), inhibition of synaptic excitation mediated by ionotropic glutamate receptors, and modulation of neurotransmitter release, particularly of glutamate, via presynaptic mechanisms (Table 4) (Macdonald and Rogawski 2007).

An accurate classification of epilepsy is important in choosing the first AED. The choice of medication should take into consideration different clinical factors, such as the individual characteristics of the patient and the drugs (Kwan and Brodie 2002). Established AEDs include barbiturates, benzodiazepines, carbamazepine, ethosuximide, phenytoin, and valproic acid. Felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, pregabaline, rufinamide, tiagabine, topiramate, vigabatrin, and zonisamide are considered as newer AEDs (Macdonald and Rogawski 2007). Carbamazepine, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, topiramate, and valproic acid are preferred first-line AEDs for the treatment of new-onset focal epilepsies, whereas lamotrigine, topiramate, and valproic acid are first-line AEDs for patients with idiopathic generalised epilepsies (Table 4). When monotherapy fails, adding a second drug and substitution monotherapy are common options. In addition to the first-line AEDs, pregabalin, zonisamide, and clobazam can be considered in refractory focal epilepsy. In refractory idiopathic generalised epilepsy, clobazam and levetiracetam are additional options (Elger and Schmidt 2008). Furthermore, adrenocorticotropic hormone and steroids have been used to treat certain seizure types and epileptic syndromes after unsuccessful trials of standard AEDs (Hrachovy and Frost 2007).

Table 4 Molecular targets of antiepileptic drugs and their actions in different seizure types (modified from Mac-Donald and Rogawski 2007)

Drug	Sodium channels	Calcium channels	GABA system	Glutamate receptors	Partial onset seizure	Primary GTC seizure	Absence seizure	Myoclonic seizure
Benzodiazepines			$GABA_A$ R		+	+	+	+
Carbamazepine	+				+			
Ethosuximide	+?	T-type					+	(+)
Felbamate	+	HVA	$GABA_A$ R	NMDA	+?			
Gabapentin		HVA α2δ	GABA turnover		+			
Lamotrigine	+	HVA			+	+	(+)	+
Levetiracetam		HVA			+	(+)	(+)	+
Oxcarbazepine	+				+			
Phenobarbital		HVA	$GABA_A$ R	AMPA	+	+		+
Phenytoin	+				+			
Pregabalin		HVA α2δ	GABA turnover		+			
Tiagabine			GABA transporter		+			
Topiramate	+	HVA	$GABA_A$ R	AMPA	+	+		+
Valproic acid	+	+	GABA-T		+	+	+	+
Vigabatrin			GABA-T		+			
Zonisamide	+	T-type			+	+	(+)	+

GABA = γ-aminobutyric acid; GTC = generalised tonic-clonic; $GABA_A$ R = $GABA_A$ receptor; HVA = high voltage activated; NMDA = N-methyl-D-aspartate; AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; GABA-T = GABA aminotransferase.

2.1.4.2 Surgery

Presurgical evaluation should be performed without delay if focal epilepsy proves to be refractory to AED treatment (Cascino 2004). There are proposals for the consideration of surgery or combination AED therapy after the failure of the first two AEDs due to a lack of efficacy or poor tolerability (Kwan and Brodie 2002).

Surgical procedures to treat epilepsy include lesionectomy, lobectomy, corticectomy, multiple subpial transection, corpus callosotomy, and combinations of these procedures (Duchowny et al. 2007). Amygdalohippocampal resection is an established method for the treatment of TLE+HS, demonstrating a 75% chance of remission in well-selected patients (Spencer 2002). In other aetiologies often considered for surgery (CD, AVM and tumour), the response to surgery depends on multiple factors, including the possibility of removing the entire lesion and the epileptogenic zone (Yeh et al.

1990, Palmini et al. 1991, Dodick et al. 1994, Zentner et al. 1997, Luyken et al. 2003). In a cryptogenic partial epilepsy, the chance of remission after surgery is considered <50% (Engel 1996).

2.1.4.3 Vagus nerve stimulation

For patients who are refractory to both AED therapy and surgical intervention, the goal of treatment is often no longer seizure freedom, but a reduction in the number of seizures or a reduction in seizure severity while maintaining tolerability. Such patients may benefit from the application of a vagus nerve stimulator (VNS). The mechanism of action of VNS is not fully known, but it is probably mediated by competition between different neuronal networks in the brain (Faingold 2008).

2.1.5 Refractory epilepsy

Although a majority of patients with epilepsy can be successfully treated with AEDs, 25%-30% of the patients continue to have seizures, with extensive effects on their health as well as psychosocial, cognitive, and economic consequences (Table 5) (Kwan and Brodie 2002). There are no single predictive markers of refractoriness, but a combination of risk factors together may aid in evaluating the outcome of the epilepsy (Arts et al. 1999, Regesta and Tanganelli 1999, Arroyo et al. 2002). However, new prognostic factors, such as different biomarkers, are needed to identify which patients are at risk of developing refractory epilepsy.

Table 5 Dimensions of refractory epilepsy (modified from Kwan and Brodie 2002)

Intractable seizures
Excessive drug burden
Neurobiochemical plastic changes
Cognitive decline
Psychosocial problems
Dependent behaviour
Restricted lifestyle
Unsatisfactory quality of life
Increased mortality

2.1.5.1 Prognosis of epilepsy

The response to the first antiepileptic drug (AED) has a significant impact on the prognosis of epilepsy. In a large hospital-based study of 780 newly diagnosed epilepsy patients, 50.4% of the patients became seizure free with the first AED, 10.7% with the second, and only 2.7% with the third. There were no significant differences between different AEDs with regards to the responder rates (Mohanraj and Brodie 2006). Three studies evaluated the possibility of remission in an adult population with refractory epilepsy. In a study of 246 patients with refractory epilepsy (defined as having at least 1 seizure per month after two AEDs), 15% obtained seizure remission (Callaghan et al. 2007). Of the 15%, 4.5% of the patients became seizure free with surgery. Among patients treated only medically, 12% became seizure free. 81% of the patients presented localisation-related epilepsy, 6.9% had primary generalised epilepsy, 11% had symptomatic generalised epilepsy, and 1.6% had other epilepsy syndromes. Seizure type and epilepsy syndrome were not predictive of seizure remission.

In a study of 155 patients with chronic epilepsy (defined as epilepsy active at least 5 years after the initiation of therapy, no epilepsy surgery candidates), 28% achieved remission using a new AED (Luciano and Shorvon 2007); 21% of the patients had primary generalised seizures and 79% had partial-onset seizures. Those with idiopathic epilepsy had a greater chance of becoming seizure free. Another study evaluated the outcome of drug treatment in 34 patients with medically refractory localisation-related epilepsy who were evaluated for possible epilepsy surgery but were deemed to be inadequate surgical candidates. In that study, 21% of the patients achieved seizure freedom for an average of 2.5 years (Selwa et al. 2003).

Neuroimaging studies have shown progressive volume loss of mesial temporal lobe structures to be associated with the duration of epilepsy (Bernasconi et al. 2005, Salmenperä et al. 2005). Conversely, in a large hospital-based study, the duration of epilepsy was not a prognostic factor of the response to an AED (Mohanraj and Brodie 2006). There are controversial aspects regarding the concept that “seizures beget seizures”. In a retrospective study, patients with an increasing number of seizures had an increased risk of subsequent seizures (Hauser and Lee 2002), and MRI has revealed greater hippocampal volume reduction in TLE-patients with frequent seizures compared with patients with well-controlled epilepsy (Kälviäinen et al. 1998). The initial seizure frequency may predict a poor prognosis (Berg et al. 2001). Conversely, an apparently benign state in the beginning does not always indicate a good long-term outcome (Berg et al. 2003). Some studies have

suggested that abnormal EEG activity is associated with a poorer outcome (Caviedes and Herranz 1998, Altunbaşak et al. 1999, Arts et al. 1999).

The chances of seizure freedom with AEDs are better among adolescents and the elderly (Mohanraj and Brodie 2006). This may reflect the aetiologies of different age groups. Young patients have epileptic syndromes with good prognoses, such as juvenile myoclonus epilepsy (JME), while post-stroke epilepsy is one of the major aetiologies of epilepsy in elderly patients, a condition with a rather high chance of remission with drug therapy (Gupta et al. 1988, Stephen et al. 2006).

A few studies have evaluated the effect of epilepsy aetiology on remission. In a hospital-based study comprising 2200 adult epilepsy patients, TLE+HS was the most refractory symptomatic epilepsy, as only 11% of the patients with TLE+HS achieved seizure freedom (Semah et al. 1998). Dual pathology (HS associated with another brain lesion) as an aetiology decreased the percentage of seizure freedom to 3% (Semah et al. 1998). Acquired epilepsies, such as those following stroke or those due to the development of a vascular malformation or tumour, seemed to be more responsive to treatment (Semah et al. 1998). In a study including 550 adult patients with partial epilepsy, 42% of the patients with HS, 54% of the patients with CD, and 35% of the patients with a brain tumour were seizure free. The location of the lobar epileptogenic zone did not have a significant effect on the chances of seizure freedom (Semah et al. 1998).

Finally, patients may be labelled as refractory because they are on the wrong drug, taking the wrong dose, or not taking a drug at all (French 2007).

2.1.5.2 Definition of refractory epilepsy

Unifying criteria of refractoriness in epilepsy are still lacking. Definitions of refractoriness are always artificial because the patient population is heterogeneous and the need of a definition depends on the purpose for which the definition is being used (Berg 2006, French 2006). However, the response to the first two drugs often predicts the response to subsequent AEDs (Kwan and Brodie 2000, Mohanraj and Brodie 2006). In clinical practice, the failure of two AEDs at maximum tolerated doses should lead to the consideration of epilepsy surgery in patients with focal epilepsy (Spencer 2002, Berg 2006, Mohanraj and Brodie 2006). An additional approach to defining refractoriness is to consider the duration of non-responsiveness to medications. The duration of persistent

seizures can be 6 months or 1 to 2 years, provided a certain number of AEDs have been demonstrated ineffective (Berg 2006).

2.1.5.3 Biological processes in refractory epilepsy

Epileptogenesis is a cascade of events starting from a known or unknown initial precipitating event, e.g., perinatal hypoxia or anoxia, stroke, traumatic brain injury, CNS infection, or status epilepticus. This initial precipitating event triggers critical modulators that produce long-lasting structural and functional changes, including inflammation. Such changes may eventually be expressed as recurrent seizures in susceptible individuals, particularly if the individuals are subjected to another neurological insult - "a second hit" (Sander and White 2001, Pitkänen and Sutula 2002, Walker et al. 2002). The neuronal reorganisation continues to result in seizure control or refractory epilepsy, depending on multiple risk factors (Pitkänen and Sutula 2002).

Two main hypotheses have emerged to explain the failure of AED therapy: target and transporter hypotheses. The target hypothesis suggests that drugs fail because of alterations in the properties of their usual targets. The drugs may also fail because multidrug transporter mechanisms limit their concentrations at their targets (transporter hypothesis) (Sisodiya 2007).

Thus far, there is no convincing evidence favouring the neuroprotective effects of any AED (Löscher 2002). Furthermore, whether the mechanisms of refractoriness are identical to those of epileptogenesis has not been demonstrated (Sisodiya 2007).

2.2 Epilepsy and the immune system

The CNS has long been regarded as an immunoprivileged site with no immunological activity. This opinion was based on the presence of the blood brain barrier (BBB), the lack of major histocompatibility complex 1 (MHC1) antigens, and the absence of lymphatic drainage in the CNS (Barker and Billingham 1977). Currently, the CNS is considered an immunologically specialised site with both innate and acquired immunity (Wekerle et al. 1986). In epilepsy, the first studies concerning immunology focused on the immunosuppressive side effects of AEDs, such as IgA deficiency (Sorrell and Forbes 1975, Aarli 1976). However, it was later found that epilepsy is more common in some patients with autoimmune diseases than in the normal population (Mackworth-Young and

Hughes 1985). Epilepsy patients were found to have disorders of the immune system that were not explained by the use of AEDs alone (Bassanini et al. 1982, Bostantjopoulou et al. 1994).

Rasmussen´s encephalitis (RE), a prototype of "immunological epilepsy", is a disease characterised by progressive unilateral brain dysfunction and epilepsia partialis continua. The histopathology reveals perivascular lymphocytic cuffing and scattered microglial nodules. The first experimental immunological study in epilepsy was conducted with rabbits in which seizures and an RE-like condition were induced by injecting glutamate receptor 3 (GluR3) antibodies (Rogers et al. 1994). Furthermore, sera from patients with RE were found to contain GluR3 antibodies (Rogers et al. 1994).

The use of Ig in epilepsy was first observed 1977 when it was given to children with recurrent upper respiratory tract infections (Pèchadre et al. 1977). Those patients with concomitant epilepsy experienced beneficial effects from Ig, with a reduction of seizure frequency and severity. Since then, several reports of the anticonvulsant effects of non-steroidal anti-inflammatory drugs and glucocorticoids have emerged. Numerous case reports and small uncontrolled studies have suggested the benefit of intravenous Ig (IVIG) in certain catastrophic childhood epilepsies (Villani et al. 2007).

Some epilepsy patients with autoantibodies have undergone immunotherapy trials, with remarkable benefits in single cases (Table 6). The mechanism of action of immunotherapy in epilepsy is not fully understood, but both immunologic and non-immunologic effects have been suggested (Villani et al. 2007). Currently, however, immunological therapy is not an established treatment option for epilepsy.

Since the findings obtained for RE, a growing amount of both experimental and clinical evidence for the role of immunological factors in some patients with epilepsy has been obtained. Histological evidence has indicated immunological activity, especially in refractory TLE. Increased receptor and protein expression of cytokines have been observed in experimental models of epilepsy and in resected human temporal lobes (Sheng et al. 1994, de Simoni et al. 2000, Crespel et al. 2002, Ravizza et al. 2008). Many autoantibodies have been found to be associated with different forms of epilepsies. Additional, although controversial, evidence of the immune system's involvement in the aetiopathogenesis of certain forms of epilepsy has been provided by immunogenetic studies. An association between TLE+HS and homozygotism for the IL-1β-511 allele 2 (Kanemoto et al. 2000), as well as increased frequencies of the IL-1α allele 1 and IL-1β allele 2 in patients with focal epilepsy compared with control subjects have been reported (Peltola et al. 2001). However, other au-

thors have not been able to confirm these findings. Studies have found a significant association between TLE+HS and the occurrence of HLA-DQ2, -DR4, and –DR7 alleles, and a combination of alleles HLA-DR4-DQ2 and DR7-DQ2 (Özkara et al. 2002).

Support for an inflammatory aetiology for certain forms of epilepsy comes from the association of certain autoimmune diseases with an increased incidence of epilepsy. In addition, several other initial precipitating events may activate epileptogenesis and a chronic inflammation. Epileptic seizure can disrupt the BBB, and thus make it possible for immune cells to invade the brain (Zucker et al. 1983). Activated T lymphocytes can penetrate the BBB with the help of adhesion molecules (Wekerle et al. 1986). Despite the lack of conventional lymphatic drainage, an immune response may be activated through the blood-subarachnoidal space. Conversely, brain-derived immune mediators may activate the immune system in the blood through the hypothalamus-pituitary-adrenal axis (for a review see Esch and Stefano 2002, Vezzani et al. 2007).

2.2.1 Cytokines in epilepsy

Inflammatory cytokines are a group of soluble mediators that either are products of cells of the immune system or act upon them. The cytokines include interleukins (ILs), tumour necrosis factors (TNFs), chemokines, and growth factors (Janeway et al. 2005). A complex cytokine network is active in the CNS both during normal conditions and in pathological states (Jankowsky and Patterson 1999). The balance between proinflammatory and anti-inflammatory immune responses depends on the differentiation of T helper (Th) cells into either proinflammatory Th1 or anti-inflammatory Th2 cells (Abbas et al. 1996).

2.2.1.1 IL-1 family of cytokines

IL-1 was one of the first cytokines to be described (Dinarello 1984). It is secreted by antigen-presenting phagocytic cells, such as dendritic cells and macrophages. There are two forms of IL-1, IL-1α and IL-1β, both of which are proinflammatory cytokines and agonists of the type I IL-1 receptor. Their actions are antagonised by the IL-1 receptor antagonist (IL-1RA), which has no other known function than blocking the functions of IL-1α and IL-1β. There is also an IL-1 receptor type II, which acts as a decoy receptor (Janeway et al. 2005).

2.2.1.2 IL-6 family of cytokines

IL-6 is a proinflammatory cytokine secreted by T cells and macrophages. It is one of the most important mediators of fever and the acute phase response (Janeway et al. 2005). IL-6 signals through a cytokine receptor complex consisting of its specific receptor (IL-6 receptor) and a signal-transducing component, gp130. Binding of IL-6 to this receptor complex leads to activation of the intracellular tyrosine kinases of the Janus kinase family (JAKs) (Gadient and Otten 1997).

2.2.1.3 The cytokine network in human epilepsy

Several cytokine messenger ribonucleic acids (mRNAs) and related proteins have been shown to be induced in rodents after chemoconvulsant or electrical stimulation (Minami et al. 1991, Eriksson et al. 1998, de Simoni et al. 2000). The activation of inflammation is specifically increased in those brain areas involved in seizure onset and spread (de Simoni et al. 2000). IL-1β significantly exacerbates seizure activity when applied before the induction of seizure (Vezzani et al. 1999). Conversely, intracerebral injection of IL-1RA has powerful anticonvulsant effects (Vezzani et al. 2002), and mice overexpressing IL-1RA have a reduced risk of seizures (Vezzani et al. 2000). Intranasally applied exogenous IL-6 has a proconvulsive effect on mice (Kalueff et al. 2004). Transgenic mice overexpressing IL-6 in glia have seizures and neurodegeneration (Campbell et al. 1993, Penkowa et al. 2001), suggesting the proinflammatory nature of IL-6 in the chronic state.

Nuclear factor kappa B (NFκB) is a transcriptional factor that mediates inflammatory reactions (Crespel et al. 2002). Overexpression of NFκB and increased production of IL-1α (Sheng et al. 1994) have been found in resected hippocampi of epileptic adults. Increased expression of the IL-1β system has been demonstrated in patients with CD and refractory epilepsy (Ravizza et al. 2006). The number of IL-1β and IL-1RI+ cells has been positively correlated with the frequency of seizures, whereas the number of IL-1RA cells has been negatively correlated with the duration of epilepsy (Ravizza et al. 2006).

There is clinical evidence of a temporal association of cytokine network activation with seizures in adult patients with epilepsy. The concentration of IL-6 increases rapidly and transiently after single seizures in patients with new-onset epilepsy, along with a trend toward an increased IL-1RA concentration (Peltola et al. 1998, Peltola et al. 2000a). These findings were observed both in plasma

and the CSF, although the concentrations in the CSF were ten times higher than those in the plasma, suggesting an intrathecal origin of the cytokines. The increase in IL-6 was positively correlated with the severity of epilepsy (Lehtimäki et al. 2004), and the increased concentrations of IL-6 and IL-1RA have also been observed interictally (Lehtimäki et al. 2004, Lehtimäki et al. 2007). In children with febrile seizures (FSs), there is a significant increase in IL-1β production in monocytes stimulated with lipopolysaccharide, suggesting the susceptibility of peripheral immune cells to produce proinflammatory cytokines (Helminen and Vesikari 1990). A high plasma IL-1RA/IL-1β ratio and increased plasma levels of IL-6 have also been detected in patients with FS (Virta et al. 2002). These findings indicate that inflammatory cytokines may be associated with an active epileptic process.

2.2.2 Autoantibodies and epilepsy

Evidence has demonstrated the presence of autoantibodies against brain antigens and, in some circumstances, their association with seizures (Aarli 1993). In addition to GluR3 antibodies in RE, increasing evidence for the association of autoantibodies and epilepsy has emerged (Table 6). In experimental studies, antibodies against monosialogangliosides (GM1) have induced seizures (Karpiak et al. 1981), and anti-GM1 antibodies have been found in patients with focal epilepsy (Bartolomei et al. 1996).

Limbic encephalitis (LE) is a rare disorder that is characterised by memory loss, confusion, psychic symptoms, and seizures. Some patients with the non-paraneoplastic form of LE harbour voltage-gated potassium channel (VGKC) antibodies (Vincent et al. 2004). Immunotherapy in these patients has proven effective, with decreases in the levels of VGKC antibodies (Vincent et al. 2004). Interestingly, antibodies against the dietary protein gluten were recently detected in patients with TLE+HS (Peltola et al. 2009).

Table 6 Neurological diseases characterized by epilepsy and autoantibodies (modified from Bernasconi et al. 2002)

Disease	Antibody target	Immunotherapy
RE	GluR3	IVIG, plasma exchange or protein A
	Munc18-1	immunoadsorption in GluR3
Systemic lupus erythematosus (SLE)	Phospholipid	Not reported
Primary generalised before SLE onset	Cardiolipin	
Focal or generalised-tonic during SLE	β_2-glycoprotein I	
Therapy-resistant localisation-related epilepsy	Cardiolipin	IVIG in GM1
	Nuclear	Corticosteroids, azathioprine, cyclo-
	β_2-glycoprotein I	phosphamide in GAD
	GAD	
	GM1 ganglioside	
Newly diagnosed seizure	Cardiolipin	Not reported
	Nuclear	
	β_2-glycoprotein I	
Generalised epilepsy syndromes	Cardiolipin	Not reported
West´s syndrome		Corticosteroids, intravenous therapy
Cryptogenic Lennox-Gastaut syndrome	Haemocyanin	IVIG
Controlled focal epilepsy	GAD	Not reported
Limbic encephalitis with seizures	Voltage-gated potassium	Corticosteroids, plasma exchange,
	channel	IVIG
TLE+HS	Gluten	Not reported
Partial epilepsy	NMDA receptor	Not reported

RE = Rasmussen´s encephalitis; TLE+HS = temporal lobe epilepsy with hippocampal sclerosis; GluR3 = glutamate receptor 3 antibody; Munc18-1 = a member of the Sec/Munc family of syntaxin-binding proteins; GAD = glutamic acid decarboxylase; IVIG = intravenous immunoglobulin; NMDA = N-methyl-D-aspartate.

2.2.2.1 Antiphospholipid and antinuclear antibodies in epilepsy

Cardiolipin is an important component of the inner mitochondrial membrane. Anticardiolipin antibodies belong to the family of phospholipid antibodies and can be classified in two ways: IgM, IgG, and IgA or β_2-glycoprotein-dependent or -independent. β_2-glycoprotein I (β_2GP I) has been identified as apolipoprotein H (Apo-H) and is required for the recognition of anticardiolipin antibodies in autoimmune diseases. Anticardiolipin antibodies that bind Apo-H are associated with increased thrombosis.

Antinuclear antibodies are a group of autoantibodies that have the ability to attack structures in the nucleus. Antiphospholipid and antinuclear antibodies have conventionally been found in systemic lupus erythematosus (SLE), which is an autoimmune disorder characterised by systemic and organ-specific inflammation. Antiphospholipid antibodies are also associated with antiphospholipid syndrome (APS), an acquired autoimmune prothrombotic disease presenting with venous and arterial thrombosis, recurrent miscarriages, and thrombocytopenia.

The prevalence of epilepsy in SLE is remarkably high, 17%-37%, and in some patients, seizures begin many years before the onset of other symptoms of SLE. In this situation, epilepsy is typically primary generalised, whereas seizures after the clinical onset of SLE may be either focal or generalised (Aarli 2000). In SLE, epilepsy is associated with the presence of antiphospholipid antibodies (Herranz et al. 1994). Antiphospholipid and antinuclear antibodies have also been documented in epilepsy patients without signs of SLE (Table 7). On the basis of the results, the antiphospholipid antibodies would be associated with complicated epilepsy.

2.2.2.2 GAD antibodies and epilepsy

GAD is the enzyme that catalyses the conversion of glutamic acid to GABA, the main inhibitory neurotransmitter in the CNS. GAD exists in two isoforms, GAD_{65} and GAD_{67}, with molecular weights of 65 and 67 kDa. GAD_{65} and GAD_{67} have different distributions within the neural cytoplasm, with a preponderance of GAD_{65} in nerve terminals. Apart from its presence in the central and peripheral nervous system, GAD is observed within β cells of the pancreas, epithelial cells of the fallopian tube, and the spermatozoa of the testes. (Ellis and Atkinson 1996)

Table 7 Studies reporting on the associations of epilepsy with antiphospholipid or antinuclear antibodies in patients without SLE (modified from Billiau et al. 2005)

Authors	Type of epilepsy	Number of patients	Main findings (patients vs control subjects)
Andrews et al. 1990	RE	1	ANA +
Verrot et al. 1997	Epilepsy	163	IgG ACL Ab: 19% vs 3%; ANA: 35% vs 10%
Angelini et al. 1998	Cryptogenic focal epilepsy	23	APL in 3 patients
Peltola et al. 2000	New onset, focal and generalised epilepsy	152	IgG ACL: 21-14-8% vs 7%
Eriksson et al. 2001	Epileptic children	50	APL: 44% vs 10%
Yoshimura et al. 2001	Epileptic children, benign infantile convulsions	9	ACL: 89%
Cimaz et al. 2002	Epilepsy	142	ACL: 10% ; Aβ_2GPI: 17.6%; APT: 12.6%; no control subjects
Debourdeau et al. 2004	Epilepsy	81	ACL: 4% vs 7%; Aβ_2GPI: 5% vs 6%; ANA: 17% vs 11%
Ranua et al. 2004	Epileptic adults	960	ACL: 4.5% vs 5% (increased prevalence with long duration and uncontrolled epilepsy); ANA: 17% vs 17%
Markić et al. 2007	Epileptic children	40	ACL: 7.5% vs 0%; Aβ_2GPI: 2.5% vs 0%; ANA: 0% vs 0%

RE = Rasmussen´s encephalitis; ANA = antinuclear antibody; ACL = anticardiolipin antibody; APL = antiphospholipid antibody; Aβ_2GP1 = anti-β_2 glycoprotein 1 antibody; APT = antiprothrombin antibody.

Increased titres of antibodies against GAD (GADA) were first observed in type 1 diabetes (T1D). GADAs are present in approximately 80% of newly diagnosed patients with T1D and can be detected many years before the clinical onset of the disease (Baekkeskov et al. 1990). In T1D, titres are typically low, whereas high titres have been found in some rare neurological diseases, such as Stiff Person Syndrome (SPS) (Solimena et al. 1990) and late onset cerebellar ataxia (LOCA) (Honnorat et al. 2001). SPS is a neurological disorder characterised by progressive muscular rigidity of central origin involving mainly axial and proximal skeletal muscles with superimposed spasms (Lorish and Thorsteinsson 1989). GADAs are present at remarkably high titres in the serum and

CSF of 90% of the patients with a typical SPS (Solimena et al. 1990, Brown and Marsden 1999). Most patients respond to an immunomodulative treatment (Dalakas 2005). Oligoclonal IgG bands occur frequently in the CSF, and autoimmune diseases and organ-specific autoantibodies are present in a majority of the patients with GADA and SPS or LOCA (Solimena and De Camilli 1991, Honnorat et al. 2001, Saiz et al 2008), suggesting an autoimmune-mediated pathogenesis of these diseases.

The presence of GADA in epilepsy is supported by several case reports and a few clinical studies (Solimena et al. 1988, Nemni et al. 1994, Giometto et al. 1998, Peltola et al. 2000c, McKnight et al. 2005, Yoshimoto et al. 2005, Vulliemoz et al. 2007, Kanter et al. 2008) (Table 8). Single case reports show varying responses to immunotherapy. A recent report describes a patient with an extremely drug-resistant, long-term status epilepticus with the need for prolonged mechanical ventilation. Intensive AED therapy, high-dose steroids, IVIG, and plasma exchange demonstrated no effect, but cyclophosphamide resulted in lasting seizure control (Kanter et al. 2008). In a new-onset T1D, the injection of recombinant human GAD may contribute to the preservation of residual insulin secretion (Ludvigsson et al. 2008).

Although GABA is the principal inhibitory neurotransmitter in the brain and the impairment of GABA activity may lead to a predominance of excitatory neurotransmitters such as glutamic acid, the pathogenic significance of GADA in neurological diseases is not fully known. There is evidence that GADA in patients with SPS, LOCA, or epilepsy reduces GAD enzyme activity and GABA synthesis or transmission (Dinkel et al. 1998, Ishida et al. 1999, Mitoma et al. 2000, Takenoshita et al. 2001, Vianello et al. 2008). Antibody epitopes differ in different diseases with GADA and may have different pathophysiological significance and clinical sequelae, depending on their effects on GABA-glutamic acid conversion (Butler et al. 1993, Ellis and Atkinson 1996, Dinkel et al. 1998, Vianello et al. 2005).

Table 8 Prevalence studies and case reports on glutamic acid decarboxylase antibodies in refractory epilepsy (modified from Kanter et al. 2008)

Authors	Type of epilepsy	Number of patients	Main findings	Other features
Nemni et al. 1994	CPS	1	S-GADA	
Giometto et al. 1998	CPS	1	S-GADA 1:64000, CSF GADA 1:1500	Transient remission with corticosteroid
Kwan et al. 2000	Focal and generalised	105	No association between GADA and duration/seizure frequency	4 patients with high GADA and uncontrolled epilepsy
Peltola et al. 2000	Therapy-resistant	100	Significant association between GADA and localisation-related epilepsy	2 patients with high GADA had TLE and IS
Sokol et al. 2004	Uncontrolled focal epilepsy with MTS	31	No significant prevalence of GADA	
McKnight et al. 2005	Mixed syndromes	139	GADA associated with young onset chronic drug-resistant epilepsy and normal MRI	VGKC in 11%
Yoshimoto et al. 2005	CPS	1	S-GADA 41700 U/ml, CSF GADA 311 U/ml, GADA-index 1.62	
Vulliemoz et al. 2007	CPS	1	GADA-index (IS) 28.8, cerebellar ataxia	Limited effect on seizures with methylprednisolone and azathioprine
Kanter et al. 2008	Status epilepticus of CPS with secondarily generalisation	1	S-GADA 6500 U/ml, CSF GADA 997 U/ml, GADA-index (IS) 18.2, recovery with cyclophosphamide	

CPS = complex partial seizure; GADA = glutamic acid decarboxylase antibody; TLE = temporal lobe epilepsy; IS= intrathecal synthesis; MTS = mesial temporal sclerosis; MRI = magnetic resonance imaging; VGKC = voltage-gated potassium channel antibody.

3 PURPOSE OF THE STUDY

In the present thesis, we investigated clinical and immunological markers in patients with refractory epilepsy. We attempted to find patients with clinically significant immunological alterations in their epilepsy. The more specific purposes of the study were:

1. To evaluate the effect of epilepsy aetiology in patients with refractory focal epilepsy on the chances of seizure remission.

2. To determine the clinical characteristics of patients with antiphospholipid, antinuclear, and anti-β2 glycoprotein 1 antibodies and refractory focal epilepsy.

3. To determine the clinical characteristics of patients with refractory focal epilepsy associated with increased cytokine production.

4. To identify the clinical determinants of patients with refractory focal or generalised epilepsy and increased titres of antibodies against glutamic acid decarboxylase.

4 MATERIALS AND METHODS

The study patients were treated at the Outpatient Clinic of Neurology and Rehabilitation, Tampere University Hospital. Blood and CSF samples were taken after informed consent was received from the study patients. Patients were fully informed of the risks and benefits of the CSF sampling (study IV). The study was approved by the Ethics Committee of Tampere University Hospital. The number of patients in each study varied depending on the time of the analyses and the number of blood samples available. The study patients in studies I-III comprised a varying proportion of all of the patients in study IV.

4.1 Control subjects

The control samples were obtained from blood donors (studies II-III) and non-diabetic organ donors without a history of epilepsy (study IV) (Table 10). Information regarding the clinical status, sex, and accurate age of the control subjects was lacking in studies II and III. According to the Finnish guidelines, common autoimmune diseases are not generally contraindications for blood donation, except diabetes with drug therapy. The blood donors displayed no signs of infection during a 2-week period before the blood sampling. The organ donors did not have any chronic diseases or organ dysfunction preventing donation: subjects with a clinical diagnosis of diabetes were excluded. There were no subjects with SPS in study IV.

4.2 Study patients

Study I included 119 patients with refractory focal epilepsy defined as persistent seizures after the administration of two different AEDs (sequential maximally tolerated doses or in combination) and a duration of epilepsy of at least 2 years (Table 9). Study II included 95 patients, and study III included 86 patients with refractory focal epilepsy. In addition, studies II and III included 10 and 5 patients, respectively, with the use of only one AED and an epilepsy duration of at least 2 years (Table 10). In addition to patients with refractory focal (184) and controlled epilepsy (25), study IV also included 44 patients with primary generalised seizures (Table 10). Patients with dementia, moderate or severe mental retardation, or malignant high-grade brain tumours and epilepsy were excluded. Patients with post-stroke epilepsy were excluded in studies I-III, but included in study IV.

We followed each patient for at least two years. All patients had scheduled outpatient visits. The visits consisted of a patient interview (including seizure frequency between previous and current visits from a seizure diary), clinical and laboratory examinations to determine liver, renal, and endocrine functions, and drug concentrations when considered important. No seizures were evident for 24 hours prior to sampling. At the end of the follow-up, two blood samples were collected at a three-month interval from patients with recurring seizures to evaluate the stability of the prevalence of antibodies or cytokines (studies II and III). In patients with one-year seizure freedom, only the first blood sample was obtained (studies II and III). In study IV, analyses were carried out using the first samples.

The majority of the study patients had undergone high-resolution brain MRI with a specific epilepsy protocol (Commission 1997) evaluated by an experienced neuroradiologist. The majority of the patients with refractory epilepsy had been evaluated for the possibility of epilepsy surgery. Epilepsy was classified according to the International League Against Epilepsy (ILAE) guidelines (Commission 1989) based on the electroclinical findings (seizure semiology and EEG/video-EEG) and aetiology. In patients with focal epilepsy, epilepsy types were categorised into temporal (TLE), frontal (FLE), parietal (PLE), occipital lobe (OLE), multifocal, and unclassified epilepsies (studies I-IV). In patients with primary generalised epilepsy (PGE), epilepsy syndrome types were classified as follows: childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), JME, and idiopathic generalised epilepsy (IGE) (study IV).

The aetiology of epilepsy was defined based on the results of the imaging studies, histological analysis of tumours (if available), and medical history according to the following categories: cryptogenic (probably symptomatic), tumour, vascular malformation (AVM, venous angioma, or cavernous angioma), perinatal vascular lesion (brain infarction, intracerebral haemorrhage, or anoxia), trauma, HS, other hippocampal abnormality (vascular malformation, demyelination, or atrophy), CD (cortical dysgenesis, heterotopia, or tuberosis sclerosis), CNS infection (encephalitis, meningitis, or abscess), dual pathology, and other (local or diffuse atrophy, non-specific signal change, demyelination, or non-specific gliosis). If the medical history revealed head trauma with loss of consciousness or a confirmed CNS infection prior to the onset of epilepsy, it was considered to be the cause of the epilepsy despite normal imaging results. We compared the symptomatic aetiologies according to the possibilities of surgical treatment (studies I, III, and IV). The aetiologies often con-

sidered for surgical treatment included HS, CD, vascular malformation, tumour, and dual pathology. The remaining symptomatic aetiologies were classified as not often considered for surgery.

Data concerning age, sex, duration of epilepsy, surgical treatment (epilepsy surgery or other lesional surgery) (studies I-IV), most often-used AEDs (studies I and III), all previous and current AEDs (study III), and concomitant autoimmune diseases (including diabetes) were collected (studies II and IV). Data concerning other diseases were not systematically collected. Current AEDs were recorded for patients with GADA (study IV). All modern AEDs during the time of the studies were available except pregabaline, zonisamide, and rufinamide, which were not licensed in Finland at that time. The most common, current AEDs (in monotherapy or in combination) were carbamazepine, lamotrigine, topiramate, and valproic acid. Seizure frequency during the 2-year follow-up was recorded in the aetiology study, as was the seizure frequency during the last 1, 3, and 12 months prior to blood sampling (studies II-IV).

Table 9 Clinical characteristics of the study patients for study I

N	119
Median age (years) (range)	40 (15-75)
Sex female/male (n)	67/52
Epilepsy surgery (n)	14
High resolution brain MRI (n)	113
Proportion of abnormal EEG (n)	108
Video-EEG (n)	79
Median duration of epilepsy (years) (range)	22 (2-66)
Number of AEDs 2-3/4-7/≥8 (n)	27/56/36

MRI = magnetic resonance imaging; EEG = electroencephalogram; AED = antiepileptic drug.

Table 10 Clinical characteristics of patients and control subjects for studies II-IV

	Study II		Study III		Study IV	
	Controls	Patients	Controls	Patients	Controls	Patients
Total (N)	70	105	63	91	200	253
Age group (n)						
16-25	14	14	8	10	22	58
26-35	14	23	12	19	12	55
36-45	14	27	15	22	52	57
46-55	15	24	15	24	72	41
56+	13	17	13	16	42	42
Sex (n)						
Female	-	61	-	55	77	135
Male	-	44	-	36	123	118
Surgery (n)						
No surgery	-	80	-	71	-	213
Epilepsy surgery	-	13	-	10	-	19
Other lesional surgery	-	12	-	10	-	21
Epilepsy type (n)						
TLE	-	70	-	63	-	139
Extra-TLE	-	35	-	28	-	80
IGE	-	0	-	0	-	34
Aetiology (n)						
HS + dual pathology	-	19	-	17	-	34
Cortical dysplasia	-	13	-	9	-	30
Cryptogenic/idiopathic	-	33	-	31	-	97
Other causes	-	40	-	34	-	92
Seizure status (n)						
1-year free	-	16	-	17	-	50
1-11 per year	-	13	-	9	-	75
≥12 per year	-	76	-	65	-	128
Seizure frequency (/year)						
mean	-	68	-	66	-	58
range	-	0-720	-	0-720	-	0-730
Epilepsy duration (years)						
mean	-	25	-	25	-	23
range	-	3-68	-	2-67	-	0-68

TLE = temporal lobe epilepsy; extra-TLE = extra-temporal lobe epilepsy; IGE = idiopathic generalised epilepsy; HS = hippocampal sclerosis.

4.3 Methods

Blood samples were collected between 09:00 and 14:00 during scheduled outpatient visits. Cytokines are secreted in a biphasic circadian pattern, but we considered the time period acceptable. The exact time of blood sampling was not known for the control subjects. The samples were stored frozen at -70°C. Laboratory analyses were performed at the Centre of Laboratory Medicine, Tampere University Hospital (antiphospholipid, anti-β2-glycoprotein 1, antinuclear, anti-gastric parietal cell, Langerhans cell, thyroperoxidase, thyroglobulin, and GM1 antibodies), at the Neuroimmunological Laboratory, University of Tampere (IL-6 and IL-1RA), at the Scientific Laboratory, Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland (GADA), and at the Service of Neurology and the Institut d´Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Hospital Clínic, Barcelona, Spain [GADA IHC and immunoblot (WB)].

4.3.1 Antiphospholipid antibodies

IgG and IgM class anticardiolipin antibodies were detected by a commercial enzyme immunoassay (Quanta Lite ACA IgG and IgM, INOVA Diagnostics, San Diego, California). Results were expressed in GPL and MPL units, respectively. According to the manufacturer's and the laboratory's own reference materials, values from 15-20 GPL for the IgG class and 12.5-20 MPL for the IgM class were considered as low positive, values from 21-80 GPL/MPL as moderately positive, and values over 80 GPL/MPL as high positive. Antibodies against β2- glycoprotein I were also measured using a commercial enzyme immunoassay (Quanta Lite B2 GPI, IgG, INOVA Diagnostics, San Diego, California). Values ≥ 20 SGU were considered as positive.

4.3.2 Antinuclear antibodies

IgG class antinuclear antibodies were measured by a standard indirect immunofluorescence method using Hep-2 cells as antigens (INOVA Diagnostics, San Diego, California). Sera were diluted 1:80 and 1:160 in phosphate-buffered saline (PBS) and incubated on substrate slides along with the appropriate controls. Fluorescence-conjugated anti-human globulin antiserum was used as the conjugate. The slides were examined using fluorescence microscopy and the titres of the positive sera were determined.

4.3.3 IL-6 and IL-1RA

IL-6 concentrations were measured using enzyme-linked immunosorbent assay (ELISA) kits (Pelikine Compact, Sanquin, Amsterdam, The Netherlands). IL-1RA concentrations were also determined using an ELISA kit (R&D Systems, Minneapolis, MN). All kits were used according to the manufacturers' instructions. The sensitivity of the assays for IL-6 and IL-1RA were 0.4 pg/ml and 22 pg/ml, respectively. Samples were considered positive if the level of IL-6 or IL-1RA was higher than +3 standard deviation (SD) above the mean value of the control group.

4.3.4 Antibodies to glutamic acid decarboxylase

4.3.4.1 Radioimmunoassay (RIA)

GADA in sera was analysed with a radiobinding assay (RIA), as previously described (Savola et al. 1998). The cut-off limit for GADA positivity was based on the 99th percentile in more than 370 non-diabetic subjects and corresponded to 5.36 RU/ml. The GADA samples with initial values between the 97.5th and 99.5th percentiles were reanalysed to confirm the autoantibody status.

The cut-off limit for high GADA levels was defined based on the results of the patients with positive GADA titres. There were two distinct groups; one with high titres (>1000 RU/ml) associated with polyendocrinopathies and autoimmune diseases and the other with low titres without associated diseases (GADA <1000 RU/ml).

4.3.4.2 Immunohistochemistry (IHC)

The positive samples of 11 out of 15 patients with GADA in RIA were confirmed by IHC of sera and CSF (study IV). Wistar rats were anesthetised and perfused with saline, followed with 4% paraformaldehyde in PBS. The cerebellum was fixed with 4% paraformaldehyde for 4 hours and cryoprotected with 20% sucrose in PBS overnight. Frozen sections (4 μm) were air-dried and, after inhibition of endogenous peroxidases with 0.3% hydrogen peroxide in PBS, were sequentially incubated with 10% normal goat serum (NGS) for 20 minutes, the patient's serum (screening dilution 1:500) for 3 hours at 37°C, biotinylated goat anti-human IgG for 30 minutes, and the avidin-biotin immunoperoxidase complex (Vector Labs, USA) for 30 minutes. The reaction was developed with

0.05% diaminobenzidine tetrahydrochloride (Sigma) with 0.01% hydrogen peroxide in PBS and 0.5% Triton X-100. Antibody dilutions were made in PBS containing 0.3% Triton X-100.

4.3.4.3 WB

WBs of serum and CSF were performed for 11 of 15 patients with GADA in RIA (study IV). Human GAD_{65} recombinant protein (Diamyd Medical AB, Stockholm, Sweden) (0.12 μg/lane) was electrophoretically separated in a 4%-12% sodium dodecyl sulfate-polyacrylamide gel and transferred to nitrocellulose. After blocking with 5% dry carnation milk and 10% NGS, the strips were incubated with patient serum (1:1,000 dilution) or CSF (1:5 dilution) or serum from an SPS patient with GADA as a positive control for 12 hours at room temperature. The strips were then washed with PBS, incubated with biotinylated goat anti-human IgG (diluted 1:2,000) or horse anti-mouse IgG (diluted 1: 1,000) in 10% NGS for 1 hour, and processed as described for the IHC technique.

4.3.4.4 CSF measurements (study IV)

CSF samples were obtained from 11 of 15 patients with GADA to measure the cell count, lactate and protein content, IgG index, immunoelectrophoresis (IEF), IHC, and WB. IHC and WB were performed for the sera of 11 of 15 patients with GADA. Intrathecal synthesis (IS) of GADA was measured using the following formula: CSF GADA titre/Serum GADA titre:CSF albumin (mg/l)/Serum albumin (mg/l). Values higher than the IgG index, in particular those higher than 1, were considered as strong indicators for IS of antibody-specific IgG (Dalakas et al. 2001).

4.3.5 Polyautoimmunity measurements

In patients with increased levels of GADA and in 47-56 randomly selected GADA-negative patients with focal epilepsy (study IV), the following autoantibodies were measured in the serum samples: IgG class anticardiolipin antibodies, anti-β2-glycoprotein 1 antibodies, antinuclear antibodies, anti-gastric parietal cell antibodies, Langerhans cell antibodies, thyroperoxidase antibodies, thyroglobulin antibodies, and IgM and IgG class GM1 antibodies.

4.3.6 Statistical methods

4.3.6.1 Study I

To estimate differences between 12-month remission patients and other subjects, the frequencies were calculated using cross-tabulations. By fitting a logistic regression model to the data, the odds ratios (OR), the confidence intervals (CI), and p-values (p) of persistent seizures were obtained. The importance of predictors was shown using unstandardised coefficients. All analyses were performed with SPSS software.

4.3.6.2 Studies II-IV

A logistic regression analysis was performed for all binary outcome variables to search for the odds ratios. Five covariates (age, sex, epilepsy syndrome diagnosis, duration of epilepsy, and aetiology in patients) were considered in the fully-adjusted regression models. The only available variables for control subjects were age group and level of antibodies (study II), cytokines (study III), and GADA (study IV). The Fisher's exact test was applied to calculate the p-value when the chi-square test was not applicable. All analyses were performed using STATA statistical software.

5 RESULTS

5.1 Aetiology of refractory epilepsy

The most common aetiologies of refractory epilepsy were cryptogenic, CD, HS, vascular malformation, brain tumour, and trauma. 25% of the patients had achieved a 12-month seizure remission (Table 11). The possibility of remission was significantly higher in patients with a cryptogenic aetiology compared with symptomatic aetiologies (age-adjusted OR 3.74, 95% CI 1.54-9.07, p=0.004). Epilepsies with aetiologies often considered for surgery (epilepsy surgery or lesional surgery) displayed a significantly higher risk of persistent seizures (88.5%) compared with cryptogenic epilepsy (60.0%) (age-adjusted OR 5.85, 95% CI 2.00-17.11, p=0.001). The symptomatic aetiologies not often considered for surgery carried an increased risk of persistent seizures compared to the cryptogenic aetiology (age-adjusted OR 1.73, 95% CI 0.55-5.46, p=0.347).

In patients with a 12-month remission, 40% had experienced seizures for <15 years, 30% for 15-29 years, and a total of 30% for ≥30 years before attaining remission. In the logistic regression analysis, sex was not associated with the possibility of achieving remission. Patients 20-64 years of age exhibited a 5-fold risk of having persistent seizures compared to patients <20 years of age (OR 5.06, 95% CI 1.32-19.42, p=0.018). Patients that had used at least 8 AEDs displayed an almost 5-fold risk of having persistent seizures compared to patients with 2-3 AEDs (age-adjusted OR 4.72, 95% CI 1.25-17.75, p=0.022). Patients with 4-7 AEDs had an almost 2-fold risk of having persistent seizures compared to patients with 2-3 AEDs (age-adjusted OR 1.95, 95% CI 0.72-5.32, p=0.19).

Table 11 Aetiology of epilepsy in patients with 12-month seizure remission and persistent seizures

	12-month remission (n=30[3])	Persistent seizures (n=89[11])	Total (n=119[14])
Effect of aetiology (n)			
Symptomatic	12	62	74
Often considered for surgery	6	46	52
Not often considered for surgery	6	16	22
Cryptogenic	18	27	45
Aetiology (n)			
Trauma	2[1]	6[1]	8[2]
HS	1[1]	10[2]	11[3]
Other hippocampal pathology	2	5[2]	7[2]
Vascular lesion	1	3	4
Tumour	1	7[1]	8[1]
CD	2	12[2]	14[2]
CNS infection	2[1]	4	6[1]
Dual pathology	0	4	4
Vascular malformation	0	8	8
Other cause	1	3	4
Cryptogenic	18	27[3]	45[3]

HS = hippocampal sclerosis; CD = cortical dysplasia; CNS = central nervous system. Superscripts present the number of patients undergone epilepsy or other lesional surgery.

In addition, 4 patients with vascular malformation had undergone an embolisation procedure.

5.2 Antiphospholipid and antinuclear antibodies in patients with refractory focal epilepsy

Patients exhibited a significantly greater prevalence of IgG class anticardiolipin antibodies (25.7%) compared to control subjects (12.9%; p=0.043) (Tables 12 and 13). The mean titre of IgG class anticardiolipin in patients was 11.7 GPL (range, 7.5-80 GPL), and that in control subjects was 9.7 GPL (range, 7-35 GPL) (Table 13). Patients with recent seizures (one or more seizures in the month prior to blood sampling) displayed a greater prevalence of IgG class anticardiolipin antibodies

(29%) compared to patients with no recent seizures (11%; age-adjusted OR 4.00, 95% CI 0.84-19.02) and control subjects (12.9%; age-adjusted OR 3.09, 95% CI 1.30-7.34). The patients with recent seizures demonstrated an increased prevalence of moderate positive IgG class anticardiolipin antibodies compared to the control subjects and to patients with no recent seizures (age-adjusted OR 4.45, 95% CI 1.14-17.36). The patients with recent seizures also displayed an increased prevalence of low-positive IgG class anticardiolipin antibodies compared to the patients with no recent seizures and to the control subjects (age-adjusted OR 2.78, 95% CI 1.07-7.22). There was no significant difference between control subjects and the patients with no recent seizures (age-adjusted OR 0.72, 95% CI 0.14-3.72).

The prevalence of IgG class anticardiolipin antibodies in the patients with HS and CD was twice that of patients with cryptogenic and other causes of epilepsy, but the results were not significant (OR for HS: 2.17, 95% CI 0.62-7.57; OR for CD: 2.32, 95% CI 0.58-9.36). The prevalence of IgM class anticardiolipin antibodies was significantly higher in the patients with CD compared to the patients with cryptogenic epilepsy (age-adjusted OR 5.04, 95% CI 1.22- 20.89). The prevalence of antinuclear or anti-β_2-glycoprotein I antibodies was not associated with certain aetiologies. Age, gender, and duration of epilepsy were not associated with the prevalence of IgG or IgM class anticardiolipin, anti-β_2-glycoprotein I, or antinuclear antibodies. The prevalence of IgM class anticardiolipin or antinuclear antibodies was not significantly different between patients with recent seizures, patients with no recent seizures, or control subjects. All patients with positive anti-β_2-glycoprotein I antibody titres had experienced recent seizures. We did not find any significant difference in the prevalence of antibodies between the patients with TLE and those with extra-TLE ($p>0.825$) (Table 12). Changes in the seropositivity of IgG and IgM class anticardiolipin antibodies were not related to seizure frequency in the three-month interval between the two samples, aetiology, epilepsy type, age at diagnosis of epilepsy, sex, or age.

5.3 Cytokines in refractory focal epilepsy

The prevalence of increased levels of IL-6 was higher in epilepsy patients (11%) compared to control subjects (0%) ($p=0.007$) (Table 12). The mean level of IL-6 in patients was 2.5 pg/ml (range, 0.4-10.4), and the mean level in control subjects was 2.1 pg/ml (range, 0.7-5 pg/ml) (Table 13). The 10 patients with increased concentrations of IL-6 had TLE (including one patient with multifocal epilepsy involving the temporal lobe), whereas none of the patients with extra-TLE demonstrated

elevated levels (p=0.028). The mean and median serum levels of IL-6 were also higher in the patients with TLE compared to the patients with extra-TLE [p=0.042, Wilcoxon rank-sum (Mann-Whitney) test].

Seven patients with increased levels of IL-6 had frequent seizures, whereas three patients had not experienced seizures for at least one year. The aetiology or categorised aetiology was not significantly associated with abnormally high levels of IL-6. Age, sex, seizure frequency, a history of epilepsy surgery, and duration of epilepsy were not associated with the level of IL-6. Among patients with TLE, those with increased levels of IL-6 tended to be older than those with a normal IL-6 concentration (OR 1.02, 95% CI 0.97-1.07); they also demonstrated a longer duration (in years) of epilepsy (OR 1.03, 95% CI 0.98-1.08) and 80% of them were women ($OR_{female\ vs.\ male}$ 2.84, 95% CI 0.55-14.68), although none of these differences were statistically significant.

The patients with TLE tended to exhibit smaller increases in the levels of IL-1RA (OR 0.42, 95% CI 0.08-2.21) (Table 12), and the mean and median serum levels of IL-1RA were lower in these patients compared to the patients with extra-TLE [p=0.664, Wilcoxon rank sum (Mann-Whitney) test] (Table 12), although none of these findings were statistically significant. The prevalence of IL-1RA was not significantly different between patients with recurrent seizures, patients with one year seizure remission, or control subjects.

The changes in the levels of IL-6 or IL-1RA during a three-month interval between the two samples were not related to seizure frequency, the aetiology category, epilepsy type, duration, sex, or age.

We combined the data from studies II and III to detect associations between anticardiolipin and antinuclear antibodies and IL-6 and IL-1RA (unpublished data). No statistically significant associations between IgG or IgM class anticardiolipin or antinuclear antibodies and IL-6 or IL-1RA were observed, either by logistic or by linear regression analysis. A positive association was determined between anti-β_2 glycoprotein 1 antibody (normal/positive) and IL-6 (OR 8.11, 95% CI 0.47-141.21), but it was not statistically significant.

5.4 Glutamic acid decarboxylase antibodies in chronic epilepsy

The clinical characteristics of patients with GADA in RIA are presented in Table 14. Fifteen patients with epilepsy (5.9%) exhibited GADA in the serum, whereas only three of the control sub-

jects (1.5%: p=0.026) tested positive for GADA (Table 12). Seven (2.8%) patients had high GADA titres [≥1000 relative units (RU)/ml], six of whom had temporal lobe epilepsy (TLE). All three GADA-positive control subjects demonstrated low titres. The mean GADA level in patients was 1,694.7 RU/ml (range, 0-207,770 RU/ml), and the mean level in control subjects was 0.6 RU/ml (range, 0-52 RU/ml) (Table 13). One patient with high GADA levels had been classified as having IGE, although the classification of her epilepsy was challenging as features of both primary generalised and focal seizures were present. All patients with high GADA levels and focal epilepsy had TLE ($p_{\text{Fisher's exact test}}$=0.182). The prevalence of GADA was 1.5 times higher in the patients with TLE than that in the patients with extra-TLE (OR 1.32, 95% CI 0.39-4.42; p=0.657). All patients with high GADA titres had several organ-specific autoantibodies and autoimmune diseases (Table 14); the number of autoantibodies was significantly higher in the patients with high GADA titres compared to both the patients with low GADA titres (p=0.001) and the patients with normal GADA titres (p<0.001) (Figure 1).

The epilepsy type (TLE vs. extra-TLE) or the presence of high GADA titres (>1000 RU/ml) were not significantly associated with age, sex, epilepsy duration, number of seizures in the month or the year before blood sampling, aetiology, or epilepsy surgery. In the univariate or multivariate logistic regression analyses, GADA positivity was not significantly associated with age, sex, seizure frequency before sampling, duration, epilepsy type (TLE vs. extra-TLE), or the aetiology of the epilepsy.

Six of the seven serum samples from the patients with high GADA levels and all five available CSF samples exhibited a typical GAD pattern by IHC. All of these serum and CSF samples recognised linear epitopes of GAD_{65} in the WB. GADA IS was demonstrated in two patients (Table 14).

IS of GADA was found in Patients 6 and 7 (Table 14). Patient 6 (GADA 4569 RU/ml) had celiac disease, IgA deficiency, and juvenile rheumatoid arthritis. Patient 7 (GADA 1680 RU/ml) had been diagnosed with T1D. She also had demyelinating disease with typical MRI and CSF findings, but no symptoms other than epilepsy. Patient 3 (38,138 RU/ml) exhibited oligoclonal bands in the CSF. Patient 2 was included in our earlier study when he was found to have a high serum GADA titre (107,000 RU/ml) and a positive IS of GADA (Peltola et al. 2000c). He has been in remission since undergoing an amygdalohippocampal resection, but his serum GADA titre has remained high (142,520 RU/ml), although there is currently no evidence of IS of GADA.

No consistent findings were detected by neuroimaging in relation to the GADA levels. The 10 patients with GADA also participated in study II; 2 patients with a high GADA level also had an increased IL-6 level, whereas 8 patients with GADA had normal IL-6 levels. In 5 patients with GADA, IL-6 was not measured (unpublished data).

Table 12 Percentages of positive levels of antiphospholipid and antinuclear antibodies, cytokines and glutamic acid decarboxylase antibodies in patients and control subjects

	Study II									Study III					Study IV				
		ACL IgG		ACL IgM		Aβ2GPI		ANA			IL-6		IL-1RA			GADA			
	Total	Positive		Positive		Positive		Positive		Total	Positive		Positive		Total	Positive		High positive	
	n	% of n	P value	% of n	P value	% of n	P value	% of n	P value	n	% of n	P value	% of n	P value	n	% of n	P value	% of n	P value
Control subjects	70	12.9		20.0		0.0		7.1		63	0.0		3.2		200	1.5		0.0	
Patients	105	25.7	0.043	30.5	0.125	2.9	0.276	13.3	0.204	91	11.0	0.007	6.6	0.358	253	5.9	0.026	2.8	0.019
TLE	70	25.7	1.000	31.4	0.764	2.9	1.000	14.3	1.000	63	15.9	0.028	4.8	0.303	139	6.5	0.773	4.3	0.088
Extra-TLE	35	25.7		28.6		2.9		12.9		28	0.0		10.7		80	5.0		0.0	.
IGE	0	-	-	-	-	-	-	-	-	0	-	-	-	-	34	5.9	1.000	2.9	0.298

APL = antiphospholipid antibody; ACL = anticardiolipin antibody; $A\beta_2GPI$ = anti-β_2-glycoprotein I antibody; ANA = antinuclear antibody; IL-6 = interleukin-6; IL-1RA = interleukin-1 receptor antagonist; GADA = glutamic acid decarboxylase antibody; TLE = temporal lobe epilepsy; extra-TLE = extra-temporal lobe epilepsy. TLE includes multifocal epilepsy involving temporal lobe; extra-TLE includes patients with unknown epilepsy type (all the patients with unknown epilepsy type had focal epilepsy). Significance compared to control subjects, in patients significance compared to extra-TLE.

Table 13 Serum levels of antiphospholipid, antinuclear antibodies, cytokines and glutamic acid decarboxylase antibodies in patients and control subjects

	Control subjects								Patients								*p* value*
	n	mean	SD	min	Q1	median	Q3	max	n	mean	SD	min	Q1	median	Q3	max	
Study II																	
ACL IgG (GPL)	70	9.7	4.0	7	7.5	7.5	12	25	105	11.7	10.0	7.5	7.5	7.5	15	80	0.643
ACL IgM (MPL)	70	10.0	13.1	6	6	6	6	102	105	9.8	7.2	6	6	6	14	52	0.146
Aβ2GPI (SGU)	70	10.0	0.0	10	10	10	10	10	105	10.7	4.1	10	10	10	10	38	0.155
ANA (titre)	70	50.9	72.0	40	40	40	40	640	105	59.0	122.4	40	40	40	40	1280	0.195
Study III																	
IL-6 (pg/ml)	63	2.1	1.0	0.7	1.3	1.8	2.7	5	91	2.5	1.9	0.4	1.3	2	2.9	10.4	0.564
IL-1 RA (pg/ml)	63	282.8	152.5	42	200	250	330	970	91	340.8	296.1	110	195	250	380	2300	0.833
Study IV																	
GADA (RU/ml)	200	0.6	4.2	0	0	0	0	52	253	1694.7	16,049.5	0	0	0	0	207,770	0.001

SD = standard deviation; Q = quartile; ACL = anticardiolipin antibody; Aβ_2GPI = anti- β_2-glycoprotein I antibody; ANA = antinuclear antibody; IL-6 = interleukin-6; IL-1RA = interleukin 1 receptor antagonist; GAD = glutamic acid decarboxylase antibody. * Wilcoxon rank-sum (Mann-Whitney) test.

Table 14 Clinical characteristics of patients with glutamic acid decarboxylase antibodies in serum

Patient	Gender	Age	Seizure frequency per year	Epilepsy type	Aetiology	Autoimmune diseases	GAD S-RIA (RU/ml)	Other autoantibodies	IS of GADA
1	F	42	1	TLE	Cryptogenic	No	207,770	TPO, IgM GM1, LANGAB	No
2	M	54	0	TLE	HS	CD	142,520	TPO, TYGL, IgM GM1, LANGAB	No
3	F	32	365	TLE	Cryptogenic	Thyroiditis, psoriasis	38,138	TPO, TYGL, IgM GM1, LANGAB	No
4	F	72	1	IGE	Idiopathic	Asthma bronchiale	24,124	ANA, AB2GP1, IgM GM1, LANGAB	Not done
5	M	71	26	TLE	HC atrophy	No	8534	TPO, IgG GM1, LANGAB	Not done
6	F	31	48	TLE	Cryptogenic	CD, IgA deficiency, juvenile RA	4569	ANA, IgG GM1	Yes
7	F	45	60	TLE	Other cause	T1D, demyelinating disease	1680	TPO, AB2GP1, IgM GM1, IgG GM1	Yes
8	F	58	72	TLE	HS	No	800	AGPC, IgM GM1	No
9	M	57	0	Unknown	Trauma	No	22.5	IgG GM1	Not done
10	F	60	4	FLE	Trauma	No	14.1	No antibodies	No
11	F	18	1	JME	Idiopathic	No	12.3	IgG GM1	Not done
12	F	40	24	TLE	HC atrophy	No	10.9	No antibodies	No
13	F	24	1	TLE	Cryptogenic	No	7.6	IgM GM1, IgG GM1	No
14	M	37	24	FLE	Trauma	No	6.4	No antibodies	No
15	M	48	365	FLE	Cryptogenic	No	6.3	IgG GM1	No

F = female; M = male; TLE = temporal lobe epilepsy; IGE = idiopathic generalised epilepsy; FLE = frontal lobe epilepsy; JME = juvenile myoclonic epilepsy; HS = hippocampal sclerosis; HC = hippocampus; CD = celiac disease; IgA = immunoglobulin A; T1D = type 1 diabetes; RA = rheumatoid arthritis; S = serum; RIA = radioimmunoassay; IS = intrathecal synthesis; TPO = thyroperoxidase antibody; GM1 = GM1 antibody; LANGAB = Langerhans cell antibody; TYGL = thyroglobulin antibody; ANA = antinuclear antibody; Aβ2GP1 = anti-β2glycoprotein 1 antibody; AGPC = anti-gastric parietal cell antibody.

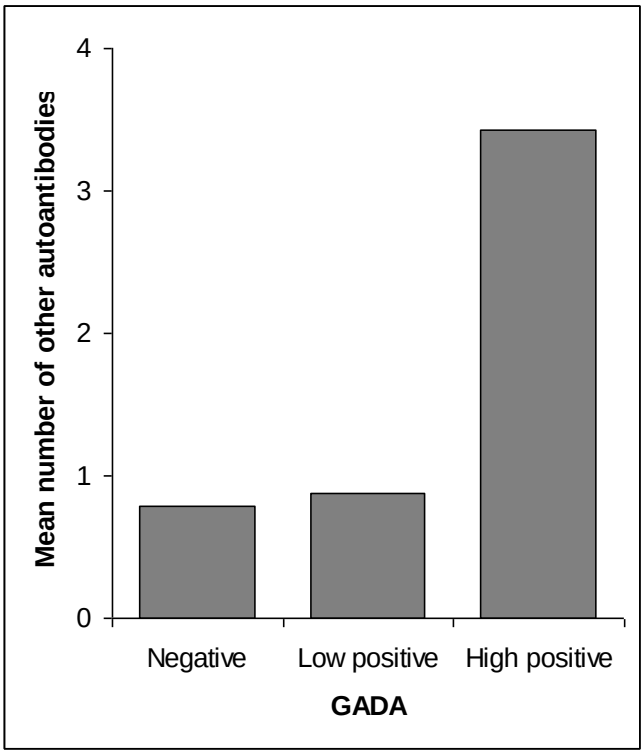

Figure 1 Mean number of other autoantibodies in patients with negative, low positive and high positive GADA-titres.

6 DISCUSSION

Epilepsy is a significant neurological disorder that is characterised by recurrent seizures. Currently, there are more than 20 different AEDs on the market. Despite increasing possibilities for drug therapy and drug combinations, approximately one third of patients have recurrent seizures. This may have an enormous impact on the patient's life. There is a need for better characterisation of the clinical determinants in those patients in order to find prognostic factors and new treatment options for their epilepsy. In this dissertation, we studied the effects of aetiology and different immunological markers on the clinical features of refractory epilepsy.

The strength of our studies was a comprehensive, well-examined patient population with precise classification of epilepsies. The classification of epilepsies has improved with the aid of developed techniques, such as video-EEG and high-resolution MRI. Previous studies have mostly lent on differentiation to partial or generalised epilepsies with a higher proportion of unclassified epilepsy types. In our studies, the proportion of unclassified epilepsies was remarkably low, at only a few percent. We have been able to accurately classify epilepsy types into temporal and frontal lobe epilepsies instead of non-specific frontotemporal lobe epilepsies. The study design was prospective with the utility of exact-source document information. Seizure frequencies were collected from a reliable seizure register. For the autoantibody measurements, standardised and well-controlled assays were employed.

Our study possessed certain weaknesses. Clinical characterisation for control subjects was incomplete, with data missing for the exact time of sampling, age, sex, and autoimmune diseases (in studies II and III), as well as a lack of exact data concerning other diseases (study IV). However, the significance of the missing data was reduced by statistical differences between the patient groups. We excluded patients with moderate or severe mental retardation due to local practices (these patients are not followed in our centre). This may have reduced the applicability of the results to the whole patient group with refractory epilepsy.

6.1 The effect of aetiology on refractoriness

In this dissertation, we studied patients with refractory focal epilepsy and found that a significant proportion of them (25%) achieved seizure remission during the follow-up, some after a remarkably

long duration of epilepsy. The chances of remission were significantly higher in patients with cryptogenic aetiology compared to patients with symptomatic aetiology, especially to those with aetiologies often considered for surgical intervention.

Earlier studies have shown that a very small percentage of patients with epilepsy become seizure free after two or three AEDs (Kwan and Brodie 2000, Mohanraj and Brodie 2006). Overall response rates for the first AED are considered to be 50%, whereas response rates with the second and the third AEDs decrease significantly. The present study and three recent studies (Selwa et al. 2003, Callaghan et al. 2007, Luciano and Shorvon 2007) provide hope for patients with refractory epilepsy, since there are always individuals that achieve remission after administration of a new AED, no matter how many AEDs have failed in the past. Nevertheless, it is not always clear whether remission is due to changes in the AEDs or the natural fluctuation of seizure frequencies in some patients. However, patients with a 12-month seizure remission usually continue to be seizure free throughout longer follow-up (Callaghan et al 2007).

All of our study patients with vascular malformation and dual pathology, as well as an outstanding majority of the patients with HS and CD, had recurrent seizures, which is in agreement with the previous studies. For patients with TLE+HS, surgical removal of the epileptogenic zone is associated with seizure remission in 2/3 of patients, whereas only 8% remit with AED treatment (Wiebe et al. 2001). Less is known about remission rates in patients with refractory epilepsy without the possibility of epilepsy surgery. Selwa et al. (2003) observed that 21% of patients with refractory partial epilepsy who were not eligible for surgical management were seizure free during follow-up. In our study, a majority of the patients had seizures after epilepsy surgery or other lesional surgery, and a majority of the remitted patients attained remission with only AED treatment. However, the patient selection in these two studies was somewhat different.

6.2 Recurrent seizures and immunological markers

Earlier studies have demonstrated an increased prevalence of antiphospholipid and antinuclear antibodies in patients with different forms of epilepsy compared to healthy control subjects. In the present study, we evaluated the effects of clinical characteristics in patients with refractory epilepsy on the prevalence of anticardiolipin, anti-β_2 glycoprotein 1, antinuclear, and GAD antibodies, as well as IL-6 and IL-1RA. We found that the increased prevalence of the IgG class anticardiolipin anti-

bodies was associated with recurrent seizures. We also detected that titres of IgG class anticardiolipin antibodies were higher in patients with recurrent seizures compared to those with no recent seizures and the control subjects. The results parallel those observed in previous studies in which anticardiolipin antibodies were detected especially in patients with focal or complicated epilepsy.

The increased prevalence of IgG class anticardiolipin antibodies in patients with active epilepsy suggests the possible role of immunological factors in some patients with refractory epilepsy. The pathophysiology between antiphospholipid antibodies and epilepsy is unknown, but several mechanisms of action have been suggested. Although anti-β_2-glycoprotein I antibodies have been regarded as specific for thrombosis-mediated antiphospholipid events, they and other antiphospholipid antibodies also have the potential to bind to brain tissue (Kent et al. 1997, Caronti et al. 1998, Chapman et al. 1999). Furthermore, signs of cerebral thrombosis in patients with antiphospholipid antibodies and epilepsy without SLE are rare (Angelini et al. 1998). In our study, patients with post-stroke epilepsy were excluded. Other possible pathophysiological mechanisms between antiphospholipid antibodies and epilepsy include GABA receptor-mediated chloride current dysfunction (Liou et al. 1994), as well as epileptogenic and apoptotic effects of autoantibodies (Bordron et al. 1998, Greaves 1999).

Although AED treatment can induce SLE-like disorders (Jain 1991, Park-Matsumoto and Tazawa 1996, Scheinfeld 2004), there is evidence for a stronger association between antiphospholipid/antinuclear antibodies and epilepsy than with AEDs (Peltola et al. 2000b). In patients with SLE, antiphospholipid antibodies are potent risk factors for brain injury, as measured by magnetic resonance spectroscopy, independent of stroke (Sabet et al. 1998). In stroke, cerebrovascular events are associated with high titres of anticardiolipin antibodies (Verro et al. 1998). In our study, the titres of anticardiolipin antibodies were, at most, moderate; we did not find any high titres in our patients. Whether anticardiolipin antibodies are causative or reflect neuronal damage remains unknown, but they may participate in the chronic immunological process in refractory epilepsy.

No statistically significant associations were detected between seizure frequencies and the positivity of antinuclear antibodies, IgM class anticardiolipin antibodies, cytokines, or GADA. All three patients with anti-β_2-glycoprotein I antibodies had experienced recent seizures, but the number of patients was too small to draw definite conclusions. Some patients in remission exhibited increased levels of cytokines or GADA, suggesting that these parameters are not direct signs of seizures.

6.3 Epilepsy type and immunological markers

Experimental and clinical studies have demonstrated the immediate release of cytokines, especially IL-6, after seizures. In previous studies, basal levels of IL-6 have tended to be higher in patients compared to control subjects (Lehtimäki et al. 2004, 2007). In the present dissertation, we investigated for the first time a comprehensive population of patients with refractory epilepsy at the onset of the study and found chronically increased serum levels of IL-6 in patients with TLE compared to patients with extra-TLE and control subjects. The concentrations of IL-1RA were not significantly different in patients compared with control subjects, although there was a tendency for patients with TLE to have decreased levels of IL-1RA compared to patients with extra-TLE and control subjects.

The finding of increased levels of IL-6 in patients with TLE suggests that the epilepsy type is important for determining the chronic overproduction of IL-6 in epilepsy. Increased levels of possibly proconvulsive IL-6 and a tendency toward decreased levels of anticonvulsive IL-1RA suggest that some patients with TLE, but not extra-TLE, possess a proinflammatory blood profile. This is supported by the previous finding of a highly proinflammatory profile of plasma cytokines in patients with therapy-resistant epilepsy (Hulkkonen et al. 2004), but that study did not evaluate the effect of epilepsy type on cytokine levels.

We found a distinct subgroup of patients with increased serum levels of GADA. All but one patient with a high level of GADA had TLE. Our findings are in line with previous studies in which the majority of patients with GADA and practically all patients with a high GADA level had TLE. One of our patients with a high level of GADA had been classified as having IGE, but, in this particular patient, the classification was difficult, as there were features of both focal and primary generalised seizures. However, no statistically significant differences were detected between patients with TLE and those with extra-TLE regarding the high levels of GADA. This result is probably due to the infrequency of high GADA levels as a phenomenon. Our patients with high levels of GADA demonstrated organ-specific autoimmune diseases and polyendocrinopathies, supporting the autoimmune origin of their epilepsy. These patients also had GADA in their CSF, as demonstrated by IHC and WB. Two patients presented IS of GADA, suggesting a CNS origin of autoantibodies in these patients. One additional patient with a high GADA level had an increased IgG index in the CSF.

In the present dissertation, we demonstrated that immunological markers, such as IL-6, GADA and multiple other autoantibodies, are activated specifically in TLE. The temporal lobe is particularly vulnerable in traumatic brain injury, infection, and neurodegenerative diseases, and is characterised

by an abundance of projections to other brain areas. Based on our results, TLE - especially TLE+HS - can be considered as its own entity with distinct clinical, anatomical, and immunological characteristics.

Inflammation and inflammatory responses are modulated by a bidirectional communication between the brain and the immune system. The CNS signals the immune system through hormonal pathways, including the hypothalamic-pituitary-adrenal axis and the hormones of the neuroendocrine stress response, as well as through neuronal pathways, including the autonomic nervous system. The immune system signals the CNS via immune mediators and cytokines (Eskandari et al 2003). Previous clinical studies have demonstrated an immediate increase in immunological mediators after seizures. Prolactin has important immunoregulatory properties, and it may play a role in the pathogenesis of certain autoimmune diseases (Buskila et al. 1999). Increases in prolactin levels have been found to be greatest in patients with TLE, possibly reflecting a close relationship between the mesial temporal lobe and the sympathetic nervous system (Meierkord et al. 1992, Bauer et al. 2008). Activation of the IL-1β system and the presence of monocytes and macrophages have been found in both experimental epileptogenesis models and in human TLE hippocampi (Ravizza et al. 2008). In patients who had undergone anterior temporal lobectomy for an intractable TLE, interictal serum prolactin levels were presurgically elevated and declined to normal after resection (Lin et al. 1997). This finding suggests a sustained increase of prolactin release in refractory TLE. In the present study, chronically increased levels of IL-6 were detected in patients with TLE, but not in patients with extra-TLE, which is in line with the earlier observations.

Antiphospholipid, antinuclear, and anti-β_2-glycoprotein I antibodies were not associated with epilepsy type. No correlation was found between IL-6, IL-1RA, and antiphospholipid antibody levels in the present dissertation. This does not exclude the possibility of interrelationships between mediators of the immune system, since the activation cascade is very complex, especially in the chronic state, and the factors regulating different immunological mediators are diverse.

In the present study, one of our TLE-patients with a very high level of GADA had achieved seizure freedom after epilepsy surgery. He was included in an earlier study (Peltola et al. 2000c) prior to his epilepsy surgery, when IS of GADA was detected in addition to a high level of GADA. By the time of his enrolment in the current study, IS was no longer present. He presented multiple polyendocrinopathies and celiac disease, suggesting an autoimmune basis of epilepsy, but the lack of IS in the

present measurement suggests that the previous IS of GADA might have been a sign of an ongoing immune response in the brain.

The mechanism of action of GADA in neuroinflammation remains obscure, but a functional impairment of GABAergic transmission is suggested. The serum of epilepsy patients with GADA display a significant increase in the frequency of inhibitory post-synaptic potentials on hippocampal neurons (Vianello et al 2008), and in rats, the cerebellar and spinal cord activities are also affected (Manto et al. 2007). Synthesis of GABA is decreased in knock-out mice that lack GAD_{65}, leading to spontaneous seizures (Kash et al. 1997). In chronic epilepsy, GADA may serve as a sign of immunological damage that occurred at the onset of epilepsy similar to that considered in diabetes. It is unlikely that GADAs are only epiphenomena, since high titres of GADA are rare in other neurological diseases (Meinck et al. 2001).

6.4 The effect of aetiology on immunological markers

In many patients, no aetiology of epilepsy can be found. Even if a clear structural lesion can be found in an MRI, the initial insult is often unknown. Many different CNS diseases, such as viral infections, may trigger seizures and contribute to epilepsy. In viral encephalitis, immune system activation may play a role in triggering seizures (Getts et al. 2008). Evidence of viral infection has been found in resected brain with HS (Theodore et al 2008). In addition to infectious aetiologies, LE, a subacute disorder with behavioural symptoms and cognitive disturbances, often causes seizures. It is usually considered to be paraneoplastic in origin, and many reported cases are associated with specific autoantibodies. A proportion of patients suffering from LE have features of a non-paraneoplastic form of LE (NPLE), with evidence of antibodies to VGKCs, and some may also harbour GADA. Immunotherapy has been shown to be effective in NPLE. It is therefore not excluded that (subclinical) infectious or inflammatory aetiologies could contribute to the epilepsy of some patients (Bien et al. 2007, Levite and Ganor 2008).

We found that anticardiolipin antibodies were more prevalent in HS and CD, although the finding was not statistically significant. No associations were detected between the aetiologies of epilepsy and the prevalence of antinuclear antibodies, cytokines, or GADA. The present results are in line with experimental observations, which showed that epileptic activity may induce inflammatory mediators in many different situations (Vezzani and Granata 2005). Increased expression of proin-

flammatory molecules has been demonstrated in resected epileptic brain samples with HS and CD, but also for other aetiologies (Vezzani and Granata 2005), suggesting that the aetiology may not be the main differentiating cause of chronic immune activation in epilepsy.

6.5 Clinical significance of aetiology on refractory epilepsy

In the present dissertation, we studied the effect of aetiology on the changes of remission in refractory focal epilepsy and found that a remarkable proportion of patients achieved remission during follow-up. In particular, patients with cryptogenic epilepsy demonstrated a significantly higher number of remissions compared to patients with symptomatic epilepsy. The findings can already be implicated in the diagnostic stage, where the classification of aetiology may help in the evaluation of the prognosis of epilepsy. A high-resolution brain MRI with an epilepsy protocol is needed for diagnosis in patients with focal epilepsy, and the repetition of imaging is essential for those with continuous seizures to facilitate the recognition of surgery candidates. The development of imaging techniques will improve the evaluation of aetiology in patients with refractory focal epilepsy and hopefully aid in preventing the development of refractoriness in newly diagnosed patients.

6.6 Clinical significance of immunological markers in epilepsy

Increased levels of IL-6 and autoantibodies were detected in patients with refractory epilepsy. The immunological markers could serve as biomarkers of refractoriness. In the present thesis, we could clarify clinical features in epilepsy that were associated with immunological activity. IL-6 and high levels of GADA were increased only in TLE, but not in extra-TLE, offering a possibility to use these biomarkers for distinguishing epilepsy types into TLE and extra-TLE in the same way as has been found in the case of prolactin (Lin et al. 1997). The increased prevalence of polyendocrinopathies in patients with GADA titres further reinforces the significance of immunological activity in some patients with epilepsy.

The identification of patients with possible immune-mediated epilepsy would offer new treatment options for patients with this disease. Immunotherapies have been successfully used in some catastrophic epileptic syndromes of childhood and in single cases of adult epilepsies. IS of GADA may be a marker of an ongoing immune response, and its measurement could be useful for identifying patients in whom a trial of immunosuppressive therapy might be warranted. Recent examples have

been obtained from patients with IS of GADA and refractory epilepsy, for whom steroids, IVIG, plasma exchange, and cyclophosphamide showed significant effects on seizure frequencies. One of our patients with IS of GADA and demyelinating disease had received IVIG without any effects on her seizure frequency. However, this does not exclude the possibility that her epilepsy is immune-mediated, since the dose or the drug may have been too weak to suppress the chronic immune process. Conversely, other immunomodulative or immunosuppressive drugs have not been indicated for patients with rather controlled epilepsy.

A future goal is to study patients with new-onset epilepsy to define associations between immune biomarkers and the onset of epilepsy. There is a need for large-scale follow-up studies to better define the correlation of these phenomena with different clinical features in epilepsy. Since the mesial temporal lobe is exceptionally vulnerable to different damaging agents, the differentiation of TLE into mesial and cortical TLEs might be useful in future studies. Experimental studies are needed to more precisely study the pathogenetic factors associated with immune-mediated epilepsy.

SUMMARY AND CONCLUSIONS

In this dissertation, we studied the underlying causes of refractory focal epilepsy in terms of the possibility of seizure freedom. In the second section of the present dissertation, we studied immunological factors of refractory epilepsy. We measured antiphospholipid and antinuclear antibodies as well as cytokines in patients with refractory focal epilepsy to discover the effects of clinical determinants on the prevalence of these immunological markers. We measured serum levels of GADA in patients with controlled or refractory focal epilepsy, as well as in patients with primary generalised epilepsy.

In the first part of the dissertation, we examined the aetiology of epilepsy in 119 patients with refractory focal epilepsy during a two-year follow-up. The aetiology was based on high-resolution brain MRI and patient history. A significant proportion of the patients attained remission during the follow-up. The chances of remission were significantly higher in patients with cryptogenic aetiology compared to patients with symptomatic aetiology. Some patients had attained remission after a long period of epilepsy. The present study demonstrates that aetiology is important in determining the prognosis of epilepsy. Furthermore, it shows that the prognosis of patients with refractory focal epilepsy might be better than previously reported.

In the second part of the dissertation, we measured serum levels of anticardiolipin, anti-β2-glycoprotein I, and antinuclear antibodies in 95 patients with refractory focal epilepsy and in 10 patients with controlled focal epilepsy. Clinical determinants included the patient history, electroclinical classification, and high-resolution brain MRI. Seventy healthy blood donors served as control subjects. Blood samples were drawn after the two-year follow-up. One sample was drawn from patients with no recent seizures, and two samples were drawn at a three-month interval from patients with recent seizures. The prevalence of IgG class anticardiolipin antibodies was increased in the patients with recurrent seizures compared to those with no recent seizures and the control subjects. The patients with recent seizures demonstrated an increased prevalence of moderate positive IgG class anticardiolipin antibodies compared to the control subjects and the patients with no recent seizures. No differences in the levels of IgM anticardiolipin or antinuclear antibodies were detected between patients and control subjects. All three patients with anti-β_2-glycoprotein I antibodies had recurrent seizures. The prevalence of IgG class anticardiolipin antibodies was not associated with the epilepsy type, duration, or aetiology. These results suggest that the presence of antiphospholipid antibodies is associated with seizure frequency in refractory epilepsy.

In the third study, we measured serum levels of IL-6 and IL-1RA in 86 well-evaluated patients with refractory focal epilepsy and in 5 patients with controlled focal epilepsy. Epilepsy was evaluated based on the patient history, electroclinical findings, and high-resolution brain MRI. Sixty-three healthy blood donors served as control subjects. Two blood samples obtained with a three-month interval were drawn similarly to that described for the antiphospholipid study. IL-6 concentrations were chronically increased in epilepsy patients compared with control subjects. All patients with increased levels of IL-6 had TLE. Furthermore, the mean and the median serum levels of IL-6 were higher in patients with TLE than in patients with extra-TLE. The concentrations of IL-1RA were not significantly different in patients compared with control subjects, although the patients with TLE tended to demonstrate smaller increases in the levels of IL-1RA. Age, sex, epilepsy surgery, and duration of epilepsy were not associated with the level of IL-6. These findings demonstrate the importance of epilepsy type in determining the chronically increased levels of IL-6 in patients with refractory focal epilepsy.

In the last part of the dissertation, we studied GADA with an RIA in the serum of 253 well-evaluated patients with refractory focal (184 patients), controlled focal (25), or primarily generalised epilepsy (44). The positive samples of patients were confirmed by IHC and WB in serum and CSF. The control samples for RIA were obtained from 200 non-diabetics without a history of epilepsy. In the patients with increased levels of GADA, serum was screened for polyendocrinopathies. Epilepsy was carefully classified based on patient histories, seizure semiology, and high-resolution brain MRI. A homogeneous subgroup of patients with increased levels of GADA was discovered. The majority of the patients with high levels of GADA had TLE, and all exhibited polyendocrinopathies, indicating an immune-mediated origin of epilepsy. IS in a few patients with high levels of GADA supports the CNS origin of GADA.

The aetiology of epilepsy is important for determining the prognosis of epilepsy. In patients with cryptogenic refractory epilepsy, continuous new drug trials are suggested because there are always patients who may benefit from them. The presence of IL-6 and a high level of GADA are increased in patients with TLE, but not in those with extra-TLE. In the GADA study, the polyendocrinopathies in patients with high levels of GADA, and IS of GADA in a few patients, strongly suggest an immunological background of epilepsy in these patients. IS of GADA might be a marker of an ongoing immunological process in the brain. These findings suggest that immunological mediators, such as antiphospholipid antibodies, cytokines, and GADA may serve as clinically significant

inflammatory markers in refractory epilepsy. Measurements of IL-6 and GADA could be used to distinguish TLE from extra-TLE. In patients with epilepsy and specific autoantibodies or cytokines, alternative therapeutic interventions such as immunotherapy could be offered. Further studies are needed to determine the cause and effect relationship of seizures and immunological changes, as well as the effects of immunotherapeutic drugs, especially in patients with new-onset epilepsy.

YHTEENVETO

Asianmukaisesta lääkityksestä huolimatta 25-30%:lla epilepsiapotilaista esiintyy kohtauksia, mikä aiheuttaa erittäin runsaasti inhimillistä kärsimystä. Vaikeahoitoisen epilepsian tutkimisen tavoitteena on tunnistaa tekijöitä, jotka voisivat ennakoida kohtauksien jatkumista. Näiden tekijöiden tarkempi tunteminen voisi mahdollistaa uusia hoitokeinoja.

Väitöskirjan tavoitteena oli kartoittaa vaikeahoitoisen epilepsian syitä ja immunologisia tekijöitä. Ensimmäisessä osatyössä tutkittiin vaikeahoitoisen paikallisalkuisen epilepsian etiologian vaikutusta kohtauksien jatkumiselle. Aikaisempien tutkimusten perusteella epilepsian etiologia voi vaikuttaa epilepsialääkityksen vasteeseen. Sen sijaan vaikeahoitoista epilepsiaa sairastavien potilaiden epilepsioiden etiologioiden ennusteesta on vain vähän tietoa.

Väitöskirjan kolmessa muussa osatyössä kartoitettiin immunologisten tekijöiden merkitystä vaikeahoitoisessa paikallisalkuisessa epilepsiassa. Sekä kokeellisissa että kliinisissä tutkimuksissa on saatu runsaasti näyttöä immunologisten tekijöiden merkityksestä epilepsiassa. Tiettyjä autoimmuunisairauksia potevilla potilailla on runsaammin epilepsiaa kuin normaaliväestöllä. Immunologisista hoidoista on merkittävää hyötyä eräissä lapsuusiän vaikeahoitoisissa epilepsioissa. Epilepsiapotilaiden seerumista on mitattu useita spesifejä autovasta-aineita, ja epilepsiakohtaukset aktivoivat proinflammatoristen sytokiinien tuotantoa.

Ensimmäisessä osatyössä tutkittiin vaikeahoitoisen paikallisalkuisen epilepsian etiologiaa 119 potilaalla. Etiologia luokiteltiin sairaushistorian ja aivojen korkeakenttämagneettitutkimuksen perusteella. Merkittävä osa alun perin vaikeahoitoista epilepsiaa sairastavista potilaista tuli kahden vuoden seurannan aikana kohtauksettomiksi. Kohtauksettomuus oli todennäköisempää niillä potilailla, joiden epilepsian etiologia oli kryptogeeninen verrattuna potilaisiin, joiden epilepsia oli symptomaattinen. Osalla potilaista oli ollut kohtauksia erittäin pitkään ennen kohtauksettomuuden saavuttamista.

Toisessa osatyössä mitattiin antikardiolipiini-, anti-β_2-glykoproteiini I- ja tumavasta-aineet 105 paikallisalkuista epilepsiaa sairastavan potilaan seerumista. Jatkuvasti kohtauksia saavilla potilailla todettiin runsaammin IgG-luokan antikardiolipiinivasta-aineita kuin terveillä kontrolleilla tai potilailla, joilla ei ollut jatkuvasti kohtauksia. Jatkuvasti kohtauksia saavien potilaiden IgG-luokan antikardiolipiinivasta-aineiden tiitterit olivat myös muiden ryhmien tiittereitä korkeampia. IgM-luokan antikardiolipiini- ja tumavasta-aineiden tiittereissä ei ollut eroja eri ryhmien välillä. Kaikki kolme potilasta, joilla todettiin anti-β_2-glykoproteiini I-vasta-aineita, kärsivät jatkuvista kohtauksista.

Kolmannessa osatyössä 91 paikallisalkuista epilepsiaa sairastavan potilaan seerumista mitattiin IL-6- ja IL-1RA-pitoisuudet. Kontrollihenkilöihin verrattuna epilepsiapotilaiden IL-6-pitoisuudet olivat kroonisesti korkeammat. Kaikilla potilailla, joilla IL-6-pitoisuus oli koholla, oli temporaaliepilepsia. Myös IL-6-pitoisuuksien keskiarvot ja mediaanit olivat temporaaliepilepsiaa sairastavilla potilailla korkeammat kuin ekstratemporaaliepilepsiaa sairastavilla potilailla. Temporaaliepilepsiaa sairastavien potilaiden IL-1RA-pitoisuudet olivat ekstratemporaaliepilepsiaa sairastavien potilaiden pitoisuuksia matalammat, mutta tulos ei ollut tilastollisesti merkitsevä.

Viimeisessä osatyössä 253 epilepsiapotilaalta mitattiin seerumin GAD-vasta-aineet. GAD-vasta-aineet olivat merkittävästi koholla osalla potilaista kontrollihenkilöihin verrattuna. Kaikilla potilailla, joilla GAD-vasta-ainetiitteri oli korkea, seerumissa havaittiin useita autovasta-aineita, mikä viittaa immuunivälitteiseen epilepsiaan; lähes 90%:lla oli temporaaliepilepsia. Kahdella potilaalla todettiin intratekaalinen GAD-vasta-ainetuotanto.

Tulosten perusteella vaikeahoitoisen epilepsian ennuste riippuu sen etiologiasta. Tutkimuksesta voidaan myös päätellä, että vaikeahoitoisen paikallisalkuisen epilepsian ennuste saattaa olla parempi kuin aikaisemmin on ajateltu. Jatkuvat lääkeponnistelut saattavat olla hyödyksi erityisesti kryptogeenista epilepsiaa sairastavien potilaiden hoidossa.

IgG-luokan antikardiolipiinivasta-aineet olivat koholla osalla niistä potilaista, joilla on jatkuvia kohtauksia. IL-6-pitoisuudet olivat koholla osalla temporaaliepilepsiaa sairastavista potilaista. Lähes 90%:llä niistä potilaista, joilla todettiin korkea GAD-vasta-ainetiitteri, oli temporaaliepilepsia. Niillä potilailla, joilla todettiin korkea GAD-vasta-ainetiitteri ja useita autovasta-aineita, epilepsia on todennäköisesti immuunivälitteinen. Intratekaalinen GAD-vasta-ainetuotanto saattaa viitata käynnissä olevaan immunologiseen keskushermoston prosessiin.

Immunologisia mittareita, kuten fosfolipidi- ja GAD-vasta-aineita sekä sytokiineja, voitaisiin käyttää vaikeahoitoisen epilepsian inflammatorisina biomarkkereina. IL-6:n ja GAD-vasta-aineiden mittaaminen saattaa auttaa epilepsiatyyppien arvioimisessa erottelemaan temporaaliepilepsia ekstratemporaaliepilepsiasta. Väitöskirjan löydöksiä voitaisiin hyödyntää jatkossa tunnistamalla ne epilepsiapotilaat, joille immunoterapeuttisista hoidoista saattaisi olla apua. Autovasta-aineiden ja sytokiinien patogeneettinen merkitys epilepsiassa on silti vielä avoin. Jatkossa on syytä kartoittaa immunologisten tekijöiden syy-seuraussuhteita ja immunoterapeuttisten lääkehoitojen tehoa erityisesti vastasairastuneilla epilepsiapotilailla.

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The benefit of active drug trials is dependent on aetiology in refractory focal epilepsy

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ABSTRACT

Background: Earlier studies have shown that aetiology makes a difference in the outcome of epilepsy, but there is a paucity of follow-up studies to evaluate the possibilities of achieving seizure freedom in initially refractory epilepsy.

Methods: We evaluated the cause of epilepsy based on high-resolution brain MRI and patient history in 119 consecutive thoroughly examined adult patients with refractory focal epilepsy followed up in our centre. We also evaluated the influence of aetiology and duration of epilepsy in this patient cohort on the chances of achieving 12-month remission in a 2-year follow-up.

Results: The major finding was that a substantial group of patients achieved remission; 30 (25%) initially refractory patients achieved at least 12 months remission during follow-up. A total of 40.0% of the patients with cryptogenic aetiology had achieved 12-month remission compared with the 16.2% patients with symptomatic aetiologies (age-adjusted OR 3.74, 95% CI 1.54 to 9.07, $p = 0.004$). Aetiologies often considered for surgical treatment (hippocampal sclerosis, cortical dysplasia, vascular malformation, tumour and dual pathology) carried an almost six-fold risk of persistent seizures compared with cryptogenic epilepsy (age-adjusted OR 5.85, 95% CI 2.00 to 17.11, $p = 0.001$).

Conclusions: Patients with vascular malformation and dual pathology as aetiology were most refractory, none being in remission for 12 months. There were also patients achieving 12-month remission after a long period of active epilepsy. These results encourage physicians to continue with new drug trials, especially on patients with no possibilities of epilepsy surgery, as well as on those still having seizures after epilepsy surgery.

Epilepsy is a multifactorial and heterogeneous group of disorders that demands active treatment from the beginning. Despite appropriate antiepileptic medication, nearly one-third of patients with epilepsy continue to have refractory seizures.[1 2] Response to a first antiepileptic drug (AED) seems to predict the outcome on subsequent AEDs.[1–3] In a recent study, 50% of newly diagnosed patients became seizure-free during treatment with a first AED, 11% during treatment with a second and 3% with a third drug.[2] On the other hand, there is a report from the UK in which 28% of the patients with drug-resistant epilepsy gained seizure freedom by adding a previously unused AED.[4] An established definition of refractory epilepsy requires failure of two or more AEDs, with maximally tolerated doses sequentially or in combination, and lack of seizure control after 2 years of AED therapy.[1 5 6]

There are two previous studies addressing the importance of aetiology for possibilities of seizure freedom. In a large, hospital-based study including more than 2000 patients, the aetiology of epilepsy was evaluated as a prognostic factor determining the chances of remission.[7] Remission was achieved in 82% of patients with idiopathic generalised epilepsy, in 45% of patients with cryptogenic focal epilepsy and in 35% of patients with symptomatic focal epilepsy. Temporal lobe epilepsy (TLE) with hippocampal sclerosis (HS), in particular, seemed to carry a poor prognosis, with 90% of the patients having persistent seizures. If the aetiology was dual pathology, 97% had persistent seizures. Stephen *et al.* analysed 550 patients with partial seizures and found that 42% of the patients with TLE + HS, 54% of the patients with cortical dysplasia (CD) and 63% of the patients with brain tumour were seizure-free.[8] These two studies did not evaluate the chances of achieving seizure freedom in initially refractory patients.

Previous studies have not addressed the clinically relevant issue of possibility for remission in patients with initially refractory epilepsy not suitable for epilepsy surgery, including patients with failed epilepsy surgery. In this study, we assessed the underlying causes of refractory epilepsy and evaluated its effects on chances for achieving 12-month remission. According to local treatment guidelines, all adult patients with refractory epilepsy—except patients with moderate or severe mental retardation, those elderly patients with controlled epilepsy and patients with post-stroke epilepsy—in Pirkanmaa Hospital District (population of 440 000) were treated and followed-up in our institution. We also act as a tertiary referral centre for neighbouring hospital regions, but these referral patients undergoing preliminary evaluation for possibilities of epilepsy surgery are not included in the present study population.

METHODS

This was a prospective cross-sectional cohort study in 119 consecutive adult patients with localisation-related refractory seizures that occurred between 1 January 2003 and 30 November 2004 and that fulfilled the inclusion criteria. The patients were followed-up for 2 years. All the study patients have been treated and followed-up in our clinic for years and were under active drug treatment, including new AEDs, at the time of study. The patients fulfilled the following inclusion criteria: persistency of seizures after trials of at least two AEDs with maximally tolerated doses (sequentially or in combination therapy), and sustained AED treatment for at least 2 years before the beginning of the study. All the patients had seizures in the

beginning of the study. Some of the patients had had epilepsy surgery or other lesional surgery or were under presurgical evaluation, but none underwent operation during the follow-up. No children were enrolled in the study, but adolescents were. On the other hand, no strict upper age limits were used. Patients with epilepsy caused by perinatal vascular attack (anoxia, haemorrhage or brain infarction) were included, but those with post-stroke epilepsy of adulthood were excluded due to the usually good response to AED therapy and due to the local practise of following-up by general practitioners. In addition, patients with dementia or malignant brain tumour and epilepsy are treated elsewhere, but those with stable chronic low-grade brain tumour and epilepsy are treated in our clinic and were recruited in the present study. The follow-ups of these brain tumours were performed either by neurosurgeons or in our clinic by scheduled cranial MRI studies.Those with primarily generalised seizures were excluded. The ethics committee of Tampere University Hospital approved the study, and all the patients gave written informed consent.

A total of 95% of the patients had undergone 1.5 Tesla cranial MR imaging using the epilepsy protocol[9] by experienced neuroradiologists (table 1). The patients without MRI had either a clear lesion in a cranial CT scan or had claustrophobia. Some 91% of the patients had abnormal electroencephalography (EEG); 66% of the patients had had long-term video EEG monitoring.

Aetiology was classified as remote symptomatic or probably symptomatic (cryptogenic), depending on the results of imaging studies, histological analysis in some brain tumours and the medical history. Brain imaging results were classified as follows: 1) tumour (dysembryoplastic neuroepithelial tumour (DNET), ependymoma, hamartoma, meningioma, low-grade oligoastrocytoma); 2) vascular malformation (arteriovenous malformation, venous angioma, cavernous angioma); 3) vascular lesion (perinatal brain infarction, intracerebral haemorrhage, anoxia); 4) trauma; 5) HS; 6) other hippocampal abnormality (vascular malformation, demyelination or atrophy); 7) CD (cortical dysgenesis, heterotopia, tuberosis sclerosis); 8) central nervous system (CNS) infection (encephalitis, meningitis, abscess); 9) dual pathology (HS associated with another brain lesion); and 10) other (local or diffuse atrophy, non-specific signal change, demyelination, non-specific gliosis). If the patient history revealed a clinically relevant head trauma with loss of consciousness of more than 30 minutes or a confirmed CNS infection prior to onset of epilepsy, it was considered to be the cause of epilepsy in spite of normal imaging results. We compared the symptomatic aetiologies according to the possibilities of surgical treatment. The aetiologies often considered for surgical treatment included HS, CD, vascular malformation, tumour and dual pathology. The rest of the symptomatic aetiologies were classified as not often considered for surgery. If no cause of epilepsy was found, epilepsy was classified as cryptogenic. The duration of epilepsy was recorded. Surgical treatment, if performed, was divided into two groups: epilepsy surgery (amygdohippocampal resection) and other lesional surgery. The effect of age on the possibility of remission was evaluated, categorizing age into three classes: <20 years of age, 20–64 years of age and ⩾65 years of age.

For the outcome, the patients were divided into two groups based on seizure frequency: patients having continuous seizures (patients with persistent seizures) and patients having had seizures in the beginning of the study but having achieved 12 months seizure remission during the follow-up (12-month remission).

To estimate differences between 12-month remission patients and other subjects, the frequencies were calculated using cross-tabulations. By fitting a logistic regression model to data, the odds ratios (ORs), the confidence intervals (CIs) and p values of persistent seizures were obtained. The importance of predictors was shown by non-standardised coefficients. All statistical analyses were performed using SPSS software version 12.0.1.

Table 1 Clinical characteristics of study patients

N	119
Median age in years (range)	40 (15–75)
Sex	
Female	56%
Male	44%
Median duration of epilepsy in years (range)	22 (2–66)
Epilepsy surgery	12%
Video EEG	66%
MRI	95%
EEG (proportion of abnormal)	91%

EEG, electroencephalography; MRI, magnetic resonance imaging.

RESULTS

The clinical characteristics of the patients are presented in table 1. The most common aetiologies in patients with remote symptomatic epilepsy included CD, HS, vascular malformation, brain tumour and trauma (fig 1). The aetiology was unknown (cryptogenic) in 45 patients. There were 30 patients (25%) that achieved at least 1-year remission during follow-up, whereas the remaining 89 patients (75%) had persistent seizures (table 2, fig 1). Altogether, three of the 30 patients with 12-month remission had undergone epilepsy surgery before entering the study; the rest had only received pharmacological treatment. The duration of epilepsy in the total study group was long—up to 66 years (mean 23.17, SD 14.40, median 22, range 2–66 years). A total of 24.3% of the patients with 12-month remission had had active disease for ⩾30 years before the remission (table 3).

The aetiology was important in determining the possibility for remission. A total of 40% of the patients with cryptogenic epilepsy, 27.3% of the patients with symptomatic aetiology not often considered for surgical treatment and 11.5% of the patients with symptomatic aetiology often considered for surgical treatment had achieved 12-month remission (table 2). The chances of achieving the remission were significantly higher in the patients with cryptogenic aetiology compared with symptomatic aetiologies (age-adjusted OR 3.74, 95% CI 1.54 to 9.07, p = 0.04). Epilepsies with aetiologies often considered for surgery had significantly higher risk of having persistent seizures compared with cryptogenic epilepsy (age-adjusted OR 5.85, 95% CI 2.00 to 17.11, p = 0.001). The symptomatic aetiologies not often considered for surgery carried an increased risk of persistent seizures compared with cryptogenic aetiology, but the result was not statistically significant (age-adjusted OR 1.73, 95% CI 0.55 to 5.46, p = 0.347). In logistic regression analysis, sex was not associated with possibility of achieving remission. The longer the duration of epilepsy, the higher the possibility of persistent seizures, but the difference was not statistically significant (OR 1.01, 95% CI 0.98 to 1.04, p = 0.44). Ten patients were <20 years of age, 105 patients were 20–64 years of age and 4 were ⩾65 years of age. The patients who were aged 20–64 years had a fivefold risk of having persistent seizures compared with the patients <20 years of age (OR 5.06, 95% CI 1.32 to 19.42, p = 0.018).

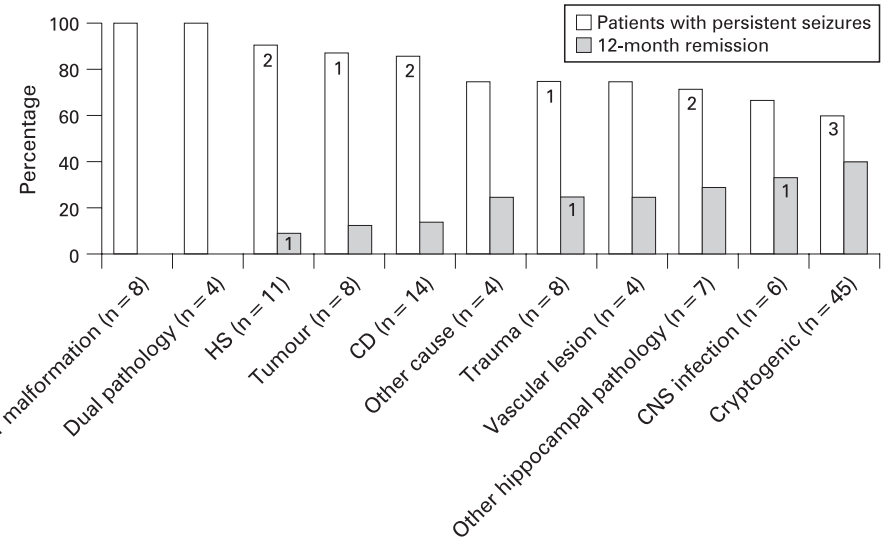

Figure 1 Influence of aetiology of epilepsy on 12-month remission. HS, hippocampal sclerosis; other hippocampal path, other hippocampal pathology; CD, cortical dysplasia; CNS, central nervous system.

*Numbers inside bars are the number of patients who have undergone epilepsy surgery

The patients who had used at least eight AEDS had an almost fivefold risk of having persistent seizures compared with the patients with 2–3 AEDs (age-adjusted OR 4.72, 95% CI 1.25 to 17.75, p = 0.022). Respectively, the patients with 4–7 AEDs had an almost twofold risk of having persistent seizures compared with the patients with 2–3 AEDs, but the result was not statistically significant (age-adjusted OR 1.95, 95% CI 0.72 to 5.32, p = 0.19). The most often used AEDs (in monotherapy or combination) were carbamazepine (in 47 patients), lamotrigine (in 40), topiramate (in 38), leveritacetam (in 28), tiagabine (in 25), oxcarbazepine (in 24) and valproic acid (in 19). The most often used AED combinations were carbamazepine + tiagabine (in 8 patients), carbamazepine + topiramate (in 6), lamotrigine + topiramate (in 6), carbamazepine + leveritacetam (in 5), leveritacetam + oxcarbazepine (in 5), lamotrigine + valproic acid (in 5) and oxcarbazepine + tiagabine (in 5). All the new AEDs during the time the study was performed were available except pregabaline and zonisamide, which were not licenced in Finland at that time. In the patients with persistent seizures, seizure frequencies were $\leqslant 1$ per month in 26% patients, 2–4 per month in 36% patients and $\geqslant 5$ per month in 38% patients.

One patient with HS in remission had undergone epilepsy surgery just before entering the study. All the patients with vascular malformation and dual pathology, and 85.7% of the patients with CD, had persistent seizures (fig 1). A total of 50% of the patients with vascular malformation had undergone an embolisation procedure, but all were still having seizures. Many had had multiple procedures, indicating a complicated situation. Two patients with CD were operated, neither of them in remission. The group of other brain pathologies was heterogeneous, 75% of the patients with persistent seizures. Altogether, surgery (amygdalohippocampectomy or other lesional surgery) was performed in 3 patients with HS (1 in remission), in 1 patient with brain tumour (with persistent seizures), in 2 patients with CD (both with persistent seizures), in 2 patients with trauma (1 in remission and 1 with persistent seizures), in 2 patients with other hippocampal pathology (both with persistent seizures), in 1 patient with CNS infection (in remission) and in 3 patients with cryptogenic epilepsy (all with persistent seizures).

DISCUSSION

This is the first adult study to address the specific population of initially refractory epilepsy with predefined criteria in a well-characterised group of patients. The major finding was that there were patients with previously intractable epilepsy achieving 12-month remission, some with a remarkably long duration of active disease. In particular, 40% of the patients with cryptogenic epilepsy had 12-month remission. Patients with

Table 2 Percentages of 12-month remission in three different patient groups with respect to possibility for resective surgery

	Often considered for surgical treatment	Not often considered for surgical treatment	Cryptogenic	Total
Persistent seizures				
N	46	16	27	89
%	88.5%	72.7%	60.0%	74.8%
12-month remission				
N	6	6	18	30
%	11.5%	27.3%	40.0%	25.2%
Total				
N	52	22	45	119
%	100%	100%	100%	100%

The group defined as often considered for surgical treatment includes hippocampal sclerosis, cortical dysplasia, vascular malformation, tumour and dual pathology; those not often considered for surgical treatment includes trauma, other hippocampal pathology, CNS infection, other causes and vascular lesion; cryptogenic means that the aetiology is unknown.

Table 3 Influence of the duration of epilepsy on the possibility of remission

	Duration of epilepsy in years			
	<15	15–29	⩾30	Total
Persistent seizures				
N	25	36	28	89
%	67.6%	80.0%	75.7%	74.8%
12-month remission				
N	12	9	9	30
%	32.4%	20.0%	24.3%	25.2%
Total				
N	37	45	37	119
%	100%	100%	100%	100%

TLE+HS have previously been extensively studied, but the possibility for remission after long duration of medical refractoriness has not been evaluated in the other groups of initially refractory patients. The results represent an overview of the aetiologies of the refractory epilepsy population followed-up in the clinic, many patients with follow-up for a remarkably long time. At present, the concept of refractoriness is not fully developed. An essential problem lies in the heterogeneity of refractory epilepsy, which makes increasing knowledge of different factors associated with refractoriness important in identifying patients at risk of developing uncontrolled epilepsy.[10 11]

A fourth of the initially refractory patients achieved at least 12-month remission. In the patients with HS, seizure freedom had demanded operation. Two other patients obtained 12-month remission with surgery, but the rest of the patients with remission (27) had only received AED therapy. The possibilities for remission in patients with epilepsy who fail to improve with two or three drugs have been regarded limited. However, the recent report from the UK,[4] together with our results, show that the medical treatment with continuing drug trials may be effective as well as surgery, depending on the MRI findings in a given patient. The power of this study is not enough to evaluate the effects of single AEDs, but the study shows the effect of active treatment during the given period of time, both in those with prior surgery as well as among those without the possibility of resective surgery. In this initially refractory group of patients, aetiologies often considered for epilepsy surgery have a higher risk of persistent seizures even after surgery, so it is important to recognise this group as early as possible. Although the possibility of achieving a remission is reduced after administering the first two drugs, new drug trials should be encouraged because there are patients who may benefit from them.[2 4 12]

The aetiologies of epilepsy fell into small, heterogeneous groups, thus only descriptive analysis was possible in our study. However, interesting differences emerged. The most intractable aetiologies (vascular malformation, dual pathology, HS and CD) were in the definite remote symptomatic groups. Ten of the 11 patients with HS had persistent seizures and the only patient with HS with remission had undergone an epilepsy surgery procedure, for which the efficacy has been well documented.[13 14] Still, two out of three of the operated patients with HS continued to have seizures. These results do not reflect the operated patients with HS in general because, in this study, we included only those operated patients with seizures after surgery. Furthermore, this was not a study of a population of different aetiologies, but a study of refractory patients with these aetiologies.

In addition to HS, there were two other remarkably refractory aetiologies: vascular malformation and dual pathology. None of the patients with these aetiologies had 12-month remission. The result of postoperative seizure control in vascular malformations is usually good if total excision is possible,[15–17] which was not the case in the present study. Only two out of 12 patients with CD had achieved remission. In CD, surgery can sometimes help to achieve seizure control,[15] but surgery was not possible in most of our patients—mostly due to multiple lesions or inoperable location. Two patients had been operated but were still having seizures. In general, the percentages of different aetiologies were comparable with the previous study,[7] although all our patients were initially refractory. The epilepsy patients with at least moderate mental retardation known to have a relative high incidence of refractory epilepsy were excluded from the present study, which may partly explain the achieved remission rate. The exclusion of the patients with post-stroke epilepsy from our study may also affect the results but, in that particular group of patients, remission is often achieved.[18] In a retrospective study, 88% of 90 patients with post-stroke epilepsy could be managed with monotherapy.[19]

The aim in the future is to recognise patients at risk of refractory epilepsy as early as possible in order to evaluate the chance of long-term seizure remission and to prevent the development and progression of intractability.[3 5 7 20–23] The aetiology seems to influence the outcome in patients with epilepsy, which makes high-resolution MRI important for diagnosis of patients with focal epilepsy; repetition of imaging is essential in refractory patients. The specific imaging protocol is needed; this has certain sequences and thin slices to find the smallest abnormalities, especially in patients failing to achieve remission after two consecutive drugs.[9 24 25] Patients with lesional aetiologies, including HS, are potential candidates for resective surgery in medically refractory patients.[3 5 13 15–17 26–29] There is no need to hesitate in evaluating the possibilities of surgery in such cases because otherwise the prognosis seems to be poor. In patients who do not have the possibility of epilepsy surgery, as well as patients with seizures after epilepsy surgery, drug trials should be continued even after several years of refractoriness.[4]

Competing interests: None declared.

Ethics approval: Ethics approval was obtained.

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CORRECTION

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S P Liimatainen, J A Raitanen, A M Ylinen, *et al*. *J Nerol Neurosurg Psychiatry*, 2008; **79**:808–12. The benefit of active drug trials is dependent on aetiology in refractory focal epilepsy. There is an error in the results section in the second paragraph: ''The chances of achieving the remission were significantly higher in the patients with cryptogenic aetiology compared with symptomatic aetiologies (age-adjusted OR 3.74, 95% CI 1.54 to 9.07, p = 0.04).'' The correct p-value should be 0.004, not 0.04.

Interleukin-6 Levels are Increased in Temporal Lobe Epilepsy but not in Extra-Temporal Lobe Epilepsy

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Key words: refractory focal epilepsy, cytokines, epilepsy type

Column title: Interleukin-6 in temporal lobe epilepsy

ABSTRACT

Background: Previous studies have reported activation of inflammatory cytokines in seizures but clinical characteristics of epilepsy associated with cytokine activation have not been well established.

Methods: In this study serum levels of interleukin-6 (IL-6) and interleukin-1 receptor antagonist (IL-1RA) were measured and clinical characteristics of epilepsy were assessed in 86 well-evaluated patients with refractory focal epilepsy and in 5 patients with controlled focal epilepsy. Epilepsy was evaluated based on patient histories, electroclinical findings, and high resolution brain MRI scans. Sixty-three healthy blood donors served as controls.

Results: IL-6 concentrations were chronically increased in epilepsy patients (11%) compared with healthy controls (0%) ($p = 0.007$). Increased levels of IL-6 were more prevalent in patients with temporal lobe epilepsy (TLE) compared to patients with extra-TLE ($p = 0.028$). Also the mean and the median serum levels of IL-6 were higher in patients with TLE than in patients with extra-TLE ($p = 0.042$). Concentrations of IL-1RA were not significantly different in patients compared with controls.

Interpretation: Indicated by increased levels of IL-6 in TLE, epilepsy type is important in determining chronic overproduction of cytokines in refractory focal epilepsy. The results may reflect a chronic immunological process in the brain in patients with refractory epilepsy.

Cytokines such as interleukins (IL) are a heterogeneous group of molecules that are involved in inflammation, immune activation, and cell differentiation or death. Increasing evidence supports the role of cytokines also in seizures. Interleukin-6 (IL-6) knockout mice are more susceptible to seizures induced by kainic acid compared to controls (1), and exogenously applied IL-6 increase the severity of pentylene tetrazole-induced seizures in rats (2). IL-1β prolongs kainic acid induced seizures whereas its natural antagonist interleukin-1 receptor antagonist (IL-1RA) seems to possess anticonvulsive effect (3-6). In experimental studies, activation of cytokines is observed especially in areas of onset of seizure and spreading of discharge (3, 7). In humans, IL-6 concentration rises rapidly in cerebrospinal fluid (CSF) after tonic-clonic seizures (8). In clinical studies, cytokine levels in blood seem to reflect the central nervous system´s production of cytokines (9), mediated through hypothalamus-pituitary-adrenal (HPA) axis and sympathetic nervous system (10, 11). In patients with refractory epilepsy, elevated levels of IL-1 and IL-6, and decreased level of IL-1RA have been detected without evidence of increased production of these molecules from peripheral mononuclear cells (12).

There is histological evidence of immunological alterations in TLE (3, 13-15), and in cortical dysplasias associated with epilepsy (16). The prevalence of autoantibodies is increased in refractory epilepsy (17-19). However, the significance of clinical characteristics of epilepsy has not been well established in patients with possible immunological alterations. In our previous studies, titres of IL-6 measured at the beginning of video-EEG recordings before documented seizures and those measured between documented seizures during the recording have tended to be higher in patients with chronic epilepsy compared with healthy controls (20, 21). We have previously demonstrated an immediate effect of seizures on concentrations of IL-6 and to a lesser extent IL-1RA, the duration of this effect was less than 24 hours. In this study, we aimed to assess long term effects of seizure frequency on prevalence of IL-6 and IL-1RA. IL-β levels were not measured here due to negative results in the previous studies (8, 9, 21). In addition to seizure frequency, we evaluated the

influence of epilepsy type, aetiology, and duration of epilepsy on the prevalence of elevated concentrations of IL-6 and IL-1RA in refractory focal epilepsy.

METHODS

Subjects

This was a prospective 2 year follow-up study in 91 consecutive adult patients with focal epilepsy, 86 patients fulfilling the following inclusion criteria for refractory epilepsy: persistence of seizures after trials of at least two antiepileptic drugs (AEDs) with maximally tolerated doses sequentially or in combination, and duration of AED treatment of at least two years (22, 23). In 5 patients with focal epilepsy the duration of epilepsy was at least two years but they had used only one AED. Patients with moderate or severe mental retardation, dementia or malignant brain tumor were excluded but patients with chronic stable low grade brain tumor were included. Patients with post-stroke epilepsy were excluded due to a local practice according to which they are followed up by general practitioners. The study took place in the outpatient clinic of the Department of Neurology and Rehabilitation in the Tampere University Hospital. All patients gave a written informed consent before blood sampling. The study was approved by the Ethics Committee of the Tampere University Hospital.

Epilepsy types were categorized based on anatomical origin into temporal (TLE), frontal (FLE), parietal (PLE), occipital lobe (OLE) or multifocal epilepsies according to International League Against Epilepsy (ILAE) guidelines (24), based on electroclinical findings (seizure semiology and EEG/video-EEG) and aetiology. Aetiology was defined based on high resolution brain MRI, histological analysis of tumours (if available), and medical history into the following categories:

tumour, vascular malformation (arteriovenous malformation, venous angioma or cavernous angioma), perinatal vascular lesion (brain infarction, intracerebral hemorrhage or anoxia), trauma, hippocampal sclerosis (HS), other hippocampal abnormality (vascular malformation, demyelination or atrophy), cortical dysplasia (CD) (cortical dysgenesis, heterotopia or tuberosis sclerosis), central nervous system (CNS) infection (encephalitis, meningitis or abscess), dual pathology (hippocampal sclerosis associated with another brain lesion), and other (local or diffuse atrophy, non-specific signal change, demyelination or non-specific gliosis). If medical history revealed a clinically relevant head trauma with loss of consciousness or a confirmed central nervous system infection prior to the onset of epilepsy, it was considered to be the cause of epilepsy despite normal imaging results. If no aetiology was found, epilepsy was classified as probably symptomatic (cryptogenic). Categorizing aetiology into four groups was done as follows: HS (including dual pathology), cortical dysplasia (CD), cryptogenic and other causes (trauma, other hippocampal pathology than HS, vascular lesion, tumour, CNS infection, vascular malformation and other causes). All but one patient had undergone a 1.5 Tesla brain MRI with a specific epilepsy protocol (25). One patient had had only brain CT due to claustrophobia. Patients with epilepsy surgery (amygdohippocampal resection or occipital resection) or other lesional surgery were recorded, as well as duration of epilepsy and diagnosed autoimmune diseases. The possibility of brain atrophy in MRI was evaluated in the patients with increased levels of IL-6 or IL-1RA.

Measurements

Blood samplings were performed at the end of the 2 year follow-up between January 2003 and January 2004. From patients with a 12 month remission only the first sample was taken, and from those with seizures in previous 12 months two samples were taken with a three month interval. Seizure frequencies during the month prior to blood sampling (called recent seizures) were recorded in order to detect immune stimulation related to seizures. Average frequencies of seizures during

the last 12 months before blood sampling were classified as infrequent (<1 per month) or frequent (≥1 per month). No seizures were evident in 24 hours before sampling in any patient to rule out immediate effects of seizures. Sixty-three healthy blood donors served as unmatched healthy controls - they had not any sign of infection during a 2-week period before the blood sampling.

Blood samples were obtained between 09:00 and 14:00 during scheduled outpatient visits. Blood was collected in a Vacutainer EDTA vacuum tube and centrifuged at 3000 rpm for 10 min and the samples were stored frozen at -70ºC. IL-6 concentrations in the samples were measured using ELISA kits (Pelikine Compact, Sanquin, Amsterdam, The Netherlands). IL-1RA concentrations were also determined using an ELISA kit (R&D Systems, Minneapolis, MN). All kits were used according to manufacturers' instructions. The sensitivity of the assay for IL-6 was 0.4 pg/ml and for IL-1RA 22 pg/ml. Samples were considered positive if the level of IL-6 or IL-1RA was higher than +3 standard deviations (SD) above mean value of the control group.

Statistical methods

Logistic regression analysis was performed to assess the association of the binary dependent variables, IL-6 and IL-1RA (cut-off=mean+3SD) with explanatory variables, such as epilepsy type, aetiology, age, sex, duration of epilepsy, epilepsy surgery and current/past/ever exposure to each AED. Five covariates (age, sex, epilepsy type, duration of epilepsy and aetiology in patients) were considered in fully-adjusted regression models when applicable. The only available variables for controls were age group and level of IL-6 and IL-1RA. Fisher's exact test was applied when logistic regression or chi-square test was not applicable. All analyses were done using STATA statistical software, 8th version.

RESULTS

Clinical characteristics of patients and controls are presented in Table 1. The prevalence of increased levels of IL-6 was higher in epilepsy patients (11%) compared to healthy controls (0%) (p=0.007). All 10 patients with increased concentrations of IL-6 had TLE (including one patient with multifocal epilepsy involving temporal lobe) whereas none of the patients with extra-TLE had elevated levels (p = 0.028) (Figure 1). The mean and the median serum levels of IL-6 were also higher in the patients with TLE than in patients with extra-TLE [p = 0.042, Wilcoxon rank-sum (Mann-Whitney) test] (Table 2, Figure 2).

Seven patients with increased levels of IL-6 had frequent seizures, whereas three patients had not had seizures for at least one year. Four patients with increased levels of IL-6 had cryptogenic epilepsy, two had HS, one had CNS infection, one had tumour, one had hippocampal atrophy, and one patient had non-specific signal change as aetiology. Aetiology or categorized aetiology was not significantly associated with abnormally high levels of IL-6. Four patients with increased levels of IL-6 had atrophy in brain MRI (two with parietal atrophy, one with mild cortical atrophy and one with local cerebellar atrophy). 10 patients had undergone epilepsy surgery: 9 had had amygdohippocampal resection and 1 had had occipital resection during years 1978-2000. No one had undergone epilepsy surgery during the study or shortly before it.

Age, sex, epilepsy surgery, and duration of epilepsy were not associated with the level of IL-6 in the multivariable and univariable analyses. Among patients with TLE, those with increased levels of IL-6 tended to be older than those with normal IL-6 concentration (OR 1.02, 95% CI 0.97-1.07); they also had longer duration of epilepsy in years (OR 1.03, 95% CI 0.98-1.08) and 80% of them were women ($OR_{\text{female vs. male}}$ 2.84, 95% CI 0.55-14.68) although none of these differences were significant. The overall range for IL-6 was 0.4-10.4 pg/ml in the whole study group. The most

common current AEDs were carbamazepine, lamotrigine, and topiramate. Current use of clonazepam was associated with higher level of IL-6, but the finding was not statistically significant (OR 8.89, 95% CI 0.51-154.62). There were no statistically significant associations between the levels of IL-6 and other current or previous AEDs, or number of current or previous AEDs.

The patients with TLE tended to have less increased levels of IL-1RA (OR 0.42, 95% CI 0.08-2.21) and the mean and the median serum levels of IL-1RA were somewhat lower in them compared to patients with extra-TLE [p = 0.664, Wilcoxon rank sum (Mann-Whitney) test] (Figure 3, Table 2), but none of these findings were significant. The prevalence of IL-1RA was not significantly different between patients with continuous seizures, patients with one year seizure remission and healthy controls. Age, sex, epilepsy surgery, and the duration of epilepsy were not associated with the level of IL-1RA in the multivariable and univariable analyses. The overall range for IL-1RA was 42-2300 pg/ml in the whole study group. Those patients on clonazepam had higher prevalence of increased levels of IL-1RA in their serum samples than other patients (OR 16.80, 95% CI 0.91-309.92; age-adjusted OR 41.97, 95% CI 1.33-1322.52). There were no statistically significant associations between levels of IL-1RA and other current or previous AEDs, or number of current or previous AEDs.

Changes in the levels of IL-6 or IL-1RA during the three month interval between the two samples were not in relation with seizure frequency, aetiology category, type, duration, sex and age. Measurements of IL-6 and IL-1RA were repeated after three months for 98.6% of the patients with a seizure in the month before the first sample. The agreement percentages between the two samples were 92.8% for both IL-6 (Kappa=0.626, $p<0.0001$) and IL-1RA (Kappa=0.631, $p<0.0001$).

DISCUSSION

This study shows the importance of epilepsy type in determining the chronic overproduction of IL-6 in refractory epilepsy, whereas our previous studies have demonstrated the acute activation of cytokine network following seizures (8, 9, 20, 21). All patients with increased levels of IL-6 had TLE which is known to be the most common epilepsy type refractory to drugs. Furthermore, concentrations of IL-6 were higher in patients with TLE compared with patients with extra-TLE. There were no significant differences in levels of IL-1RA between patients with TLE and patients with extra-TLE, although TLE patients tended to have lower prevalence of increased levels and a lower mean concentration of IL-1RA compared with patients with extra-TLE.

There is accumulating histological evidence of activation of immune mediated processes in human temporal lobe epilepsy. Our study provides additional evidence of systemic activation of IL-6 in TLE as an inflammatory biomarker. In previous studies elevated levels of β-amyloid precursor protein and IL-1α immune reaction have been found in hippocampi in patients with TLE (13). Nuclear Factor kappa B, (NFκB), a transcription factor responsible for activation of numerous genes encoding inflammatory mediators, is over expressed in TLE+HS (14). A chronically increased IL-1β and IL-1 receptor type I (IL-1RI) expression has been detected in rat and human TLE tissue (15), and also in resected epileptic tissue of patients with focal cortical dysplasias (16). There are studies supporting the association of certain IL-1β allele polymorphism and TLE (26, 27), but this association is still controversial (28).

Medial temporal lobe structures have been shown to be extremely vulnerable during global ischemia (29) and excessive excitatory activity (30). In viral encephalitis, seizures are probably caused by the activation of the immune system (31). Patients with subacute form of non-paraneoplastic limbic encephalitis and temporal lobe seizures have evidence of chronic

inflammation (19, 32). The structures prone to specific plastic phenomena during memory trace formation and retrieval may, in the case of epilepsy, contribute to progressive severity of the disease (33, 34). Peripheral activation of IL-6 may reflect this process, especially because there is new data linking the activation of inflammatory biomarkers such as IL-6 and the total loss of brain volume in general population (35) as well as in dementia patients (36, 37). In this study we did not perform volumetric analysis of brain MRIs. However, in qualitative scale, brain atrophy was found in 40% of the patients with increased levels of IL- 6. Aetiologies in the present study were diverse in patients with increased levels of cytokines. Patients with cryptogenic epilepsy tended to have an increased prevalence of IL-6, but the sample size in our study was too small to analyze this conclusively. The evaluation of hippocampal structures was categorical in this study. In the future, volumetric analyses of the hippocampus and the total brain volume could help in more detailed characterization of the temporal lobe and general brain pathology in the group of patients with immunological activation. Furthermore, aetiology may not be the main differentiating cause in chronic immunological processes in an epileptic brain. This is supported by the finding that epilepsy surgery was not associated with increased levels of cytokines.

Production of IL-6 in human epilepsy is well-documented demonstrating a temporary effect with return to a basal level in 24 hours. In this study the increase cannot be explained by acute effects of single seizures. In previous studies cytokine levels tended to be higher in epilepsy patients compared with controls (12, 20, 21). In the present study we are able to confirm this finding in a statistically significant way. Most of the patients with increased levels of IL-6 had frequent seizures although there where also patients with seizure remission. The chronic overproduction of cytokines may be due to an active epileptic process in the brain, a finding that is not explained with a direct response to seizures. IL-6 may have both proconvulsive and anticonvulsive properties possibly depending on its concentration, the duration of its action and the complex effect of other inflammatory mediators. Transgenic mice with overexpression of IL-6 develop a severe neurologic

syndrome with seizures (38). Chronic stimulation of IL-6 may lead to structural changes that may predispose to refractory seizures (1, 39).The previous studies have shown a correlation between levels of IL-6 and the severity of seizures (20, 21, 38). Peripheral expression of cytokines has been shown to be a consequence of activation of the central nervous system. In monkeys, intracerebroventricular injection of IL-1β has raised concentrations of IL-6 and IL-1RA in CSF and blood more than intravenous injection (40). In addition, concentrations of adrenocorticotropic hormone (ACTH) and cortisol have risen following the IL-1β injection indicating activation of HPA axis (40). The abundance of projections from the medial temporal lobe to other areas in the brain (including the hypothalamus) may explain the activation of cytokine cascade specifically in TLE. An older finding of increased secretion of prolactin in TLE may support this hypothesis (41).

There were no significant differences in the concentrations or the prevalence of IL-1RA between different groups. The levels of IL-1RA tended to be higher in patients compared with controls, but interestingly there was some indication of decreased concentration of IL-1RA in patients with TLE compared with extra-TLE suggesting a possible imbalance between protective (IL-1RA) and destructive (IL-6) cytokine concentrations in TLE compared with extra-TLE. IL-1RA is a molecule competing for receptor binding with IL-1α and IL-1β by blocking their actions. In mice, intrahippocampal injection of recombinant IL-1RA or its overexpression have reduced bicuculline-induced seizures indicating anticonvulsant properties of IL-1RA (4). In experimental studies the production of IL-1RA has been lower in the brain than in the peripheral inflammation (6). However, the production and the kinetics of cytokines are not fully understood in patients with epilepsy and the findings concerning IL-1RA need to be studied in a larger group of patients.

Most commonly used AEDs did not have a significant effect on the levels of IL-6 and IL-1RA in patients with epilepsy. The current use of clonazepam was associated with increased level of IL-1RA. Peripheral-type benzodiazepines such as midazolam have been shown to suppress the release

of IL-6 (42) that is difficult to apply to our findings. It is possible that our finding was only coincidental, especially since this association was based on only a small number of patients.

This study demonstrated a strong association between the type of epilepsy and the chronic overproduction of IL-6. The results suggest a role for cytokines in the chronic epileptic process in TLE. In the future, more detailed studies of neuromodulatory effects of cytokines on both experimental and clinical epilepsy are needed to assess the significance of this activation. The present study raises a possible role for IL-6 as a clinically significant inflammatory biomarker in epilepsy.

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