Epilepsy Research (2014) 108, 592—596

journal homepage: www.elsevier.com/locate/epilepsyres

SHORT COMMUNICATION

Lack of antibodies to NMDAR or VGKC-complex in GAD and cardiolipin antibody-positive refractory epilepsy

Suvi Liimatainen[a,b,*], Jukka Peltola[a], Aki Hietaharju[a], Lidia Sabater[c], Bethan Lang[d]

[a] Department of Neurosciences and Rehabilitation, Tampere University Hospital, Tampere, Finland
[b] Department of Emergency Medicine, Tampere University Hospital, Tampere, Finland
[c] Service of Neurology and Institut d'Investigacions Biomèdiques August Pi i Sunyer, Hospital Clínic, Barcelona, Spain
[d] Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital, Oxford OX3 9DS, UK

Received 1 August 2013; received in revised form 29 November 2013; accepted 5 December 2013
Available online 6 January 2014

KEYWORDS
Neuronal antibody;
Seizure;
Autoimmune encephalitis;
New-onset epilepsy;
Refractory epilepsy;
Polyautoimmunity

Summary
Background: Over the last few years autoantibodies against neuronal proteins have been identified in several forms of autoimmune encephalitis and epilepsy. NMDA receptor (NMDAR) and voltage gated potassium channel (VGKC) complex antibodies are mainly associated with limbic encephalitis (LE) whereas glutamic acid decarboxylase antibodies (GADA) and anticardiolipin (ACL) antibodies are more commonly detected in patients with chronic epilepsy. Clinical features vary between these antibodies suggesting the specificity of different neuronal antibodies in seizures.
Methods: Serum samples of 14 GADA positive and 24 ACL positive patients with refractory epilepsy were analyzed for the presence of VGKC or NMDAR antibodies.
Results: No positive VGKC or NMDAR antibodies were found in these patients.
Conclusions: The results confirm the different significance of these neuronal antibodies in seizure disorders. Different autoantibodies have different significance in seizures and probably have different pathophysiological mechanisms of actions.

Abbreviations: Ab, antibody; ACL, anticardiolipin; AED, antiepileptic drug; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CASPR2, contactin-associated protein-like 2; DTX, dendrotoxin; FLE, frontal lobe epilepsy; GABA B, gamma amino butyric acid B; GAD, glutamic acid decarboxylase; GADA, glutamic acid decarboxylase antibody; Ig, immunoglobulin; IGE, idiopathic generalized epilepsy; JME, juvenile myoclonic epilepsy; LE, limbic encephalitis; LGI1, leucine-rich, glioma inactivated 1; NMDA, N-methyl-D-aspartate; NMDAR, N-methyl-D-aspartate receptor; OLE, occipital lobe epilepsy; SPS, stiff person syndrome; T1D, type 1 diabetes; TLE, temporal lobe epilepsy; TLE + HS, temporal lobe epilepsy associated with hippocampal sclerosis; VGKC, voltage-gated potassium channel.

* Corresponding author at: Department of Neurosciences and Rehabilitation, Tampere University Hospital, P.O. Box 2000, 33521 Tampere, Finland. Tel.: +358 3 31166339; fax: +358 3 31164351.
E-mail address: suvi.liimatainen@fimnet.fi (S. Liimatainen).

0920-1211/$ — see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.eplepsyres.2013.12.015

Introduction

There is rapidly increasing amount of evidence on the importance of autoimmunity in the etiology for refractory epilepsy. Several autoantibodies such as antibodies against components of the voltage-gated potassium channel (VGKC) complex, glutamatergic N-methyl-D-aspartic acid receptor (NMDAR) and glutamic acid decarboxylase antibodies (GADA) have been found in patients with different seizure disorders (Palace and Lang, 2000; Peltola et al., 2000a,b; Vincent et al., 1999; Ranua et al., 2004; Liimatainen et al., 2009; Bien and Scheffer, 2011; Vincent and Bien, 2008; Suleiman et al., 2011; Quek et al., 2012; Brenner et al., 2013; Hacohen et al., 2013). However, a question regarding the specificity of these autoantibodies has been raised because clinical features vary in different antibody-associated disorders such as acute onset encephalitis, new-onset epilepsy and long-standing refractory epilepsy. In our previous studies GADA have shown association with refractory temporal lobe epilepsy (TLE), and patients with epilepsy and GADA have been positive for several other autoantibodies suggesting the presence of polyautoimmunity of these patients (Peltola et al., 2000b; Liimatainen et al., 2010).

VGKC complex and NMDAR antibodies have mainly been observed in limbic encephalitis (LE) with seizures. In these cases immunotherapeutic agents have been shown to be effective in diminishing the symptoms and decreasing the titer of antibodies supporting the role of the autoantibodies in pathophysiology of these disorders (Vincent et al., 2004; Dalmau et al., 2008; Irani et al., 2008). High titer GADA antibodies have also been detected in cohorts of new-onset TLE initiated by LE where they appear to define a cohort of hard-to-treat nonparaneoplastic LE patients (Malter et al., 2010). However, variance of antibody titers as a result of therapeutic interventions has also been demonstrated LE with GADA (Kanter et al., 2008).

VGKC complex and NMDAR antibodies belong to surface antigens whereas antibodies to GADA are thought to be directed against intracellular epitopes with a diagnostic significance but without known pathophysiological mechanisms (Bien et al., 2012).

Although the suggested therapeutic interventions are currently quite similar regardless of the different autoantibodies, there are emerging differences (e.g. GAD vs. NMDAR antibodies) underlying the important issues of specificity of these autoantibodies. In another set of studies we have demonstrated that patients with recurring seizures or with various developmental disorders and epilepsy had more immunoglobulin (Ig) G class anticardiolipin (ACL) antibodies compared to healthy controls subjects (Liimatainen et al., 2009; Lehtimäki et al., 2011; Peltola et al., 2000a). The significance or specificity of these ACL antibodies is far less established compared with GADA, NMDAR or VGKC antibodies.

The purpose of this study was to analyze serum samples from our previously published data on ACL (Liimatainen et al., 2009) or GADA (Liimatainen et al., 2010) positive patients for the presence of NMDAR or VGKC antibodies to evaluate the significance of these antibody-positive patients with refractory epilepsy.

Materials and methods

We have previously analyzed GADA titer in 253 patients with refractory epilepsy (Liimatainen et al., 2010). One hundred eighty-six patients had refractory focal epilepsy with persistent seizures after administrating two antiepileptic drugs (AEDs) sequentially or in combination and a duration of epilepsy at least two years (Kwan et al., 2010). All available modern antiepileptics were used in these patients. 209 patients had focal epilepsy, 34 patients had generalized epilepsy and in 10 patients focal epilepsy type was unknown. In this study 15 patients had positive GADA titer, seven of them with high GADA [$\geq$1000 relative units (RU)/ml]. Only 1 had type 1 diabetes (T1D) (GAD titer 1680 RU/ml). In another set of study we measured ACL antibodies in 105 patients with refractory focal epilepsy, this study cohort was a part of GADA study cohort; positive ACL IgG titer was observed in 27 patients (Liimatainen et al., 2009). No post-stroke epilepsy patients were included.

We measured VGKC-complex and NMDAR antibodies in patients with either GADA or IgG ACL to rule out whether these antibodies were only concurrent antibodies in the setting of a more complex autoantibody response. The serum samples of 13 patients with GADA and 24 patients with IgG ACL antibodies were tested for antibodies to VGKC-complex antibodies by immunoprecipitation of 125I-α-dendrotoxin (DTX)-labelled rabbit whole brain extract, and were considered positive if the individual titer exceeded 100 pM; 3 standard deviations above the mean titer observed in healthy controls (McKnight et al., 2005). Antibodies to NMDAR and to VGKC-complex proteins (LGI1 and CASPR2) were determined by a cell-based assay as previously described (Brenner et al., 2013).

The study was approved by the local Ethics Committee, and all the patients gave a written informed consent. The work was carried out in accordance with Declaration of Helsinki and Uniform Requirements for manuscripts submitted to Biomedical journals.

Results

Clinical characteristics of the study patients are presented in Tables 1 and 2 (see for details Liimatainen et al., 2009, 2010). We did not find any increased concentrations of NMDAR or LG1/CASPR2-antibodies in our patients. High GADA values were consistent with our previous results supporting the reliability of these different analysis methods. Two ACL positive patients had positive GADA titer, one of them with very high titer GADA (142,520 RU/ml).

Discussion

Several autoantibodies have been associated with different seizure disorders. Antibodies to neuronal proteins such as NMDAR and VGKC are typically associated with subacute form of LE. There are also LE patients with high titer GADA. In new-onset epilepsy increased titers of ACL and antinuclear antibodies were observed before antiepileptic treatment (Peltola et al., 2000a). An autoimmune pattern of patients with chronic refractory epilepsy seems to be