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Special Communication

Complement system dysregulation in patients affected by Idiopathic Generalized Epilepsy and the effect of antiepileptic treatment

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ABSTRACT

Complement system dysregulation has been hypothesized as a possible pathogenetic factor triggering epileptogenesis in both animal models and human studies. The aim of the present study is to evaluate the complement system in adult patients affected by idiopathic generalized epilepsy (IGE), either untreated or treated by antiepileptic drugs (AEDs).

Thirty-seven IGE patients were compared to a population of 20 matched healthy controls. IGE patients underwent neurological investigation, epilepsy diary, 24-h EEG recording, and blood sample for the assessment of the complement factors C3 and C4, fibrinogen, and C-reactive protein (CRP) serum levels. We excluded patients with clinical and subclinical seizures in the 24 h before obtaining the blood sample. We observed decreased C3 and C4 serum levels in IGE patients with respect to controls ($p < 0.05$), and in untreated compared to treated IGE patients ($p < 0.05$). We found significant correlations in the IGE group linking C3 to C4 ($R = 0.34$), CRP ($R = 0.49$), and fibrinogen serum levels ($R = 0.61$).

This study proved a significant alteration of the complement system in IGE patients not related to ictal conditions. The hyperactivation of the complement cascade was more significant in untreated than in treated IGE patients. Hence, this study documented the complement factors dysregulation in patients affected by IGE. However, the impact of complement system alteration in the epileptogenetic process needs to be clarified.

1. Introduction

Converging experimental and human studies suggest that the immune system may play a critical role in epileptogenesis (Vezzani and Friedman, 2011). Despite progresses in pharmacological and surgical treatments of epilepsy, the complex and multifactorial mechanisms leading to the generation of seizures are not completely understood, mainly in idiopathic generalized epilepsies (IGE) (Vezzani and Friedman, 2011). Recent findings propose the involvement of inflammatory mediators in both the origin of individual seizures and the epileptogenic process (Aronica et al., 2007). Accordingly, neuroinflammation has been identified as a possible crucial mechanism in the pathophysiology of seizures and epilepsy (Vezzani and Granata, 2005; Riazi et al., 2010; Choi et al., 2009). In keeping with this hypothesis, several inflammatory mediators, such as interleukines, chemokines, and adhesion molecules, have been evaluated in both epileptic patients and experimental models of epilepsy (Vezzani and Friedman, 2011). Among them, also the complement cascade has been investigated in rat models of temporal lobe epilepsy (TLE) (Aronica et al., 2007). In particular, increased expression of the complement factors C1q, C3, and C4 has been proved in the hippocampal and entorhinal cortex of animal tissues (Xiong et al., 2003). Moreover, the upregulation of C1q and C3d protein expression has been documented in TLE tissues of drug-resistant epileptic patients affected by hippocampal sclerosis (HS) (Aronica et al., 2007). On these bases, it has been demonstrated a prominent activation of the complement cascade in patients affected by TLE due to HS (Aronica et al., 2007). Therefore, the complement system may be altered in patients affected by idiopathic or secondary focal epilepsies (Başaran et al., 1994; Bostantjopoulou et al., 1994).

The complement system represents the backbone of the innate immune system (McGeer et al., 2016). It is constituted by more than 25 components working together in a clockwork way in order to discriminate and eliminate foes. Complement exerts its function by priming key proteins. Among them, C3 and C4 are considered central

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Table 1
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		IGE (n = < 37) mean ± SD	Controls (n = < 20) mean ± SD	P value
Age		26.6 ± 9.49	24.6 ± 8.16	NS
Sex		17F 20M	10F 10M	NS
Age of seizure onset		13.8 ± 4.3	NA	
Seizure type	TA	6	NA	NA
	MS	9		
	GTCS	22		
AEDs	VPA	15	NA	NA
	LEV	12		
	PB	4		
	LTG	2		
	Drug-naive	9		
Fibrinogen (mg/dL) r.i. 200–400 mg/dL		291.6 ± 68.48	379.2 ± 61.42	< 0.05
C-Reactive Protein (mg/L) r.i. 0–3 mg/L		1.1 ± 2.60	0.3 ± 0.22	< 0.05
C3 (mg/dL) r.i. 90–180 mg/dL		107.6 ± 17.9	117.2 ± 7.99	< 0.01
C4 (mg/dL) r.i. 10–40 mg/dL		22.2 ± 5.77	24.85 ± 4.53	< 0.05
IGE (n = 37)				
		Therapy (n = 28) mean ± SD	Drug-naive (n = 9) mean ± SD	
Age		27.7 ± 9.79	19.9 ± 5.69	NS
Sex		12F 16M	5F 4M	NS
Fibrinogen (mg/dL)		294.1 ± 70.56	273.8 ± 59.08	NS
C-Reactive Protein (mg/L)		1.2 ± 2.82	0.5 ± 0.09	NS
C3 (mg/dL)		109.1 ± 17.17	99 ± 15.75	< 0.05
C4 (mg/dL)		23 ± 5.52	17.7 ± 4.92	< 0.05
		Seizure Free (n = 24) mean ± SD	Persistent Seizures (n = 13) mean ± SD	
Age		27 ± 8.98	26.1 ± 10.32	NS
Sex		11F 13M	6F 7M	NS
Fibrinogen (mg/dL)		275.5 ± 54.24	314.6 ± 81.46	NS
C-Reactive Protein (mg/L)		0.7 ± 1.46	1.7 ± 3.66	NS
C3 (mg/dL)		107.3 ± 15.84	108.1 ± 19.4	NS
C4 (mg/dL)		21.7 ± 6.52	22.8 ± 4.73	NS

Abbreviations: IGE, idiopathic generalized epilepsy; AEDs, antiepileptic drugs; VPA, valproic acid; LEV, levetiracetam; PB, phenobarbital; LTG, lamotrigine; TA, typical absences; MS, myoclonic seizures; GTCS, generalized tonic–clonic seizures;NS, not significant; NA, not admitted; SD, standard deviation; r.i., reference interval.

players of the complement system, since these proteins have an important role in the complement activation pathways (McGeer et al., 2016). Considering the complexity of this system, it could happen that a deranged complement response may mistakenly attack host tissue. Such attacks are known to contribute to the pathology of a spectrum of autoimmune diseases (McGeer et al., 2016).

Although complement system dysregulation has been investigated in TLE, evidence of complement system hyperactivity in IGE is still missing. IGE is a group of epileptic syndromes with no identifiable causes other than a possible genetic/familial predisposition, which may involve ion channels or neurotransmitter receptors (Caraballo and Dalla Bernardina, 2013). The pathogenesis of IGE remains poorly understood, although the clinical and electroencephalografic (EEG) features have been well recognized. Therefore, researches about the mechanisms at the basis of epileptogenis in IGE are constantly encouraged.

Hence, this report is aimed at investigating the possible role of inflammation and complement system dysregulation in a population of IGE patients.

2. Methods

In our study, we included consecutive adult outpatients affected by IGE admitted at the Epilepsy Centre of the University Hospital of Rome "Tor Vergata", undergoing history, epilepsy diary, 24-h EEG recording, and blood sample. IGE was diagnosed according to the International League Against Epilepsy proposed classification, through observation of EEG and clinical hystory (Engel, 2001).

All IGE patients underwent a 24-h EEG monitoring, and the following morning the blood sample for the assessments of serum levels of complement factors C3 and C4, fibrinogen, and C-reactive protein (CRP). IGE patients were compared to a population of controls undergoing EEG and serum sample for research purposes.

Exclusion criteria for IGE patients and controls were: chronic liver disease; use of corticosteroids and any drugs over the 4 weeks preceding blood sample; autoimmune disorders; symptoms or signs of acute or chronic inflammatory disorders; recent infections. Moreover, specific inclusion criterion for IGE patients was the absence of clinical and subclinical seizures in the 24 h before the blood sample, in order to exclude complement factors changes owing to ictal conditions. As previously reported, generalized spike-and-wave or poly spike-and-wave discharges with a duration longer than 6 s during the 24-h EEG monitoring were considered ictal (Niedermayer and Lopes da Silva, 1987; Fattouch et al., 2012).

We took venous blood samples from IGE patients and controls between 08:00 and 09:00 am after overnight fasting. Quantitative measurements of serum complement C3 and C4, fibrinogen and CRP were analyzed by an immunonephelometric method, performed on a Dimension System Vista (Siemens Healtcare Diagnostics). The intensity of light scattering was measured at 840 nm and it is proportional to the concentration of the respective analytes in the sample. Reference intervals for serum biomarkers levels were the following: i) C3, 90–180 mg/mL; ii) C4, 10–40 mg/dL; iii) CRP, 0–3 mg/L; iv) fibrinogen, 200–400 mg/L.

On the basis of some clinical features, IGE patients were distributed in subgroups: untreated and antiepileptic drugs (AEDs)-treated IGE patients; seizure free patients and patients with persistent seizures (> 1 seizure/year).

The study protocol, approved by the Independent Ethical Committee of University Hospital of Rome "Tor Vergata", was conducted according to the STROBE statement.

A commercial software (Statistica 10.0 program; Statsoft Inc, Tulsa, OK, USA) was used for the statistical analysis. The Kolmogorov-Smirnov test was used to control the normal distribution of the obtained data. The Student *t*-test was used to compare C3, C4, CRP, and fibrinogen in IGE patients vs controls. The same test was used to compare data between untreated vs AEDs-treated IGE patients, and between seizure free