Anti-inflammatory strategies during epileptogenesis

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Publication date
2013

Chapter 4

Complement protein 6-deficiency in PVG/c rats does not lead to neuroprotection against seizure induced cell death

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Neuroscience 2011; 188:109-16

Abstract

Purpose: Since the membrane attack complex (MAC), an end product of the activated complement cascade, has been shown to play a role in neurodegeneration, we investigated to which extent MAC contributes to structural reorganization, neuronal cell death and seizure development.
Methods: We used the kindling model and the electrically induced status epilepticus (SE) model in C6-deficient animals (that are unable to form MAC) and wild-type (WT) female PVG/c animals. Structural reorganization was investigated using hilar cell counts and mossy fiber sprouting. Seizure development was monitored using electroencephalographic (EEG) recordings.
Conclusion: No difference in hilar cell counts were observed between C6-deficient and WT kindled rats. Kindling development was not different between C6-deficient and WT rats. All rats exhibited generalized seizures within 34 stimulations; seizure duration was not affected. Four weeks after SE, hilar cell counts were not different between the 2 groups; also no difference in seizure development could be observed during the 4 week observation period.
Discussion: The lack of effect of C6 deficiency on hilar cell death and mossy fiber sprouting after kindling or electrically induced SE suggests that MAC formation does not significantly contribute to structural changes in this region.

Introduction

Inflammatory processes in the brain are implicated in epilepsy (Gorter, et al. 2006, Vezzani 2005, Vezzani, et al. 2008). Additionally, two studies provide direct evidence that IL-1, which plays a major role early in the induction of inflammation, is involved in epileptogenesis (Auvin, et al. 2010b, Ravizza, et al. 2008b), indicating the relevance of inflammation in epilepsy and the development of epilepsy.

One of the inflammatory pathways that might be involved is the complement system which belongs to the innate immune response. The ultimate role of complement activation is removal of pathogens such as bacteria, virus-infected cells, cell debris or apoptotic cells. The central nervous system (CNS) constitutively expresses complement proteins (Yu, et al. 2002). In the developing CNS, the complement system is also involved in synaptic organization (Stevens, et al. 2007). Upregulated expression of complement proteins has been found in numerous CNS pathologies such as Alzheimer's disease, Huntington's disease, traumatic brain injury (TBI) (Bellander, et al. 2001, Gasque, et al. 2000) and epilepsy (Aronica, et al. 2007, Gorter, et al. 2006, Jamali, et al. 2006, Jamali, et al. 2010). Complement activation is associated with neurodegeneration (Bonifati and Kishore 2007, Francis, et al. 2003, Rus, et al. 2006, van Beek, et al. 2003), which is supported by several studies that have actually shown a neuroprotective effect of complement inhibition in animal models for TBI (Leinhase, et al. 2006, Leinhase, et al. 2007) and stroke (Arumugam, et al. 2009). C1q knock-out mice develop spontaneous absence-like seizures due to impaired synaptic pruning. (Chu, et al. 2010).

Complement activation may contribute to cell death and seizure generation. The main contributor to complement-induced cell death is believed to be the membrane attack complex (MAC). MAC is the end product of complement activation and is composed of several complement proteins, forming a pore-shaped structure that is inserted into the cell membrane to cause lysis by colloid osmosis. When MAC is inserted into the cell membrane, the first event that can be measured is an influx of

extracellular Ca^{2+}, which eventually can cause the cell to die (Cole and Morgan 2003). Sub-lytic amounts of MAC formation in the cell membrane may cause membrane depolarization (Wiedmer and Sims 1985), however this effect has been found in blood platelets and has not been reproduced in neuronal cultures or in brain slice recordings.

Interestingly, artificially induced formation of the membrane attack complex (MAC) in the hippocampus, by sequential infusion of C5b6, C7, C8, and C9 proteins, induces neurodegeneration and seizures (Xiong, et al. 2003). In a previous study we demonstrated that the complement proteins are overexpressed and MAC is formed in human as well as experimental epilepsy (Aronica, et al. 2007). In the present study we investigated the relevance of MAC formation in two animal models for epilepsy and epileptogenesis in more detail. To this aim we used a rat strain with a hereditary deficiency in complement protein 6 (C6) (Bhole and Stahl 2004, Leenaerts, et al. 1994, van Dixhoorn, et al. 1997). Since C6 is essential for MAC formation, C6-deficient rats are unable to form MAC.

First, we used the kindling model for epileptogenesis so that we could investigate the contribution of MAC on progression of seizure severity during subsequent kindling stimulations. In this model for epileptogenesis, where cell loss is minor, the behavior and duration of the evoked seizures progress over time. We expected that C6-deficient rats would exhibit a slower progression of seizure severity compared to wild-type (WT) animals. Since cell death and structural reorganization is more extensive after status epilepticus (SE), we also used the electrically induced status epilepticus model (Gorter, et al. 2003). In this model we expected that MAC would contribute to cell death after tetanic electrical stimulations. C6-deficiency would then have a pronounced neuroprotective effect, as previously shown in a peripheral nerve crush model (Ramaglia, et al. 2007). We hypothesized that a neuroprotective effect could also lead to a slowing down of seizure development. Since the complement system has also been implicated in synaptic organization (Stevens, et al. 2007) we also investigated the extent of mossy fiber sprouting after kindling and after SE.

Material and Methods

Experimental animals

Adult female PVG/c (Harlan Netherlands, Horst, The Netherlands) and C6-deficient PVG/c$^-$ (Amsterdam Medical Center, Amsterdam, The Netherlands) rats weighing 150-200 grams were used for this study, which was approved by the University Animal Welfare committee. The rats were housed individually in a controlled environment (21±1°C; humidity 60%; lights on 08:00 a.m. - 8:00 p.m.; food and water available *ad libitum)*.
C6-deficient rats are a substrain of WT PVG rats and do not form C6 protein, most likely due to a post-transcriptional error (Leenaerts, et al. 1994, van Dixhoorn, et al. 1997).

Electrode implantation

In order to record hippocampal EEG and to induce kindling stimulations or SE, insulated stainless steel electrodes were implanted in the left dentate gyrus and angular bundle respectively, as described previously (Gorter, et al. 2001). Two weeks after recovery from the operation, rats were connected to a stimulation and recording system (NeuroData Digital Stimulator, Cygnus Technology Inc, Delaware Water Gap, PA, USA), with a shielded multistrand cable and electrical swivel (Air Precision, Le Plessis Robinson, France). After habituation to the new condition, rats were either stimulated according to a kindling protocol or according to a protocol to induce SE.

Daily kindling stimulations

Animals were kindled twice a day by electrical stimulation of the angular bundle, with a stimulus of 2 seconds (biphasic pulses, 0.5 ms pulse duration intensity 500 µA, frequency of 50 Hz). This stimulus was sufficient to induce an afterdischarge (AD), which is defined by a burst of EEG-activity lasting at least 10 seconds. Behavior was recorded by the Racine scale for seizure severity (Racine 1972).

Stage 1: behavioral arrest, eye closure, facial clonus and 'wet dog shakes'.
Stage 2: head nodding associated with more severe facial clonus.
Stage 3: forelimb clonus
Stage 4: bilateral clonus accompanied by rearing.
Stage 5: generalized tonic-clonic seizures.
It was assumed that the transition from a focal to a generalized seizure occurred between a stage 4 and a stage 5 seizure.

SE induction

Rats underwent tetanic stimulations (50 Hz) of the angular bundle until a SE was evoked, as described previously (Gorter, et al. 2001). SE was characterized by periodic epileptiform discharges (PEDs) of 1-2 Hz, which was accompanied by behavioral and electroencephalographic (EEG) seizures. Four hours after onset, the SE was controlled with an intraperitoneal injection of 10 mg/kg diazepam (1.4 mg/ml diazepam in a 10% ethanol solution). EEG was recorded continuously (24 hours/day), as described previously (Gorter, et al. 2001) until 4 weeks after SE induction, when the animals were sacrificed. The EEG recordings were reviewed off-line. EEG seizures were defined by alteration of the EEG signal with minimum of two-fold increase of amplitude and an increase in
As a control, we used non implanted C6-deficient animals.

Histology and hilar cell count

For the kindling study, the animals were sacrificed 24 hours after the last kindling stimulation, when they experienced at least 5 generalized seizures. The animals that underwent SE, were sacrificed 4 weeks after SE induction, following the same protocol. Non-implanted animals (n=6) were used as controls and sacrificed following the same protocol.
Briefly, rats were deeply anesthetized with pentobarbital (Nembutal, intraperitoneally, 60 mg/kg) and perfused via the ascending aorta as described before (Gorter, et al. 2001).

Brains were dissected and the left hemisphere was kept on EDTA (0.3 M, pH 6.7) until further processing for embedding in paraffin. The right hemisphere was frozen in isopentane (-32°C) and sectioned on a sliding microtome into 40 µm sagittal sections. Sections were processed for a staining with cresyl violet (Nissl) as described previously (Gorter, et al. 2001). Sections 3.75-3.95 mm from the midline (Paxinos and Watson 1998) were used for cell counts. The hilar region was defined by the border with the granule cell layer and by straight lines drawn from the ends of the granule cell layer to the proximal end of the CA3 pyramidal cell layer. Per section, the number of neurons (cells larger than 10 µm in size) (Gorter, et al. 2003) was counted by two independent observers.

A number of sections (3.75-3.95 mm from the midline) (Paxinos and Watson 1998), was mounted on superfrost plus slides (Menzel-Gläser, Brunswick, Germany) for a modified Timm's staining of the mossy fibers (Sloviter 1982). Sections were processed at the same time with the same development time (60 minutes). The extent of synaptic reorganization of the mossy fibers was evaluated by two observers according to a standardized 0-5 scale according to the extent and density of zinc-stained granules (Cavazos, et al. 1994). 0: No zinc staining between the tips and crest of the dentate gyrus (DG). 1: Sparse zinc staining in the supragranular region in a patchy distribution between the tips and crest of the DG. 2: More abundant staining in the supragranular region with patchy distribution between tips and crest of the DG and a continuous pattern near the tips. 3: Prominent staining in the supragranular region in a continuous pattern between the tips and crest of the DG. 4: Prominent staining in the supragranular region that forms a confluent dense laminar band in the inner molecular layer (IML) but does not completely fill it. 5: Confluent dense laminar band of staining in the supragranular region that completely occupies the IML. Intermediary values were scored in case the suprapyramidal blade had a different score than the infrapyramidal blade. Nissl and Timm stained sections were photographed using bright-field illumination on a Zeiss Axiophot microscope, equipped with a digital

camera (MicroFire, Optronics, Golate, CA, USA), using StereoInvestigator software (version 6) and imported into Adobe Photoshop (version 8.0). This program was used to optimize contrast and brightness, but not to enhance or change the image content in any way.

Immunocytochemistry, NeuN+ counts and GFAP scoring

The left hemispheres were kept on EDTA (0.3 M, pH 6.7) after dissection and embedded in paraffin. Tissue was sectioned (6 µm) in the sagittal orientation, sections were mounted on organosilane-coated slides (SIGMA, Saint-Luis MO, USA) and used for immunocytochemistry. Sections were deparaffinated in xylene (3x 3 min), rinsed in ethanol (100%, 96%, 70%, 3 min each) and incubated in 0.03% H_2O_2 in methanol for 20 min. Slides were washed 3x with PBS (0.01 M, pH 7.4), placed in citrate buffer (0.01M, pH 6) and autoclaved (120°C, 10 min) for antigen retrieval. Primary antibodies (rabbit-anti-GFAP polyclonal 1:4000, DAKO, Glostrup, Denmark; mouse-anti-NeuN 1:2000, Millipore, Billerica, MA, USA) were diluted in PBS and incubated overnight at 4ºC. After washing with PBS 3x, slides were incubated with the secondary antibody (polyclonal rabbit-anti-mouse biotinylated 1:200 or polyclonal swine-anti-rabbit-biotinylated 1:300, DAKO, Glostrup, Denmark) in PBS 30 min at room temperature. Slides were washed with PBS 3x and thereafter incubated with AB-mix of the ABC-kit from Vectastain (Vector Laboratories, Brunschwig Chemie, Amsterdam, The Netherlands) diluted 1:50 in PBS containing 10% human serum and incubated at room temperature for 30 min and then washed 3x with PBS. Sections were stained with a DAB solution (0.5 mg/ml DAB in 0.05 M Tris-HCl pH 7.6 with 0.2 % H_2O_2) at room temperature for 5-10 min. Thereafter, sections were counterstained with cresyl violet.

Stained sections were photographed using bright-field illumination on a Zeiss Axiophot microscope, equipped with a digital camera (MicroFire, Optronics, Golate, CA, USA), using StereoInvestigator software (version 6) and imported into Adobe Photoshop (version 8.0). This program was used

to optimize contrast and brightness, but not to enhance or change the image content in any way.
From sections 3.75-3.95 mm from the midline (Paxinos and Watson 1998) the ventral horn of the hippocampus was used for counts of NeuN+ cells and GFAP scoring. For NeuN+ cell counts, the hilar region was defined by the border with the granule cell layer and by straight lines drawn from the ends of the granule cell layer to the proximal end of the CA3 pyramidal cell layer. Per section, the number of NeuN+ cells was counted by two independent observers.
GFAP scores were given for the dentate gyrus (DG), CA3 and CA1 area of the ventral horn of the hippocampus. GFAP immunoreactivity was defined as follows: 1: resting astrocytes that stain lightly, soma and processes just visible; 2: resting astrocytes with clearly, darker stained processes and a clearly visible soma; 3: reactive astrocytes, bigger, with a swollen morphology (soma and processes) and/or a dense network of processes. GFAP frequency was defined as follows: 1: sparse occurrence of GFAP+ cells; 2: moderate occurrence of GFAP+ cells, with a regular distribution; 3: GFAP+ cells occur with a high frequency; 4: very high frequency of GFAP+ cells, cluster formation.

Statistical comparisons

Figure 1a represents median values. Data was analyzed with a Mann-Whitney U test, with a post-hoc Bonferroni correction.
The number of ADs required to evoke the first generalized seizure is tested with a Mann-Whitney U test, where $p<0.05$ was assumed to be significantly different.
Figure 1b represents mean values ± sem. Significance is tested with an two-way independent ANOVA, with a post-hoc Bonferroni correction.
The number of hilar cells are representend as mean values ± sem, significance is tested with a student's t-test, $p<0.05$ was assumed to be significantly different.

The scores for mossy fiber sprouting and GFAP were tested with a Mann-Whitney U test, $p<0.05$ was assumed to be significantly different.

Results

Effects of C6-deficiency on kindling epileptogenesis, cell death and mossy fiber sprouting

The behavior of evoked seizures developed over time from a stage 1 seizure after the first few kindling stimulations to a generalized seizure (stage 5) after the last stimulations. Both WT (n=10) and C6-deficient rats (n=8) experienced generalized seizures within 34 ADs. The number of ADs required to evoke the first generalized seizure (stage 5) was not different between C6-deficient rats (range 15-34, median 22.5) and WT rats (range 9-24, median 18.1).

The development of seizures was not different between the two groups (figure 1A). C6-deficient animals appear to develop more slowly into generalized seizures, however this difference does not reach significance. Seizure durations increased over time after each stimulation but the progression of AD duration was similar in WT and C6-deficient rats (figure 1B).

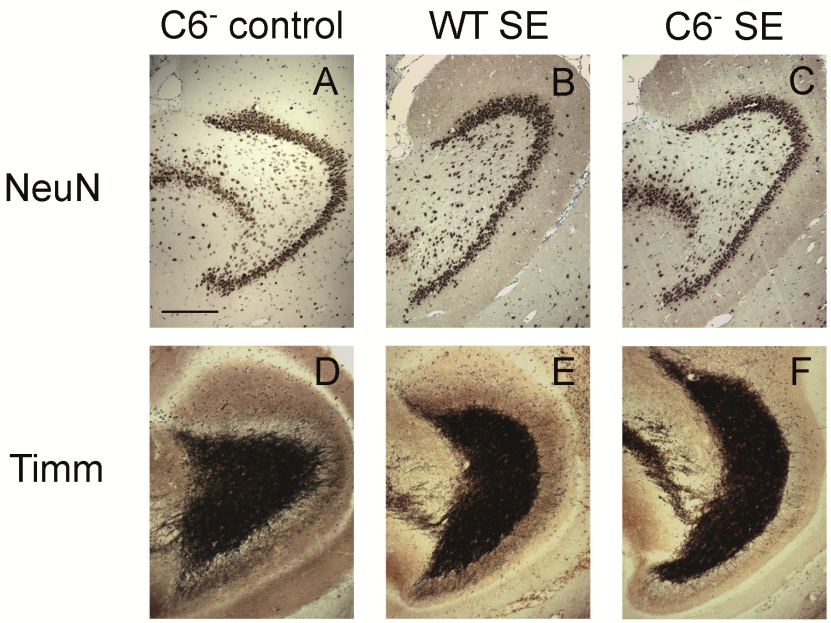

Figure 1: *NeuN and Timm's stained sagittal hippocampal sections 4 weeks after SE. NeuN stainings of C6-deficient controls* ***(A)*** *C6-deficient SE* ***(B)*** *and WT SE* ***(C)*** *animals. Loss of neurons in the ventral hilus of the hippocampus is clear in C6-deficient SE and WT SE animals, however no difference in neuronal loss between C6-deficient (C6-) SE and WT SE animals was observed.*
Timm stainings show moderate zinc staining of the mossy fibers in the ventral horn of the hippocampus in C6-deficient controls ***(D)****, C6-deficient SE* ***(E)****, and WT SE* ***(F)*** *animals, although no difference between groups was observed. Scale bar: 250 µm.*

Rats were sacrificed 24 hours after the last AD. Non-implanted C6-deficient animals were used as controls. Neurons (cells with a diameter of >10 µm) in the hilar region of the right hippocampus were counted from Nissl-stained sections. The number of hilar neurons was not different between the C6-deficient controls (267±13, n=6) and C6-deficient kindled (231±11, n=5) and WT (270±21, n=8) kindled animals (figure 2A-2C).
The extent of mossy fiber sprouting did not differ between C6-deficient (range 0.5-1.5, median 0.5) and WT (range 0.5-1.5, median 0.75) rats or between C6-deficient control (range 1-1.5, median 1) and kindled animals.

The sections showed either no or sparse zinc staining in the supragranular region (figure 2D-F).

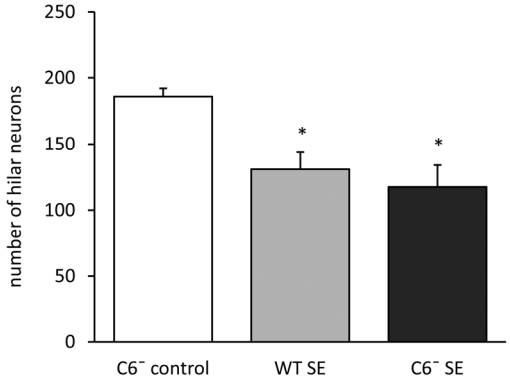

Figure 2: *Quantification of hilar NeuN-positive cells 4 weeks after SE. The number of NeuN-positive hilar cells was significantly lower in C6-deficient and WT animals that experienced SE, compared to C6-deficient controls (ANOVA and post-hoc t-test, * = p<0.05) but the extent of neuronal damage was not different between C6-deficient (C6⁻) SE and WT SE animals.*

Effects of C6-deficiency on cell death and mossy fiber sprouting after SE.
Rats were sacrificed 4 weeks after electrically induced SE. Contrary to what we normally observe in post-SE rats we recorded only one electrographic seizure in 3 out of 10 C6-deficient rats during this period. None of the WT rats (n=6) experienced seizures during this period. To determine the extent of hippocampal cell death, neurons from NeuN stained, sagittal hippocampal sections were counted. As expected, rats that experienced SE (C6-deficient SE 118±17, n= 10; WT SE 131±13, n=5) exhibited a significant ($p<0.05$) lower number of neurons non-implanted controls (186±6, n=6) (figure 3A-3C and figure 4). However, C6-deficient SE rats did not differ from SE WT rats ($p=0.64$; figure 4).

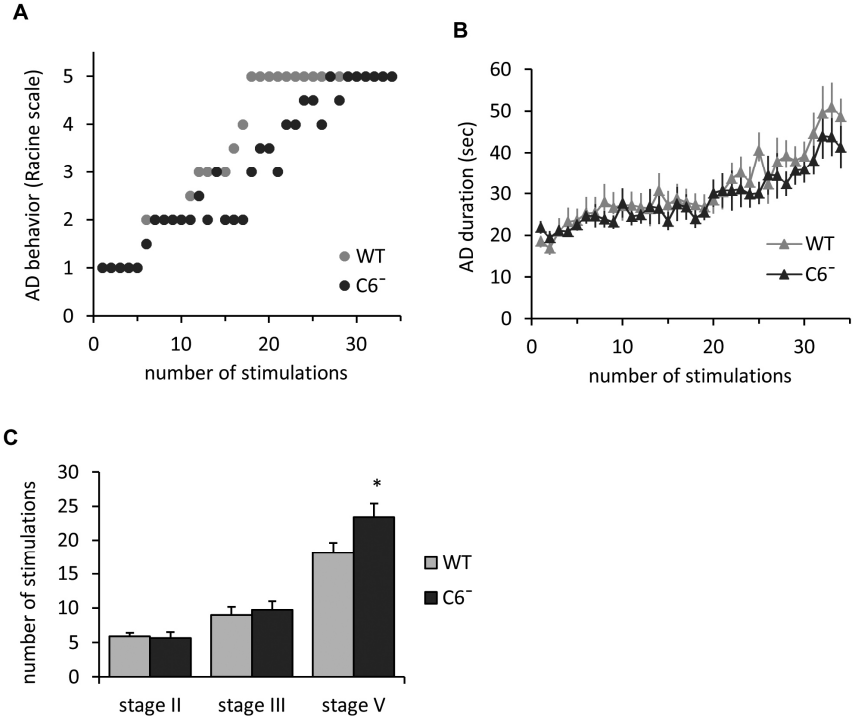

Figure 3: *Seizure development during kindling epileptogenesis. Median values are shown. Kindling epileptogenesis in C6-deficient rats (C6⁻, n=8) appeared slower between stimulation number 16 to 24 than WT (n=10) but this was not significant (Mann Whitney, p>0.05)* ***(A)****. Development of seizure duration (average values) in C6-deficient and WT rat showed a similar increase over time* ***(B)****. The number of stimulations required to reach the first stage II or III seizure was not different between C6-deficient and WT rats, whereas the number of stimulations required to reach the first stage V seizure was higher in C6-deficient rats.*

The extent of mossy fiber sprouting did not differ either between control animals (range 1-1.5, median 1) and SE animals, or between C6-deficient (range 0.5-2.5, median 1.25) and WT (0.5-2.5, median 1.5) rats (table 2). In both SE-groups, sparse to moderate zinc staining in the supragranular region was observed (figure 3).

GFAP immunoreactivity (IR) and frequency (fr) scores in the dentate gyrus (DG), CA3 and CA1 area of the ventral hippocampus were not different between experimental groups (table 1).

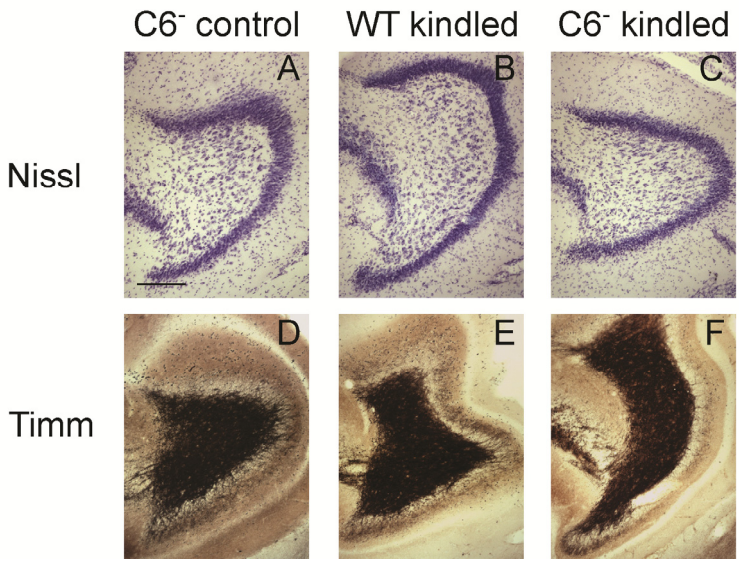

Figure 4: *Nissl and Timm's stained sagittal hippocampal sections. Nissl stained hippocampal sections from C6-deficient controls* ***(A)****, C6-deficient kindled* ***(B)*** *and WT kindled* ***(C)*** *animals show no apparent hilar cell loss.*
Timm's stained sections show moderate sprouting of the ventral part of the hippocampus, which is similar between C6-deficient controls ***(D)****, C6-deficient kindled* ***(E)*** *and WT kindled* ***(F)*** *animals. Scale bar: 250 µm*

Discussion

In this study we found that rats that lack complement protein C6 and are therefore not able to form the membrane attack complex (MAC), do not differ from wild-type (WT) animals in the development of kindling. Histological and immunochemical analysis did not reveal a difference in C6-deficient animals after kindling.

Status epilepticus (SE) induced hippocampal pathology was not different between C6-deficient animals and WT-animals.

Table 1: *GFAP immunoreactivity (IR) and frequency (fr) scores in the dentate gyrus (DG), CA3 and CA1 area of the ventral hippocampus in control and in WT and C6 deficient rats after SE.*

	C6 def control		WT		C6 def	
GFAP	**IR**	**fr**	**IR**	**fr**	**IR**	**fr**
DG	2 (2-2)	2 (2-2)	2 (1-2)	2 (2-2)	2 (1-3)	2 (2-2)
CA3	1.5 (1-2)	2 (2-2)	2 (1-2)	2 (2-2)	2 (1-3)	2 (1-2)
CA1	1.5 (1-2)	2 (2-2)	2 (1-2)	2 (2-2)	2 (1-3)	2 (2-2)

Median values and range are given. No difference between experimental groups was observed.

Kindling in C6-deficient animals

The development of kindling is widely used as a measure for epileptogenesis. The progression from stage 1 behavioral seizures in the first stimulation to generalized stage 5 seizures was not different between C6-deficient and WT-animals. A tendency of a delayed development of stage 5 seizures seems apparent in C6-deficient animals; however this is not significantly different. Both C6-deficient as well as WT animals experienced generalized stage 5 seizures after stimulation at the time of sacrifice. C6-deficient and WT kindled animals did not have hilar cell loss when compared to non-kindled controls. It is a matter of debate whether cell loss occurs in the kindling model (Bertram 2007, Sutula and Ockuly 2006) but if it occurs, it is far more subtle than is seen after SE induced cell loss.

Both C6-deficient and WT kindled animals show sparse mossy fiber staining, which was not different from control animals. Mossy fiber sprouting is correlated to hippocampal cell loss (Gorter, et al. 2001). We did not observe a difference between C6-deficient and WT animals in the extent of mossy fiber staining after kindling. In both groups, animals reached the fully kindled stage, where they experienced stage 5 generalized seizures after stimulation. Thus, at time of sacrifice the animals were not at a different stage of kindling development, which may explain why we did not find differences based on the histopathological analysis.

SE induced alterations in C6-deficient animals

Both C6-deficient as WT animals were electrically stimulated to evoke a SE. Four weeks after SE induction, animals were sacrificed. Within this time, few animals developed spontaneous seizures (as detected by continuous EEG recordings), which is different from what is observed in this post-SE model (Gorter, et al. 2001). The C6-deficient rats are from another strain (PVG) that the rat strain that is usually used in our studies (Sprague Dawley). For mice, strain differences in response to intraperitoneal injections of kainic acid has been reported repeatedly (Schauwecker 2002) (McKhann, et al. 2003). For rats kindling rate may differ between strains (Racine, et al. 2003), but also after SE induction, strain differences are reported (Xu, et al. 2004). Although no studies on PVG rats on epilepsy models have been reported previously, it may be possible that the PVG strain is less sensitive to the development of spontaneous seizures.

Despite the few spontaneous seizures at four weeks after SE induction, we did observe SE-induced hippocampal cell loss in both C6-deficient and WT animals. Hippocampal cell loss was not different between the two groups, indicating that C6-deficiency is not neuroprotective against SE-induced hippocampal cell loss. Neither did we observe a difference in mossy fiber sprouting.

The complement system is implicated in epilepsy (Aronica, et al. 2007, Gorter, et al. 2006, Jamali, et al. 2010) and several studies have also shown a role for the complement system in the development of epilepsy and seizures.

A genetic association of C3 in the development of temporal lobe epilepsy patients with a history of febrile seizures has been found, indicating that the development of temporal lobe epilepsy involves the complement system (Jamali, et al. 2010). Also, complement activation is involved in the development of seizures following viral infection of the CNS (Libbey, et al.) without affecting neuronal cell loss or microglia activation, indicating that the complement system may be involved in epileptogenesis.

Studies focusing on the role of complement activation in other neurodegenerative disorders such as TBI (Leinhase, et al. 2006, Leinhase, et al. 2007, Longhi, et al. 2009), stroke (Arumugam, et al. 2009) and ischemia (Storini, et al. 2005) show neuroprotective effects of complement inhibition, indicating that the complement system is involved in neurodegeneration.
From our study it appears that SE induced hippocampal cell loss does not critically involve activation of C6 and subsequent formation of MAC, but could still be controlled by complement proteins more upstream of C6.
Formation of MAC in the cell membrane may lead to cell lysis, thus cell death, but a sub-lytic effect of MAC (i.e. MAC is formed, but the effect of the pores is not sufficient to induce cell death) is described as well. Due to formation of MAC in the cell membrane, membrane potentials are altered (Wiedmer and Sims 1985). This observation has been made in cultured blood platelets, but it may also affect membrane potentials in neurons as MAC may function as a non-selective ion-channel. This could lead to depolarization thereby contributing to neuronal firing and spread of neuronal activity. Indeed, sequential infusion of components of the MAC complex in the hippocampus results in seizures (Xiong, et al. 2003), indicating that formation of MAC can indeed induce seizures, probably due to the sublytic-effect of MAC on membrane potentials. In an epileptic brain, with a neuronal network prone to synchronization of neuronal activity leading to seizures, the additional sub-lytic effect of MAC may contribute to epileptogenesis and the occurrence of spontaneous seizures.
Because we did not observe a difference between C6-deficient animals and WT animals after SE, we do not expect that sub-lytic effects of MAC play a prominent role in our study.

In conclusion, C6 is not critically involved in kindling development and SE induced cell loss. Whether other complement components upstream of the MAC complex play a more critical role in seizure progression needs to be further investigated.

Acknowledgements

This work was supported by the “Nationaal Epilepsie Fonds” grant 07-19 (JAG), The Netherlands Organization for Scientific Research (NWO) grant 863.08.017 (Veni) (EAvV) and EU FP7 project NeuroGlia, Grant Agreement N° 202167 (EA).

Disclosure: We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors have any conflict of interest to disclose.