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Acute and prolonged complement activation in the central nervous system during herpes simplex encephalitis

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ABSTRACT

Herpes simplex encephalitis (HSE) is characterized by a pronounced inflammatory activity in the central nervous system (CNS). Here, we investigated the acute and prolonged complement system activity in HSE patients, by using enzyme-linked immunosorbent assays (ELISAs) for numerous complement components (C). We found increased cerebrospinal fluid concentrations of C3a, C3b, C5 and C5a in HSE patients compared with healthy controls. C3a and C5a concentrations remained increased also compared with patient controls. Our results conclude that the complement system is activated in CNS during HSE in the acute phase, and interestingly also in later stages supporting previous reports of prolonged inflammation.

1. Introduction

Herpes simplex virus type 1 (HSV-1) infects the central nervous system (CNS) as part of its natural infection and in rare cases, a severe manifestation in form of herpes simplex encephalitis (HSE) occurs. With an incidence rate of 2–4 cases per million inhabitants per year (Granerod et al., 2013; Hjalmarsson et al., 2007; Studahl et al., 2013), HSE is the most common sporadic viral encephalitis in the Western world. Antiviral treatment markedly reduces HSE mortality from 70% to approximately 15% (Hjalmarsson et al., 2007; McGrath et al., 1997; Raschilas et al., 2002; Skoldenberg et al., 1984), but neurological sequelae are still frequently encountered (McGrath et al., 1997; Raschilas et al., 2002). In HSE patients, long-term intrathecal inflammation with increased proinflammatory factors in cerebrospinal fluid (CSF) (Aurelius et al., 1993, 1994; Skoldenberg et al., 2006) has been observed. This could suggest that a chronic, residual inflammation resulting in neurodegeneration might be part of the pathogenesis behind the persistent neurological damage seen in HSE survivors.

The complement system, a family of over 30 fluid-phase proteins and cell surface receptors (Shastri et al., 2013), is an important part of the innate immune response, where activation can lead to inflammation and other innate immune functions and can also enhance adaptive immunity (van Beek et al., 2003; Veerhuis et al., 2011). The pathways for complement activation in CNS have not been extensively explored, but activation in the blood generally occurs via three partly overlapping pathways. Although other routes of activation have been reported (Huber-Lang et al., 2006; Neher et al., 2011), the classical, the lectin and the alternative pathways are the three general pathways. For the general circulation, most complement components are produced in the liver, but the CNS might have a separate production (Veerhuis et al., 2011; Woodruff et al., 2010), where both glial cells and neurons have been reported to serve as sources for complement protein production (Veerhuis et al., 2011).

In various clinical studies of CNS infections caused by bacteria, such as bacterial meningitis where neurological sequelae also are seen (van de Beek, 2012), activation of different complement components in CSF has been reported (Brouwer et al., 2013; Henningsson et al., 2007; Mook-Kanamori et al., 2014; Skattum et al., 2011). In contrast to bacterial infections, clinical data of complement activation is scarce for viral CNS infections. However, complement activity in response to such infections has recently been demonstrated in cell cultures as well as in animal studies (Chen and Reiss, 2002; Crisci et al., 2016; Fuchs et al., 2011; Kotwal et al., 2014; Rhoades et al., 2011; Speth et al., 2002). Moreover, invading microorganisms have developed different strategies to evade the complement participation in combating their invasion (Veerhuis et al., 2011). For HSV-1, glycoprotein C (gC) has been shown to bind complement component 3b (C3b) and thereby reduce or inhibit activation of the later stages in the complement cascade (Friedman et al., 1984; Fries et al., 1986). While some studies have suggested a role for the classical pathway in humoral response to HSV-1 (Da Costa et al., 1999), other studies have indicated that gC binding to C3b argues for

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activation via the alternative pathway (Hung et al., 1994). Therefore, investigating CNS complement activity during HSE could be valuable.

2. Materials and methods

2.1. Patients and diagnostics

One or several CSF sample and corresponding serum samples (when available) were obtained from HSE-diagnosed patients admitted to the Infectious Diseases Clinic at Sahlgrenska University Hospital in Gothenburg (n = 35) between 1995 and 2014. Along with clinical symptoms and diagnosis reported in their medical records, patients were included based on presence of HSV-1 DNA in at least one CSF sample which was determined through positive qPCR results of HSV-1 DNA in CSF as described previously (Namvar et al., 2005). Briefly, extraction of viral DNA was conducted in a MagNA Pure LC Robot (Roche Diagnostics, Mannheim, Germany) using the MagNA Pure LC DNA isolation kit 1 (Roche). Amplification of HSV-1 DNA was performed over 45 PCR cycles using TaqMan 7300 RealTime PCR system (Applied Biosystems, Foster City, CA), where a 118-nucleotide segment of the highly conserved gB region of HSV-1 was targeted with HSV-1 specific primers (forward 5′ GCAGTTTACGTACAACCACATACAGC′3; reverse 3′AGCTTGCGGGCCTC GTT′5) and a probe (FAM-5′-CGGCCCAACATATCGTTGACATGGC-3′-TAMRA). Resulting cycle threshold (C_t) values were related to an HSV-1 standard curve to determine HSV-1 DNA copies/mL in each sample.

For selected patients, days after onset of neurological symptoms were noted and outcomes were reported according to the Glasgow outcome scale (GOS) measured after 6 months (Jennett and Bond, 1975), a scale of 1–5 where 1 indicates death and 5 indicates low or no disability where the patients can return to the life they had before the infection.

Ethical approval was obtained from the Regional Research Ethics Committee in Gothenburg, in accordance to local regulations and the Declaration of Helsinki.

Decoded control samples of serum and CSF were obtained from healthy controls (HC; n = 11) and patient controls (PC; n = 28; individuals seeking medical care for headache and/or psychoneurotic symptoms in whom CNS infection initially was suspected but later excluded based on lack of pleocytosis in the CSF) (Table 1). Analysis of all control samples revealed normal CSF cell count and, for most, normal CSF albumin concentration.

Frozen samples were thawed at room temperature.

Table 1
Clinical data/demographics of herpes simplex encephalitis (HSE) patients, healthy controls (HC) and patient controls (PC).

	HSE (n = 35)	HC (n = 11)	PC (n = 28)
Male:female	17:18	11:0	19:9
Mean age in years (SD)	61.1 (16.5)	31.5 (10.7)	46.8 (11.9)
Outcome according to GOS			
1	5		
2	0		
3	7		
4	8		
5	11		
Unknown	4	n/a	n/a
Mean number of days after onset of neurological symptoms and sample	29.2 (±58.8)	n/a	n/a
Number of CSF samples tested per individual			
1 sample	13	11	28
2 sample	12		
3 samples	6		
4 samples	1		
5 samples	2		
10 samples	1		

Age is summarized as mean ± standard deviation (SD). Outcomes are listed according to the Glasgow outcome scale (GOS) (Jennett and Bond, 1975), where 1 = death, 2 = persistent vegetative state (unresponsiveness and speechlessness), 3 = severe disability (daily support dependency due to mental or physical disability), 4 = moderate disability (disability but independency), 5 = good recovery (might be minor neurological and psychological deficits).

2.2. Quantification of complement factors

Using commercially available ELISA kits, concentrations of complement factor B (CFB), C1q, C3a, C4b2a, C5 and C5a (all from Cloud-Clone Corp., purchased through Nordic Diagnostica, Billdal, Sweden) and C3b (Cusabio Biotech, purchased through Nordic Biosite, Täby, Sweden) were analyzed in CSF and serum samples from included subjects, according to the instructions supplied by the manufacturers. Characterization of specificity and sensitivity of the respective kits, provided by the manufacturers, are found in Supplementary Table A.1 and assay linearity in Supplementary Table A.2.

2.3. Statistical analysis

GraphPad Prism version 6.04 (GraphPad Software Inc., La Jolla, USA) and IBM SPSS Statistics version 22 (IBM Corporation, Armonk, USA) were used for statistical analysis and graph construction. Mann–Whitney U tests were performed for statistical comparison using a two-tailed p-value, where $p < 0.05$ was considered significant. Descriptive statistics are presented as median value with interquartile range (IQR). Non-parametric Spearman correlation coefficients r_s were calculated to investigate the correlation between the complement factors and age, where 95% confidence interval (CI) and p-values were recorded.

3. Theory

Assuming a potential involvement for complement activation in neurological sequelae, the objective of our study was to examine the complement activity in CNS during acute and later stages of HSE, including search for possible activation pathway(s), which may be involved in the pathogenesis of the disease.

4. Results

4.1. Study population

In all patients with HSE (n = 35, serum samples only available for 32 patients), HSV-1 DNA was detected in early CSF samples by qPCR, but rarely in late samples. We defined acute infection (early samples) as day 0–10 after onset of neurological symptoms and samples after day 10 were defined as prolonged inflammation/late infection (late samples). HSV-1 DNA was neither detected in CSF of the HC group nor in the PC group. Summarizing demographics regarding study participants can be found in Table 1 and individual subject details are found in Supplementary Table A.3.

Almost all subjects in our PC group had CSF albumin concentrations below the normal value of 320 mg/l. The five subjects exceeding the normal value were not associated with any extreme results in complement analysis, but concentrations of different components were rather gathered around the median values.

Of our 35 HSE cases, the neurological sequelae measured by GOS after 6 months were 5 in 11 patients and 4 in 8 patients. GOS score 4 and 5 combined are considered a good outcome by McGrath et al. (1997), and correspond to 54.3% of the HSE cases in our study.

4.2. Complement activity in HSE patients

In our study, HSE patient samples and control samples were analyzed for the activity of seven different complement components, namely C1q (representing the classical pathway), C4b2a (representing the classical and the lectin pathway), CFB (representing the alternative pathway), C3a and C3b (representing both the alternative pathway and