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Complement Receptor 2 is increased in cerebrospinal fluid of multiple sclerosis patients and regulates C3 function

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ABSTRACT

Besides its vital role in immunity, the complement system also contributes to the shaping of the synaptic circuitry of the brain. We recently described that soluble Complement Receptor 2 (sCR2) is part of the nerve injury response in rodents. We here study CR2 in context of multiple sclerosis (MS) and explore the molecular effects of CR2 on C3 activation.

Significant increases in sCR2 levels were evident in cerebrospinal fluid (CSF) from both patients with relapsing-remitting MS (n = 33; 6.2 ng/mL) and secondary-progressive MS (n = 9; 7.0 ng/mL) as compared to controls (n = 18; 4.1 ng/mL). Furthermore, CSF sCR2 levels correlated significantly both with CSF C3 and C1q as well as to a disease severity measure. In vitro, sCR2 inhibited the cleavage and down regulation of C3b to iC3b, suggesting that it exerts a modulatory role in complement activation downstream of C3.

These results propose a novel function for CR2/sCR2 in human neuroinflammatory conditions.

1. Introduction

The complement system, an important part of the innate immune system, is activated in conditions of neuroinflammation where it conveys a range of effects comprising cell-lysis, chemotaxis, opsonization and immune cell stimulation [1,2], but also contributes to tissue damage [3–6]. All these functions result in clearance of debris and foreign materials. The complement system consists of a large number of components, many of which derive from the liver, but where some are also expressed by immune/inflammatory cells such as macrophages, other cells in various epithelium, endothelium, and intrinsic cells of the central nervous system (CNS) such as neurons and glia [7,8]. Complement activation is the result of a cascade of interacting processes, a structure that enables fine-tuning and adaptation, but also introduces multiple levels where activation can be dysregulated [9].

A factor crucial for the dexterity of the complement system is cellular responses mediated by several complement receptors present on a range of cell types including macrophages [10], T- and B-lymphocytes [11,12], microglia and astrocytes [13,14], which are either constitutively or conditionally present in the neurological system. Many of the complement receptors belong to a superprotein family (regulators of complement activation, RCA) that contains the main regulators of complement e.g. factor H and C4BP [9]. Also, many of the receptors exist in both secreted and membrane bound forms, for instance complement receptor 1 (CR1) and 2 (CR2) exist in soluble forms, e.g. sCR1 and sCR2 (also known as sCD21) [15,16]. This has functional implications, since soluble complement receptors can function as inhibitors instead of activators [9], which are applied in complement directed therapies [17].

The RCA proteins can act as regulators by influencing the convertases by either decay acceleration of the convertases and/or by acting as co-factors to factor I, which leads to downregulation of

Abbreviations: CR2, Complement Receptor 2; CSF, cerebrospinal fluid; EDSS, Extended Disability Status Scale; fI, factor I; fH, factor H; MSSS, Multiple Sclerosis Severity Score; NFL, neurofilament-light; OND, other neurological disease; RCA, regulator of complement activation; RRMS, relapsing-remitting MS; SPMS, secondary progressive MS.

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convertase activity. As co-factors they provide help to factor I to cleave C3b to iC3b and thereafter iC3b to C3d,g. C3b is the only fragment that can trigger activation of C5 (and subsequent generation of the membrane attack complex, C5b-9). iC3b works mainly as a ligand to CR3 and CR4, while C3d,g is a ligand to CR2.

In the CNS an increasing body of evidence suggests that certain parts of the complement system play important roles for shaping synaptic networks during normal development and ageing, as well as being implicated in different disease processes. For example, transgenic mice lacking either C1q or C3 display aberrant innervation of visual pathways and levels of C1q are greatly increased in both the ageing mouse and human brain and correlate to cognitive decline [18–20]. However, the intricate interplay between different complement components and their interacting partners both during physiological conditions and in different disease states is far from clarified in detail.

We previously found considerable strain-dependent differences in the local expression of several complement components in the spinal cord of nerve-injured rats, and identified distinct regulatory pathways [21,22]. Recently, using the same standardized rat nerve injury model, we also demonstrated strain-dependent differences in the local expression of several complement receptors in the spinal cord [23]. Interestingly, the most conspicuous finding was that of large differences in CR2 both regarding tissue mRNA expression and presence of soluble protein in cerebrospinal fluid (CSF). A possible functional role for CR2 was suggested by a reduced elimination of synaptic connections as a result of axonal injury in mice lacking functional protein. The aim of the current study was to extend these observations to human neuroinflammatory disease and further characterize the function of CR2 at the molecular level.

2. Material and methods

2.1. Ethics, consent and permissions

The study was approved by the regional ethical committee in Stockholm (ethical permit 2009/2107-31/2) and written informed consent was obtained from all patients.

2.2. Patients and CSF CR2/C3 determinations

CSF samples were collected during routine visits to the neurology clinic at Karolinska University Hospital. Samples were centrifuged immediately after lumbar puncture at 440*g* for 10 min at room temperature to separate cells from the CSF supernatant. The supernatants were subsequently batched and stored at −80 °C until use. Patients were subdivided into relapsing-remitting MS (RRMS), secondary progressive MS (SPMS) and other neurological disease (OND) controls lacking signs of inflammatory components on magnetic resonance imaging and established markers of immune activation in the CSF (pleocytosis, oligoclonal bands, increased albumin quotient and/or increased IgG index). The OND controls were; psychosis n = 6; functional paresthesia n = 4; peripheral mononeuropathy n = 3; vertigo n = 1; lumbago = 1; syringomyelia n = 1; epilepsy n = 1; fatigue n = 1. For detailed patient characteristics see Table 1. All clinical examinations were performed by a board certified specialist in neurology, and all patients diagnosed with MS fulfilled the McDonald criteria [24]. An Expanded Disability Status Scale (EDSS) score for degree of neurological disability was determined at time of sampling by a certified rater. Together with estimated disease duration the EDSS score was used to calculate the MS Severity Score (MSSS), a measure of disease severity [25]. One patient in the RRMS group and three patients in the SPMS group received disease modifying treatment with beta-interferon at time of sampling. There were no significant concomitant diseases, such as infections, in any of the subjects and corticosteroids had not been given within three months of sampling.

Levels of sCR2 (sCD21) were determined in undiluted CSF using a human ELISA kit (USCN Life, Wuhan, China, cat.nr E0750h), according to the manufacturer's instructions. The detection limit of the assay was 0.3 ng/mL. All samples were analyzed in duplicates with a resulting intra-assay variability of 10%.

Complement protein C3 levels were determined in 1:100 diluted CSF using an in house sandwich ELISA as previously described [26,27]. In brief, wells of microtitre plates were coated with rabbit anti-C3c (A0062, Dako, Glostrup, Denmark) diluted 1:3000, recognizing C3, C3b, iC3b, and C3c. Detection of the bound C3/C3-fragments was carried out with biotinylated anti-C3c diluted 1:3200, followed by streptavidin-HRP diluted 1:500 (Amersham, Little Chalfont, UK). The concentration of C3, in each sample was determined using DeltaSoft (BioMetallics Inc, Princeton NJ, USA) software. A positive control of pooled plasma from five blood donors was included, and a sample with known concentrations of C3 was used as a standard. The inter-assay variability was 19%. Also the levels of neurofilament-light (NFL) was determined using a commercial ELISA kit (UMAN Diagnostics AB, Umeå, Sweden) in undiluted CSF according to the manufacturer's instructions. The levels of C3 and NFL for some of the patients have previously been reported [27].

In order to avoid complement activation in vitro, 10 mM EDTA (final concentration) was added to the CSF samples immediately after thawing. For C1q determination microtitre plates were coated with anti-C1q (A0136, Dako) diluted 1/3000. Detection of bound C1q was performed using biotinylated anti-C1q, followed by streptavidin-horseradish peroxidase (HRP) diluted 1/500 (Amersham). The inter-assay variability was 7.1%.

2.3. Co-factor activity of CR2

C3b, factor I (fI), and factor H (fH) were prepared from human plasma as previously described [28] and recombinant soluble CR2 was purchased from R & D Systems (Minneapolis, MD). The potential ability of CR2 to act as a co-factor for fI mediated cleavage of C3b was investigated by incubating C3b (10 μg), fI (0.6 μg) together with CR2 in various concentrations (2.8–8.4 μg), for 60 min at 37 °C. FH (0.5 to 2 μg) is well-established to mediate C3 cleavage [29] and served as positive control. In additional experiments, iC3b (available for CR2 to bind to) was generated by incubating C3b with fI and fH in the presence of CR2 (1.4–5.6 μg). After incubation all samples were subjected to SDS-PAGE under reducing conditions and the gels were stained using Coomassie. In addition, the generated C3 fragments were visualized by western

Table 1
Demographics and clinical characteristics of the patient cohort.

Diagnose	n	Sex (% females)	Mean age years (SD)	Disease duration at sampling average years (range)	EDSS median (range)	MSSS median (range)
OND	18	55.6	30.4 (7.9)	NA	NA	NA
RRMS	33	72.7	36.6 (9.6)	5.4 (0–19)	2 (0–4.5)	4.9 (0.7–7.9)
SPMS	9	55.6	52.0 (10.1)	17.0 (4–25)	5.5 (4–6.5)	2.7 (1.0–5.5)
All MS	42	69.0	39.9 (11.5)	7.9 (0–25)	2.5 (0–6.5)	3.3 (0.7–7.9)

RRMS, relapsing-remitting Multiple Sclerosis; SPMS, secondary progressive MS; OND, other non-inflammatory neurological/psychiatric conditions; EDSS, Extended Disability Status Scale; MSSS, MS Severity Score.