Clinical Immunology 166–167 (2016) 89–95

Contents lists available at ScienceDirect

Clinical Immunology

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

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

Rickard P.F. Lindblom [a,b,c,*,1,2,3], Shahin Aeinehband [a,1], Mikael Ström [a,1], Faiez Al Nimer [a,1], Kerstin Sandholm [e,4], Mohsen Khademi [a,1], Bo Nilsson [d,5], Fredrik Piehl [a,1], Kristina N. Ekdahl [d,e,4,5]

[a] Department of Clinical Neuroscience, Neuroimmunology Unit, Karolinska Institutet, Sweden
[b] Department of Cardiothoracic Surgery and Anaesthesia, Uppsala University Hospital, Sweden
[c] Department of Surgical Sciences, Uppsala University, Sweden
[d] Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Sweden
[e] Linnæus Center of Biomaterials Chemistry, Linnæus University, Kalmar, Sweden

ARTICLE INFO

Article history:
Received 23 March 2016
accepted with revision 8 April 2016
Available online 13 April 2016

Keywords:
Complement Receptor 2
Complement system
Multiple sclerosis
Neurodegeneration
Neuroinflammation

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.

* Corresponding author at: Neuroimmunology Unit L8:04 CMM, Karolinska University Hospital, 171 76 Stockholm, Sweden.
E-mail address: rickard.lindblom@ki.se (R.P.F. Lindblom).
[1] Postal address: Neuroimmunology Unit L8:04 CMM, Karolinska University Hospital, 171 76 Stockholm, Sweden.
[2] Postal address: Department of Cardiothoracic Surgery and Anaesthesia, Entrance 50, 4th Floor, Uppsala University Hospital, 751 85 Uppsala.
[3] Postal address: Uppsala University Hospital, 751 85 Uppsala.
[4] Postal address: Kerstin Sandholm, Linnæus Center of Biomaterials Chemistry, Linnæus University, 391 82 Kalmar, Sweden.
[5] Postal address: Rudbeck Laboratory C5, 751 85 Uppsala, Sweden.

http://dx.doi.org/10.1016/j.clim.2016.04.003

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.

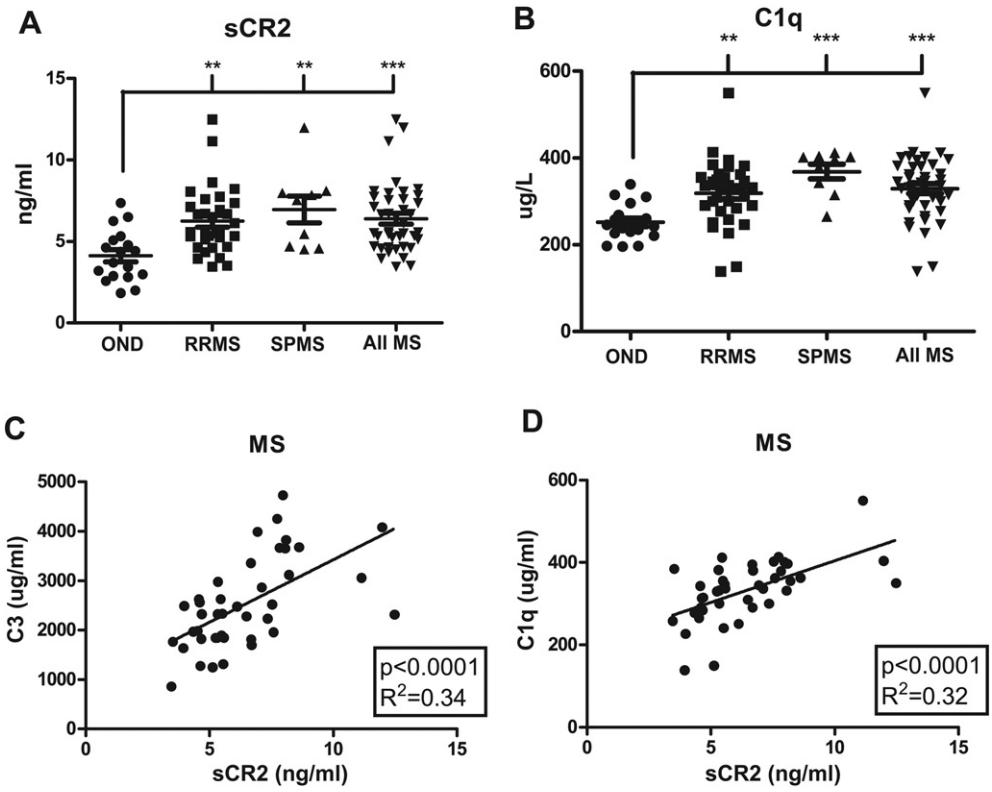

Fig. 1. Levels of soluble CR2 (sCR2) are increased in cerebrospinal fluid from patients with Multiple Sclerosis and correlate with levels of C3 and C1q. Levels of soluble CR2 (sCR2) were quantified in the CSF in a well characterized cohort of MS patients and a control group consisting of patients with non-inflammatory neurological/psychiatric diseases (OND). This demonstrated significantly increased levels of sCR2 in patients compared to controls (All MS: 6.40 ± 0.33, OND: 4.12 ± 0.37) (A). The levels of C1q were also increased in the MS group (329.4 ± 11.4 μg/L) compared to controls (251.6 ± 9.8 μg/L) (B). CSF levels of sCR2 correlated with C3, suggesting that increased it may serve a counter-regulatory role in situations where C3 activation is increased (C). The levels of sCR2 also correlated with C1q (D).

blotting using biotinylated antibody directed to C3c (Dako). Visualization was performed using streptavidin-HRP (GE-Healthcare, Buckinghamshire, UK) and detection by chemiluminescence with ECL Plus Western Blotting Detection system (GE-Healthcare).

2.4. Statistical analysis

The software program R 2.6.0 was used to carry out statistical analyses and create all graphs. One-way ANOVA calculated with GraphPad Prism 5.0 (San Diego, CA) were carried out on protein data, results are represented as mean ± SEM. Correlations between protein levels in clinical samples, were calculated using Pearson's algorithm assuming equal distribution, and visualized graphically using linear regression plots, also in GraphPad Prism 5.0. $p < 0.05$ was considered statistically significant.

3. Results

3.1. CR2 exists in a soluble form in the intrathecal compartment

In order to replicate our previous finding from rats, we determined levels of sCR2 in CSF from 33 RRMS patients, 9 SPMS patients and a control group consisting of 18 individuals without signs of intrathecal inflammation (for patient characteristics see Table 1). The mean level of sCR2 in OND controls was 4.1 ng/mL compared to 6.2 ng/mL in RRMS and 7.0 ng/mL in SPMS patients (Fig. 1A, Table 2). The levels of C3 were 1853 ± 171 μg/L in the OND group and significantly higher (2498 ± 139 μg/L) in the MS group (Table 2), these data have in part been published previously [27]. The levels of C1q were also higher in the MS patients compared to the controls; 251.6 ± 9.8 μg/L in the OND group and 329.4 ± 11.4 μg/L in the MS group, with the highest levels seen in SPMS patients (fig. 1B, Table 2).

3.2. CR2 correlates with levels of C3 as well as to Multiple Sclerosis Severity Score

Furthermore, we found that sCR2 levels displayed a strong positive correlation to both C3 (Fig. 1C) and C1q (Fig. 1D). sCR2 also correlated with albumin quotient (Fig. 2A, Table 2). In contrast, there was no significant correlation between sCR2 and NFL (Fig. 2B, Table 2), a commonly used surrogate marker of ongoing neuroaxonal degeneration in MS [30]. When correlating the sCR2 levels with the clinical parameters at time of sampling we saw a significant correlation between Multiple Sclerosis Severity Score (MSSS), a measure of disease severity, and sCR2 (Fig. 3A, Table 1). The pattern was similar, but not significant for sCR2 and Extended Disability Status Scale (EDSS), a measure of neurological disability, (Fig. 3B, Table 1). However, when stratifying into the group with higher EDSS (4 and above) the levels of sCR2 were significantly higher in the group with higher EDSS (Fig. 3C).

3.3. CR2 inhibits generation of iC3b by interfering with factor H

C3b is cleaved by the plasma protease fI in three positions. The first two cleavages give rise to iC3b and a third subsequent cleavage to C3d,g [31]. The cleavage process occurs only in the presence of a co-factor binding to C3b and thereby causing a conformational change, which makes the cleavage sites accessible for fI. The co-factor activity can be measured by the relative reduction in the band corresponding to the 101 kDa α-chain of C3b and the corresponding increase of the cleaved product, the 67 kDa band of iC3b. The third cleavage will reduce

Table 2
Biochemical analysis of cerebrospinal fluid from patients and controls.

		sCR2 average (SEM)	C3 average (SEM)	C1q average (SEM)	NFL average (SEM)	Albumin quotient (SEM)
Diagnosis	n	ng/mL	μg/mL	μg/mL	μg/mL	
OND	18	4.12 (0.37)	1853 (170.8)	251.6 (9.8)	233.2 (26.85)	4.53 (0.51)
RRMS	33	6.24 (0.36)	2320 (142.9)	318.7 (13.2)	2019 (334.0)	4.84 (0.36)
SPMS	9	6.96 (0.81)	3148 (313.1)	368.5 (16.9)	1378 (457.6)	6.50 (1.22)
All MS	42	6.40 (0.33)	2498 (139.4)	329.4 (11.4)	1882 (280.8)	5.18 (0.39)

RRMS, relapsing-remitting Multiple Sclerosis; SPMS, secondary progressive MS; OND, other non-inflammatory neurological/psychiatric conditions. The normal reference value for the CSF albumin quotient is <7.

the iC3b 67 kDa form to further smaller fragments, including a 40 kDa C3d,g band.

To assess the ability of CR2 to modify cleavage of the α-chain of C3b to iC3b, C3b was first incubated with 0.6 μg fI and increasing concentrations of CR2, however, with no discernible effect on the C3b/iC3b relation on SDS-PAGE (Fig. 4A, lanes 2–4). In contrast, the positive control using C3b incubated with fI and increasing concentrations of fH resulted in almost total disappearance of the 101 kDa band and appearance of a strong 67 kDa iC3b band, as well as smaller break-down products (Fig. 4A, lanes 6–8).

As a subsequent step we studied the effect of CR2 in the presence of both fI and fH. Interestingly, increasing concentrations of CR2 now resulted in decreased degradation of C3b, as well as less generation of iC3b and the 40 kDa band likely representing C3d,g, suggesting that CR2 blocks the interaction between C3b and factor H (Fig. 4B, lanes 3–5, Fig. 4C, lanes 3–5).

4. Discussion

We here demonstrate that sCR2 is detectable in human CSF and that levels are upregulated in patients with MS compared to non-inflammatory controls. At the molecular levels we find evidence that CR2 regulates the function of C3. These findings extend our previous observations of a strong genetic influence on the local expression of CR2 in the spinal cord of rats subjected to a standardized nerve injury, which also was reflected in the levels of sCR2 in CSF [23]. Furthermore, a functional role for CR2 was suggested by the observation of an increased elimination of synaptic elements in the injured area of transgenic mice lacking functional CR1/2, which are transcribed from the same gene in mice but not rats or humans, compared to wild type [23].

The notion that the complement system may exert important regulatory functions on the shaping of neuronal networks is of recent date. Thus, in 2007 Stevens and co-workers reported that mice lacking C1q or C3 displayed defective developmental elimination of synaptic connections in the visual pathway [18]. Subsequent studies have revealed that a dynamic interplay between neurons and microglia mediated by immune factors such as C1q and C3 play an important role for the shaping and homeostatic plasticity of the brain synaptic circuitry [19]. Thus, microglia have been implicated in the removal of synapses occurring after CNS injury, where complement is known to increase their phagocytic properties through receptors such as CR3 (CD11b/CD18), the expression of which characterizes microglia with phagocytic potential [19,32]. The relevance of C3-CR3 communication is supported by the finding that mice deficient for CR3 display reduced loss of synapses during development of the visual system, i.e. a phenotype that is similar to that of mice lacking C3 [18,19].

Complement mediated synaptic plasticity is not limited to physiological functions during normal development, but may also constitute an important biological substrate for chronic neurodegenerative diseases, since failure to regulate the system may lead to excessive loss of synaptic connectivity, in turn representing an early disease related phenomenon in models of neuroinflammation and neurodegeneration [33,34]. Signs of complement activation are readily evident in neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases, but also in MS [5,35]. An important question is if dysregulation of the complement system merely reflects downstream effects of an inherent neurodegenerative process. However, the fact that genetic variability in both *Clusterin* and *CR1* genes are associated to risk of late-onset AD [36,37] suggests that this is not the case, but rather that excessive activation may indeed trigger or exaggerate neurodegenerative processes.

CR2 has mostly been studied in context of B cell and follicular dendritic cell immunology, where CR2 forms a co-receptor complex together with CD19 and CD81, and CR2 binds opsonized C3d,g and antigen-bound IgM that in turn results in a more efficient humoral immune response [38]. In contrast, any possible function of CR2 in the CNS has received little attention, even if expression of CR2 by activated astrocytes has been reported [14]. More recently CR2 has also been shown to regulate neurogenesis in the mouse [39]. In another recent study CR1/CR2

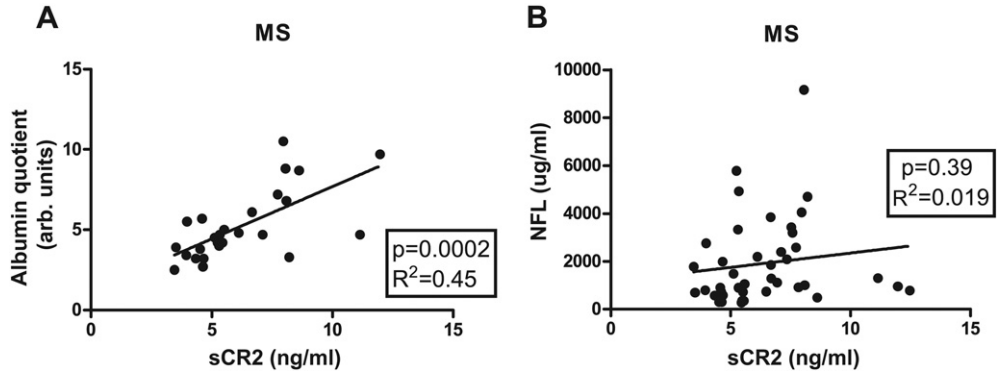

Fig. 2. Correlations between sCR2, the albumin quotient and NFL in MS patients. At the time of sampling, the levels of soluble CR2 (sCR2) correlated well with the albumin quotient (A), often used to illustrate blood-brain barrier integrity however not with NFL (B), a commonly used surrogate marker of ongoing neuroaxonal degeneration in MS.

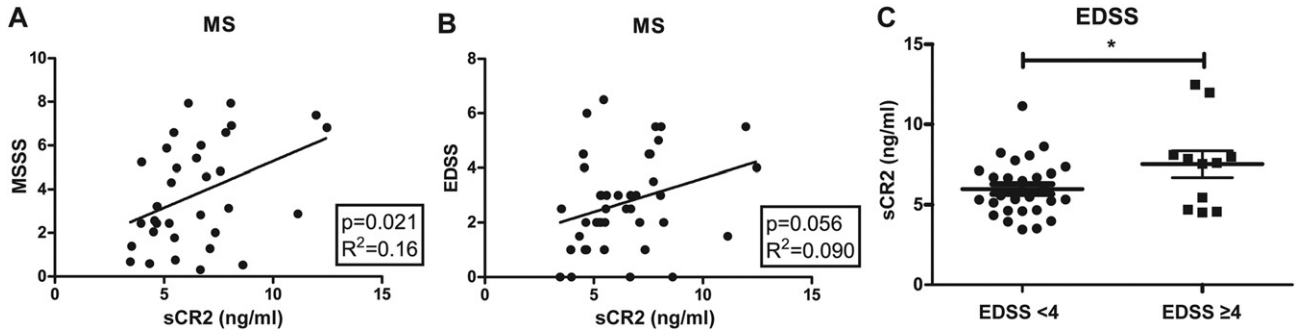

Fig. 3. Correlations between sCR2 and clinical parameters in MS patients. The levels of soluble CR2 (sCR2) at the time of sampling were correlated with the two most frequently used clinical scoring scales in MS; Multiple Sclerosis Severity Score (MSSS) and Extended Disability Status Scale (EDSS). sCR2 levels correlated with MSSS (A) but not with EDSS (B). However, when stratified into high and low EDSS, the high EDSS group had increased sCR2 levels (C). n = 18 OND; 33 RRMS; 9 SPMS patients respectively. The results are represented as mean ± SEM.

knock-out mice displayed improved neurological outcomes in an experimental traumatic brain injury model compared to wild-type mice [40]. The authors also reported that the deposition of C3 in the brain tissue was reduced in animals lacking CR1/CR2. The fact that both receptors are transcribed from the same gene in mice, however, makes extrapolations to human conditions uncertain. Still, the findings provide some support for a functional role for CR2 in the response to nerve injury.

The existence mainly of a soluble form of CR2 in the CNS could explain why immunohistochemical detection has proven difficult [39]. In fact, while tissue stainings were weak in the rat, sCR2 was readily detectable in CSF and levels increased following a standardized nerve injury leading to localized inflammatory activation in the spinal cord, but with very little influx of blood derived immune cells [23]. Possibly increased sCR2 may not only be regulated at the transcriptional levels, but can also be the result of increased shedding of the CR2 ectodomain due to oxidative stress [41,42]. We here demonstrate the presence of sCR2 also in human CSF and that levels increase in conditions of inflammation. Interestingly, decreased serum sCR2 levels have been reported in MS [43], alike multiple other autoimmune diseases [44–46]. If this is due to reduced production or increased consumption during autoimmune inflammation is not known.

We also found a significant correlation between CSF C1q/C3 and sCR2 levels. The role of complement in MS has received increasing attention, with a suggested role in the neurodegenerative processes [47]. For example, certain types of MS lesions are characterized by prominent complement activation [48], and elevated complement levels have been demonstrated in serum of MS patients [35,49], as well as in the CSF in protein profiling studies [4,50]. In addition, we previously reported that C3 levels in CSF of MS patients correlate with degree of neurological disability as well as CSF levels of NFL [27].

C3 is a large and complex molecule, with multiple active breakdown products. Therefore, elevated sCR2 could reflect an intrinsic regulatory mechanism in the CNS, where up regulation and/or increased shedding of CR2 serves to modulate increased C3 activity. In fact, we here found molecular evidence supporting this notion, since CR2 in vitro inhibited cleavage of C3b into iC3b. This is likely of importance, since C3b (in contrast to iC3b) is able to amplify the alternative pathway amplification loop leading to deposition of C3b at sites of inflammation and to trigger C5 activation and C5a/C5b-9 associated damage and inflammation, i.e., anaphylaxis and cell lysis. Reduced generation of iC3b will impair the clearance of debris from the inflammatory site, since this fragment is the primary ligand for CR3 (CD11b/CD18) [51], whereas C3b is not bound by CR3 [52] but instead by CR1 [53]. Thus, it may be speculated that increased levels of sCR2 in context of nerve injury limit iC3b generation, in turn reducing iC3b-CR3 mediated microglial synaptic removal [19]. This hypothesis is interesting in the light of previous postulations of the neurodegenerative disease progression, where loss of synaptic connectivity constitutes an early process [33,34].

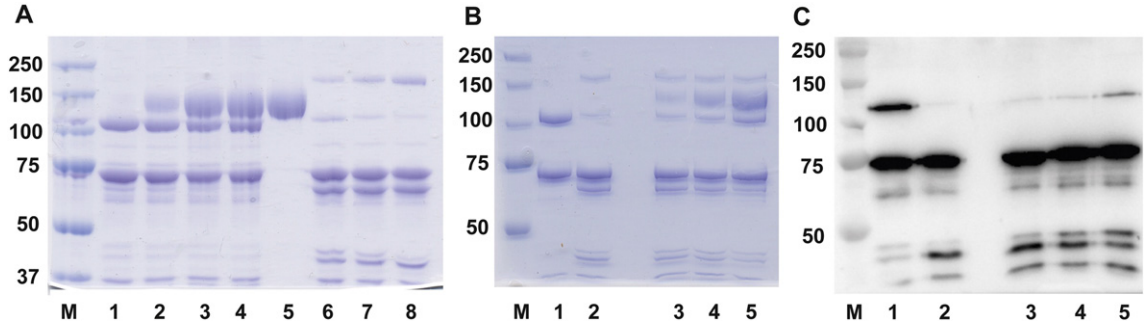

Fig. 4. CR2 does not contribute cleavage of C3b but inhibits generation of iC3b. A: To assess the function of CR2 and its ability to cleave C3b alpha chain (101 kDa) to iC3b (67 kDa), 10 μg C3b was incubated together with either 0.6 μg factor I (fI) only (lane 1) or with 0.6 μg factor I and increasing concentrations of CR2 (2.8/5.6/8.4 μg) (lanes 2–4) using SDS-PAGE. There was no discernible effect of CR2 on C3b cleavage as judged from the intensity of the 101 kDa band. As a positive control C3b was incubated with 0.6 μg factor I with increasing concentrations of factor H (0.5/1/2 μg) (lanes 6–8), which demonstrated cleavage of C3b to iC3b with disappearance of the 101 kDa band and appearance of a new band at around 67 kDa, as well as smaller break-down products. The band at 75 kDa is the C3b beta chain which is unaffected in all conditions. B: To assess the function of CR2 in the presence of both fI and factor H (fH) 10 μg C3b was incubated with; 0.6 μg fI (lane 1); 0.6 μg fI together with 0.5 μg fH (lane 2); or 0.6 μg fI together with 0.5 μg fH and increasing concentrations of CR2 (1.4/2.8/5.6 μg) (lanes 3–5). This demonstrated decreased degradation of the 101 kDa C3b band, as well as decreased generation of iC3b, suggesting that CR2 blocks the interaction between C3b and fH. The band at 40 kDa is also weaker with increasing concentrations of CR2, which likely represents C3dg. C: The experiment was repeated using western blot and with an antibody recognizing C3b cleavage products using the same conditions as in (B), i.e. 10 μg C3b was incubated with; 0.6 μg fI (lane 1); 0.6 μg fI together with 0.5 μg fH (lane 2); or 0.6 μg fI together with 0.5 μg fH and increasing concentrations of CR2 (1.4/2.8/5.6 μg) (lanes 3–5). The results again showed less breakdown of the alpha chain C3b, with increasing concentrations of CR2. Again, there is less C3dg with increasing CR2. M = molecular weight marker.

5. Conclusions

We here find that MS patients display elevated CSF levels of sCR2, which correlate both with C3 and C1q, but less well with biomarkers of nerve injury. We also provide novel evidence that sCR2 impairs the cleavage of C3b into active metabolites in vitro suggesting that it has a modulatory function in situations of complement activation. Further work is needed to explore if CR2/sCR2 treatment is feasible and beneficial in conditions characterized by loss of nerve terminals and dysregulated expression of complement, for example chronic neurodegenerative disorders.

Competing interests

None of the authors has any potential financial conflict of interest related to this manuscript.

Acknowledgements

This work was supported by the 6th Framework Program of the European Union, NeuroproMiSe, LSHM-CT-2005-018637, by the Swedish Research Council, the Swedish Brain Foundation, the King Gustaf V:s 80-years foundation, Dr. Åke Olssons Stiftelse för utbildning, faculty grants from the Linneaus University and the Swedish Association of Persons with Neurological Disabilities. The funders had no role in study design, data collection and analysis, preparation of the manuscript or decision to publish.

References

[1] M.C. Carroll, The complement system in regulation of adaptive immunity, Nat. Immunol. 5 (2004) 981–986.
[2] D. Ricklin, G. Hajishengallis, K. Yang, J.D. Lambris, Complement: a key system for immune surveillance and homeostasis, Nat. Immunol. 11 (2010) 785–797.
[3] J.J. Alexander, A.J. Anderson, S.R. Barnum, B. Stevens, A.J. Tenner, The complement cascade: Yin-Yang in neuroinflammation–neuro-protection and -degeneration, J. Neurochem. 107 (2008) 1169–1187.
[4] Y. Li, Z. Qin, M. Yang, Y. Qin, C. Lin, S. Liu, Differential expression of complement proteins in cerebrospinal fluid from active multiple sclerosis patients, J. Cell. Biochem. 112 (2011) 1930–1937.
[5] Y. Wang, A.M. Hancock, J. Bradner, K.A. Chung, J.F. Quinn, E.R. Peskind, D. Galasko, J. Jankovic, C.P. Zabetian, H.M. Kim, J.B. Leverenz, T.J. Montine, C. Ginghina, K.L. Edwards, K.W. Snapinn, D.S. Goldstein, M. Shi, J. Zhang, Complement 3 and factor h in human cerebrospinal fluid in Parkinson's disease, Alzheimer's disease, and multiple-system atrophy, Am. J. Pathol. 178 (2011) 1509–1516.
[6] B.M. Bellander, S.K. Singhrao, M. Ohlsson, P. Mattsson, M. Svensson, Complement activation in the human brain after traumatic head injury, J. Neurotrauma 18 (2001) 1295–1311.
[7] P. Gasque, M. Fontaine, B.P. Morgan, Complement expression in human brain. Biosynthesis of terminal pathway components and regulators in human glial cells and cell lines, J. Immunol. 154 (1995) 4726–4733.
[8] A. Thomas, P. Gasque, D. Vaudry, B. Gonzalez, M. Fontaine, Expression of a complete and functional complement system by human neuronal cells in vitro, Int. Immunol. 12 (2000) 1015–1023.
[9] P.F. Zipfel, C. Skerka, Complement regulators and inhibitory proteins, Nat. Rev. Immunol. 9 (2009) 729–740.
[10] F.M. Griffin Jr., P.J. Mullinax, Augmentation of macrophage complement receptor function in vitro. III. C3b receptors that promote phagocytosis migrate within the plane of the macrophage plasma membrane, J. Exp. Med. 154 (1981) 291–305.
[11] A. Chen, S. Gaddipati, Y. Hong, D.J. Volkman, E.I. Peerschke, B. Ghebrehiwet, Human T cells express specific binding sites for C1q. Role in T cell activation and proliferation, J. Immunol. 153 (1994) 1430–1440.
[12] A.K. Matsumoto, J. Kopicky-Burd, R.H. Carter, D.A. Tuveson, T.F. Tedder, D.T. Fearon, Intersection of the complement and immune systems: a signal transduction complex of the B lymphocyte-containing complement receptor type 2 and CD19, J. Exp. Med. 173 (1991) 55–64.
[13] J.D. Sedgwick, S. Schwender, H. Imrich, R. Dorries, G.W. Butcher, V. ter Meulen, Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 7438–7442.
[14] P. Gasque, P. Chan, C. Mauger, M.T. Schouft, S. Singhrao, M.P. Dierich, B.P. Morgan, M. Fontaine, Identification and characterization of complement C3 receptors on human astrocytes, J. Immunol. 156 (1996) 2247–2255.
[15] R. Khera, N. Das, Complement receptor 1: disease associations and therapeutic implications, Mol. Immunol. 46 (2009) 761–772.
[16] A. Isaak, J. Prechl, J. Gergely, A. Erdei, The role of CR2 in autoimmunity, Autoimmunity 39 (2006) 357–366.
[17] E. Wagner, M.M. Frank, Therapeutic potential of complement modulation, Nat. Rev. Drug Discov. 9 (2010) 43–56.
[18] B. Stevens, N.J. Allen, L.E. Vazquez, G.R. Howell, K.S. Christopherson, N. Nouri, K.D. Micheva, A.K. Mehalow, A.D. Huberman, B. Stafford, A. Sher, A.M. Litke, J.D. Lambris, S.J. Smith, S.W. John, B.A. Barres, The classical complement cascade mediates CNS synapse elimination, Cell 131 (2007) 1164–1178.
[19] D.P. Schafer, E.K. Lehrman, A.G. Kautzman, R. Koyama, A.R. Mardinly, R. Yamasaki, R.M. Ransohoff, M.E. Greenberg, B.A. Barres, B. Stevens, Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner, Neuron 74 (2012) 691–705.
[20] A.H. Stephan, D.V. Madison, J.M. Mateos, D.A. Fraser, E.A. Lovelett, L. Coutellier, L. Kim, H.H. Tsai, E.J. Huang, D.H. Rowitch, D.S. Berns, A.J. Tenner, M. Shamloo, B.A. Barres, A dramatic increase of C1q protein in the CNS during normal aging, J. Neurosci. 33 (2013) 13460–13474.
[21] M. Swanberg, K. Duvefelt, M. Diez, J. Hillert, T. Olsson, F. Piehl, O. Lidman, Genetically determined susceptibility to neurodegeneration is associated with expression of inflammatory genes, Neurobiol. Dis. 24 (2006) 67–88.
[22] F. Al Nimer, M. Strom, R. Lindblom, S. Aeinehband, B.M. Bellander, J.R. Nyengaard, O. Lidman, F. Piehl, Naturally occurring variation in the glutathione-S-transferase 4 gene determines neurodegeneration after traumatic brain injury, Antioxid. Redox Signal. 18 (2013) 784–794.
[23] R.P. Lindblom, A. Berg, M. Strom, S. Aeinehband, C.A. Dominguez, F. Al Nimer, N. Abdelmagid, M. Heinig, J. Zelano, K. Harnesk, N. Hubner, B. Nilsson, K.N. Ekdahl, M. Diez, S. Cullheim, F. Piehl, Complement receptor 2 is up regulated in the spinal cord following nerve root injury and modulates the spinal cord response, J. Neuroinflammation 12 (2015) 192.
[24] W.I. McDonald, A. Compston, G. Edan, D. Goodkin, H.P. Hartung, F.D. Lublin, H.F. McFarland, D.W. Paty, C.H. Polman, S.C. Reingold, M. Sandberg-Wollheim, W. Sibley, A. Thompson, S. van den Noort, B.Y. Weinshenker, J.S. Wolinsky, Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis, Ann. Neurol. 50 (2001) 121–127.
[25] R.H. Roxburgh, S.R. Seaman, T. Masterman, A.E. Hensiek, S.J. Sawcer, S. Vukusic, I. Achiti, C. Confavreux, M. Coustans, E. le Page, G. Edan, G.V. McDonnell, S. Hawkins, M. Trojano, M. Liguori, E. Cocco, M.G. Marrosu, F. Tesser, M.A. Leone, A. Weber, F. Zipp, B. Miterski, J.T. Epplen, A. Oturai, P.S. Sorensen, E.G. Celius, N.T. Lara, X. Montalban, P. Villoslada, A.M. Silva, M. Marta, I. Leite, B. Dubois, J. Rubio, H. Butzkueven, T. Kilpatrick, M.P. Mycko, K.W. Selmaj, M.E. Rio, M. Sa, G. Salemi, G. Savettieri, J. Hillert, D.A. Compston, Multiple Sclerosis Severity Score: using disability and disease duration to rate disease severity, Neurology 64 (2005) 1144–1151.
[26] A.J. Henningsson, J. Ernerudh, K. Sandholm, S.A. Carlsson, H. Granlund, C. Jansson, D. Nyman, P. Forsberg, K. Nilsson Ekdahl, Complement activation in Lyme neuroborreliosis–increased levels of C1q and C3a in cerebrospinal fluid indicate complement activation in the CNS, J. Neuroimmunol. 183 (2007) 200–207.
[27] S. Aeinehband, R.P. Lindblom, F. Al Nimer, S. Vijayaraghavan, K. Sandholm, M. Khademi, T. Olsson, B. Nilsson, K.N. Ekdahl, T. Darreh-Shori, F. Piehl, Complement component C3 and butyrylcholinesterase activity are associated with neurodegeneration and clinical disability in multiple sclerosis, PLoS One 10 (2015), e0122048.
[28] J. Andersson, F. Bexborn, J. Klinth, B. Nilsson, K.N. Ekdahl, Surface-attached PEO in the form of activated Pluronic with immobilized factor H reduces both coagulation and complement activation in a whole-blood model, J. Biomed. Mater. Res. A 76 (2006) 25–34.
[29] A. Sahu, J.D. Lambris, Structure and biology of complement protein C3, a connecting link between innate and acquired immunity, Immunol. Rev. 180 (2001) 35–48.
[30] C.E. Teunissen, C. Dijkstra, C. Polman, Biological markers in CSF and blood for axonal degeneration in multiple sclerosis, Lancet Neurol. 4 (2005) 32–41.
[31] U.R. Nilsson, L. Funke, B. Nilsson, K.N. Ekdahl, Two conformational forms of target-bound iC3b that distinctively bind complement receptors 1 and 2 and two specific monoclonal antibodies, Ups. J. Med. Sci. 116 (2011) 26–33.
[32] S.D. Webster, M. Park, M.I. Fonseca, A.J. Tenner, Structural and functional evidence for microglial expression of C1qR(P), the C1q receptor that enhances phagocytosis, J. Leukoc. Biol. 67 (2000) 109–116.
[33] D. Centonze, L. Muzio, S. Rossi, F. Cavasinni, V. De Chiara, A. Bergami, A. Musella, M. D'Amelio, V. Cavallucci, A. Martorana, A. Bergamaschi, M.T. Cencioni, A. Diamantini, E. Butti, G. Comi, G. Bernardi, F. Cecconi, L. Battistini, R. Furlan, G. Martino, Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis, J. Neurosci. 29 (2009) 3442–3452.
[34] K.J. Hilton, C. Cunningham, R.A. Reynolds, V.H. Perry, Early hippocampal synaptic loss precedes neuronal loss and associates with early behavioural deficits in three distinct strains of prion disease, PLoS One 8 (2013), e68062.
[35] G. Ingram, S. Hakobyan, C.L. Hirst, C.L. Harris, T.P. Pickersgill, M.D. Cossburn, S. Loveless, N.P. Robertson, B.P. Morgan, Complement regulator factor H as a serum biomarker of multiple sclerosis disease state, Brain 133 (2010) 1602–1611.
[36] D. Harold, R. Abraham, P. Hollingworth, R. Sims, A. Gerrish, M.L. Hamshere, J.S. Pahwa, V. Moskvina, K. Dowzell, A. Williams, N. Jones, C. Thomas, A. Stretton, A.R. Morgan, S. Lovestone, J. Powell, P. Proitsi, M.K. Lupton, C. Brayne, D.C. Rubinsztein, M. Gill, B. Lawlor, A. Lynch, K. Morgan, K.S. Brown, P.A. Passmore, D. Craig, B. McGuinness, S. Todd, C. Holmes, D. Mann, A.D. Smith, S. Love, P.G. Kehoe, J. Hardy, S. Mead, N. Fox, M. Rossor, J. Collinge, W. Maier, F. Jessen, B. Schurmann, H. van den Bussche, I. Heuser, J. Kornhuber, J. Wiltfang, M. Dichgans, L. Frolich, H. Hampel, M. Hull, D. Rujescu, A.M. Goate, J.S. Kauwe, C. Cruchaga, P. Nowotny, J.C. Morris, K. Mayo, K. Sleegers, K. Bettens, S. Engelborghs, P.P. De Deyn, C. Van Broeckhoven, G. Livingston, N.J. Bass, H. Gurling, A. McQuillin, R. Gwilliam, P. Deloukas, A. Al-Chalabi, C.E. Shaw, M. Tsolaki, A.B. Singleton, R. Guerreiro, T.W. Muhleisen, M.M. Nothen, S. Moebus, K.H. Jockel, N. Klopp, H.E. Wichmann, M.M. Carrasquillo, V.S. Pankratz, S.G. Younkin, P.A. Holmans, M. O'Donovan, M.J. Owen,

J. Williams, Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease, Nat. Genet. 41 (2009) 1088–1093.
[37] J.C. Lambert, S. Heath, G. Even, D. Campion, K. Sleegers, M. Hiltunen, O. Combarros, D. Zelenika, M.J. Bullido, B. Tavernier, L. Letenneur, K. Bettens, C. Berr, F. Pasquier, N. Fievet, P. Barberger-Gateau, S. Engelborghs, P. De Deyn, I. Mateo, A. Franck, S. Helisalmi, E. Porcellini, O. Hanon, M.M. de Pancorbo, C. Lendon, C. Dufouil, C. Jaillard, T. Leveillard, V. Alvarez, P. Bosco, M. Mancuso, F. Panza, B. Nacmias, P. Bossu, P. Piccardi, G. Annoni, D. Seripa, D. Galimberti, D. Hannequin, F. Licastro, H. Soininen, K. Ritchie, H. Blanche, J.F. Dartigues, C. Tzourio, I. Gut, C. Van Broeckhoven, A. Alperovitch, M. Lathrop, P. Amouyel, Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease, Nat. Genet. 41 (2009) 1094–1099.
[38] D.T. Fearon, R.H. Carter, The CD19/CR2/TAPA-1 complex of B lymphocytes: linking natural to acquired immunity, Annu. Rev. Immunol. 13 (1995) 127–149.
[39] M. Moriyama, T. Fukuhara, M. Britschgi, Y. He, R. Narasimhan, S. Villeda, H. Molina, B.T. Huber, M. Holers, T. Wyss-Coray, Complement receptor 2 is expressed in neural progenitor cells and regulates adult hippocampal neurogenesis, J. Neurosci. 31 (2011) 3981–3989.
[40] M.D. Neher, M.C. Rich, C.N. Keene, S. Weckbach, A.L. Bolden, J.T. Losacco, J. Patane, M.A. Flierl, L. Kulik, V.M. Holers, P.F. Stahel, Deficiency of complement receptors CR2/CR1 in Cr2(−)/(−) mice reduces the extent of secondary brain damage after closed head injury, J. Neuroinflammation 11 (2014) 95.
[41] A. Aichem, M. Masilamani, H. Illges, Redox regulation of CD21 shedding involves signaling via PKC and indicates the formation of a juxtamembrane stalk, J. Cell Sci. 119 (2006) 2892–2902.
[42] M. Strom, F. Al Nimer, R. Lindblom, J.R. Nyengaard, F. Piehl, Naturally occurring genetic variability in expression of Gsta4 is associated with differential survival of axotomized rat motoneurons, Neruomol. Med. 14 (2012) 15–29.
[43] N. Toepfner, S. Cepok, V. Grummel, B. Hemmer, The role of the Epstein-Barr virus receptor CD21 in multiple sclerosis, J. Neuroimmunol. (2011).
[44] M. Tomita, T. Kadono, N. Yazawa, T. Kawashima, Z. Tamaki, R. Ashida, H. Ohmatsu, Y. Asano, M. Sugaya, M. Kubo, H. Ihn, K. Tamaki, S. Sato, Serum levels of soluble CD21 in patients with systemic sclerosis, Rheumatol. Int. (2010).
[45] M. Masilamani, J. von Kempis, H. Illges, Decreased levels of serum soluble complement receptor-II (CR2/CD21) in patients with rheumatoid arthritis, Rheumatology (Oxford) 43 (2004) 186–190.
[46] A. Singh, S.J. Vastert, B.J. Prakken, H. Illges, Decreased levels of sCD21 and sCD23 in blood of patients with systemic-juvenile arthritis, polyarticular-juvenile arthritis, and pauciarticular-juvenile arthritis, Rheumatol. Int. (2011).
[47] G. Ingram, S. Hakobyan, N.P. Robertson, B.P. Morgan, Complement in multiple sclerosis: its role in disease and potential as a biomarker, Clin. Exp. Immunol. 155 (2009) 128–139.
[48] C. Lucchinetti, W. Bruck, J. Parisi, B. Scheithauer, M. Rodriguez, H. Lassmann, Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination, Ann. Neurol. 47 (2000) 707–717.
[49] G. Ingram, S. Hakobyan, C. Hirst, C. Harris, S. Loveless, J. Mitchell, T. Pickersgill, N. Robertson, B. Morgan, Systemic complement profiling in multiple sclerosis as a biomarker of disease state, Mult. Scler. (2012).
[50] C.E. Teunissen, M.J. Koel-Simmelink, T.V. Pham, J.C. Knol, M. Khalil, A. Trentini, J. Killestein, J. Nielsen, H. Vrenken, V. Popescu, C.D. Dijkstra, C.R. Jimenez, Identification of biomarkers for diagnosis and progression of MS by MALDI-TOF mass spectrometry, Mult. Scler. 17 (2011) 838–850.
[51] G. Bajic, L. Yatime, R.B. Sim, T. Vorup-Jensen, G.R. Andersen, Structural insight on the recognition of surface-bound opsonins by the integrin I domain of complement receptor 3, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 16426–16431.
[52] D.I. Beller, T.A. Springer, R.D. Schreiber, Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor, J. Exp. Med. 156 (1982) 1000–1009.
[53] D.T. Fearon, et al., J. Investig. Dermatol. 85 (1985) 53s–57s.