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BRIEF COMMUNICATION

Impact of complement activation on clinical outcomes in multiple sclerosis

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Abstract

We determined activation profiles of the classical and alternative complement pathway in 39 treatment-naïve patients with early relapse-onset MS. Plasma concentrations of complement fragments were unchanged in MS compared to 32 patients with non-inflammatory neurological diseases. Profiles in patients experiencing clinical exacerbations did not differ from patients with stable disease and did not correlate with baseline EDSS, numbers of T2 lesions and time to second relapse. Long-term EDSS outcomes 4 years after diagnosis did not significantly correlate with baseline complement levels. These data do not support the use of complement activation products as biomarkers for disease activity in early MS.

Introduction

The complement system regulates development, homeostasis and regeneration in the central nervous system (CNS) throughout life. Hyperactivation of complement pathways, as observed in several autoimmune diseases or in subjects with dysfunctional complement regulatory proteins, can drive severe inflammatory responses in numerous organs including the CNS, and systemic inhibition of complement activation is a therapeutically highly effective strategy in many neuroinflammatory diseases such as neuromyelitis optica (NMO) associated with aquaporin-4 antibodies.

Multiple sclerosis (MS) is a complex chronic inflammatory disease of the CNS. Lesion-associated deposition of complement proteins can be visualized at least in a subset of patients[1-3] and plasma levels of individual complement proteins have been described to be increased in patients with MS if compared to healthy volunteers and associated with surrogate markers for disease activity in some[4,5] but not all[6] studies. In a study including 350 patients with MS, complement regulatory factor H serum levels were found to be increased in patients with secondary and primary progressive MS compared to patients with relapsing-remitting disease (RRMS) and healthy controls, and higher in RRMS patients in relapse compared to those in remission.[7] If validated, plasma complement components and activation products could serve as serological correlates for MS disease activity and progression. In the present study, we systematically profiled complement activation pathways in treatment-naïve patients with early relapse-onset MS (RMS) and correlated complement activation with clinical outcome measures recorded over a period of 4 years.

Materials and methods

Study subjects

Plasma concentrations of complement proteins were measured cross-sectionally in a cohort of patients with RMS compared to patients with non-inflammatory neurological diseases (NIND) and patients with other inflammatory

Table 1. Demographic and clinical characteristics of patient cohorts.

	RMS	NIND	OIND
n	39	32	19
Female	30	23	6
Age (mean, median, SD)	36.1, 35.5, 11.8	42.9, 43.9, 14.1	57.7, 59, 17.8
Age range (years)	19.1 – 70.6	16.8 – 73.3	25.2 – 80.6
Last EDSS prior to spinal tap (mean, median, SD),	2, 2, 0.9	n.a.	n.a.
EDSS (range)	0-4	n.a.	n.a.
%Treatment-naïve	100	n.a.	n.a.
Status at time point of spinal tap: (%relapse/%stable)	64/36	n.a.	n.a.
Clinical Diagnoses	RMS (n = 39)	Tension type headache (n = 11), migraine and other headache entities (n = 3), symptoms related to paraesthesia, hypaesthesia and pain (5), seizure-associated (n = 3), somatoform/psychogenic disorder (n = 2), neuropsychological disorder/ depression (n = 2), obstructive sleep apnoea (n = 1), vascular leukencephalopathy (n = 1), central vestibular disturbance (n = 1), pseudotumour cerebri (n = 1)	Viral (meningo)encephalitis (n = 6), neuroborreliosis (n = 3), (meningo)encephalitis of unknown aetiology (n = 4), eosinophilic encephalitis (n = 1), giant cell arteritis (n = 1), zoster ophthalmicus (n = 1), oligoneuritis cranialis (n = 1), tuberculous meningoencephalitis (n = 1), varicella zoster-associated polyradiculitis (n = 1)

neurological diseases (OIND) (Table 1) recruited at the MS Centre, University Hospital Basel (Switzerland). Patients with RMS were followed longitudinally. Clinical examinations including EDSS scores were determined for a period of at least 4 years. The study was approved by the Ethical Committee Northwest and Central Switzerland, University of Basel, Basel, Switzerland, and followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Complement profiling

Upon venipuncture, samples were held at room temperature for 30 minutes to allow for clot retraction then centrifugation at 4°C was performed and serum specimens were immediately frozen down at −80°C. A multiplex ELISA based on chemi-luminescence was used according to the manufacturer's recommendations (Tecomedical AG, Sissach, Switzerland) to systematically profile complement proteins in plasma samples.

All samples were run in duplicates and the average value was used for statistical analysis. Each plate contained samples from different clinical cohorts (RMS, OIND, NIND) to minimize inter-plate variations. Known samples were included on each plate to assure for plate-to-plate consistency. The range of the respective complement factors was Ba (30.73-0.28 ng/ml), Bb (0.32-0.0032 µg/ml), C3a (196.82-0.20 ng/ml), C4a (51.62-0.75 ng/ml), C5a (1.89-0.0050 ng/ml), SC5b9 (458.74-1.15 ng/ml), Factor H (8.08-0.14 ng/ml) and Factor I (888.80-15.40 ng/ml).

Statistics

Mann–Whitney test was performed to compare levels of complement proteins between clinical cohorts. Correlation with clinical and paraclinical data was calculated by Spearman r. GraphPad-Prism v7.0b was used for statistical analyses. Association of plasma proteins with number of T2w lesions at baseline was assessed in a negative binomial model. The model was adjusted for age and sex. Association of plasma proteins with EDSS worsening over 4 years was assessed using a logistic regression model. The analyses were adjusted for age at baseline, gender and (any) medication start within 4 years. Association of plasma proteins with time to second event was assessed using a cox proportional hazards model. The model was adjusted for age at baseline, gender and medication start before second event.

Results

Complement activation products are not increased in early MS if compared to other neurological diseases

To systematically profile activity of the classical and the alternative complement pathway in treatment-naïve

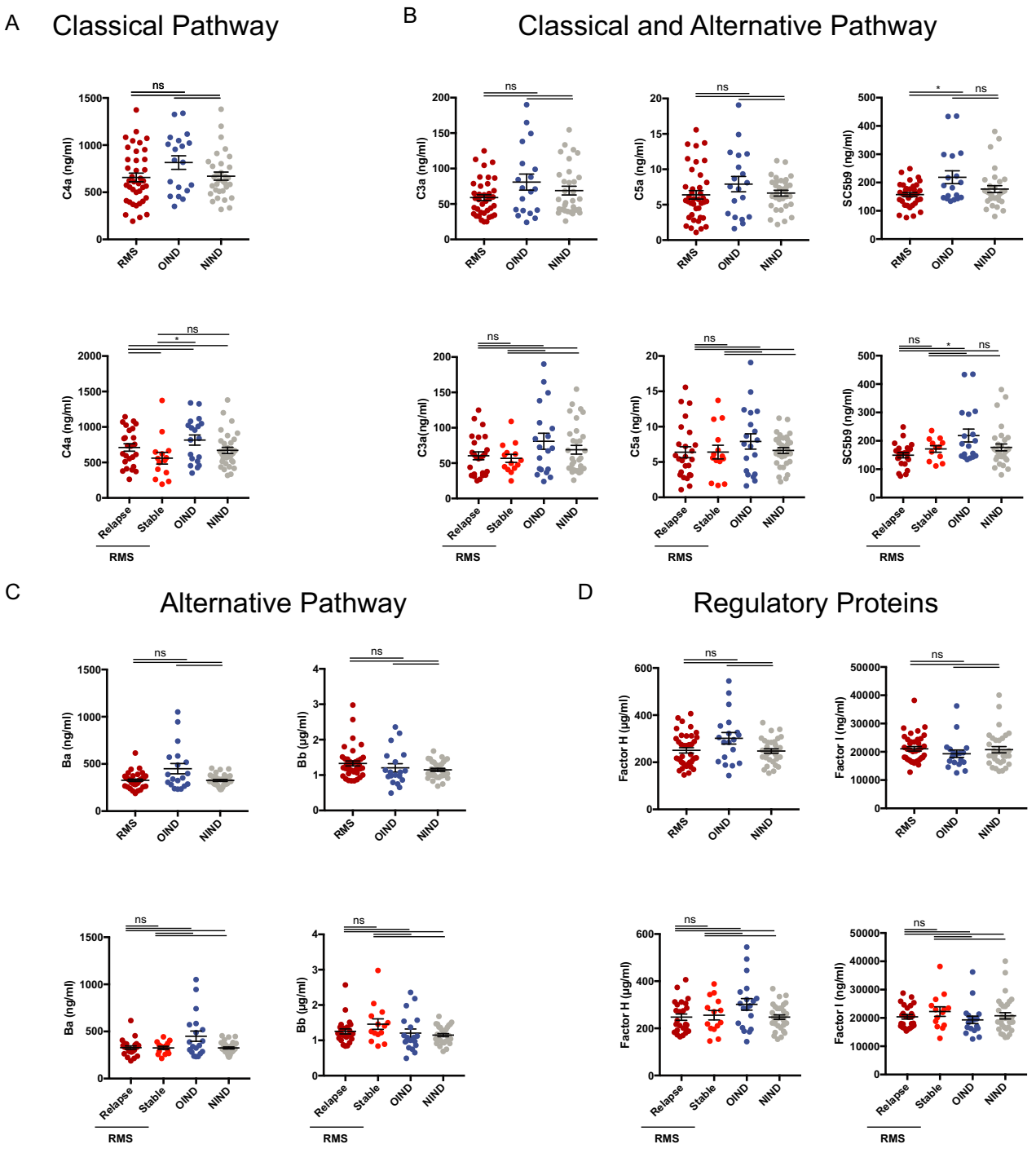

Figure 1. Plasma concentrations of complement proteins representative for distinct complement activation pathways and complement activation-inhibition and regulation pathways (A: classical pathway; B: general complement activation; C: alternative pathway; D: regulatory proteins). RMS patients compared to patients with other neurologic diseases NIND and OIND. Each dot represents an individual patient. RMS = relapsing multiple sclerosis; NIND = noninflammatory neurologic disease; OIND = other inflammatory neurologic disease. Statistics: Mann–Whitney test. RMS: n = 39 (in five patients SC5b-9 was undetectable; n = 34), OIND = 19 (in one patient Factor H was undetectable, n = 18; in one patient SC5b9 was undetectable, n = 18), NIND = 32.

patients with RMS, we simultaneously quantified plasma levels of C3a, C5a and SC5b-9, indicative for general activation of the complement system, of C4a, specific for the activation of the classical pathway, and Ba and Bb, reflective for alternative complement activation, as well as concentrations of complement activation-inhibitory and regulatory proteins factor H and factor I, found to be dysregulated in several autoimmune diseases. Levels of complement activation products were unchanged in patients with RMS if compared to patients with NIND and levels of the soluble terminal complement activation complex SC5b-9 were moderately decreased in RMS if compared to other inflammatory diseases (Figure 1). Complement activation in RMS patients experiencing clinical exacerbations did not differ from patients with stable disease. Plasma concentrations of C4a were higher in OIND as compared to stable RMS and SC5b-9 levels were higher in OIND if compared to active RMS (Figure 1A,B).

Complement activation does not correlate with clinical disease severity and long-term progression

Next, we determined whether activation of individual complement components or pathways are a biomarker for clinical disease severity and progression. Baseline EDSS scores, numbers of T2 lesions and time to second relapse in individual patients did not significantly correlate with plasma levels of complement activation products (Figure 2). Furthermore, 4 years long-term outcomes as determined by EDSS did not significantly correlate with baseline complement protein levels (Figure 2). Multivariate analyses were performed to assess the estimated association between complement protein levels and (I) number of T2w lesions at baseline, (II) EDSS progression within 4 years and (III) time to second clinical event. No statistically significant differences were found (Suppl. Table S1).

Discussion

Previous studies reported either increased or unchanged plasma or serum levels of individual or a combination of a few complement proteins in patients with MS if compared to healthy individuals.[4-6] Compared to patients with NMO, however, complement activation products appear to be decreased in MS.[8,9] Our approach to simultaneously determine eight complement activation and regulatory proteins in a single specimen allowed us to systematically interrogate the complement system at high resolution and to profile its activation at the level of complement pathways instead of individual proteins. The study indicates that deregulated overactivation of complement pathways in plasma, as observed in several inflammatory neurological diseases including NMO, is not a general feature of early RMS. In addition, levels of systemic complement activation products are neither associated with nor predictive for clinical disease activity and progression in early RMS.

Our data do not generally argue against a pathogenic role for complement activation in MS. Complement activation and deposition have consistently been identified in MS brain tissue including cortical gray matter in progressive MS cases,[2,3] suggesting that complement factors may contribute to the worsening pathology that underlies the irreversible progression of MS. Combined analysis of postmortem human MS tissue with experimental *in vivo* models demonstrated that microglia eliminates synapses through the alternative complement cascade and that inhibition of the complement cascade prevented synaptic loss in experimental demyelinating diseases.[10] Genetic variants of early complement pathway genes have been found to predispose some RMS patients to develop more rapid retinal neurodegeneration or increased susceptibility to visual function loss.[11] Moreover, Bhargava et al.[6] recently reported that complement activation products such as C5a are increased in circulating astrocytic-enriched extracellular vesicles but, in line with our data, not in total plasma samples from MS patients. Aforementioned findings clearly support a potential role of complement activation in mediating neurodegeneration in MS *in situ*. Such local activation might not necessarily translate into elevated circulating levels of complement factor fragments.

Limitations of our study include the size of the cohorts investigated and the focus on early MS. Our data do not exclude the possibility that complement activation products are systemically increased and correlate with clinical outcomes in later stages of MS including secondary-progressive MS or in cases with primary-progressive MS. Taken together, our study does not support the use of complement activation products as serological correlates for disease activity and progression in treatment-naïve patients with early RMS.

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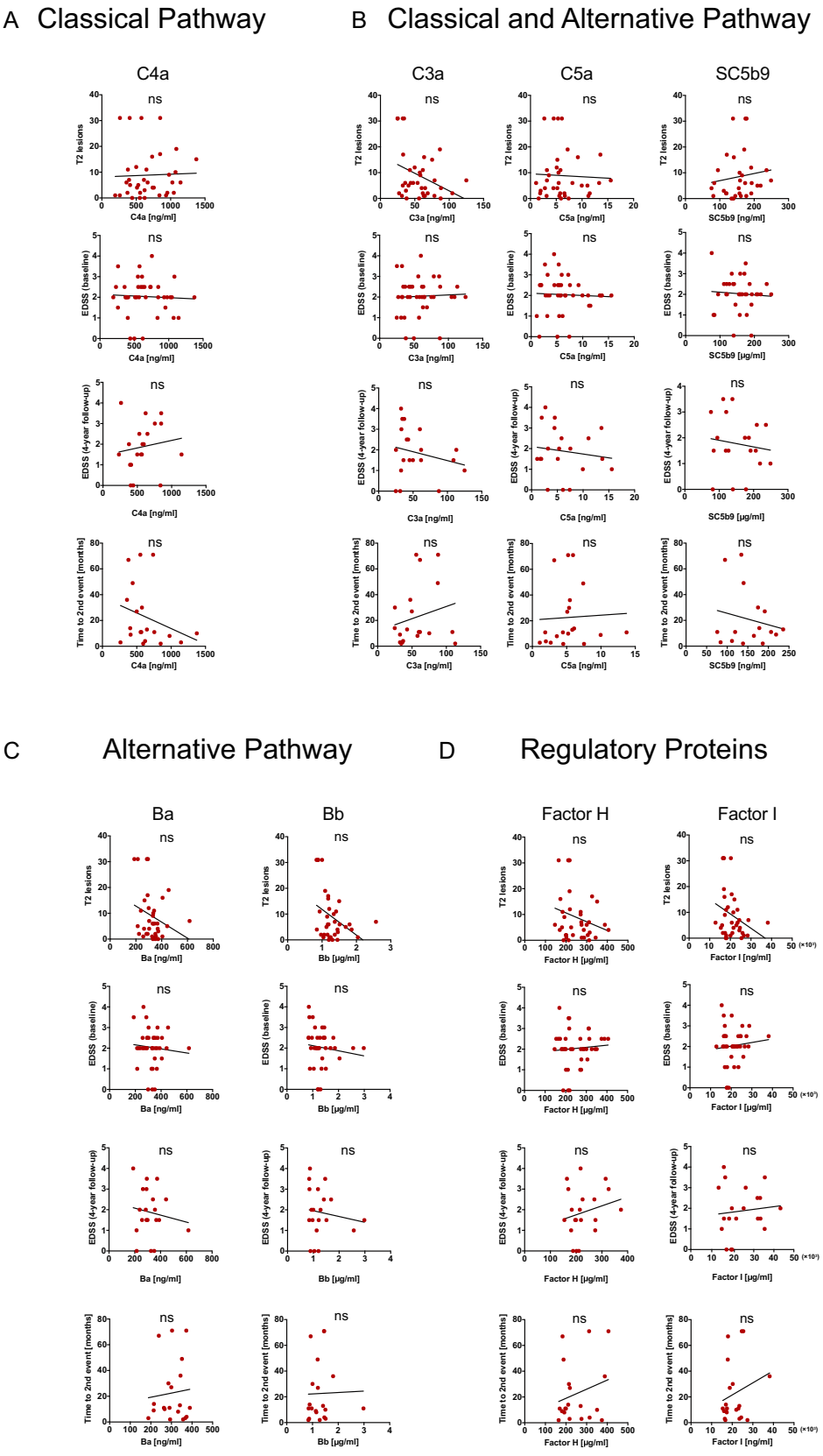

Figure 2. Plasma concentrations of complement proteins representative for distinct complement activation pathways and complement activation-inhibition and regulation pathways correlated with clinical and paraclinical parameters (A: classical pathway; B: general complement activation; C: alternative pathway; D: regulatory proteins). Statistics: Spearman r. Clinical data were available for: T2 lesions (n = 36), EDSS baseline (n = 39), time to 2nd event (n = 20), EDSS-4 year follow-up (n = 20).

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Author Contributions

C.W.K., J.O., H.W., L.K. J.K. and J.D.L. contributed to acquisition of data, analysis and interpretation of data, and drafting and revising the manuscript.

Conflict of Interest

Christian W. Keller has nothing to disclose. Johanna Oechtering has received travel grants from Bayer, Biogen and Novartis and served on a scientific advisory board for Roche, not related to this work. Heinz Wiendl received honoraria for acting as a member of Scientific Advisory Boards Biogen, Evgen, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Roche Pharma AG, and Sanofi-Aventis as well as speaker honoraria and travel support from Alexion, Biogen, Cognomed, F. Hoffmann-La Roche Ltd., Gemeinnützige Hertie-Stiftung, Merck Serono, Novartis, Roche Pharma AG, Genzyme, TEVA, and WebMD Global. Prof. Wiendl is acting as a paid consultant for Abbvie, Actelion, Biogen, IGES, Johnson & Johnson, Novartis, Roche, Sanofi-Aventis, and the Swiss Multiple Sclerosis Society. His research is funded by the German Ministry for Education and Research (BMBF), Deutsche Forschungsgemeinschaft (DFG), Else Kröner Fresenius Foundation, Fresenius Foundation, the European Union, Hertie Foundation, NRW Ministry of Education and Research, Interdisciplinary Center for Clinical Studies (IZKF) Muenster and Biogen, GlaxoSmithKline GmbH, Roche Pharma AG, Sanofi-Genzyme. Ludwig Kappos's institution (University Hospital Basel) received in the last 3 years and used exclusively for research support at the Department: steering committee, advisory board, consultancy fees and support of educational activities from: Actelion, Allergan, Almirall, Baxalta, Bayer, Biogen, Celgene/Receptos, CSL-Behring, Desitin, Excemed, Eisai,, Genzyme, Japan Tobacco, Merck, Minoryx, Novartis, Pfizer, F. Hoffmann-La Roche Ltd, Sanofi Aventis, Santhera, Teva, and license fees for Neurostatus-UHB products; the Research of the MS Center in Basel has been supported by grants from Bayer, Biogen, Novartis, the Swiss MS Society, the Swiss National Research Foundation, Innosuisse, the European Union, and Roche Research Foundations. Jens Kuhle received speaker fees, research support, travel support and/or served on advisory boards by the Swiss MS Society, Swiss National Research Foundation (320030_189140/1), University of Basel, Progressive MS Alliance, Biogen, Celgene, Merck, Novartis, Roche, Sanofi. Jan D. Lünemann received speaker fees, research support, travel support and/or served on advisory boards by the Swiss MS Society, Swiss National Research Foundation, Abbvie, Alexion, Biogen, Merck, Novartis, Roche, Sanofi.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Estimated associations between selected blood proteins and number of T2w lesions at baseline, EDSS progression within 4 years and time to second clinical event.

