Compartmentalized complement activation is associated with cytokines CXCL-13, CXCL-9, IL-12b and paramagnetic rim lesions in multiple sclerosis

Johanna Oechtering[1,2], Sabine Schädelin[2,3], Kerstin Stein[4] , Alessandro Cagol[1,2,5], Selina Leber[1,2,5], Pascal Benkert [2,3], Wayne Hu[6], Ferhan Qureshi[6], Aleksandra Maleska Maceski[1,2], Riccardo Galbusera[1,2,5], Lester Melie-Garcia[2,5], Annette Orleth[1,2], Stephanie Meier[1,2], Eline Willemse[1,2,3], Regeniter Axel[7], Özgür Yaldizli[1,2], Marcus DSouza[1,2], Bettina Fischer-Barnicol[1,2], Lutz Achtnichts[8] , Stefanie Müller[9], Anke Salmen[10], Patrice Lalive[11], Claire Bridel[11], Caroline Pot[12], Renaud Du Pasquier[12], Claudio Gobbi[13,14] Heinz Wiendl[4], Tobias Derfuss[1,2], Johanna M. Lieb[15], Ludwig Kappos[1,2], David Leppert[1,2], Marten Trendelenburg[16], Cristina Granziera[1,2,5], Jan Lünemann[4], Jens Kuhle[1,2] for the Swiss MS cohort.

[1]Department of Neurology, University Hospital and University of Basel, Basel, Switzerland. [2]Multiple Sclerosis Centre and Research Center for Clinical Neuroimmunology and Neuroscience (RC2NB), Departments of Biomedicine and Clinical Research, University Hospital and University of Basel, Basel, Switzerland. [3]Department of Clinical Research, University Hospital Basel, University of Basel, Basel, Switzerland. [4]Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany. [5]Translational Imaging in Neurology (ThINk) Basel, Department of Biomedical Engineering, Faculty of Medicine, University Hospital Basel and University of Basel, Basel, Switzerland. [6]Octave Bioscience, Menlo Park, USA.[7]Medica Laboratory, Zürich, Switzerland. [8]Department of Neurology, Cantonal Hospital Aarau, Switzerland. [9]Department of Neurology, Cantonal Hospital St. Gallen, St. Gallen, Switzerland. [10]Department of Neurology, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland. [11]Department of Clinical Neurosciences, Division of Neurology, Geneva University Hospital, Geneva, Switzerland. [12]Service of Neurology, Department of Clinical Neurosciences, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland. [13]Neurocentre of Southern Switzerland, Multiple sclerosis centre, Ospedale Civico, Lugano, Switzerland. [14]Faculty of biomedical Sciences, Università della Svizzera Italiana USI), Lugano, Switzerland.[15]Division of Neuroradiology, Department of Radiology, University Hospital Basel, University of Basel, Switzerland. [16]Division of Internal Medicine, University Hospital Basel and Clinical Immunology, Department of Biomedicine, University of Basel, Basel, Switzerland.

Introduction & Objectives

Intrathecal complement activation is associated with clinical outcomes reflecting disease severity in multiple sclerosis (MS). The cytokines CXCL-13, CXCL-9 and IL-12b are involved in B- and T-lymphocyte regulation of compartmentalized inflammation and paramagnetic rim lesions (PRLs) are supposed to play an important role in smouldering MS. Complement-receptor positive cells produce these cytokines and are also relevant for the formation of PRLs. We aimed to investigate whether intrathecal complement activation is associated with increased production of these cytokines and the occurrence of PRLs.

Methods

We measured complement components (CC) and their activation products (CAP) (Factor H and I, C1q, C3, C4, C5, Ba, Bb, C3a, C4a, C5a, sC5b-9) by multiplex assays based on chemiluminescence and CXCL-13, CXCL-9 and IL-12b levels by Octave custom assay panel in the cerebrospinal fluid (CSF) of 112 clinically isolated syndrome (CIS) and 127 MS patients (90 relapsing-remitting, 14 primary progressive, 23 secondary progressive). In 103 patients followed in the Swiss MS cohort, PRLs were quantified in susceptibility-based images cross-sectionally. We used separate linear regression models with the 12 (log2) CC/CAP levels as individual independent variables and the (log2) levels of CXCL-13, CXCL-9 and IL-12b as dependent variables, respectively. The models were adjusted for age, sex, albumin-ratio and disease-modifying treatments at lumbar puncture (platform vs oral drugs vs highly effective treatments). PRL counts were used as dependent variables in negative binomial models adjusted for age, sex, albumin-ratio and dominant disease-modifying treatment category during Follow-up (platform vs oral drugs vs highly effective treatments).

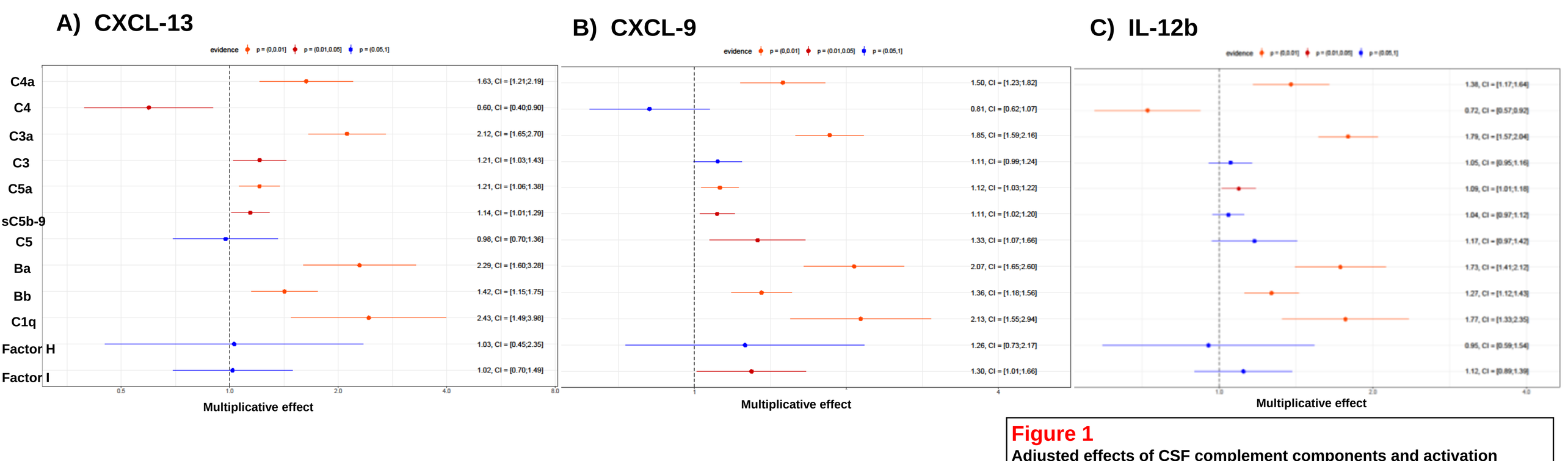

Figure 1

Adjusted effects of CSF complement components and activation products on A) CXCL-13, B) CXCL-9 and C) IL-12b cytokine levels

- Doubling CSF C4a-levels was associated with increased levels of CXCL-13 by factor 1.6 (95%-confidence intervall: 1.2-2.2), CXCL-9 by 1.5 (CI: 1.2-1.8) and IL-12b by 1.4 (CI: 1.2-1.6) (all p<0.001).
- Doubling of CSF C3a levels was associated with 2.1-fold higher CXCL-13 levels (CI 1.7-2.7), 1.9-fold CXCL-9 (CI:1.6-2.2) and 1.8-fold (CI:1.6-2.0) IL-12b levels (all p<0.001).
- Doubling of Ba levels was similarly associated with increased cytokines: CXCL-13: 2.3-fold (CI:1.6-3.3); CXCL-9: 2.1-fold (CI:1.7-2.6); IL-12b: 1.7-fold (CI:1.4-2.1) (all p<0.001).
- Doubling of C1q levels was similarly associated with increased cytokines: CXCL-13: 2.4-fold (CI:1.5-4.0); CXCL-9: 2.1-fold (CI:1.6-3.0); IL-12b: 1.8-fold (CI:1.3-2.4) (all p<0.001).

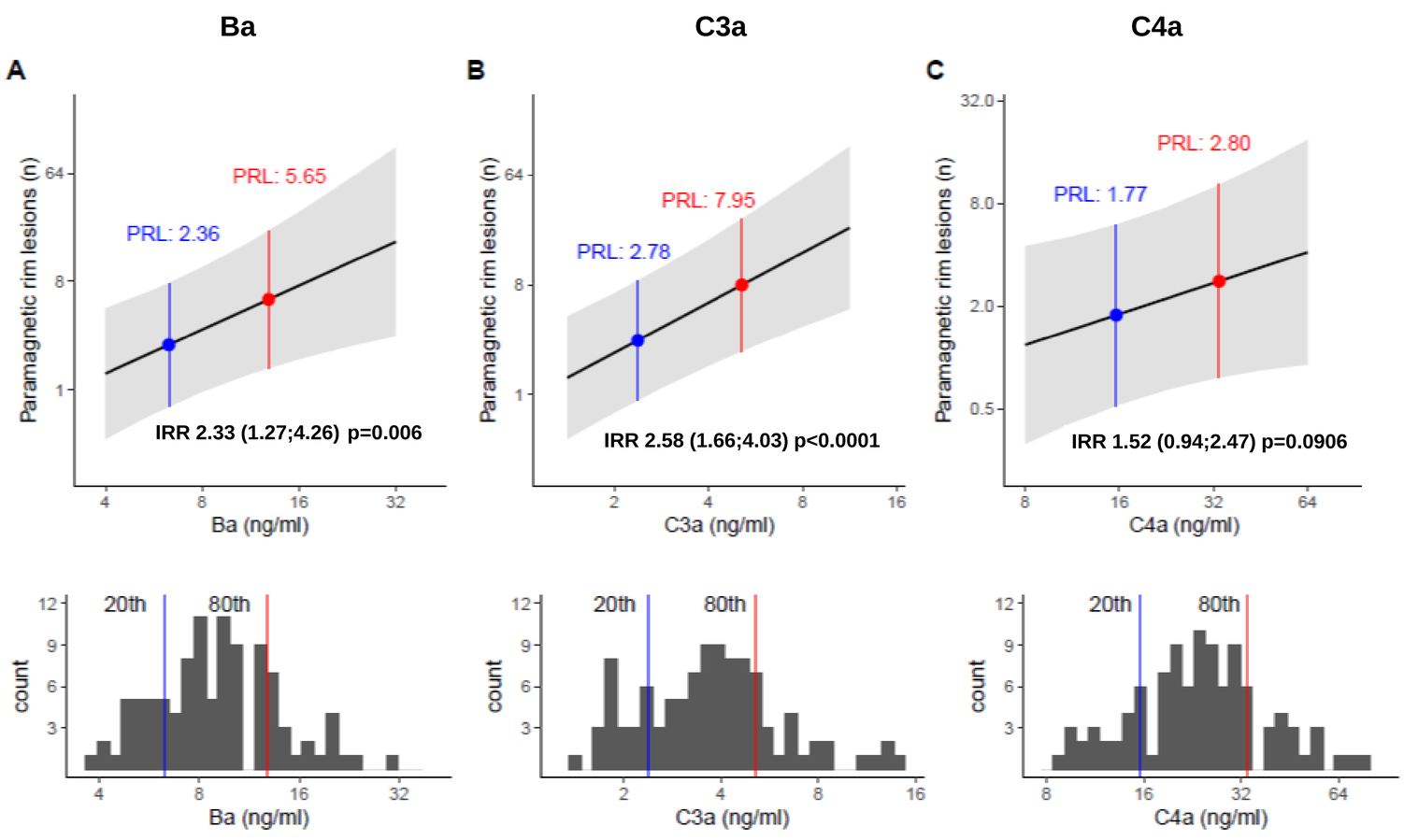

Figure 2

Adjusted effects of CSF Ba, C3a and C4a levels on PRL counts

A) Per doubling of Ba CSF levels the Incidence rate ratio (IRR) for paramagnetic rim lesions (PRLs) count is 2.3-fold (p=0.006) higher.
A patient with a Ba level (6.3 ng/ml) on the 20th percentile would have an estimated PRL count of 2.4 (blue) while one with a 80th percentile value (12.8 ng/ml) would correspond to 5.7 PRLs (red).
B) A 20th percentile C3a level (2.4 ng/ml) would correspond to 2.8 PRLs and a 80th percentile level (5.1 ng/ml) to 8.0 estimated PRLs.
C) C4a levels on the 20th percentile (15.6 ng/ml) are associated with estimated 1.8 and on the 80th percentile (33.4 ng/ml) with 2.8 PRLS (IRR 1.52; 0.94-2.47; p=0.0906).

Conclusions

Patients with increased intrathecal complement activation show a consistent pattern of higher cytokine levels and increased PRL counts. Our results support the concept that complement activation plays a crucial pathophysiological role in compartmentalized inflammation in MS.

Disclosures

JO received research support by the Swiss MS Society and served on advisory boards for Roche and Merck. SS, KS, SL, PB, AM, ER, RG, LMG, AO, SM, EW, AR, BFB have nothing to disclose. AC is supported by EUROSTAR E!113682 HORIZON2020, and received speaker honoraria from Novartis. FQ & WH are employees of Octave Bioscience, Inc. MDS received travel support from Novartis and Roche, and research support from the University Hospital Basel, is CEO of Neurostatus-UHB AG (employment by University Hospital Basel). TD received speaker fees, research support, travel support, and/or served on Advisory Boards, data safety monitoring boards, or Steering Committees of Actelion, Alexion, Celgene, Polyneuron, Novartis Pharma, Merck Serono, Biogen, Teva, Bayer-Schering, GeNeuro, Mitsubishi Pharma, MedDay, Roche, and Genzyme. MDS received travel support from Novartis and Roche, and research support from the University Hospital Basel, is CEO of Neurostatus-UHB AG (employment by University Hospital Basel). LA served on scientific advisory boards for Celgene, Novartis Pharmaceuticals, Merck, Biogen, Sanofi Genzyme, Roche and Bayer; received funding for travel and/or speaker honoraria from Celgene, Biogen, Sanofi Genzyme, Novartis, Merck Serono, Roche, Teva and the Swiss MS Society; and research support from Biogen, Sanofi, Genzyme, and Novartis. SM received honoraria for travel, honoraria for lectures/ consulting and/or grants for studies from Almirall, Alexion, Bayer, Biogen, Bristol-Myers Squibb SA/Celgene, Genzyme, Merck-Serono, Teva, Novartis and Roche. AS received speaker honoraria for activities with Bristol Myers Squibb, CSL Behring, Novartis, and Roche, and research support by the Baasch Medicus Foundation, the Medical Faculty of the University of Bern and the Swiss MS Society. PHL received honoraria for speaking and/or travel expenses from Biogen, Merck, Novartis, Roche; consulting fees from Biogen, GeNeuro, Merck, Novartis, Roche; research support from Biogen, Merck, Novartis. None were related to this work. CB served on scientific advisory boards for Biogen, Novartis and BMS. CP reports that the Lausanne University Hospital received speaker honoraria and travel grants from Novartis, Roche, Biogen, Merck none related to this work. RDP reports that the Lausanne University Hospital received speaker honoraria and travel grants for his activities with Biogen, Genzyme, Merck, Novartis, Roche, and Sanofi. None of them were related to this work. HW receives honoraria for acting as a member of Scientific Advisory Boards for Janssen, Merck, and Novartis as well as speaker honoraria and travel support from Alexion, Amicus Therapeuticus, Biogen, Biologix, Bristol Myers Squibb, Cognomed, F. Hoffmann-La Roche Ltd., Gemeinnützige Hertie-Stiftung, Medison, Merck, Novartis, Roche Pharma AG, Genzyme, TEVA, and WebMD Global. Prof. Wiendl is acting as a paid consultant for Biogen, Bristol Myers Squibb, EMD Serono, Idorsia, Immunic, Novartis, Roche, Sanofi, the Swiss Multiple Sclerosis Society, and UCB. His research is funded by the German Ministry for Education and Research (BMBF), Deutsche Forschungs-gesellschaft (DFG), Deutsche Myasthenie Gesellschaft e.V., Alexion, Amicus Therapeutics Inc., Argenx, Biogen, CSL Behring, F. Hoffmann - La Roche, Genzyme, Merck KgaA, Novartis Pharma, Roche Pharma, and UCB Biopharma. LK has received no personal compensation. His institutions (University Hospital Basel/Stiftung Neuroimmunology and Neuroscience Basel) have received and used exclusively for research support payments for steering committee and advisory board participation, consultancy services, and participation in educational activities from: Actelion, Bayer, BMS, df-mp Molnia & Pohlmann, Celgene, Eli Lilly, EMD Serono, Genentech, Glaxo Smith Kline, Janssen, Japan Tobacco, Merck, MH Consulting, Minoryx, Novartis, F. Hoffmann-La Roche Ltd, Senda Biosciences Inc., Sanofi, Santhera, Shionogi BV, TG Therapeutics, and Wellmera, and license fees for Neurostatus-UHB products; grants from Novartis, Innosuisse, and Roche. CG: The Employer Department of Neurology, Regional Hospital Lugano (EOC), Lugano, Switzerland receives financial support for speaking, educational, research or travel grants from Abbvie, Almirall, Biogen Idec, Celgene, Sanofi, Merck, Novartis, Teva Pharma, Roche. CG: The University Hospital Basel (USB), as the employer of C.G., has received the following fees which were used exclusively for research support: (i) advisory boards and consultancy fees from Actelion, Novartis, Genzyme-Sanofi, GeNeuro, Hoffmann La Roche and Siemens; (ii) speaker fees from Biogen, Hoffmann La Roche, Teva, Novartis, Merck, Jannsen Pharmaceuticals and Genzyme-Sanofi; (iii) research grants: Biogen, Genzyme Sanofi, Hoffmann La Roche, GeNeuro. MT has research collaborations with Roche, Novartis and Idorsia (all Switzerland). DL is Chief Medical Officer of GeNeuro. JDL receives honoraria for acting as a member of Scientific Advisory Boards, speaker fees, research support, travel support by Abbvie, Alexion, Argenx, Biogen, CSL Behring, Merck KgaA, Moderna, Novartis, Roche Pharma AG, Sanofi, Takeda, UCB Biopharma. His research is supported by the German Research Foundation (DFG), the French National Research Agency (ANR), the National Multiple Sclerosis Society (NMSS), and the National Institutes of Health (NIH). JK received speaker fees, research support, travel support, and/or served on advisory boards by Swiss MS Society, Swiss National Research Foundation (320030_189140/1), University of Basel, Progressive MS Alliance, Bayer, Biogen, Bristol Myers Squibb, Celgene, Merck, Novartis, Octave Bioscience, Roche, Sanofi.