

## BRIEF COMMUNICATION

**Terminal complement activation is increased and associated with disease severity in CIDP**Isaak Quast<sup>1,a</sup>, Christian W. Keller<sup>1,a</sup>, Falk Hiepe<sup>2</sup>, Björn Tackenberg<sup>3</sup> & Jan D. Lünemann<sup>1,4</sup><sup>1</sup>Department of Neuroinflammation, Institute of Experimental Immunology, University of Zürich, Zürich, Switzerland<sup>2</sup>Department of Rheumatology and Clinical Immunology, Charité-University Medicine Berlin, Berlin, Germany<sup>3</sup>Department of Neurology, Philipps-University, Marburg, Germany<sup>4</sup>Department of Neurology, University Hospital Basel, Basel, Switzerland**Correspondence**

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**Introduction**

Chronic inflammatory demyelinating polyneuropathy (CIDP) is the most prevalent chronic autoimmune neuropathy and the most common autoimmune-mediated disease of the peripheral nervous system.<sup>1,2</sup> Both cell-mediated and humoral mechanisms contribute to its pathogenesis and the rapid clinical response to plasmapheresis therapy implicates a circulating factor responsible for peripheral nerve tissue injury in patients with CIDP.<sup>1,2</sup> Consistent with this hypothesis, complement-fixing IgG and IgM deposits are found on the myelin sheath in sural nerve biopsies from patients with CIDP.<sup>3,4</sup> Furthermore, sera derived from patients who responded well to plasma exchange were shown to induce demyelination and reduction of conduction velocity if adoptively transferred to

**Abstract**

Chronic inflammatory demyelinating polyneuropathy (CIDP) is the most common chronic autoimmune neuropathy. While both cell-mediated and humoral mechanisms contribute to its pathogenesis, the rapid clinical response to plasmapheresis implicates a circulating factor responsible for peripheral nerve injury. We report that treatment-naïve patients with CIDP show increased serum and CSF levels of the anaphylatoxin C5a and the soluble terminal complement complex (sTCC). Systemic terminal complement activation correlates with clinical disease severity as determined by the Inflammatory Neuropathy Cause and Treatment (INCAT) disability scale. These data indicate that complement activation contributes to peripheral nerve injury and suggest that complement inhibition should be explored for its potential therapeutic merit in CIDP.

nonhuman primates or intraneurally injected into rat sciatic nerves.<sup>5,6</sup> Most patients with CIDP lack detectable titers of antibodies specific for major compact myelin proteins which suggests that serum constituents other than myelin-directed antibodies, such as noncompact myelin-specific antibodies, cytokines, or components of the complement cascade might contribute to peripheral nerve injury.<sup>1,2,7</sup>

Complement activation has long been thought to constitute a potential pathogenic mechanism in CIDP since the complement component C3d was found deposited on the outer surface of Schwann cells and the compact myelin in biopsies from CIDP patients.<sup>3,4</sup> Its proinflammatory function is reflected by the ability of terminal complement components such as C5a to recruit myeloid cells such as macrophages to sites of inflammation

through complement receptors and in inducing tissue injury through formation of the terminal complement complex (TCC), that is, membrane attack complex.

Koski and colleagues reported that serum TCC levels are increased in patients with Guillain-Barré syndrome (GBS), decline with clinical improvement and become undetectable 1 month after onset of symptoms.<sup>8</sup> They also reported elevated TCC serum levels in six out of seven patients diagnosed with chronic recurrent polyneuropathy.<sup>8</sup> In patients with GBS, membrane bound TCC components were found to be deposited on the abaxonal Schwann cell surface.<sup>8,9</sup> Hartung et al. reported elevated C3a and C5a CSF levels in patients with GBS if compared to patients with noninflammatory neurological diseases.<sup>10</sup> We could previously show that the presence of serum IgG antibodies enriched for IgG-Fc glycovariants that efficiently activate the complement cascade is associated with disease activity in patients with CIDP.<sup>11</sup> Here, we hypothesized that complement activation is increased in CIDP patients and associated with disease severity.

## Patients and Methods

### Patients

Samples and clinical data were collected between 2010 and 2015 at the Department of Neurology, University of Marburg, Germany. All patients with CIDP fulfilled the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) diagnostic criteria<sup>12</sup> and were newly diagnosed with an interval between onset of symptoms and blood/CSF draw of <3 months.

Patients showed symmetrical, proximal and distal sensory and motor deficits and were therefore classified as idiopathic/typical CIDP. Control samples were collected from 29 patients with noninflammatory neurological disease conditions (tension-type or hypertension-induced headache, movement disorders, stenosis of the lumbal spinal canal, noninflammatory polyneuropathies). Sera from patients with systemic lupus erythematosus (SLE) were collected in 2016 at the Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Germany. All patients met the updated and revised criteria proposed by the American College of Rheumatology (ACR) for the diagnosis of SLE<sup>13</sup> and received immunomodulatory therapy at the time of blood draw (Table 1). After blood draw, serum was separated by centrifugation at 4°C and immediately frozen at -80°C within 30 min of venipuncture. CSF collected from both patients with CIDP and controls was also frozen within 30 min of collection and stored at -80°C. To assess treatment response, the modified INCAT score<sup>14,15</sup>

was used routinely before and 4 weeks after IVIG-treatment (Gamunex-C (Grifols) and Privigen (CSL Behring); 2 g/kg body weight over 5 consecutive days). The study was approved by the local Institutional Review Boards and all subjects provided informed consent.

### ELISA

C5a and TCC levels in serum and CSF samples were quantified by ELISA (Tecommedical AG, Sissach, Switzerland) according to the manufacturer's recommendations. CSF indices for C5a and sTCC were calculated as follows:  $(C5a \text{ or } sTCC_{CSF}/C5a \text{ or } sTCC_{serum})/(\text{albumin}_{CSF}/\text{albumin}_{serum})$ .

### Statistics

Protein expression levels in serum and CSF samples were compared using the nonparametric Mann-Whitney *U* test. Correlation analyses were performed using the Spearman's rank correlation coefficient. A *P* < 0.05 was

**Table 1.** Demographic and clinical characteristics of CIDP patients and their controls.

	CIDP <i>n</i> = 21	Controls <i>n</i> = 29	SLE <i>n</i> = 25 <sup>1</sup>
Age (Years ± SD; range)	64 ± 13 (38–80)	49 ± 19 (21–81)	43 ± 14 (23–71)
Male to female ratio	1.3	1.3	0.09
Fulfilling modified AAN criteria, <i>n</i> ; %	21; 100	NA	NA
Fulfilling EFNS/PNS criteria, <i>n</i> ; %	21; 100	NA	NA
Clinical course		NA	NA
CP, <i>n</i> ; %	19; 90.5	–	NA
Monophasic <sup>2</sup> , <i>n</i> ; %	2; 9.5	–	NA
CIDP subtype		NA	NA
CIDP-I, <i>n</i> ; %	19; 90.5	–	NA
CIDP-MGUS, <i>n</i> ; %	2; 9.5	–	NA
Treatment response <sup>3</sup> , <i>n</i> ; %	16; 80	NA	NA

AAN, American Academy of Neurology; CP, chronic progressive; CIDP, chronic inflammatory demyelinating polyneuropathy CIDP-I, idiopathic CIDP; CIDP-MGUS, CIDP with monoclonal gammopathy of uncertain significance; EFNS, European Federation of Neurological Societies; NA, not applicable; PNS, Peripheral Nerve Society; SD, standard deviation; SLE, systemic lupus erythematosus.

<sup>1</sup>All patients with SLE received immunomodulatory/immunosuppressive treatment such as belimumab (*n* = 9/25), a monoclonal antibody targeting the soluble human B lymphocyte stimulator protein BlyS, low-dose glucocorticosteroids (*n* = 21/25), azathioprine (*n* = 21/25), low-dose methotrexate (*n* = 3/25), mycophenolate mofetil (*n* = 3/25), and cyclosporine (*n* = 1/25).

<sup>2</sup>But no Guillain-Barré syndrome.

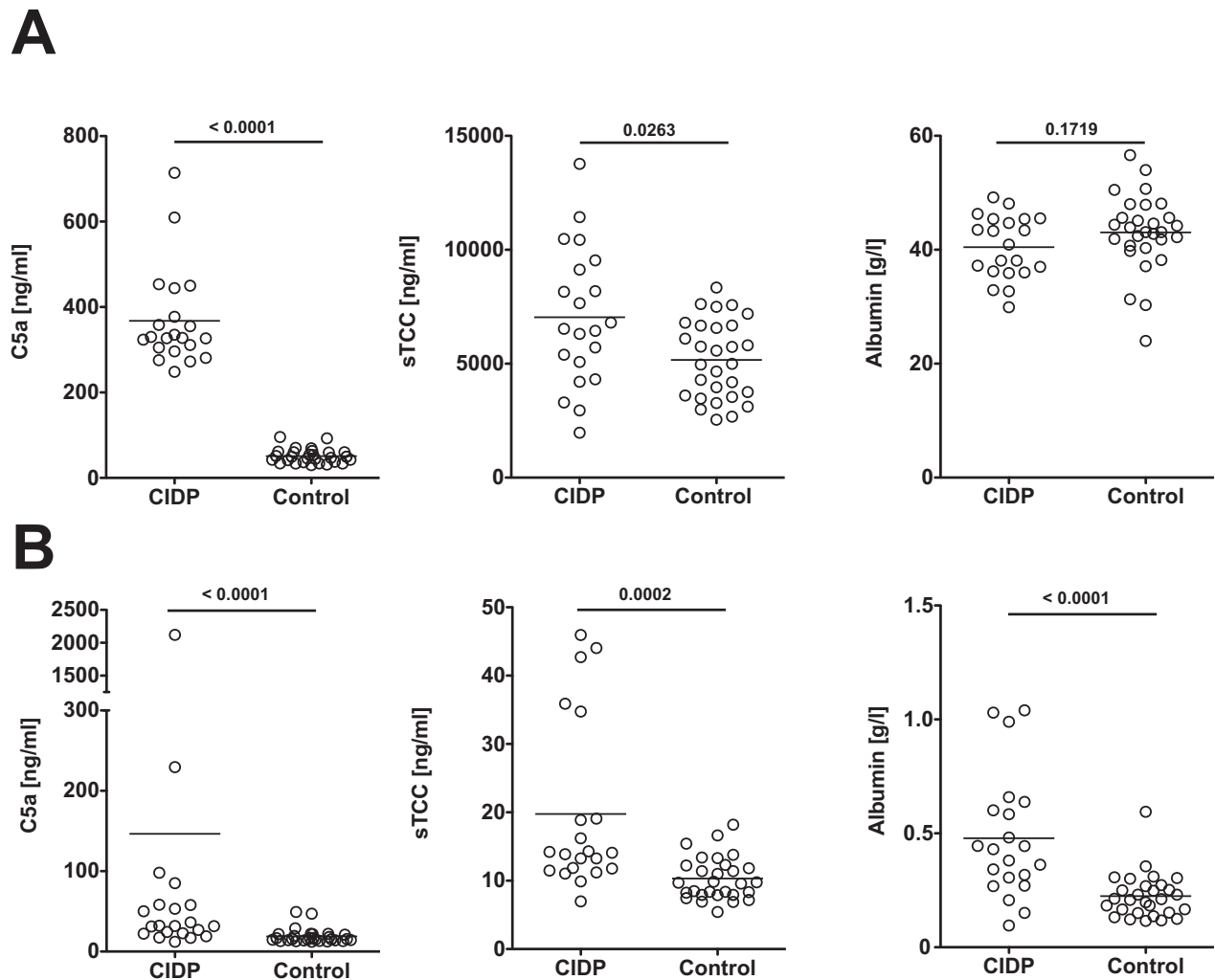
<sup>3</sup>Defined as ≥1 point decrease in the modified Rankin' scale. No pretreatment data was available for one patient.

considered significant. All graphs and statistics were done using GraphPad Prism 5 (GraphPad Software, Inc.; La Jolla, CA).

## Results

A total of 21 newly diagnosed and treatment-naïve patients with CIDP fulfilling the EFNS/PNS diagnostic criteria<sup>12</sup> and 29 controls with noninflammatory neurological disease conditions were included in the analysis (Table 1). All patients underwent neurological examination at the time of diagnosis, serum and CSF were collected before induction of immunomodulatory therapy with intravenous immunoglobulin (2 g/kg body weight over 5 consecutive days) and patients were re-examined

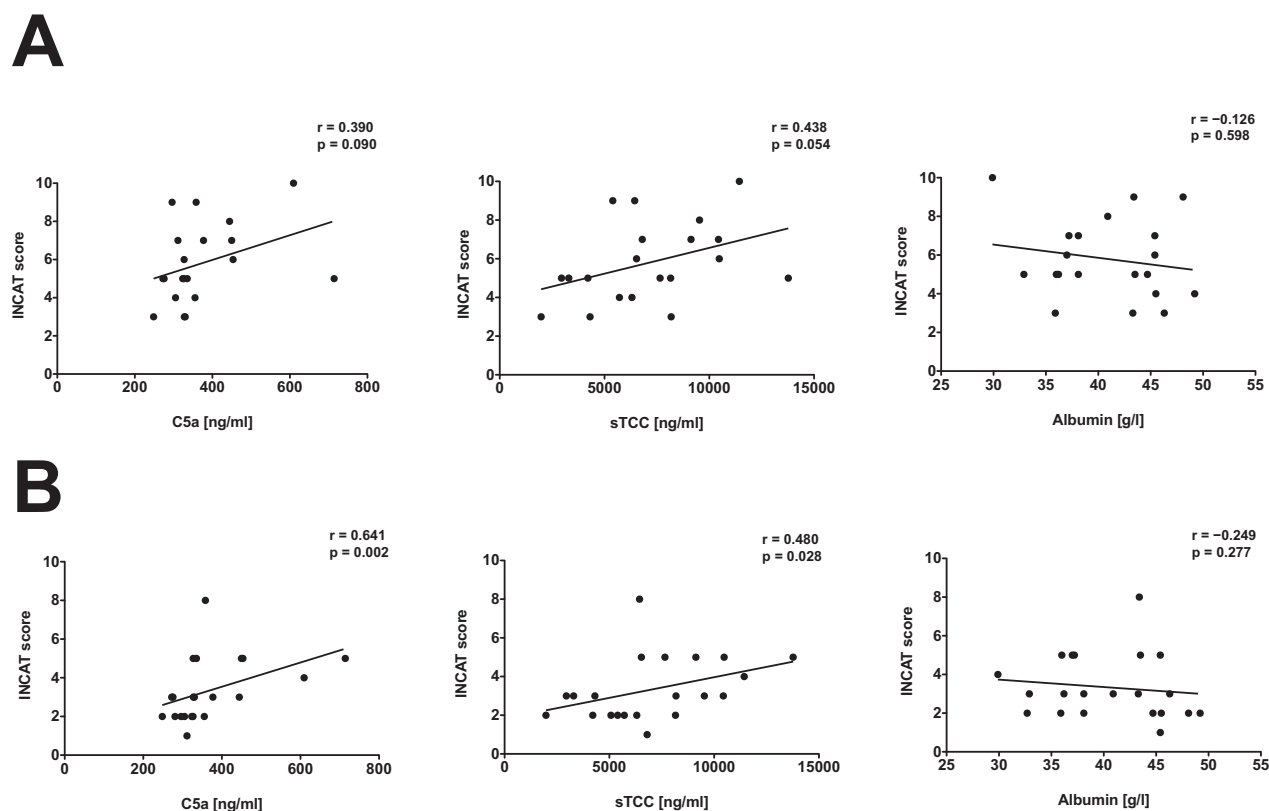
after 4 weeks. Complement activation can be triggered by various stimuli including immune complexes, extracellular matrix proteins and apoptotic/necrotic cells<sup>17</sup>. Complement activating pathways converge to the generation of the anaphylatoxin C5a and the TCC, generated by the assembly of C5b through C9. The C5b-9 that fails to assemble in the membrane forms a soluble, lytically inert complex, termed sTCC, and complement-mediated cell lysis was shown to closely correlate with the generation of sTCC levels in vitro.<sup>16</sup> Here, terminal complement activation was quantified in serum and CSF samples by ELISAs specific for C5a and sTCC. Compared to controls, patients with CIDP showed significantly higher levels of C5a and sTCC in both serum and CSF (Fig. 1). C5a serum levels higher than



**Figure 1.** Increased terminal complement activation in serum and CSF of CIDP patients. Shown are (A) serum and (B) CSF levels of C5a, sTCC and albumin in treatment-naïve patients with CIDP compared to patients with noninflammatory neurological diseases (controls). Statistics were performed by Mann-Whitney *U* test. CIDP, Chronic inflammatory demyelinating polyneuropathy; sTCC, soluble terminal complement complex.

95.6 ng/mL were exclusively observed in patients with CIDP. We additionally analyzed C5a and sTCC serum levels in patients with SLE receiving immunomodulatory therapy (Table 1). Compared to patients with CIDP, patients with SLE showed lower levels of C5a (mean: 43.18 ng/mL, SD: 22.68;  $P < 0.0001$ ) and sTCC (mean: 2782 ng/mL, SD: 1533;  $P < 0.0001$ ). Increased levels of C5a and sTCC in CSF samples of CIDP patients correlated with CSF albumin concentrations (sTCC:  $r = 0.82$ ,  $P = 0.0001$ ; C5a:  $r = 0.77$ ,  $P < 0.0001$ ) and CSF indices for C5a and TCC were lower in patients with CIDP compared to controls ( $P < 0.0001$  for C5a and  $P < 0.012$  for sTCC) suggesting a systemic increase of terminal complement activation as opposed to local production within the CSF compartment. Serum levels of albumin, chosen as control protein, were unchanged in patients with CIDP (Fig. 1). Thus, systemic terminal complement activation as reflected by C5a and sTCC and levels is increased in treatment-naïve patients with CIDP.

We next investigated whether increased terminal complement activation is associated with or anticipates clinical disease severity in patients with CIDP. Disease severity was monitored using the INCAT (Inflammatory Neuropathy Cause and Treatment) disability score<sup>14,15</sup> during the diagnostic workup and 4 weeks after initiation of IVIG therapy. INCAT disability scores were reduced in 16/21 (80%) patients 4 weeks after IVIG therapy induction. Both C5a and sTCC levels tended to be higher in patients with higher disability at the time of diagnosis (Fig. 2A) and significantly correlated with INCAT scores determined at 4 week follow-up visits (Fig. 2B). No significant correlation could be observed for clinical disease severity and albumin concentrations (Fig. 2). CSF levels of C5a and TCC at the initial presentation did not significantly correlate with INCAT disability scores at any time point (data not shown). Thus, systemic terminal complement activation is associated with residual disease severity 4 weeks after effective IVIG therapy in patients with CIDP.



**Figure 2.** Systemic terminal complement activation correlates with disease severity in patients with CIDP. Shown are Spearman's rank correlation analyses of serum C5a, sTCC and albumin levels with disease severity (defined by INCAT score) of (A) newly diagnosed, previously untreated patients with CIDP and (B) 4 weeks after immunomodulatory therapy with IVIG (2 g/kg body weight over 5 consecutive days). Each dot reflects an individual patient. Displayed are linear regression curves. Statistics were performed by Spearman test. CIDP, Chronic inflammatory demyelinating polyneuropathy; INCAT, Inflammatory Neuropathy Cause and Treatment; sTCC, soluble terminal complement complex.

## Discussion

Our study demonstrates that systemic terminal complement activation as defined by serum C5a and TCC levels is increased and associated with disease severity in patients with CIDP. Sera from CIDP patients which, upon adoptive transfer, induced conduction block and demyelination in rat sciatic nerves show IgG and C3d binding to myelin components on normal peripheral nerve tissue, indicating that their demyelinating capacity is antibody-mediated and complement-dependent.<sup>6</sup> Our data suggest that systemic and local terminal complement activation is a characteristic feature of inflammatory demyelinating polyneuropathies and support a role of complement activation in the pathogenesis of CIDP.

Deficiencies of early components of the classical complement activation pathway such as C1q, C2, and C4 are strongly associated with the development of SLE, presumably due to impaired complement-mediated clearance of dying cells,<sup>17</sup> and low concentrations of complement components are observed in a majority of patients with active and severe SLE. In contrast, terminal complement activation products such as C5a and sTCC, although reported to correlate with clinical disease activity in individual patients, are either normal or only slightly elevated in sera from patients with established SLE.<sup>18,19</sup> The finding that C5a and sTCC levels are substantially higher in patients with CIDP compared to SLE suggest differential mechanisms and functions of terminal complement activation in these diseases, although treatment-related effects cannot be excluded since all SLE patients included in our study received immunomodulatory therapy.

Terminal complement activation can be inhibited therapeutically, for example, by eculizumab, a humanized monoclonal antibody which blocks the cleavage from C5 to C5a and C5b, thereby preventing the generation of C5a and TCC formation. Eculizumab is approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, was shown to be therapeutically effective in a murine model of acute antibody-mediated neuropathy<sup>20</sup> and is currently tested for its safety and efficacy in patients with GBS. In an open-label clinical trial conducted in 13 patients with multifocal motor neuropathy (MMN), eculizumab treatment did not show objective clinical benefits, although improvement of some secondary outcomes such as patient-rated subjective scores and selected clinical and electrophysiological measurements were reported.<sup>21</sup> Our data strongly indicate that inhibition of terminal complement activation should be explored for its potential therapeutic merit in patients with CIDP.

There are limitations to our study. First, the relatively small number of patients requires validation in larger independent cohorts. Second, we did not monitor serum

C5a and TCC levels over time in order to investigate whether terminal complement activation remains increased during disease progression and under immunomodulatory therapy since transient reduction of complement proteins and/or complement activation occur after plasmapheresis,<sup>22</sup> during corticosteroid therapy<sup>23</sup> as well as following IVIG treatment,<sup>24</sup>. A small open-label clinical trial on eculizumab in patients with MMN who concomitantly received IVIG reported that most patients continued to require IVIG despite full inhibition of terminal complement function<sup>21</sup> suggesting that the therapeutic efficacy of IVIG is mediated by immunomodulatory mechanisms other than complement inhibition. Furthermore, our study was restricted to patients with idiopathic or typical CIDP characterized by symmetrical, proximal and distal sensory and motor deficits which comprise only 50–60% of patients with CIDP.<sup>1,2,7</sup> Our findings, however, provide incentive to conduct larger prospective investigations to examine terminal complement activation as a potential surrogate marker for clinical disease activity, progression, and response to immunomodulatory therapies in patients with major phenotypic variants of CIDP. It remains to be evaluated whether inhibition of terminal complement activation can be harnessed to improve the clinical outcome in patients with CIDP.

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## Author Contribution

Conception and design of the study: I.Q., J.D.L.; acquisition and analysis of data: I.Q., C.W.K., F.H., B.T., J.D.L.; drafting of the manuscript and figures: I.Q., C.W.K., F.H., B.T., J.D.L.

## Conflict of Interest

The authors declare that there are no financial or other relationships that might lead to a perceived conflict of interest.

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