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Effects of pooled human immunoglobulins in an animal model of neuromyelitis optica with chronic application of autoantibodies to aquaporin 4

C. Geis*†
*Hans-Berger Department of Neurology, Center for Sepsis Control and Care (CSCC) Jena University Hospital, Jena, Germany and
†Department of Neurology, University Hospital Würzburg, Würzburg, Germany
Correspondence: C. Geis.
E-mail: christian.geis@med.uni-jena.de

Neuromyelitis optica (NMO) is a relapsing, chronic inflammatory disease of the central nervous system (CNS) mainly affecting the spinal cord and the optic nerves. The discovery of highly specific associated autoantibodies (AB) to the osmotic-driven water channel aquaporin 4 (APQ4) led to the classification of NMO as a separate entity from multiple sclerosis [1,2]. AQP4 is an integral membrane protein, located on the abluminal side of astrocyte endfeet in the CNS. Immunopathology of typical NMO lesions show distinct deposition of immunoglobulin (Ig)G and activated complement, as well as immune cell infiltration resulting in tissue destruction. NMO lesions are also characterized by astrocyte depletion and loss of AQP4. Recent reports also suggest 'penumbra-like' regions adjacent to destructive areas, which are characterized by specific loss of AQP4 but preserved astrocytes [3]. Current treatment strategies for NMO patients consist of AB removal by plasma exchange or immunoadsorption and/or high-dose corticosteroids during acute relapses, and of B cell depletion with rituximab or immunosuppressive treatment with mitoxantrone or cyclophosphamide as an interval therapy. Furthermore, there is some clinical evidence for a favourable outcome with an interval treatment using eculizumab, a therapeutic monoclonal antibody that neutralizes complement protein C5 [4]. Together, these complex immunopathological features and the treatment response to either AB removal, B cell depletion or downstream complement inhibition point clearly towards an AB-mediated, target-directed pathomechanism. The aim of the current study was to evaluate the treatment effect of pooled human immunoglobulins (IVIg; Privigen®) in a rat model that focuses on the intrinsic pathogenic effects of AB to AQP4 induced by repetitive intrathecal (i.th.) application of purified patient IgG fractions.

In previous studies we developed an animal model that proved to be useful to study AB-dependent pathomechanisms in the CNS in the living organism [5,6]. In adult female Wistar rats, i.th. catheters were inserted through the cerebellomedullary cistern, with the internal opening of the catheter right above the lumbar enlargement of the spinal cord whereas the external ending was facing outwards of the animal's occiput. When small amounts of high-dose NMO-patient IgG (10 µl volume of 100 mg/ml concentration) purified from patient plasma exchange material were administered in three series of five daily applications and a 2-day break in between (3 weeks in total), rats developed progressive disease signs starting with motor symptoms of unilateral hind limb paresis reaching to paraplegia in severe cases. Histologically, spinal cord areas near the catheter ending were characterized by intense IgG deposition and reduction of AQP4 immunoreactivity, but without obvious cell destruction, immune cell infiltration and astrocyte depletion. Thus, in this animal model without additional co-injection of complement factors, the intrinsic effects of AB to AQP4 mediated distinct immunopathological features and led to functional motor deficits. We then aimed to evaluate the effects of IVIg in this animal model in several treatment strategies: (1) in a prophylactic strategy with systemic application of IVIg from day 1 in parallel to NMO-IgG i.th. application, (2) in a therapeutic strategy with systemic application from day 10 after start of NMO-IgG application when rats already had developed disease signs and (3) in an approach for testing direct competitive mechanisms when IVIg was applied locally to the i.th. compartment together with the pathogenic NMO-IgG fraction. All groups were tested in comparison to respective control groups receiving equal amounts of 0·9% saline systemically or i.th. in blinded conditions.

IVIg applied systemically (intraperitoneal injections) in a concentration of 0·4 g/kg/day from day 1 led to an improvement of disease signs from the beginning of injections, as measured by a disease score that was modified from the

score of experimental autoimmune encephalomyelitis (EAE) ranging from 0 (no symptoms) to 10 (animals died due to disease severity). The mean disease severity calculated over the entire experimental period was decreased significantly in the experimental group with systemic IVIg treatment with a score value of $2{\cdot}3 \pm 0{\cdot}5$ in comparison to control-treated animals with a mean disease score of $4{\cdot}5 \pm 0{\cdot}7$ [mean ± standard error of mean (s.e.m.); $P = 0{\cdot}027$; *t*-test following Shapiro–Wilk testing for normal distribution]. When IVIg was administered systemically in a therapeutic strategy from day 10 after beginning of i.th. injections, rats showed a stabilization of the disease course at that time-point without further clinical deterioration. When the mean disease scores of the experimental period before starting IVIg in the therapeutic IVIg group (before day 10) were compared with the prophylactic IVIg application group (starting from day 1), the disease score was significantly lower in the prophylactic group ($2{\cdot}1 \pm 0{\cdot}3$ *versus* $3{\cdot}4 \pm 0{\cdot}3$; $P = 0{\cdot}008$). However, in comparison to mean disease score on day 10, the therapeutic group with IVIg treatment from day 10 had a significant reduction of $0{\cdot}4 \pm 0{\cdot}2$ on the disease score in the following time–course (mean ± s.e.m. days 11–17; $P = 0{\cdot}013$), whereas the group with IVIg from day 1 showed a further slight increase in score value of $0{\cdot}3 \pm 0{\cdot}05$ in the same period.

Next, we investigated the effects when IVIg was co-administered i.th. (1 μg in 10 μl) following pathogenic NMO-IgG application to test for possible direct Ig interactions. We found a reduced total mean disease score in comparison to sham injected rats ($2{\cdot}1 \pm 0{\cdot}3$ and $3{\cdot}5 \pm 0{\cdot}4$, respectively; $P = 0{\cdot}023$).

In the animal model with chronic i.th. application of pathogenic IgG containing high titres of AB to AQP4, systemic and local treatment with IVIg led to a significant amelioration, but not to a complete reversal of disease signs. Systemic and local treatment with IVIg was well tolerated and showed no obvious side effects. This may serve as first experimental proof-of-concept data for the use of IVIg in NMO disease with AB to AQP4, which is in line with other experimental evidence using a different approach [7]. These promising experimental results are, of course, limited in their prediction to human disease, as animal models can only insufficiently represent human NMO disease, with its complex and co-operative immunoinflammatory events involving humoral factors, complement activation and cell-mediated cytotoxicity. There are some small case–series with a limited number of NMO patients and single case reports that report a beneficial outcome with a reduction of relapse rate and clinical improvement when IVIg was given in a prophylactic regimen [8,9]. The role of therapeutic IVIg in NMO should be evaluated further; larger prospective cohorts and randomized trials are warranted [10].

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