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Recombinant IgG1 Fc hexamers block cytotoxicity and pathological changes in experimental in vitro and rat models of neuromyelitis optica

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Abstract

Intravenous human immunoglobulin G (IVIG) may have therapeutic benefit in neuromyelitis optica spectrum disorders (herein called NMO), in part because of the anti-inflammatory properties of the IgG Fc region. Here, we evaluated recombinant Fc hexamers consisting of the IgM μ-tailpiece fused with the Fc region of human IgG1. In vitro, the Fc hexamers prevented cytotoxicity in aquaporin-4 (AQP4) expressing cells and in rat spinal cord slice cultures exposed to NMO anti-AQP4 autoantibody (AQP4-IgG) and complement, with >500-fold greater potency than IVIG or monomeric Fc fragments. Fc hexamers at low concentration also prevented antibody-dependent cellular cytotoxicity produced by AQP4-IgG and natural killer cells. Serum from rats administered a single intravenous dose of Fc hexamers at 50 mg/kg taken at 8 h did not produce complement-dependent cytotoxicity when added to AQP4-IgG-treated AQP4-expressing cell cultures. In an experimental rat model of NMO produced by intracerebral injection of AQP4-IgG, Fc hexamers at 50 mg/kg administered before and at 12 h after AQP4-IgG fully prevented astrocyte injury, complement activation, inflammation and demyelination. These results support the potential therapeutic utility of recombinant IgG1 Fc hexamers in AQP4-IgG seropositive NMO.

Keywords

AQP4; NMO; Fc multimer; Astrocyte; Complement; Immunoglobulin; Neuroinflammation

1. Introduction

There is a need for effective and safe therapy for neuromyelitis optica spectrum disorders (herein called NMO), an autoimmune demyelinating disease of the central nervous system characterized by astrocyte injury, inflammation and demyelination (Hengstman et al., 2007;

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Disclosure/conflict of interest

RS and ABM are employees of CSL Behring and CSL Ltd, respectively. The other authors have no conflicts of interest.

Misu et al., 2007; Papadopoulos and Verkman, 2012). Current therapeutics include immunosuppressants, plasma exchange and B cell depletion, and several drugs are under evaluation or in pre-clinical development targeting various NMO pathogenesis mechanisms such as complement, IL-6 receptors and NMO autoantibody interactions (Araki et al., 2014; Cree et al., 2005; Greenberg et al., 2012; Kageyama et al., 2013; Papadopoulos et al., 2014; Verkman et al., 2013). Most NMO patients are seropositive for IgG1 autoantibodies against aquaporin-4 (AQP4) (called AQP4-IgG or NMO-IgG), a water channel expressed on astrocytes in which AQP4-IgG binding to AQP4 causes primary injury to astrocytes by complement and cellular effector mechanisms, producing inflammation and oligodendrocyte injury (Asgari et al., 2013; Graber et al., 2008; Jarius et al., 2014; Jarius and Wildemann, 2010; Lennon et al., 2005; Lucchinetti et al., 2002; Parratt and Prineas, 2010).

Intravenous human immunoglobulin G (IVIG) is believed to have possible therapeutic benefit in NMO. IVIG has been reported to have a variety of anti-inflammatory actions including complement inhibition (Piepers et al., 2010; Yuki et al., 2011), accelerated autoantibody clearance (Li et al., 2005), cytokine neutralization, blocking of antibody-antigen and antibody-Fcγ receptor binding, and perhaps inhibition of leukocyte migration and modulation of immune cell function (Berger et al., 2013; Chaigne and Mouthon, 2017; Dalakas, 2004; Jacob, and Rajabally, 2009; Schwab and Nimmerjahn, 2013). IVIG has been used in various neuro-inflammatory demyelinating disorders including Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and myasthenia gravis (Gelfand, 2012; Lünemann et al., 2015; Nguyen et al., 2012; Winkelmann and Zettl, 2012), as well as immune disorders outside of the nervous system such as idiopathic thrombocytopenic purpura (ITP) and Kawasaki disease (Galeotti et al., 2010; Imbach et al., 1981; Katz-Agranov et al., 2015). Several clinical studies, albeit largely anecdotal, support the efficacy of IVIG in NMO (Bakker and Metz, 2004; Elsone et al., 2014; Magraner et al., 2013; Okada et al., 2007; Viswanathan et al., 2015; Wingerchuk, 2013); however, a recent controlled trial of IVIG in NMO transverse myelitis showed no benefit, though the study may have been underpowered (Absoud et al., 2017). We reported a modest, ~50% reduction in pathology in an experimental mouse model of NMO in which IVIG was administered at a dose that produced serum levels comparable to those in IVIG-treated humans (Ratelade et al., 2014). The reduction in NMO pathology by IVIG involved reduced complement- and cell-mediated AQP4-IgG astrocyte injury. Limited efficacy of IVIG was also reported recently in rats administered human NMO patient sera by an intrathecal route (Grünewald et al., 2016).

Motivated by the anti-inflammatory properties of the Fc region of human IgG in IVIG, various recombinant Fc-based therapeutics are under development, including Fc fusion and multimeric proteins, which have shown efficacy in experimental animal models of arthritis, ITP and inflammatory neuropathy (Anthony et al., 2008; Czajkowsky et al., 2015; Jain et al., 2012; Lin et al., 2007; Niknami et al., 2013; Ortiz et al., 2016; Thiruppathi et al., 2014). In a recent advance, recombinant Fc hexamers were generated by fusion of the IgM μ-tailpiece to the Fc region of human IgG1 and shown to be effective in rat models of arthritis and ITP (Spirig et al., submitted). The Fc hexamers were reported to deplete early components of the classical complement system and to have high avidity for inhibition of Fcγ receptors and hence Fcγ receptor-mediated effector functions, thus providing rationale for their potential

therapeutic utility in NMO. Here, we demonstrate the efficacy of Fc hexamers in in vitro, ex vivo and in vivo rat models of NMO and studied their mechanism of action.

2. Materals and methods

2.1. Antibodies and sera

Purified recombinant Fc preparations were generated and provided by CSL Behring (Victoria, Australia), which included: Fc-μTP (hexameric human IgG1 Fc, in which the 18 amino acid IgM μ-tailpiece was fused with 231 amino acids at the C-terminus of the constant region of human IgG1 Fc); Fc-μTP-L309C (Fc-μTP with stabilizing L309C mutation in the Fc region); and Fc monomer (monomeric recombinant IgG1Fc) (Fig. 1A). In addition, comparisons were made with clinical-grade IVIG (Privigen, CSL Behring, Bern Switzerland). Recombinant monoclonal NMO antibody rAb-53, which recognizes human and rodent AQP4, was generated as described (Bennett et al., 2009; Crane et al., 2011) and provided by Dr. Jeff Bennett (Univ. Colorado). NMO serum was obtained from a seropositive individual who met the revised diagnostic criteria for clinical disease, with non-NMO human serum as control.

2.2. Rats

Experiments were done using weight-matched female Sprague Dawley rats (250–300 g, age 9–12 weeks). Protocols were approved by the UCSF Institutional Animal Care and Use Committee (IACUC, protocol AN108511).

2.3. Cell culture

Chinese hamster ovary (CHO) cells stably expressing human AQP4-M23 (named CHO-AQP4 cells), as described (Crane et al., 2011), were cultured at 37 °C in 5% CO_2 95% air in F-12 Ham's Nutrient Mixture medium supplemented with 10% fetal bovine serum, 200 μg/ml geneticin, 100 U/ml penicillin and 100 μg/ml streptomycin. Human natural killer cells (NK cells) transfected to express the high-affinity 176 V variant of the Fcγ receptor IIIA (Yusa et al., 2002) were obtained from Fox Chase Cancer Center (Philadelphia, PA).

2.4. CDC and ADCC assays

CHO-AQP4 cells were grown in 96-well plates until confluence with 25,000 cells per well. Cells were pre-incubated with 10 μg/ml AQP4-IgG (rAb-53) or NMO serum (1:50) for 1 hat 23 °C. For assay of CDC, human or rat complement was pre-incubated for 1 h at 4 °C with specified concentrations of Fc preparations and then added to the AQP4-IgG-coated CHO-AQP4 cells for an additional 1 h at 23 °C. For analysis of kinetics, human complement was pre-incubated with Fc-μTP-L309C for specified times prior to addition to the AQP4-IgG-coated CHO-AQP4 cells. For assay of ADCC, CHO-AQP4 cells were incubated for 2 hat 37 °C with 5 μg/ml AQP4-IgG, without or with Fc preparations, and NK cells at an effector:target cell ratio of 4:1. CHO-AQP4 cells were then washed extensively in PBS and cell viability was measured by addition of 20% Alamar Blue (Invitrogen, Carlsbad, CA) for 45 min at 37 °C. Percentage cytotoxicity was determined as described (Phuan et al., 2013; Ratelade et al., 2014). Hemolysis assays using IgM-coated sheep red blood cells (for assay of classical complement pathway) and uncoated rabbit red blood cells (for assay of

alternative pathway) were done according to manufacturers' instructions (Complement Technology Inc., Tyler, TX).

2.5. Ex vivo spinal cord slice model of NMO

Spinal cords were obtained from 7-day old rats and cut at 300-μm thickness using a vibratome, as described for mice (Zhang et al., 2011). Transverse slices were placed on transparent membrane inserts (Millipore, Millicell-CM 0.4 μm pores, 30 mm diameter) in 6-well plates containing 1 ml culture medium, with a thin film of culture medium covering the slices. Slices were cultured in 5% CO2 at 37 °C for 7 days in 50% MEM, 25% HBSS, 25% horse serum, 1% penicillin–streptomycin, 0.65% glucose and 25 mM HEPES. The 7-day old slices were incubated for 24 h with AQP4-IgG (5 μg/ml) and human complement (5%) without or with Fc-μTP or Fc-μTP-L309C (50 μg/ml). The Fc preparations were pre-incubated with human complement at room temperature for 1 h prior to addition to cells. Spinal cords were immunostained for AQP4, GFAP, MBP, Iba1 and C5b-9, and photographed and scored as described (Zhang et al., 2011): 0, intact slice with normal GFAP and AQP4 staining; 1, mild astrocyte swelling and/or reduced AQP4 staining; 2, at least one lesion with loss of GFAP and AQP4 staining; 3, multiple lesions affecting >30% of slice area; 4, lesions affecting >80% of slice area (Phuan et al., 2013; Ratelade et al., 2014; Zhang et al., 2011).

2.6. AQP4-IgG and C1q binding assays

CHO-AQP4 cells were grown on 96-well plates for 24 h. After blocking with 1% BSA in PBS, cells were incubated with AQP4-IgG or control IgG without or with 100 μg/ml Fc-μTP-L309C at 23 °C for 1 h. Cells were then washed with PBS and incubated with Alexa Fluor 594-goat anti-human IgG secondary antibody, F $(ab')_2$-fragment specific (1:500; Jackson ImmunoResearch, West Grove, PA) for 1 h. Cells were then rinsed three times with PBS and fluorescence quantified using a plate reader at excitation/emission wavelengths of 591/614 nm. For human IgG and AQP4 immunostaining, cells were incubated for 1 h at 23 °C with 10 μg/ml AQP4-IgG or control IgG in the absence or presence of 100 μg/ml Fc-μTP-L309C. Cells were then fixed in 4% PFA for 15 min and permeabilized with 0.1% Triton X-100. After blocking with 1% BSA, cells were incubated for 1 h with 0.4 μg/ml polyclonal, AQP4 C-terminal-specific rabbit anti-AQP4 antibody (Santa Cruz Biotechnology, Dallas, TX). Cells were rinsed with PBS and incubated for 1 h with Alexa Fluor 594- the F $(ab')_2$ fragment-specific antibody (1:400) and Alexa Fluor-488 goat anti-rabbit IgG secondary antibody (1:400; Invitrogen). To assay C1q binding, CHO-AQP4 cells were pre-incubated with 20 μg/ml AQP4-IgG for 1 hat 23 °C and then washed with PBS. Recombinant human C1q (60 μg/ml) was pre-incubated for 1 h with Fc monomers or Fc-μTP-L309C and then added to AQP4-IgG-coated cells for 1 h. Cells were washed, fixed and C1q was stained with a rabbit FITC-conjugated anti-C1q antibody (1:50; Abcam, Cambridge, MA).

2.7. Fc-μTP-L309C pharmacodynamics

Rats were administered Fc-μTP-L309C at 3.125, 6.25, 12.5, 25 or 50 mg/kg by tail vein injection and blood was collected at 2 h. The blood was left to clot at room temperature for 30 min, centrifuged at 2000 × g for 10 min at 4 °C, and serum was collected and frozen at

20 ° C overnight. Serum was used in CDC assays, as described above, in which a 1:50 dilution of serum was added to 1.25–10 μg/ml AQP4-IgG for 1 h at 23 °C. In some studies rat blood was collected at specified times after intravenous injection of 50 mg/kg Fc-μTP-L309C and subjected to CDC assay.

2.8. Rat model of NMO

AQP4-IgG was delivered by intracerebral injection as described (Asavapanumas et al., 2014; Yao and Verkman, 2017). Briefly, rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) and then mounted onto a stereotaxic frame. Following a midline scalp incision, a burr hole of 1 mm diameter was created 0.5 mm anterior and 3.5 mm lateral of bregma. A 40-μm diameter glass needle was inserted 5 mm deep to infuse 30 or 40 μg AQP4-IgG in a total volume of 3–6 μL over 10 min by pressure injection. Fc-μTP-L309C (50 mg/kg, IV) was administered at the time of and 12 h after intracerebral injection of AQP4-IgG. At day 5 rats were deeply anesthetized, followed by a transcardiac perfusion through the left ventricle with 200 ml of heparinized PBS and then 100 ml of 4% paraformaldehyde (PFA) in PBS. Brains were fixed in 4% PFA, left overnight at 4 °C in 30% sucrose and embedded in OCT.

2.9. Immunofluorescence

Fixed brains were frozen, sectioned (10-μm thickness) and incubated in blocking solution (PBS, 1% bovine serum albumin, 0.2% Triton X-100) for 1 h prior to overnight incubation (4 °C) with primary antibodies: AQP4 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), GFAP (1:100, Millipore), myelin basic protein (MBP) (1:200, Santa Cruz Biotechnology), ionized calcium-binding adaptor molecule-1 (Iba1; 1:1000; Wako, Richmond, VA), C5b-9 (1:50, Hycult Biotech, Uden, The Netherlands) or CD45 (1:10, BD Biosciences, San Jose, CA), followed by the appropriate fluorescent secondary antibody (1:200, Invitrogen, Carlsbad, CA). Sections were mounted with VECTASHIELD (Vector Laboratories, Burlingame, CA) for visualization on a Leica fluorescence microscope.

2.10. Statistical analysis

Data are presented as mean ± S.E.M. Statistical comparisons were made using the non-parametric Mann-Whitney test when comparing two groups.

3. Results

3.1. Fc hexamers inhibit complement-dependent cytotoxicity in AQP4-expressing cell cultures

Four Fc preparations were tested, including: clinical-grade IVIG (pooled human IgG), Fc monomers, and Fc-μTP and Fc-μTP-L309C hexamers, the latter containing a stabilizing point mutation at leucine 309 in the Fc region to allow inter-Fc disulfide bonds (Sorensen et al., 1999) (Fig. 1A). Biochemical characterization and validation of the Fc hexamers is being reported separately (Spirig et al., submitted).

The Fc preparations were first tested for their efficacy in inhibiting CDC in a standard assay involving incubation of AQP4-expressing CHO cells with a recombinant anti-AQP4 NMO

autoantibody (AQP4-IgG) and human complement, with percentage cytotoxicity measured by Alamar blue assay. For these studies the Fc preparations were incubated with human complement (human serum) prior to addition to AQP4-IgG pre-incubated cells. Fc-μTP and Fc-μTP-L309C blocked cytotoxicity in a concentration-dependent manner with >500-fold greater potency than IVIG and >3000-fold greater potency than Fc monomers (Fig. 1B).

Measurement of inhibition kinetics showed rapid inhibition of CDC at a Fc-μTP-L309C concentration above its IC_{50}, though much slower inhibition at lower Fc-μTP-L309C concentration (Fig. 1C), suggesting a cooperative binding mechanism involving multivalent interaction of Fc-μTP-L309C with C1q.

Fig. 1D shows inhibition of CDC by Fc-μTP and Fc-μTP-L309C when cytotoxicity was initiated by serum from a seropositive NMO patient rather than by recombinant AQP4-IgG. Apparent IC_{50} values were similar to those in Fig. 1B (left), supporting the conclusion that the Fc hexamers act on complement rather than AQP4-IgG or its binding to AQP4.

3.2. Fc hexamers prevent pathology in a spinal cord slice model of NMO

CDC inhibition studies were also done in an ex vivo spinal cord slice model of NMO in which 7-day cultured rat spinal cord slices show astrocyte injury (loss of AQP4 and GFAP), demyelination (reduced MPB staining), inflammation (increased Iba-1 staining) and deposition of the complement terminal membrane attack complex (C5b-9) following 24 h incubation with AQP4-IgG and human complement (Phuan et al., 2013; Zhang et al., 2011). Immunofluorescence of AQP4-IgG/complement-treated spinal cord slices showed the expected pathological changes, which were largely prevented by Fc-μTP or Fc-μTP-L309C (Fig. 2A). Fig. 2B summarizes pathology scores.

3.3. Fc hexamers inhibit antibody-dependent cellular cytotoxicity in AQP4-expressing cell cultures

The Fc preparations were also tested for their efficacy in inhibition of ADCC produced by incubation of AQP4-expressing CHO cells with AQP4-IgG and NK cells (Phuan et al., 2013; Ratelade et al., 2014; Tradtrantip et al., 2012). ADCC was inhibited in a concentration-dependent manner by Fc-μTP and Fc-μTP-L309C with IC_{50} ~80 μg/ml and 50 μg/ml, respectively, with little inhibition seen for IVIG or Fc monomers in the concentration range tested (Fig. 3). In a prior study done using much higher concentrations of IVIG (Ratelade et al., 2014), the IC_{50} for inhibition of ADCC by IVIG was >5 mg/ml, more than 100-fold greater than that for Fc-μTP or Fc-μTP-L309C here.

3.4. Mechanism of action studies

Further studies focused on Fc-μTP-L309C because this was the hexamer that had greatest in vitro potency for inhibition of CDC and ADCC. NMO pathogenesis is initiated by AQP4-IgG binding to membrane-bound AQP4, followed by binding of the initial complement protein C1q to the Fc region of bound AQP4-IgG. Fig. 4A shows that Fc-μTP-L309C at 100 μg/ml did not inhibit AQP4-IgG binding to AQP4 on CHO cells, as assayed using a fluorescent secondary antibody that recognizes the F $(ab')_2$ fragment of the primary antibody. Fig. 4B shows that Fc-μTP-L309C prevented binding of purified C1q to AQP4-

bound AQP4-IgG, as assayed by C1q immunofluorescence, which is consistent with one of the actions of Fc-μTP-L309C being avid binding to aqueous-phase C1q. In standard models of erythrocyte lysis produced by activation of the classical or alternative complement pathways, Fc-μTP-L309C strongly inhibited the classical but not the alternative complement pathways (Fig. 4C).

3.5. Fc-μTP-L309C prevents pathology in an experimental rat model of NMO

In vivo efficacy studies were done using an established experimental model of NMO in rats in which NMO pathology is created by intracerebral administration of AQP4-IgG (Asavapanumas et al., 2014; Yao and Verkman, 2017). The model was done in rats rather than mice because rats have human-like complement activity whereas mice have a largely inactive classical complement system (Ratelade and Verkman, 2014). In initial studies we found that Fc-μTP-L309C was effective in inhibiting CDC produced by AQP4-IgG and rat complement (Fig. 5A), with several-fold greater potency than found with human complement in Fig. 1B.

To establish a Fc-μTP-L309C dosing regimen that produces therapeutic blood levels for efficacy studies, rats were administered different amounts of Fc-μTP-L309C intravenously, and complement activity of serum taken at 2 h was assayed in vitro by measurement of CDC in AQP4-expressing CHO cells that were preincubated with the rat serum and AQP4-IgG (Fig. 5B). Cytotoxicity was prevented in sera taken at 2 h from rats administrated Fc-μTP-L309C at a dose of 12.5 mg/kg or higher. Fig. 5C shows the time course of rat serum-induced cytotoxicity following administration of a single intravenous dose of 50 mg/kg Fc-μTP-L309C. Cytotoxicity was prevented for at least 8 h.

A short-term efficacy study was done in which Fc-μTP-L309C at 50 mg/kg was administered at the time of and 12 h after intracerebral injection of AQP4-IgG (Fig. 6A). Immunofluorescence of AQP4-IgG treated rats showed astrocyte injury (loss of astrocyte markers AQP4 and GFAP) in an area surrounding the administration site, as well as demyelination (reduced MBP immunofluorescence), inflammation (Iba-1 and CD45) and deposition of activated complement (C5b-9) (Fig. 6B). The increased GFAP expression surrounding the lesion represents reactive gliosis. Immunofluorescence of the non-injected contralateral hemisphere is shown for comparison. Remarkably reduced pathology was seen in the Fc-μTP-L309C-treated rats, in which AQP4, GFAP, MBP, C5b-9 and CD45 immunofluorescence were similar to that in untreated rats and the contralateral hemisphere of Fc-μTP-L309C-treated rats. The small increase in Iba-1 staining is due to very minor injury caused by the needle insertion as seen before (Asavapanumas et al., 2014; Yao and Verkman, 2017). In a further study, a greater amount of AQP4-IgG was injected in order to produce massive NMO pathology in nearly the whole ipsilateral hemisphere (Fig. 6C). Fc-μTP-L309C fully prevented the loss of AQP4, GFAP and MBP immunofluorescence.

4. Discussion

Several mechanisms may contribute to the potential therapeutic benefit of Fc hexamers in NMO (Fig. 7). Fc hexamers inhibit CDC and ADCC, the two major effector mechanisms involved in NMO pathogenesis following AQP4-IgG autoantibody binding to astrocyte

AQP4. The evidence for inhibition of CDC reported here included inhibition of AQP4-IgG-mediated CDC in AQP4-expressing cells in culture, and prevention of AQP4-IgG mediated NMO pathology in spinal cord slices ex vivo and in an experimental rat model of NMO. The evidence for inhibition of ADCC came from in vitro measurements of AQP4-IgG-mediated cytotoxicity following incubation of AQP4-expressing cell cultures with AQP4-IgG and NK cells. Though not studied here, other potentially beneficial mechanisms of Fc hexamers in NMO include accelerated AQP4-IgG autoantibody clearance by blocking FcRn, reduced cytokine production, inhibition of leukocyte migration, expansion of regulatory T cells, upregulation of FcγRIIB inhibitory receptors, and protection against blood-brain barrier disruption (Dalakas, 2004; Ephrem et al., 2008; Esen et al., 2012; Li et al., 2005; Spirig et al., submitted; Weishaupt et al., 2002).

The hexameric association of Fc fragments in the preparation tested here is likely responsible for its strong association with C1q by a multimeric interaction mechanism, as C1q is a six-headed protein with a suitable size for multivalent binding to Fc hexamers. Recent data showed that Fc-μTP-L309C inhibits activation of the classical complement pathway by heat-aggregated immunoglobulins in vitro (Spirig et al., submitted). It remains unclear, however, why more downstream complement proteins are not activated, as occurs, for example, with cobra venom factor, in which C3 and downstream complement proteins are activated and depleted. Another unique action of Fc hexamers is their greatly increased binding avidity to Fcγ receptors compared to monomeric Fc (Czajkowsky et al., 2015; Spirig et al., submitted), resulting in inhibition of various effector functions including ADCC and phagocytosis. The extent of undesired side-effects of Fcγ receptor binding such as granulocyte activation is unclear, though activation of human granulocytes was not seen in vitro (Spirig et al., submitted).

Inhibition of the classical complement pathway by Fc hexamers, as well as inhibition of ADCC effector mechanisms, suggest potential therapeutic benefit in NMO. Complement inhibition is recognized as a potential therapy for seropositive NMO, with the C5 convertase inhibitor eculizumab under clinical evaluation (Pittock et al., 2013) and alternative biologics such as C1q-specific antibodies (Phuan et al., 2013) in early-phase development. Additional potential actions of Fc hexamers in NMO, such as accelerated depletion of AQP4-IgG autoantibodies and modulation of T cell function, may be beneficial as well. However, there are a number of caveats. The apparent short serum half-life of the Fc hexamers and as found here in NMO-relevant pharmacodynamics measurements would limit their use in NMO to therapy of acute disease exacerbations, though long-term administration by daily or twice-daily subcutaneous injections may be possible. Another caveat is potential side-effects. Consideration is needed of the extent of undesired non-specific complement and granulocyte activation in humans administered Fc hexamers, as well as the consequences of chronic complement inhibition, as mice lacking C1q, for example, develop lupus-like autoimmunity (Botto, 1998; Adrichem et al., 2017). Further studies in non-human primates are required to investigate the influence of long-term administration of Fc hexamers on complement proteins and immune effector cells.

In summary, our data provide experimental evidence for Fc hexamers as a novel therapeutic approach in NMO that targets the major complement and cellular effector mechanisms in

NMO pathogenesis with greater efficacy and potency than IVIG. NMO treatment with Fc hexamers may be combined with therapeutics targeting different steps in NMO pathogenesis, such as immunosuppressants and B cell-depleting drugs. Notwithstanding the caveats mentioned above regarding the relative short serum half-life and potential side effects of Fc hexamers, their further evaluation in NMO seems warranted.

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Abbreviations:

ADCC	antibody-dependent cellular cytotoxicity
AQP4	aquaporin-4
AQP4-IgG	neuromyelitis optica immunoglobulin G antibody
BSA	bovine serum albumin
CDC	complement-dependent cytotoxicity
GFAP	glial fibrillary acidic protein
ITP	idiopathic thrombocytopenic purpura
IVIG	intravenous immunoglobulin
MAC	membrane attack complex
MBP	myelin basic protein
NK cell	natural killer cell
NMO	neuromyelitis optica spectrum disorders
PFA	paraformaldehyde

References

Absoud M, Brex P, Ciccarelli O, Diribe O, Giovannoni G, Hellier J, Howe R, Holland R, Kelly J, McCrone P, Murphy C, Palace J, Pickles A, Pike M, Robertson N, Jacob A, Lim M, 2017 A multicentre randomiSed controlled TRial of IntraVEnous immunoglobulin compared with standard therapy for the treatment of transverse myelitis in adults and children (STRIVE). Health Technol. Assess. 21, 1–50.

Adrichem ME, Starink MV, van leeuwen EM, Kramer C, van Schaik IN, Eftimou F, 2017 Drug-induced cutaneous lupus erythematosus after immunoglobulin treatment in chronic inflammatory demyelinating polyneuropathy: a case series. J. Peripher. Nerv. Syst 22, 213–218. [PubMed: 28480635]

Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV, 2008 Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. Science 320, 373–376. [PubMed: 18420934]

Araki M, Matsuoka T, Miyamoto K, Kusunoki S, Okamoto T, Murata M, Miyake S, Aranami T, Yamamura T, 2014 Efficacy of the anti-IL-6 receptor antibody tocilizumab in neuromyelitis optica: a pilot study. Neurology 82, 1302–1306. [PubMed: 24634453]

Asavapanumas N, Ratelade J, Verkman AS, 2014 Unique neuromyelitis optica pathology produced in naive rats by intracerebral administration of NMO-IgG. Acta Neuropathol. 127, 539–551. [PubMed: 24190619]

Asgari N, Khorooshi R, Lillevang ST, Owens T, 2013 Complement-dependent pathogenicity of brain-specific antibodies in cerebrospinal fluid. J. Neuroimmunol 254, 76–82. [PubMed: 23031833]

Bakker J, Metz L, 2004 Devic's neuromyelitis optica treated with intravenous gamma globulin (IVIG). Can. J. Neurol. Sci. 31, 265–26. [PubMed: 15198456]

Bennett JL, Lam C, Kalluri SR, Saikali P, Bautista K, Dupree C, Glogowska M, Case D, Antel JP, Owens GP, Gilden D, Nessler S, Stradelmann C, Hemmer B, 2009 Intrathecal pathogenic anti-aquaporin-4 antibodies in early neuromyelitis optica. Ann. Neurol. 66, 617–629. [PubMed: 19938104]

Berger M, McCallus DE, Lin CS, 2013 Rapid and reversible responses to IVIG in autoimmune neuromuscular diseases suggest mechanisms of action involving competition with functionally important autoantibodies. J. Peripher. Nerv. Syst. 18, 275–296. [PubMed: 24200120]

Botto M, 1998 c1q knock-out mice for the study of complement deficiency in autoimmune disease. Exp. Clin. Immunogent 15, 231–234.

Chaigne B, Mouthon L, 2017 Mechanisms of action of intravenous immunoglobulin. Transfus. Apher. Sci 56, 45–49. [PubMed: 28161150]

Crane JM, Lam C, Rossi A, Gupta T, Bennett JL, Verkman AS, 2011 Binding affinity and specificity of neuromyelitis optica autoantibodies to aquaporin-4 M1/M23 isoforms and orthogonal arrays. J. Biol. Chem. 286, 16516–16524. [PubMed: 21454592]

Cree BA, Lamb S, Morgan K, Chen A, Waubant E, Genain C, 2005 An open label study of the effects of rituximab in neuromyelitis optica. Neurology 64, 1270–1272. [PubMed: 15824362]

Czajkowsky DM, Anderson JT, Fuchs A, Wilson TJ, Mekhaiel D, Colonna M, He J, Shao Z, Mitchell DA, Wu G, Dell A, Haslam S, Lloy KA, Moore SC, Sandlie I, Blundell PA, Pleass RJ, 2015 Developing the IVIG biomimetic, Hexa-Fc, for drug and vaccine applications. Sci. Rep. 5, 9526. [PubMed: 25912958]

Dalakas MC, 2004 The use of intravenous immunoglobulin in the treatment of autoimmune neuromuscular diseases: evidence-based indications and safety profile. Pharmacol. Ther. 102, 177–193. [PubMed: 15246245]

Elsone L, Panicker J, Mutch K, Boggild M, Appleton R, Jacob A, 2014 Role of intravenous immunoglobulin in the treatment of acute relapses of neuromyelitis optica: experience in 10 patients. Mult. Scler. 20, 501–504.

Ephrem A, Chamat S, Miquel C, Fisson S, Mouthon L, Caligiuri G, Delignat S, Elluru S, Bayry J, Lacroix-Desmazes S, Cohen JL, Salomon BL, Kazatchkine MD, Kaveri SV, Misra N, 2008 Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. Blood 111, 715–722. [PubMed: 17932250]

Esen F Senturk E, Ozcan PE, Ahishali B, Arican N, Orhan N, Ekizoglu O, Kucuk M, Kaya M, 2012 Intravenous immunoglobulins prevent the breakdown of the blood-brain barrier in experimentally induced sepsis. Crit. Care Med. 40, 1214–1220. [PubMed: 22202704]

Galeotti C, Bayry J, Kone-Paut I, Kaveri SV, 2010 Kawasaki disease: aetiopa-thogenesis and therapeutic utility of intravenous immunoglobulin. Autoimmun. Rev. 9, 441–448. [PubMed: 20004744]

Gelfand EW, 2012 Intravenous immune globulin in autoimmune and inflammatory diseases. N. Engl. J. Med 367, 2015–2025. [PubMed: 23171098]

Graber DJ, Levy M, Kerr D, Wade WF, 2008 Neuromyelitis optica pathogenesis and aquaporin 4. J. Neuroinflam 5, 22.

Greenberg BM, Graves D, Remington G, Hardeman P, Mann M, Karendikar N, Stuve O, Monson N, Frohman E, 2012 Rituximab dosing and monitoring strategies in neuromyelitis optica patients: creating strategies for therapeutic success. Mult. Scler. 18, 1022–1026. [PubMed: 22261118]

Grunewald B, Bennett JL, Toyka KV, Sommer C, Geis C, 2016 Efficacy of polyvalent Human immunoglobulins in an animal model of neuromyelitis optica evoked by intrathecal anti-aquaporin 4 antibodies. Int.J. Mol. Sci 10.3390/ijms17091407.

Hengstman GJ, Wesseling P, Frenken CW, Jongen PJ, 2007 Neuromyelitis optica with clinical and histopathological involvement of the brain. Mult. Scler. 13, 679–682. [PubMed: 17548452]

Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schöni M, Vest M, Wagner HP, 1981 High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet 1, 1228–1231. [PubMed: 6112565]

Jacob S, Rajabally YA, 2009 Current proposed mechanisms of action of intravenous immunoglobulins in inflammatory neuropathies. Curr. Neuropharmacol. 7, 337–342. [PubMed: 20514213]

Jain A, Olsen HS, Vyzasatya R, Burch E, Sakoda Y, Mérigeon EY, Cai L, Lu C, Tan M, Tamada K, Schulze D, Block DS, Strome SE, 2012 Fully recombinant IgG2a Fc multimers (stradomers) effectively treat collagen-induced arthritis and prevent idiopathic thrombocytopenic purpura in mice. Arthritis Res. 14, R192.

Jarius S, Wildemann B, 2010 AQP4 antibodies in neuromyelitis optica: diagnostic and pathogenetic relevance. Nat. Rev. Neurol. 6, 383–392. [PubMed: 20639914]

Jarius S, Wildemann B, Paul F, 2014 Neuromyelitis optica: clinical features, immunopathogenesis and treatment. Clin. Exp. Immunol. 176, 149–164. [PubMed: 24666204]

Kageyama T, Komori M, Miyamoto K, Ozaki A, Suenaga T, Takahashi R, Matsumoto S, Kondo T, 2013 Combination of cyclosporin A with corticosteroids is effective for the treatment of neuromyelitis optica. J. Neurol. 260, 627–634. [PubMed: 23076828]

Katz-Agranov N, Khattri S, Zandman-Goddard G, 2015 The role of intravenous immunoglobulin in the treatment of rheumatoid arthritis. Autoimmun. Rev. 14, 651 −658. [PubMed: 25870941]

Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR, 2005 IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J. Exp. Med. 202, 473–477. [PubMed: 16087714]

Li N, Zhao M, Hilario-Vargas J, Prisayanh P, Warren S, Diaz LA, Roopenian DC, Liu Z, 2005 Complete FcRn dependence for intravenous Ig therapy in autoimmune skin blistering diseases. J. Clin. Invest. 115, 3440–3450. [PubMed: 16284651]

Lin HH, Wang MX, Spies JM, Pollard JD, 2007 Effective treatment of experimental autoimmune neuritis with Fc fragment of human immunoglobulin. J. Neuroimmunol 186, 133–140. [PubMed: 17482276]

Lucchinetti CF, Mandler RN, McGavern D, Bruck W, Gleich G, Ransohoff RM, Trebst C, Weinshenker BG, Wingerchuk DM, Parisi JE, Lassmann H, 2002 A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. Brain 125, 1450–1461. [PubMed: 12076996]

Lunemann JD, Nimmerjahn F, Dalakas MC, 2015 Intravenous immunoglobulin in neurology-mode of action and clinical efficacy. Nat. Rev. Neurol. 11, 80–89. [PubMed: 25561275]

Magraner MJ, Coret F, Casanova B, 2013 The effect of intravenous immunoglobulin on neuromyelitis optica. Neurologia 28, 65–72. [PubMed: 22841880]

Misu T, Fujihara K, Kakita A, Konno H, Nakamura M, Watanabe S, Takahashi T, Nakashima I, Takahashi H, Itoyama Y, 2007 Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. Brain 130, 1224–1234. [PubMed: 17405762]

Nguyen DH, Cho N, Satkunendrarajah K, Austin JW, Wang J, Fehlings MG, 2012 Immunoglobulin G (IgG) attenuates neuroinflammation and improves neurobehavioral recovery after cervical spinal cord injury. J. Neuroinflam 9, 224.

Niknami M, Wang MX, Nguyen T, Pollard JD, 2013 Beneficial effect of a multimerized immunoglobulin Fc in an animal model of inflammatory neuropathy (experimental autoimmune neuritis). J. Peripher. Nerv. Syst. 18, 141–152. [PubMed: 23781961]

Okada K, Tsuji S, Tanaka K, 2007 Intermittent intravenous immunoglobulin successfully prevents relapses of neuromyelitis optica. Intern. Med 46, 1671–1672. [PubMed: 17917332]

Ortiz DR, Lansing JC, Rutitzky L, Kurtegic E, Prod'homme T, Choudhury A, 2016 Elucidating the interplay between IgG-Fc valency and Fcγ R activation for the design of immune complex inhibitors. Sci. Transl. Med. 8, 365ra158.

Papadopoulos MC, Bennett JL, Verkman AS, 2014 Treatment of neuromyelitis optica: state-of-the-art and emerging therapies. Nat. Rev. Neurol. 10, 493–506. [PubMed: 25112508]

Papadopoulos MC, Verkman AS, 2012 Aquaporin 4 and neuromyelitis optica. Lancet Neurol. 11, 535–544. [PubMed: 22608667]

Parratt JD, Prineas JW, 2010 Neuromyelitis optica: a demyelinating disease characterized by acute destruction and regeneration of a perivascular astrocytes. Mult. Scler. 16, 1156–1172. [PubMed: 20823059]

Phuan PW, Zhang H, Asavapanumas N, Leviten M, Rosenthal A, Tradtrantip L, Verkman AS, 2013 C1q-targeted monoclonal antibody prevents complement-dependent cytotoxicity and neuropathology in in vitro and mouse models of neuromyelitis optica. Acta Neuropathol. 125, 829–840. [PubMed: 23677375]

Piepers S, Jansen MD, Cats EA, van Sorge NM, van den Berg LH, van der Pol WL, 2010 IVIg inhibits classical pathway activity and anti-GM1 IgM-mediated complement deposition in MMN. J. Neuroimmunol. 229, 256–262. [PubMed: 20920831]

Pittock SJ, Lennon VA, McKeon A, Mandrekar J, Weinshenker BG, Lucchinetti CF, O'Toole O, Wingerchuk DM, 2013 Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study. Lancet Neurol. 12, 554–562. [PubMed: 23623397]

Ratelade J, Smith AJ, Verkman AS, 2014 Human immunoglobulin G reduces the pathogenicity of aquaporin-4 autoantibodies in neuromyelitis optica. Exp. Neurol. 225, 145–153.

Ratelade J, Verkman AS, 2014 Inhibitor(s) of the classical complement pathway in mouse serum limit the utility of mice as experimental models of neuromyelitis optica. Mol. Immunol. 62, 103–114.

Schwab I, Nimmerjahn F, 2013 Intravenous immunoglobulin therapy: how does IgG modulate the immune system? Nat. Rev. Immunol. 13, 176–189. [PubMed: 23411799]

Sorensen V, Sundvold V, Michaelsen TE, Sandlie I, 1999 Polymerization of IgA and IgM: role of Cys309/Cys414 and the secretory tailpiece. J. Immunol. 162, 3448–3455. [PubMed: 10092800]

Spirig R, Campbell IK, Koernig S, Chen CG, Lewis BJB, Butcher R, Muir I, Taylor S, Chia J, Leong D, Simmonds J, Scotney P, Schmidt P, Fabri L, Hofmann A, Jordi M, Spycher MO, Cattepoel S, Brasseit J, Panousis C, Rowe T, Branch DR, Morelli AB, Käsermann F, Zuercher AW, Unique rIgG1 Fc hexamers inhibit antibody-mediated disease via complement and FcgRs. [Submitted].

Thiruppathi M, Sheng JR, Li L, Prabhakar BS, Meriggioli MN, 2014 Recombinant IgG2a Fc (M045) multimers effectively suppress experimental autoimmune myasthenia gravis. J. Autoimmun. 52, 64–73. [PubMed: 24388113]

Tradtrantip L, Zhang H, Saadoun S, Phuan PW, Lam C, Papadopoulos MC, Bennett JL, Verkman AS, 2012 Anti-aquaporin-4 monoclonal antibody blocker therapy for neuromyelitis optica. Ann. Neurol. 71, 314–322. [PubMed: 22271321]

Verkman AS, Phuan PW, Asavapanumas N, Tradtrantip L, 2013 Biology of AQP4 and anti-AQP4 antibody: therapeutic implications for NMO. Brain Pathol. 23, 84–695.

Viswanathan S, Wong AH, Quek AM, Yuki N, 2015 Intravenous immunoglobulin may reduce relapse frequency in neuromyelitis optica. J. Neuroimmunol. 282, 92–96. [PubMed: 25903734]

Weishaupt A, Kuhlmann T, Schonrock LM, Toyka KV, Bruck W, Gold R, 2002 Effects of intravenous immunoglobulins on T cell and oligodendrocyte apoptosis in high-dose antigen therapy in experimental autoimmune encephalomyelitis. Acta Neuropathol. 104, 385–390. [PubMed: 12200625]

Wingerchuk DM, 2013 Neuromyelitis optica: potential roles for intravenous immunoglobulin. J. Clin. Immunol. 33 (Suppl. 1), S33–S37. [PubMed: 22976554]

Winkelmann A, Zettl UK, 2012 Use of intravenous immunoglobulin in the treatment of immune-mediated demyelinating diseases of the nervous system. Curr. Pharm. Des 18, 4570–4582. [PubMed: 22612749]

Yao X, Verkman AS, 2017 Marked central nervous system pathology in CD59 knockout rats following passive transfer of neuromyelitis optica immunoglobulin G. Acta Neurolpathol. Commun 5, 15.

Yuki N, Watanabe H, Nakajima T, Spath PJ, 2011 IVIG blocks complement deposition mediated by anti-GM1 antibodies in multifocal motor neuropathy. J. Neurol. Neurosurg. Psychiatry 82, 87–91. [PubMed: 20667861]

Yusa S, Catina TL, Campbell KS, 2002 SHP-1- and phosphotyrosine-independent inhibitory signaling by a killer cell Ig-like receptor cytoplasmic domain in human NK cells. J. Immunol. 168, 5047–505. [PubMed: 11994457]

Zhang H, Bennett JL, Verkman AS, 2011 Ex vivo spinal cord slice model of neuromyelitis optica reveals novel immunopathogenic mechanisms. Ann. Neurol. 70, 943–954. [PubMed: 22069219]

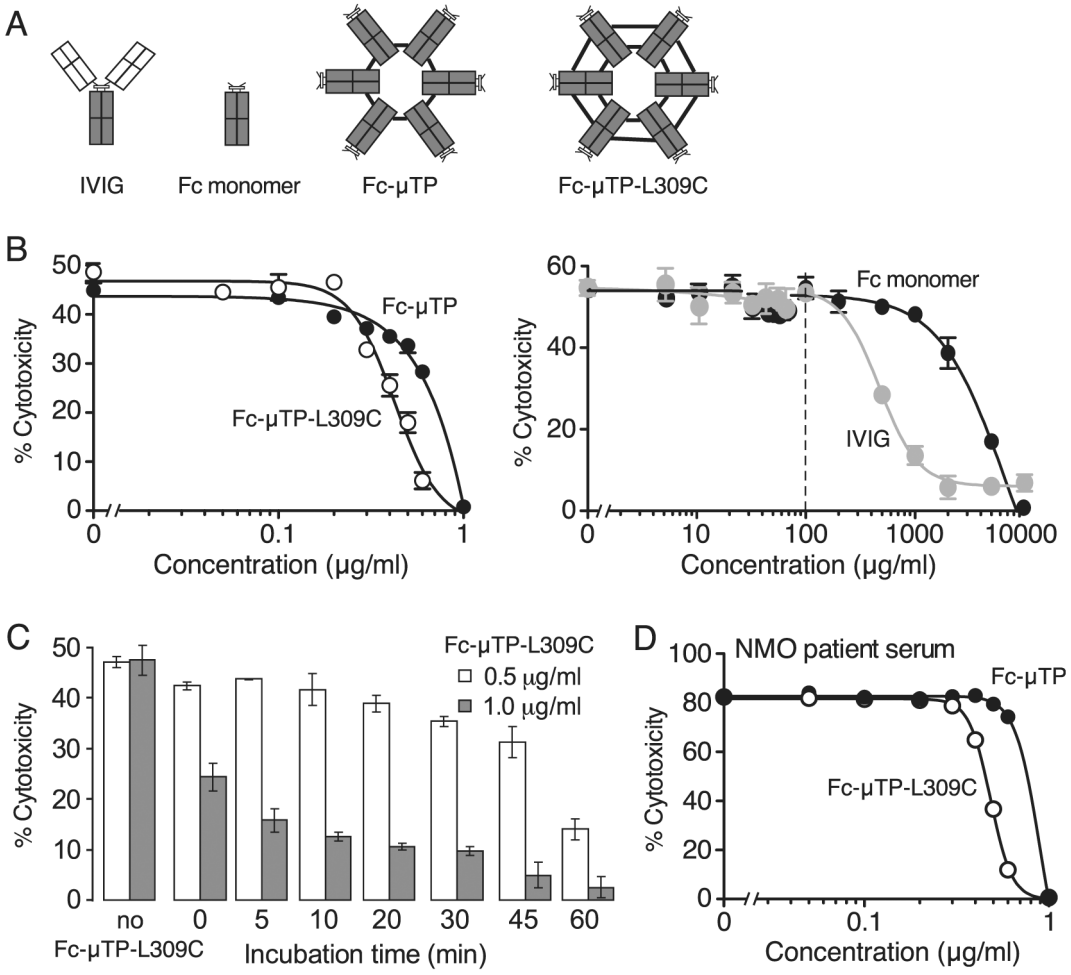

Fig. 1. Fc hexamers inhibit complement-dependent cytotoxicity (CDC) in AQP4-expressing cell cultures.

A. Diagram of Fc preparations, including: human immunoglobulin G (IVIG), monomeric Fc fragments (Fc monomers), Fc-μTP (hexameric human IgG1 Fc with 18 amino acid IgM μ-tailpiece fused to the C-terminus of the constant region); and Fc-μTP-L309C (Fc-μTP with stabilizing L309C mutation in the Fc region). B. CDC in AQP4-expressing CHO cells. AQP4-expressing CHO cells were incubated for 60 min with 10 μg/ml AQP4-IgG and washed extensively. Human complement was pre-incubated for 1 h with different concentrations of Fc-μTP-L309C or Fc-μTP (left), or IVIG or Fc monomers (right) and then 1% was added to AQP4-IgG-coated CHO-cells (mean ± S.E.M., n = 4). For higher concentrations in the right panel (to the right of dashed vertical line), IVIG or Fc monomers where added to complement after instead of before dilution. C. Time course of inhibition of CDC in which AQP4-expressing CHO cells incubated for 60 min with 10 μg/ml AQP4-IgG were exposed for 60 min to human complement after incubation for different times with Fc-μTP-L309C (mean ± S.E.M., n = 4). D. Study done as in B (left) except for replacement of recombinant AQP4-IgG by human serum (1:50 dilution) from a single seropositive NMO patient (mean ± S.E.M., n = 4).

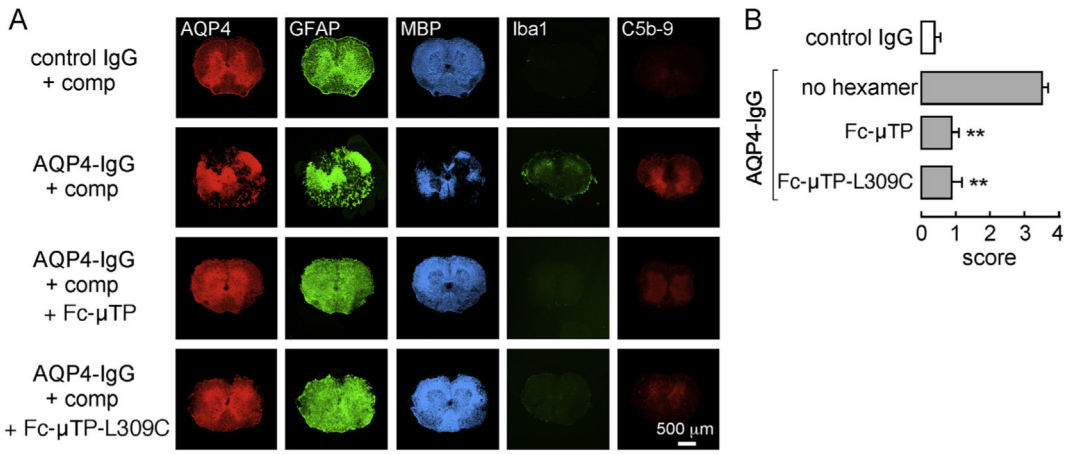

Fig. 2. Fc hexamers prevent NMO pathology in rat spinal cord slice cultures.

Spinal cord slice study in which 7-day old cultures of rat spinal cord slices were incubated with 5 µg/ml AQP4-IgG and 5% human complement for 24 h. Human complement was pre-incubated with 50 µg/ml Fc-µTP or Fc-µTP-L309C for 1 h prior to addition to slices. A. AQP4, GFAP, MBP, Iba1, and C5b-9 immunofluorescence at 24 h after treatment. B. Pathology scores (S.E.M., n ¼ 8 slices from 2 rats, **$p < 0.01$).

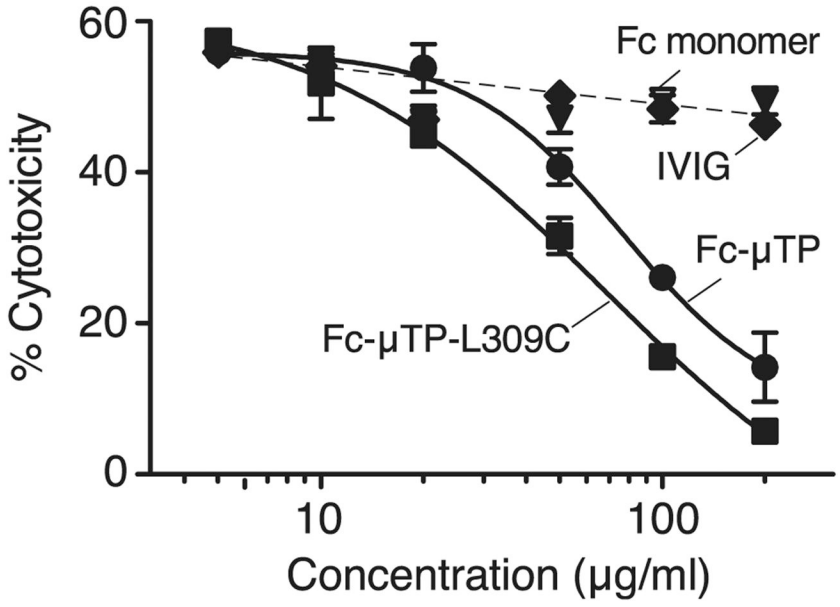

Fig. 3. Fc hexamers inhibit antibody-dependent cellular cytotoxicity (ADCC) in AQP4-expressing cell cultures.

ADCC in AQP4-expressing CHO cells incubated for 2 h with 5 µg/ml AQP4-IgG, NK-cells, and different concentrations of Fc-µTP-L309C, Fc-µTP, IVIG or Fc monomers (mean ± S.E.M., n ¼ 4).

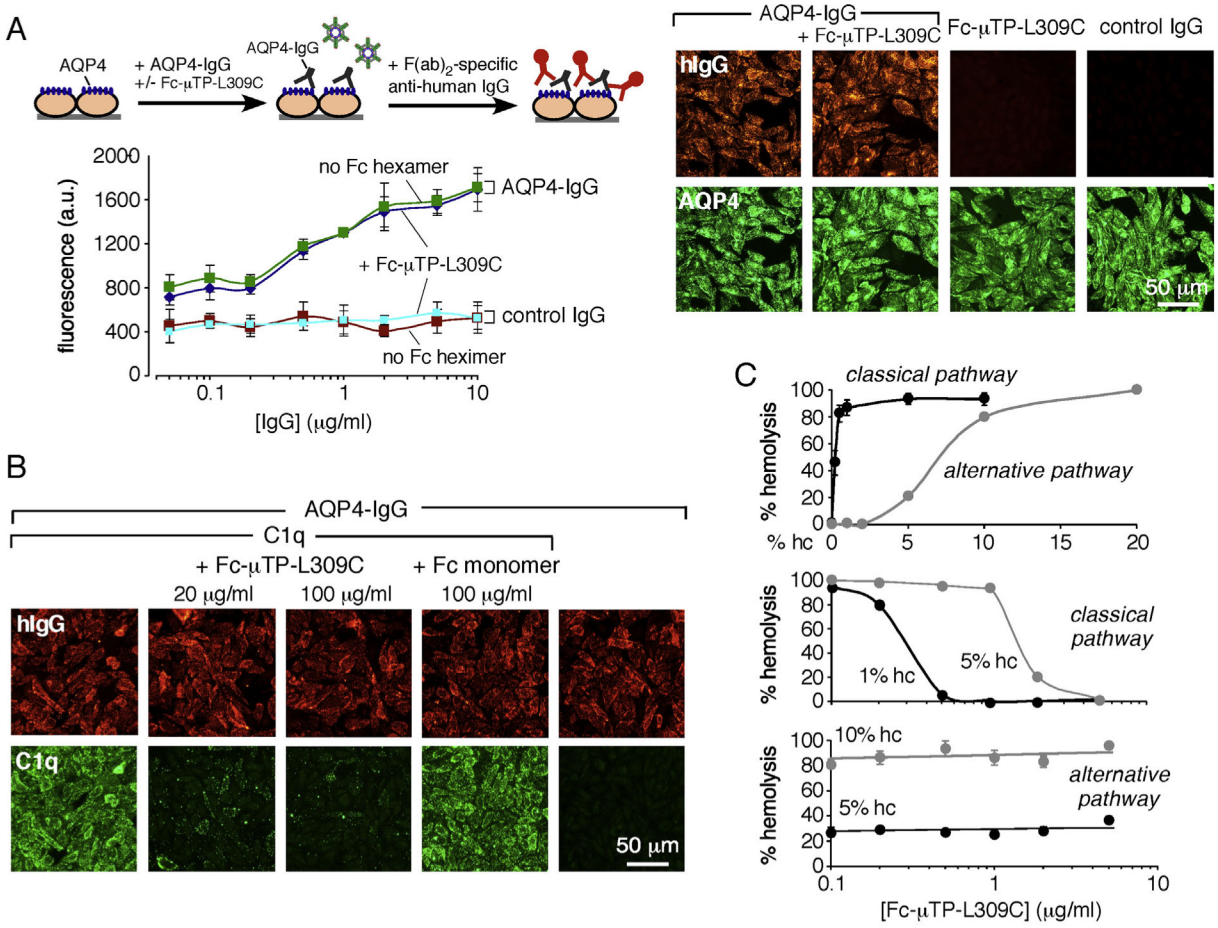

Fig. 4. Fc-μTP-L309C mechanism of action studies.

A. (Left) AQP4-IgG binding to AQP4 on AQP4-expressing CHO cells incubated with AQP4-IgG or control IgG, with or without 100 μg/ml Fc-μTP-L309C, followed by Alexa Fluor 594-conjugated antihuman IgG, F (ab')$_2$-specific secondary antibody (mean ± S.E.M., n = 4, differences not significant). (Right) F (ab')$_2$ (red) and AQP4 (green) on AQP4-expressing CHO cells incubated for 1 h with 10 μg/ml AQP4-IgG and 100 μg/ml Fc-μTP-L309C. B. Binding of recombinant human C1q (green) to AQP4-IgG (red) bound on AQP4-expressing CHO cells. Human C1q was pre-incubated for 1 h with Fc-μTP-L309C or Fc monomers before adding to the cells. C. Inhibition of the classical and alternative complement pathways by Fc-μTP-L309C. (Upper panel) Human complement (hc) concentration-dependence of hemolysis of IgM-coated sheep erythrocytes (assay of classical pathway) and uncoated rabbit erythrocytes (assay of alternative pathway). Fc-μTP-L309C concentration-dependence for inhibition of the classical (middle panel) and alternative (lower panel) complement pathways at 1% and 5% human complement (mean ± S.E.M., n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

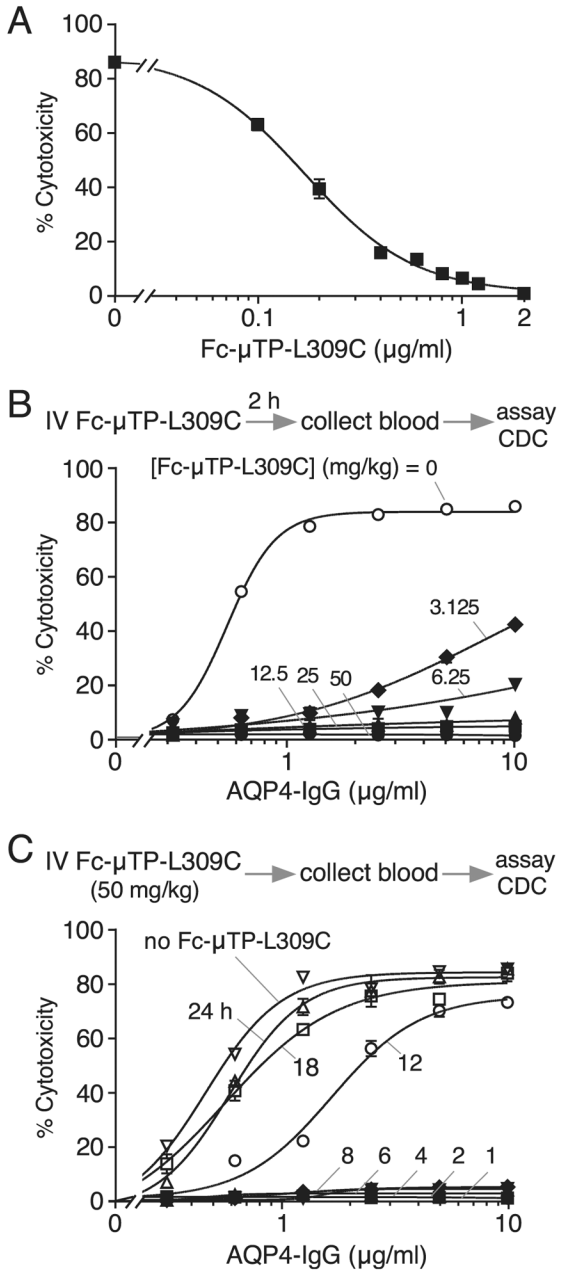

Fig. 5. Fc-µTP-L309C pharmacodynamics in rats.

A. CDC in AQP4-expressing CHO cells incubated for 1 h with 1 µg/ml AQP4-IgG, and 2% rat serum that were preincubated with different concentrations of Fc-µTP-L309C (mean ± S.E.M., n = 4). B. CDC in AQP4-expressing CHO cells incubated with different concentrations of AQP4- IgG (1.25–10 µg/ml) and a 1:50 dilution rat serum obtained 2h after intravenous administration of indicated amounts of Fc-µTP-L309C (mean ± S.E.M., 2 rats group). C. Same protocol as in B, except that serum obtained from rats at different times after administration of 50 mg/kg Fc-µTP-L309C (mean ± S.E.M., 2 rats per group).

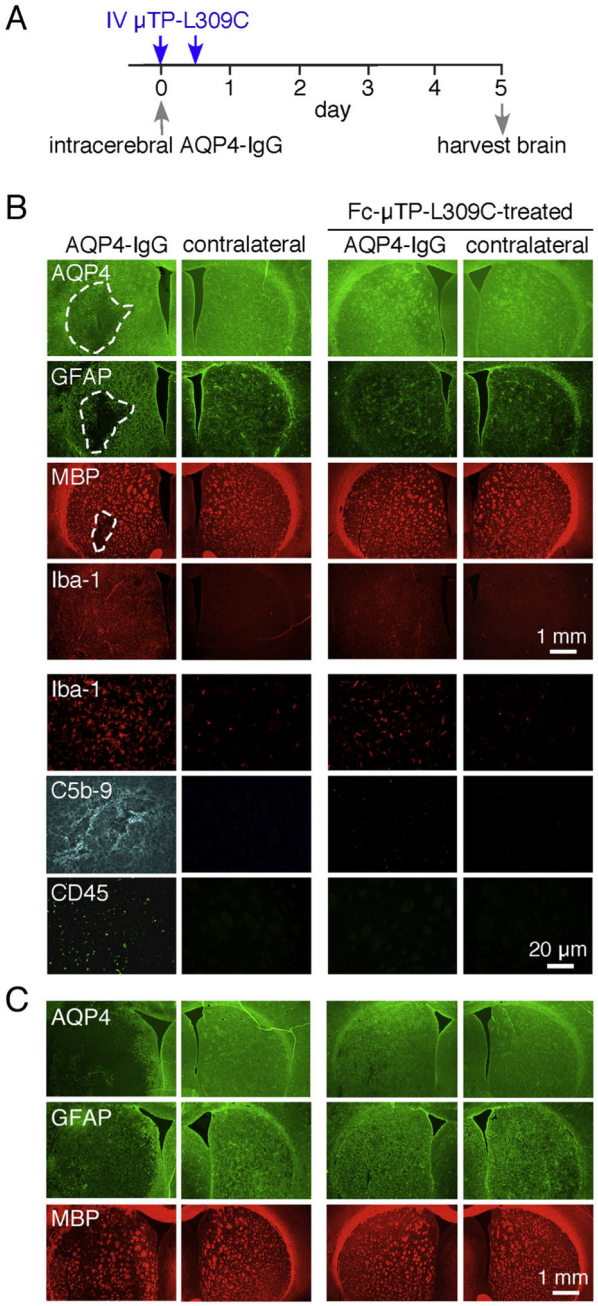

Fig. 6. Fc-μTP-L309C prevents pathology in an experimental rat model of NMO.

A. Rat model in which Fc-μTP-L309C (50 mg/kg, IV) (or untreated control) was administered at the time of and 12 h after intracerebral injection of 30 μg AQP4-IgG. B. Brain immunofluorescence of indicated markers of NMO pathology at day 5 showing AQP4-IgG-injected and control contralateral hemipheres from non-treated and Fc-μTP-L309C-treated rats. Representative of experiments on 3 rats per group. C. Same study as in B except for injection of 40 μg AQP4-IgG, which produces massive pathology of nearly the whole ipsilateral hemisphere.

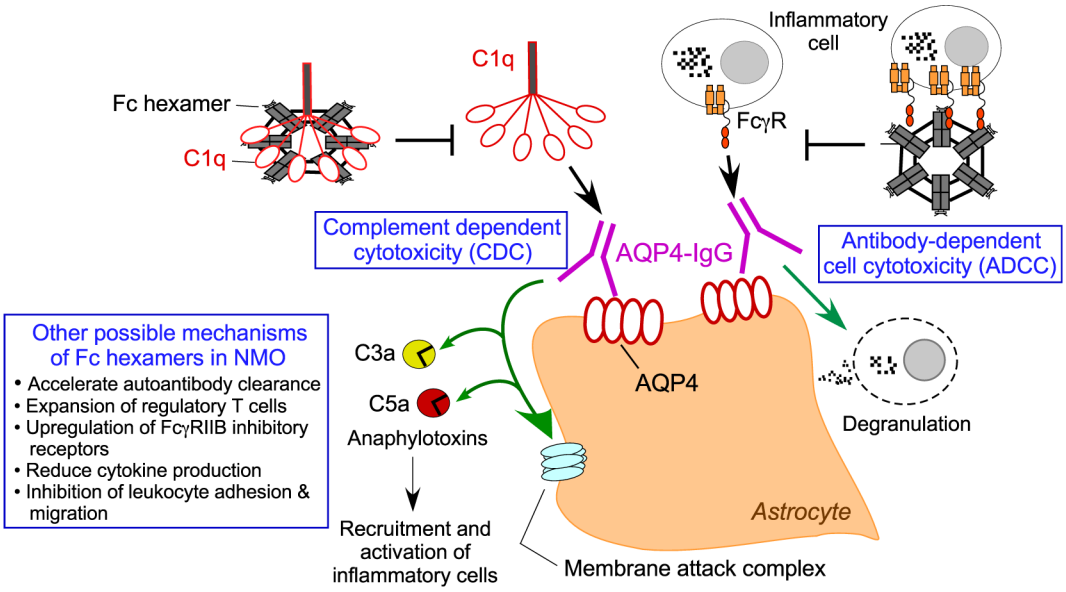

Fig. 7. Diagram showing potential beneficial mechanisms of Fc hexamers in NMO.

In addition to inhibition of AQP4-IgG-dependent CDC and ADCC, as demonstrated here, Fc hexamers may also block FcRn and accelerate AQP4-IgG clearance and have other actions as listed in the box at the left.