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Research Paper

Sialylated intravenous immunoglobulin suppress anti-ganglioside antibody mediated nerve injury

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ABSTRACT

The precise mechanisms underlying the efficacy of intravenous immunoglobulin (IVIg) in autoimmune neurological disorders including Guillain-Barré syndrome (GBS) are not known. Anti-ganglioside antibodies have been reported to be pathogenic in some variants of GBS, and we have developed passive transfer animal models to study anti-ganglioside antibody mediated-endoneurial inflammation and associated neuropathological effects and to evaluate the efficacy of new therapeutic approaches. Some studies indicate that IVIg's anti-inflammatory activity resides in a minor sialylated IVIg (sIVIg) fractions and is dependent on an innate Th2 response via binding to a specific ICAM3-grabbing nonintegrin related 1 receptor (SIGN-R1). Therefore the efficacy of IVIg, IVIg fractions with various IgG Fc sialylation status, and the involvement of Th2 pathway were examined in one of our animal model of antibody-mediated inhibition of axonal regeneration. We demonstrate that both IVIg and sIVIg ameliorated anti-glycan antibody mediated-pathological effect, whereas, the unsialylated fractions of IVIg were not beneficial in our model. Tenfold lower doses of sIVIg compared to whole IVIg provided equivalent efficacy in our studies. Moreover, we found that whole IVIg and sIVIg significantly upregulates the gene expression of IL-33, which itself can provide protection from antibody-mediated nerve injury in our model. Our results support that the SIGN-R1-Th2 pathway is involved in the anti-inflammatory effects of IVIg on endoneurium in our model and elements of this pathway including IL-33 can provide novel therapeutics in inflammatory neuropathies.

1. Introduction

In clinical practice, Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are the commonest acute and chronic inflammatory neuropathic conditions, respectively. Understanding of the pathomechanisms of nerve inflammation and related nerve injury is incomplete but a large body of work favors synergism of cellular and humoral immune elements in the pathogenesis of these inflammatory neuropathic disorders (Dalakas, 2011; Hughes and Cornblath, 2005; Hughes et al., 1999; Ilyas et al., 1988; Quarles et al., 1990; Willison and Yuki, 2002; Yuki and Hartung, 2012). It is now widely accepted that there are two major forms of GBS: the demyelinating (Asbury et al., 1969; Prineas, 1981) and axonal (Hafer-Macko et al., 1996; McKhann et al., 1993; Ogawara et al., 2000) subtypes. Anti-ganglioside/glycan antibodies (Abs) are the most commonly recognized autoimmune effectors in this disorder and are strongly associated with the axonal forms of GBS (Hughes and Cornblath, 2005; Hughes et al., 1999; Willison and Yuki, 2002; Yuki et al., 2004, 2001).

Adaptive autoimmunity uses the powerful effector functions of cells of the innate immune system including monocytes/macrophages to induce target injury in infectious and autoimmune disorders (Nimmerjahn and Ravetch, 2008; Takai, 2002). The pathologic studies in demyelinating and axonal GBS and CIDP indicate a central role for macrophage and microglia, which are the key components of endoneurial inflammation (Griffin et al., 1990; Kiefer et al., 2001; Zhang et al., 2014). Macrophage-mediated myelin stripping and nodal and periaxonal macrophage-mediated attack on axons are pathognomonic of acquired demyelinating neuropathies (GBS and CIDP) and axonal GBS, respectively. Fc-gamma receptors (FcγRs) are critical regulators of macrophage/microglia-mediated inflammation. They are classically described as activating or inhibitory FcγRs, which signal through immunoreceptor tyrosine activation or inhibitory motifs,

Abbreviations: IVIg, Intravenous immunoglobulin; GBS, Guillain-Barré syndrome; CIDP, Chronic inflammatory demyelinating polyradiculoneuropathy; SIGN-R1, Specific ICAM3-grabbing nonintegrin related 1 receptor; sIVIg, α2,6 sialic acid enriched IVIg; uIVIg, α2,6 sialic acid depleted IVIg; FcγRs, Fc-gamma receptors; CMAP, Compound muscle action potential; qPCR, Quantitative real-time PCR; BSA, Bovine serum albumin; SNL, *Sambucus nigra* lectin.

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respectively (Hogarth, 2002; Takai, 2002). We recently demonstrated, in two separate animal models, that anti-ganglioside antibody-mediated pathological effects are dependent on Ab engaging specific axonal surface gangliosides (immune complex formation) but independent of complement mediated cytolytic injury (He et al., 2015; Zhang et al., 2014). Subsequently, we showed that specific activating FcγRs on endoneurial macrophage/microglial cells are critical inflammatory elements that mediate anti-glycan Ab-induced nerve injury.

IVIg is now the most commonly used treatment in inflammatory neuropathies such as GBS and CIDP. However, the precise mechanisms underlying its protection are not completely defined. Recent studies indicate that modulation of inflammation via innate immune effectors, i.e., FcγRs, could be a mechanism of IVIg efficacy in animal models of immune arthritis and thrombocytopenic purpura (Anthony et al., 2011; Kaneko et al., 2006; Samuelsson et al., 2001). This work from Ravetch's group characterized SIGN-R1-Th2 pathway and showed that IVIg's anti-inflammatory activity resides in sialylated fractions (sIVIg) and terminal α2,6 sialic acid on IgG Fc *N*-glycan chain (IgG FcNg) and IVIg's efficacy is independent of IgG/IVIg competition for FcγR binding with autoAbs in their models (Anthony et al., 2011). Further, sIVIg and sialylated Fc (sFcs) were effective in suppressing inflammation at a 10- and 30-fold lower dose than whole IVIg, respectively. Moreover, sFcs or sIVIg trigger an innate Th2 response via production of IL-33 and Th2 cytokines including IL-4 that upregulate FcγRIIB on effector macrophages, which participate in suppression of inflammation. IgG Fc glycation consists of single N-linked glycan attached to each heavy chain in the Fc portion asparagine-297 (Anthony et al., 2012; Arnold et al., 2007). There is tremendous heterogeneity in IgG FcNg, including IVIg, with the variable addition of the bisecting *N*-acetylglucosamine, fucose to the core and sialic acid to the arms of the biantennary structure, thus affecting the efficacy of different IgG subpopulations present in the IVIg. The SIGN-R1-Th2 pathway has not been validated in neuroimmunological disorders in which IVIg is effective. Since our animal models of anti-ganglioside antibody-mediated neuropathological effects are entirely dependent on FcγR induced endoneurial inflammation, we examined the efficacy of whole IVIg and compared it with terminal α2,6 sialic acid enriched or depleted fractions of whole IVIg (sIVIg or uIVIg, respectively) in suppressing Ab-mediated nerve injury in one of our animal models. Further, we investigated the anti-inflammatory effects of IL-33 in attenuating Ab-mediated nerve injury, as this cytokine is directly induced by sIVIg and orchestrates the anti-inflammatory effects via Th2 pathway.

2. Materials and methods

2.1. Mice

Adult (8–12 weeks) wild-type C57BL/6 mice were used. All experimental procedures were complied with institutional and governmental guidelines for animal research and approved by the institutional Animal Care and Use Committee at the University of Texas Health Science Center at Houston.

2.2. Monoclonal anti-glycan antibody

Two disease relevant anti-ganglioside monoclonal antibody (mAb), GD1a/GT1b-2b (a prototypic IgG2b mAb against GD1a/GT1b) and GT1b-2b (IgG2b mAb with specificity for GT1b), were used in this study. We have demonstrated that these mAbs inhibit axon regeneration, prevent target re-innervation and induce injury to intact nerve fibers in different animal models (He et al., 2015; Lehmann et al., 2007; Zhang et al., 2011). The generation, specificity, and production, of these mAb were reported previously (Lunn et al., 2000; Schnaar et al., 2002). The hollow fiber supernatant containing anti-ganglioside mAb was used in all animal studies. An irrelevant mouse isotype matched IgG-2b mAb (Abcam, Cambridge, MA) was used as a negative control.

2.3. Preparation of sIVIg and uIVIg

Sambucus nigra lectin (SNL) chromatography (Vector Laboratories) was performed on whole IVIg (Octagam, Octapharma) to prepare terminal sialic acid-enriched (sIVIg) and sialic acid-depleted (uIVIg) IVIg fractions, as described (Kaneko et al., 2006). Briefly, IVIg (40 mg) in Tris-buffered saline (TBS) were loaded to a column of SNL resin (4 ml), and incubated for 10–15 min at room temperature. The column was then washed with TBS, and the un-bound IVIg fractions (uIVIg) were collected. The bound proteins (sIVIg; ~2–3 mg) were eluted with 0.5 M lactose in TBS. The efficiency of this lectin affinity purification is in 5–8% range. Both uIVIg and sIVIg were dialyzed against 1× PBS prior to usage.

2.4. Sciatic nerve crush model

A well-established nerve crush model was used to study the efficacy of different interventions. Nerve crush provides a convenient and well characterized system to study the regeneration of injured axons mimicking the regenerative response of degenerating axons in GBS, and it also allows us to study the factors affecting nerve repair. Briefly, the left sciatic nerve was crushed at middle thigh level on day 0, as described (Lehmann et al., 2007). Single dose of individual anti-glycan mAb or sham mAb (0.5–1 mg) was injected on day 3 via intra-peritoneal route (i.p.). Daily injection of whole IVIg (2 g/kg per day; i.p.), sIVIg (0.2 g/kg per day; i.p.), uIVIg (2 g/kg per day i.p.), IL-33 (500 ng per day; subcutaneously; R&D Systems, Minneapolis, MN) or vehicle control (subcutaneously) were given to animals 5 days/week for 2 weeks after the nerve crush. For IVIg dose-response study, 0–2 g/kg/day of whole IVIg was administered i.p. for 5 days/week for 2 weeks. Behavioral testing and electrophysiology were performed on all animals. The experiments were terminated on day 17 after the crush. All animals were perfused, sciatic and tibial nerves were harvested.

2.5. Behavioral assessment

Pinprick tests were used to evaluate sensory functional recovery. The tests were performed 1 day prior to the surgery and on indicated days post nerve crush, as described (Zhang et al., 2014). Briefly, the needle was gently applied to the lateral part of the plantar surface of the hind paw, and the animal responses to the needle prick were recorded. The Pinprick testing was performed blindly at indicated time points.

2.6. Electrophysiology

The motor function recovery after sciatic nerve crush was monitored by a sciatic nerve conduction test, which was performed on all animals, as described (Lehmann et al., 2007). Briefly, mice were anesthetized and placed on the heating pad, and their body temperature was monitored and maintained at 35–37 °C. On day 16 post sciatic nerve crush, compound muscle action potential (CMAP) amplitude was recorded from the hind paw with needle electrodes inserted in the sole of the foot and sciatic and tibial nerves were stimulated proximally at the sciatic notch.

2.7. Morphometry

The harvested sciatic and tibial nerves were post-fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde. The fixed nerves were embedded in Epon, and stained with toluidine blue. All myelinated axons in a single whole transverse section of the nerve were counted by using a motorized stage and stereotactic imaging software (Axiovision; Zeiss, Thornwood, NY), as described (Lehmann et al., 2007; Zhang et al., 2014).