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Pegylated interferon beta in the treatment of the Theiler's murine encephalomyelitis virus mouse model of multiple sclerosis

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ABSTRACT

We evaluated the effects of pegylated-interferonβ-1a (pegIFNβ) therapy on intrathecal antibody responses, disability progression, and viral load in the CNS in mice infected with the Theiler's virus (TMEV), an animal model of progressive disability in Multiple Sclerosis (MS). The lack of a direct antiviral activity in the CNS, the absence of any effect upon the intrathecal immune response, and the failure to treat disease progression, indicate that the immunomodulatory effects of pegIFNβ-1a likely occur in the systemic circulation rather than within the CNS. These results may be relevant to the relative lack of effect of IFNβ in progressive MS relative to relapsing MS.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS) and it is the most common cause of non-traumatic neurological disability in young adults. The etiology of the disease is still unknown. However, findings of many epidemiological studies suggest that MS may possibly have an infectious component (Ascherio, 2013; Emmer et al., 2014; Fernandez-Menendez et al., 2016; Leibovitch and Jacobson, 2014; Olival et al., 2013). MS usually starts as a relapsing remitting (RR) course, and in most patients, it evolves into a chronic progressive disorder characterized by continuous accumulation of neurological deficits (Fitzner and Simons, 2010). While treatment of RR-MS has improved significantly over the last years, we are still in need of therapeutic options effective in chronic progressive MS.

In 1993 interferon beta (IFNβ) was the first immunomodulatory therapy approved for the treatment of MS (Paty and Li, 1993), and it remains a mainstay of MS treatment. To date, several large clinical trials have demonstrated that therapy with IFNβ reduces the relapse rate in patients with RR-MS (Panitch et al., 2005; Paty and Li, 2001; Polman et al., 2003; Saida et al., 2005). However, the use of this drug in clinical practice may be complex, especially because its activity may be inhibited by the development of neutralizing antibodies (Kappos et al., 2005; Malucchi et al., 2004; Pachner et al., 2009), and compliance is variable from patient to patient. Moreover, its effectiveness in improving long-term disability progression has been shown to be either absent or minimal (Derfuss and Kappos, 2012; Hughes, 2003; Kuhle et al., 2016; Li et al., 2001).

To improve treatment adherence and effectiveness, a pegylated form of IFNβ-1a (Plegridy®, Biogen, Cambridge, MA) that can be administered once every 2 weeks, was approved by FDA in 2014 (Khan et al., 2015). Pegylated IFNβ (pegIFNβ) is a subcutaneous injectable therapy, in which the IFNβ-1a molecule is conjugated with polyethylene glycol to extend its half-life in circulation and permit a less frequent dosing schedule, as well as an increased, sustained and prolonged biological activity. In addition, the incidence of pegIFNβ neutralizing antibodies is reduced to < 1% (White et al., 2016). Although effective in reducing relapses (Khan et al., 2015) and short-term disability (Calabresi et al., 2014), it still needs to be clarified whether pegIFNβ has any impact on the progressive stage of MS. Thus, in the present study, we investigated the effect of pegIFNβ in a viral model of chronic progressive disability in MS, i.e. the Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD).

TMEV is a single-stranded picornavirus (Lipton, 1975). When injected intracerebrally into susceptible strains of mice, the virus causes a biphasic disease characterized by an acute encephalitis followed by chronic demyelination and viral persistence in the white matter (Lipton, 1975). Several features of progressive MS, including the intrathecal production of immunoglobulins (Igs), the development of progressive neurologic impairment without relapses,

the progressive axonal loss, the partial independence of disability from demyelination, and the epitope spread from viral to myelin epitopes, have all been demonstrated in this model (Denic et al., 2011; Lipton, 1975; Pachner et al., 2007). TMEV-IDD also feature several MRI findings of the human disease (Denic et al., 2011; Gilli et al., 2016a;

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Pirko et al., 2004, 2011, 2009).

Although a causative pathogen of MS has not yet been definitively identified, an infectious etiology for this disease is still an open hypothesis. Therefore, TMEV-IDD may also represents a useful model for understanding the possible viral etiology of MS as well as the effect of therapies on CNS infections. Hence, in the present study we focused on the effects of pegIFNβ therapy on intrathecal production of IgG, disability progression, and viral load in the CNS in TMEV-IDD mice.

2. Materials and methods

2.1. Animals and TMEV infection

SJL mice are highly susceptible to the development of chronic demyelinating disease following infection with TMEV. Sixty SJL/J mice (4 to 6-week old from Jackson Laboratory, Bar Harbor, ME) were infected intra-cranially with 2×10^5 plaque forming units (PFU) of TMEV (DA, Daniel's strain) in a 10 μL final volume. The virus was grown in BHK cells and titered by plaque assay as previously described (Trottier et al., 2002). A further group of 20 mice was sham treated, i.e. saline solution was injected intracranially, and served as control group. The animals were housed under standard conditions with water and food *ab libitum*. Their clinical signs were monitored over time. Blood was collected from the retro-orbital plexus at 24 and 108 days post infection (dpi); serum was isolated and stored at − 20 °C until use. Mice were then necropsied at an average of 190 dpi. All techniques were performed as previously described (Pachner et al., 2011; Pachner et al., 2007), including anesthesia, intracranial injections, perfusion with phosphate-buffered saline (PBS), blood, and cerebrospinal fluid (CSF) collection, and the collection of CNS tissues.

The animals and protocol procedures were approved and supervised by the Institutional Animal Care and Use Committee (IACUC) of Dartmouth College.

2.2. Pegylated IFNβ treatment protocol

Infected mice were allocated at random into two groups for treatment with either pegylated murine interferon beta (pegIFNβ) or vehicle control. PegIFNβ and vehicle control were obtained from Biogen (Cambridge, MA). Vehicle treatment was PBS pH 7.0 containing 236 μg/mL of serum albumin (MSA), whereas pegIFNβ treatment was PBS pH 7.0 containing 236 μg/mL MSA and 2 μg/mL (1×10^5 units/mL) of mPEG-propionaldehyde-modified murine IFNβ. Treatments were begun at 30 dpi, with a single weekly subcutaneous injection of 100 μL, and were continued in each animal until necropsy. PegIFNβ-treated animals were thus given 1×10^4 units of pegIFNβ per injection, a dose deemed optimal from pharmacokinetic studies in the mouse of the murine pegIFNβ and close to the per kilogram dosing for human pegIFNβ (Pepinsky et al., 2001). Importantly, experimenters were masked as to whether the treatment was pegIFNβ or vehicle, as Biogen provided Dartmouth with drug aliquots in tubes labeled as A and B only. Groups were unmasked at the end of the experiment.

2.3. Isolation and preparation of RNA

Whole blood, spinal cord and brain were obtained from each mouse at necropsy. Spinal cords and brains were snap frozen and stored at − 80 °C, whereas whole blood was processed for RNA extraction immediately after collection. Total RNA was extracted using the Trizol™ reagent (Invitrogen, Foster City, CA) following the manufacturer's instructions for either whole blood or solid tissue. The amount and quality of the extracted RNA were assessed by spectrophotometry using the NanoDrop (NanoDrop Technologies Inc., Rockland, DE).

2.4. Real time quantitative reverse transcription (RT)-PCR

Real time RT-PCR was used to assess 1) viral load, 2) in situ IgG expression, and 3) pegIFNβ bioactivity. Viral load was measured through the quantification of TMEV RNA copy number in brain and spinal cord, whereas in situ IgG expression was evaluated by quantifying IgG1 mRNA in spinal cords. PegIFNβ bioactivity was estimated by measuring transcript levels of the interferon stimulated gene (ISG) Myxovirus resistance protein A (MxA) in whole blood. Because the up-regulation of ISG transcripts was shown to be longer following dosing with pegIFNβ than with IFNβ (Allaire et al., 2013), MxA mRNA was measured ≈ 24 h after the last injection.

Total RNA (0.5 μg) was reverse transcribed using the qScript cDNA SuperMix kit (Quanta Biosciences, Gaithersburg, MD, USA). cDNA was then used as a template for real time RT-PCR analysis based on the 5′ nuclease assay. Mouse glyceraldehyde phosphate dehydrogenase (GAPDH) was used as a reference gene. RT-PCR of the desired genes was performed in a StepOne plus sequence detection system (Applied Biosystems, Foster City, CA) using PCR mastermix (Perfecta® FastMix® II, ROX™, Quanta Biosciences, Beverly, MA), along with specific primers and probes. A TaqMan real time PCR assay (Lifetechnologies, Grand Island, NY) was used as primers and probes for GAPDH and MxA, whereas custom primers and probes were used for amplification of IgG1 and TMEV as reported previously (Pachner et al., 2007; Trottier et al., 2002).

Relative mRNA expression levels of IgG1 were analyzed by the $2^{-\Delta\Delta Ct}$ method, in which ΔCt is $= (Ct_{target} - Ct_{GAPDH})$ and $\Delta\Delta Ct$ is $= (\Delta Ct_{sample} - \Delta Ct_{calibrator})$. The calibrator was the average value of the sham-treated group. Conversely, to quantify viral load, a standard curve was utilized and the threshold cycle (Ct) values of the samples were plotted against known TMEV concentrations.

2.5. Quantification of total IgG and anti-TMEV binding antibody (BAb)

Capture ELISA was used to quantitate total IgG in both CSF and serum. Briefly, a goat anti-mouse IgG was used as coating antibody, whereas a horseradish peroxidase (HRP)-conjugated donkey anti-mouse IgG was used as detecting antibody. A purified murine IgG was serially diluted as a standard positive control on each plate for final quantification. Intrathecal synthesis of IgG was then evaluated based on the IgG CSF/serum ratio. A cutoff value for determining positive vs. negative intrathecal IgG production was established as the mean IgG concentration + 2 standard deviations (SD) obtained from the sham animals.

Anti-TMEV BAb levels were determined in serum specimens by using TMEV lysate as coating solution, and a HRP-conjugated donkey anti-mouse IgG as detecting antibody. Each sample was assigned an arbitrary unit of anti-TMEV binding activity based on a standard curve derived from a known positive control in the anti-TMEV ELISA.

All reagents were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

2.6. Neutralizing antibody (NAb) assay

Levels of serum anti-TMEV neutralizing antibody (TMEV-NAb) were measured by a modified cytopathic effect assay (CPE) after heat inactivation of complement. Briefly, 1500 PFU of TMEV was incubated with serial 2-fold dilutions of mouse serum for 2 h, followed by a 48-hr incubation on BHK cell monolayers growing in 96-well plates. To quantify the CPE, cells were stained with crystal violet in 20% ethanol, followed by elution of dye taken up by the cells with 33% acetic acid. Changes in absorbance at 620 nm were detected by using an ELISA reading spectrophotometer. Neutralizing titers were expressed as the reciprocal of the dilution at which 50% of TMEV was neutralized.