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Research paper

A modified immune tolerant mouse model to study the immunogenicity of recombinant human interferon beta

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ABSTRACT

Interferon beta may induce antibodies in multiple sclerosis patients and the incidence of immunogenicity depends on the type of product. These antibodies can reduce the efficacy of interferon beta. Two transgenic immune tolerant mouse models for human interferon beta (hIFNβ) (C57Bl/6, and C57Bl/6 × FVB/N F1 hybrid mice) have been developed previously for studying immunogenicity. These models, however, may not be used for every interferon beta product because of the lack of immunogenicity in the wildtype genetic background. We therefore developed a modified transgenic mouse model by backcrossing the F1 hybrid C57Bl/6 × FVB/N transgenic mice with wildtype FVB/N for 10 generations. These F10 offspring (referred to hear as FVB/N) have a genetic background consisting of mostly FVB/N (99.9%) and very little C57Bl/6 (0.1%), and are expected to have the more sensitive antibody producing phenotype of the parental FVB/N strain. The newly generated "FVB/N" strain was assessed for antibody formation against different rhIFNβ formulations compared to the C57Bl/6, and C57Bl/6 × FVB/N transgenic mouse models.

The new FVB/N transgenic mouse model was more sensitive for all tested rhIFNβ products, and the difference in antibody titers between the transgenic and non-transgenic mice of the FVB/N strain was much bigger compared to the antibody levels of the C57Bl/6, and C57Bl/6 × FVB/N strains.

1. Introduction

Recombinant human interferon beta (rhIFNβ) is used widely to treat relapsing–remitting multiple sclerosis and inhibit disease progression (Woodcock, 1993; Paty and Li, 1993). However, rhIFNβ may lead to an antibody response interfering with its therapeutic effect and causing other side effects (Deisenhammer et al., 1999; Bertolotto et al., 2003). Among rhIFNβ products, Betaferon® (rhIFNβ-1b) has been shown to be the most immunogenic (Ross et al., 2000; Bertolotto et al., 2004; Hermeling et al., 2005; Van Beers et al., 2010a; Antonelli and Dianzani, 1999). Although aggregates are the main factors causing immunogenicity of different rhIFNβ products, other variables, such as the protein structure, formulation, and treatment factors may modulate the development of anti-drug antibodies (ADAs) (Ross et al., 2000; Bertolotto et al., 2004; Hartung et al., 2007; Runkel et al., 1998; Kijanka et al., 2013; van Beers et al., 2010b).

Immune tolerant transgenic animal models are important tools to assess which product-related characteristics contribute most to the immunogenicity of rhIFNβ. To date, transgenic immune tolerant mouse models for hIFNβ with two different

Abbreviations: rhIFNβ, recombinant human interferon beta; hIFNβ, human interferon beta; i.p., intraperitoneally; LU, laboratory units; MS, multiple sclerosis; ELISA, enzyme linked immunosorbent assay; HSA, human serum albumin; Tg, transgenic; non-Tg, non-transgenic; PCR, polymerase chain reaction; ADAs, anti-drug antibodies; SD, standard deviation.

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genetic backgrounds have been used to study the immunogenicity of rhIFNβ products (Hermeling et al., 2005; Ottesen et al., 1994). The hIFNβ transgenic immune tolerant mice developed by Hermeling et al. (2005) showed that protein aggregates play an important role in breaking immune tolerance for this protein. However, the C57Bl/6 genetic background limited the sensitivity of the mice to form antibodies against rhIFNβ. As the immunogenicity of human proteins differs between mouse strains with distinct genetic background (Ottesen et al., 1994), the hIFNβ transgenic immune tolerant mice with a C57Bl/6 background (developed by Hermeling et al., 2005) were crossbred with FVB/N mice to try to improve sensitivity for antibody formation (van Beers et al., 2010b). The C57Bl/6 × FVB/N mouse model showed an improved antibody response against all three commercial rhIFNβ products; however, genetic variability between the siblings appeared to lead to increased variability in antibody responses (van Beers et al., 2010b). To avoid large differences in genetic background as observed in the C57Bl/6 × FVB/N mouse model, and to obtain a higher sensitivity, we bred a transgenic immune tolerant mouse strain with a predominant FVB/N background. To evaluate the sensitivity of the new model, different rhIFNβ products were assessed for immunogenicity in all three genetic backgrounds.

2. Materials and methods

2.1. Interferon beta

Betaferon® (rhIFNβ-1b) was purchased from BAYER (Groningen, The Netherlands). Lyophilised powder containing 300 μg of rhIFNβ-1b, 15 mg of human serum albumin (HSA), and 15 mg of mannitol was reconstituted in 1.2 ml of 10 mM sodium phosphate pH 7.4, 137 mM sodium chloride (PBS). Avonex® drug substance (rhIFNβ-1a) was obtained from Biogen Idec Inc. and was formulated at a concentration of 270 μg/ml in 100 mM sodium phosphate, pH 7.2, and 200 mM sodium chloride. Interferon beta-1b (rhIFNβ-1b) drug substance was supplied by Zistdaru Danesh Co. Ltd. (Tehran, Iran) and was formulated at a concentration of 250 μg/ml in 10 mM sodium acetate buffer pH 7.4, 200 mM L-Arginine, and 0.1% (w/v) N-Dodecyl-β-D-Maltoside. Betaferon®, Avonex® drug substance, and formulated rhIFNβ-1b were diluted to 50 μg/ml in PBS before injection.

2.2. Mouse breeding

Heterozygous C57Bl/6 transgenic mice immune tolerant for hIFNβ developed by Hermeling et al. (2005) were bred at the Central Laboratory Animal Institute (Utrecht University, The Netherlands). To generate the C57Bl/6 × FVB/N mice, transgenic C57Bl/6 mice were crossed with wild type FVB/N mice (Janvier, BioServices, Uden, The Netherlands). The FVB/N transgenic mice were developed by backcrossing F1 C57Bl/6 × FVB/N hybrid transgenic males with wild type FVB/N females for 10 generations. These F10 offspring have a genetic background consisting of 99.9% FVB/N and 0.1% C57Bl/6 (Table 1). The genotype of the offspring from all three mouse models was determined using PCR showing the presence or absence of the hIFNβ gene in chromosomal DNA isolated from ear tissue. Both PCR positive (transgenic) and PCR negative (non-transgenic) littermates of the three mouse models (C57Bl/6, C57Bl/6 × FVB/N, or FVB/N) of 12–16 weeks of age were used for immunogenicity testing.

2.3. Animal experiments

All animal experiments were approved by the National and Institutional Ethical Committee and were in agreement with the European guidelines on animal experiments. Using power analysis, a group size of 4 was calculated for this experiment (www.cs.uiowa.edu/~rlenth/Power). Mice were housed in standard perspex cages and given access to food (Hope Farms, Woerden, The Netherlands) and water (acidified) ad libitum. The mice (4 per group) were injected intraperitoneally (i.p.) with 5 μg of rhIFNβ (Betaferon®, Avonex® drug substance, or formulated rhIFNβ-1b) on days 0–4, 7–11, and 14–18. Blood was collected from cheek puncture (3 times per animal, approximately 60 μl per time point, at least 7-days between each drawing) before immunisation on day 0, and on days 7 and 14 during immunisation. On day 21, the mice were sacrificed by decapitation under isoflurane anaesthesia and the final blood was collected. Plasma was isolated by centrifugation (3000 *g* at 4 °C for 10 min) and was stored at −20 °C until analysis.

2.4. Antibody assay

ADA titers were determined using a modified direct sandwich ELISA as described previously (Hermeling et al., 2005; Kijanka et al., 2013) using rhIFNβ-1a (Avonex® drug substance) to coat ELISA plates, peroxidise-labelled anti-mouse IgG (Invitrogen, Bleiswijk, The Netherlands) as the detecting antibody, and TMB [3,3′,5,5;-TetraMethylBenzidine] (Invitrogen, Bleiswijk, The Netherlands) as the substrate. 100-fold diluted plasma was screened and defined positive if the background-corrected absorbance values at 450 nm were ten times higher

Table 1
F1 hybrid transgenic males backcrossed with wild type FVB/N females for 10 generations to develop FVB/N transgenic mice tolerant to hIFNβ.

Generation	Backcross	% of genome
F1	Tg C57Bl/6 (male) × wt FVB/N (female)	50% C57Bl/6–50% FVB/N
F2	Tg F1 (male) × wt FVB/N (female)	25% C57Bl/6–75% FVB/N
F3	Tg F2 × wt FVB/N (female)	12.5% C57Bl/6–87.5% FVB/N
F4	Tg F3 × wt FVB/N (female)	6.25% C57Bl/6–93.75% FVB/N
F5	Tg F4 × wt FVB/N (female)	3.12% C57Bl/6–96.88% FVB/N
F6	Tg F5 × wt FVB/N (female)	1.56% C57Bl/6–98.44% FVB/N
F7	Tg F6 × wt FVB/N (female)	0.78% C57Bl/6–99.22% FVB/N
F8	Tg F7 × wt FVB/N (female)	0.39% C57Bl/6–99.61% FVB/N
F9	Tg F8 × wt FVB/N (female)	0.19% C57Bl/6–99.81% FVB/N
F10	Tg F9 × wt FVB/N (female)	0.1% C57Bl/6–99.9% FVB/N