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Anti-BlyS antibody reduces the immune reaction against enzyme and enhances the efficacy of enzyme replacement therapy in Fabry disease model mice

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ABSTRACT

Formation of antibodies against a therapeutic enzyme is an important complication during enzyme replacement therapy (ERT) for lysosomal storage diseases. Fabry disease (FD) is caused by a deficiency of alpha-galactosidase (GLA), which results in the accumulation of globotriaosylceramide (GL-3). We have shown immune tolerance induction (ITI) during ERT in FD model mice by using an anti-B lymphocyte stimulator (anti-BlyS) antibody (belimumab). A single dose of the anti-BlyS antibody temporarily lowered the percentage of B cells and IgG antibody titer against recombinant human GLA. Administration of a low maintenance dose of the anti-BlyS antibody suppressed the B cell population and immunotolerance was induced in 20% of mice, but antibody formation could not be prevented. We then increased the maintenance dose of the anti-BlyS antibody and immunotolerance was induced in 50% of mice. Therapeutic enzyme distribution and clearance of GL-3 were also enhanced by a high maintenance dose of the anti-BlyS antibody.

1. Introduction

Antibody formation against a therapeutic enzyme is a serious problem in several diseases that require protein replacement therapy, such as hemophilia, adenosine deaminase deficiency, and lysosomal storage diseases (LSDs) [1]. Fabry disease (FD) is an LSD caused by a deficiency of alpha-galactosidase (GLA), which leads to the accumulation of globotriaosylceramide (GL-3), resulting in cardiac hypertrophy, chronic neuropathic pain, and chronic kidney disease. Enzyme replacement therapy (ERT), that is, biweekly intravenous administration of recombinant human GLA (rhGLA), has been approved for FD, and >4000 patients with FD are currently receiving ERT worldwide. In the clinical setting, the emergence of a neutralizing antibody against rhGLA has been observed in the majority of the patients receiving ERT, which might weaken therapeutic efficacy in FD [2–8]. However, only a few studies have investigated induction of immune tolerance (ITI) to rhGLA. Chronic administration of methotrexate (MTX) was shown to be effective at reducing antibody titer, but it failed to induce immunotolerance [9].

In LSDs, the most striking negative effect of an antibody against a therapeutic enzyme is weakened therapeutic efficacy of ERT. A number of studies have investigated methods to induce immunotolerance to a therapeutic enzyme for an LSD, mainly in Pompe disease [10–14], but few of them have become clinically available due to their low efficacy and side effects. The current strategy for ITI is the administration of an immunosuppressant, such as corticosteroid, immunoglobulin, and anti-lymphocytic agents. Another promising strategy is the induction of oral immune tolerance, which might induce regulatory T-cell activation and immune tolerance [15].

The majority of B cell-depletion agents, including anti-CD20 monoclonal antibodies, have cytotoxic effects and there is a risk of severe infections. In addition, it is difficult to induce antigen-specific immune tolerance by B cell depletion. An ITI protocol that minimizes the risk of immune suppression and induces antigen-specific tolerance with a high efficacy is warranted during ERT. An anti-B lymphocyte stimulator (anti-BlyS) antibody that has been approved for systemic lupus erythematosus (SLE) is a promising anti-B cell therapeutic agent because of its specificity and safety [16,17]. The anti-BlyS antibody reduced antibody formation in a Pompe disease murine model receiving ERT [18]. Single administration of anti-BlyS antibodies reduced antibody titer and enhanced the efficacy of ERT in these model mice. However, its long-term administration was not discussed in this previous study. In the present study, we have

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evaluated the efficacy of the anti-BlyS antibody in a FD mouse model to prevent the formation of IgG antibody against the therapeutic enzyme.

2. Materials

2.1. Animals

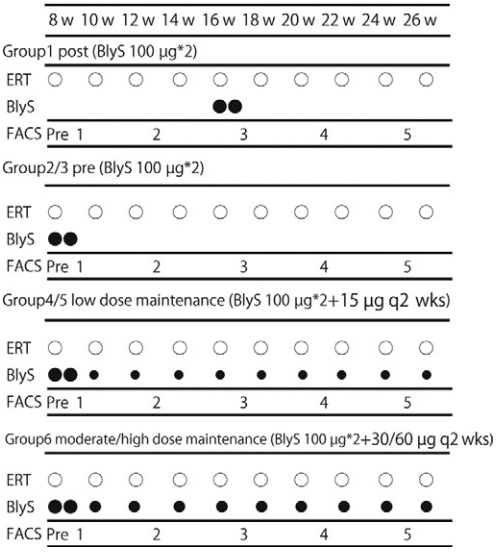
FD model mice (B6;129-Gla^{tm1}Kul/J) were a gift from Dr. Roscoe O. Brady at the National Institutes of Health (Bethesda, MD). C57B6/J mice were used as wild-type control. Eight-week-old FD mice received 1 mg/kg agalsidase beta (Fabrazyme; Genzyme, Cambridge, MA) bi-weekly for a total of 10 times. Peripheral blood cells (PBCs) were collected before and monthly after the initiation of ERT. At 2 weeks after the 10th ERT, the mice were sacrificed for tissue analysis of GLA enzyme activity, GL-3 accumulation, and histopathology. The anti-BlyS antibody (10F4) was kindly provided by Dr. Sara J. Brett (GlaxoSmithKline) and administration was performed according to the sub-group (Table 1). Hamster IgG was used as negative control in each experiment.

- Group 1: Anti-BlyS antibody (100 μg, twice, 5 days apart) was administered at 8 weeks after the initiation of ERT. (n = 10, each)
- Group 2: Anti-BlyS antibody (100 μg, twice, 5 days apart) was administered prior to the initiation of ERT. (n = 10, each)
- Group 3: Anti-BlyS antibody (100 μg, twice, 5 days apart) was administered prior to the initiation of ERT, and MTX (1 mg/kg) was administered at 48 h after ERT. (n = 10, each)
- Groups 4 and 5: Anti-BlyS antibody (100 μg, twice, 5 days apart) was administered prior to the initiation of ERT, and low-dose anti-BlyS antibody (15 μg) was added every 2 weeks combined with ERT. (n = 10, each)
- Group 6: Anti-BlyS antibody (100 μg, twice, 5 days apart) was administered prior to ERT, and anti-BlyS antibody (30 and 60 μg) was added every 2 weeks combined with ERT. (n = 10, each)

2.2. Flow cytometry (FACS)

PBCs were taken from the mice and collected into a heparinized microtainer. Lysis buffer (BD Bioscience, Franklin Lakes, NJ) was added to the PBCs and the cells were suspended in FACS buffer (PBS, 1% FCS, 0.01% sodium azide). The cells were transferred to a 96-well plate and incubated for 10 min with Fc block (anti-CD16/32 antibody; BD Bioscience). Anti-B220 antibody conjugated with PE (eBioscience, Santa Clara, CA), anti-IgM antibody conjugated with FITC (BioLegend, San Diego, CA), anti-CD21 antibody conjugated with APC (BioLegend), and anti-CD138 antibody conjugated with FITC (Miltenyi Biotech, Bergisch Gladbach, Germany) were added to the PBCs and incubated for 30 min. Isotype control antibody was used as a negative gating control in each experiment. The cells were then analyzed by FACS (MACSQuant; Miltenyi Biotech).

Table 1
Experimental design.



2.3. Enzyme-linked immunosorbent assay (ELISA)

IgG antibody titer against agalsidase beta (Fabrazyme) was analyzed by ELISA. Briefly, 96-well plates were coated with 10 μg agalsidase beta overnight at 4 °C. The plates were blocked with 100 μL PBS/1% bovine serum albumin for at least 2 h at 37 °C. Diluted serum, anti-rhGLA monoclonal antibodies produced in mouse (kindly provided by Genzyme), and rabbit IgG (GeneTex, Inc., Irvine, CA) were added to the wells and incubated for 1 h at 37 °C. Then a 10,000-fold diluted anti-mouse IgG antibody (Kirkegaard & Perry Labs, Gaithersburg, MD) was added and incubated for 30 min at 37 °C. TMB (Kirkegaard & Perry Labs) was added and incubated at 37 °C for 10 min. Finally, absorbance (450 nm) was measured by spectrometry. IgG antibody titer was calculated using a monoclonal anti-rhGLA antibody serial dilution curve. An IgG antibody titer <500 ng/mL was defined as "immune tolerant."

2.4. GLA enzyme activity

Tissue enzyme activity of GLA was measured using a previously described protocol [19]. Briefly, 20 mg tissue were homogenized in 400 μL distilled water and the supernatant was collected after centrifugation at 14000 rpm for 10 min at 4 °C. Protein concentration of the supernatant was measured by the BCA protein assay. Then 50 μL homogenate and 50 μL 4-MU buffer containing 10 mM 4-methylumbelliferyl α-D-galactopyranoside (Sigma-Aldrich, St. Louis, MO) were incubated at 37 °C for 30 min. After 100 μL of 0.15 M glycine carbonate stop buffer (pH 10.4) was added, fluorescent intensity was measured using a spectrophotometer and enzyme activity (nmol/h/mg protein) was calculated according to the intensity and protein concentration.

2.5. Thin layer chromatography (TLC)

Tissue content of GL-3 was evaluated by TLC [19]. Briefly, 20 mg tissue were homogenized in 1 mL distilled water. Then, 5 mL of chloroform and methanol (2:1) was added and the sample was centrifuged at 1500 rpm for 5 min at room temperature. The lower phase containing GL-3 was evaporated under nitric oxide and washed with chloroform, methanol, and distilled water (4:48:47). Then, the lower phase was evaporated under nitric oxide and finally dissolved in chloroform and methanol (2:1). The sample was analyzed by TLC. Detected bands were semi-quantified by ImageJ software (National Institutes of Health).

2.6. Mass spectrometry (GL-3 measurement)

Tissue content of GL-3 was also measured by mass spectrometry [20]. Briefly, 20 mg tissue was homogenized in 1 mL distilled water and protein concentration was measured by the BCA protein assay. Then, 5 mL chloroform and methanol (2:1) was added and the sample was centrifuged at 1500 rpm for 5 min at room temperature. The lower phase was evaporated under nitric oxide and washed with chloroform, methanol, and distilled water (4:48:47). Then, the lower phase was evaporated under nitric oxide and finally dissolved in chloroform and methanol (2:1). GL-3 concentration was measured by LC/MS/MS and compensated by protein concentration.