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BAFF blockade prevents anti-drug antibody formation in a mouse model of Pompe disease

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Abstract Antibodies formed against the therapeutic protein are a life-threatening complication that arises during enzyme replacement therapy for Pompe disease (acid α-glucosidase deficiency; GAA). To provide an effective alternative to current practices, we investigated the capacity of anti-B-cell activating factor (BAFF) as a novel drug candidate to prevent antibody formation in a Pompe disease mouse model. A BAFF-neutralizing antibody was administered prophylactically and with maintenance doses in association with enzyme replacement therapy using recombinant human GAA in *Gaa*$^{-/-}$ mice. BAFF blockade delayed antibody production and increased GAA activity within tissues with protection from anaphylaxis. Anti-BAFF also resolved antibody formation during an immune response and precluded the maturation of antibody secreting cells from entering the bone marrow compartment. This treatment modality may therefore be a viable alternative for the clinical management of antibody formation for Pompe disease and has potential use against antibody formation in other protein replacement therapies.

1. Introduction

Pompe disease is an autosomal recessive metabolic myopathy caused by deficiency of acid α-glucosidase (GAA), the lysosomal enzyme responsible for degrading glycogen [1–3]. Pathology includes early cardiac, respiratory and neuromuscular dysfunction. Enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) is the only approved therapy, which, although beneficial, may result in life-threatening infusion associated reactions (IAR) as a result of anti-drug antibody (ADA) formation against the therapeutic enzyme [4–9].

Abbreviations: ADA, Anti-drug antibody; BAFF, B-cell activating factor; ERT, Enzyme replacement therapy; FO, Follicular B-cell; GAA, Acid α-glucosidase; MZ, Marginal zone B-cell; rhGAA, Recombinant human GAA; TR, Transitional B-cell.

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Current treatments to alleviate immune responses during ERT for Pompe disease include cyclophosphamide, bortezomib, rituximab, sirolimus (rapamycin) and methotrexate [10–13]. Patients are currently put on long-term immune suppression in order to safely and effectively receive ERT which constitutes of 20- to 40-mg/kg infusions of rhGAA weekly to once every 2 weeks throughout the lifetime of the patient. Although IgE-mediated hypersensitivity reactions have been documented in acute responses, anti-GAA IgG formation is primarily responsible for the prolonged complications associated with ERT [7–9]. B-cell depletion with rituximab is a critical component of these regimens to prevent antibody formation but it has global effects on B-cell immunity. Therefore, development of an alternative approach that is less detrimental is desirable. In lieu of B-cell depletion or chemotherapy drugs, which carry substantial side effects [14], disrupting the maturation of antigen-specific B-cells while retaining natural and acquired humoral immunity would stand as a more attractive therapeutic intervention. The beneficial results obtained with FDA-approved anti-B-cell activating factor (BAFF) antibody, belimumab, in reducing disease manifestations in systemic lupus erythematosus (SLE) patients [15,16], as well as in mouse models of transplant tolerance [17,18], type 1 diabetes [19] and atherosclerosis [20], predict the efficacy of anti-BAFF immunotherapy for diseases complicated by ADA.

BAFF, in conjunction with a proliferation-inducing ligand (APRIL), serves as the primary survival and maturation cytokines for B-cells, with less well-defined effects on T-cell survival and functions [21,22]. BAFF is a survival factor most necessary for the maturation of follicular (FO) and marginal zone (MZ) B-cells, without which B-cells are arrested at the transitional stage (TR). This is of significance as TR B-cells are subject to clonal deletion and are incapable of responding to T-cell help (a critical component of the immune responses observed during ERT), and may therefore become tolerogenic [23]. To our knowledge, BAFF depletion has never been investigated to block antibody formation resulting from ERT for genetic diseases. As the blockade of B-cell development at the TR stage would leave pre-existing immunity intact [24], we hypothesized that humoral tolerance may be achieved using BAFF-directed immunotherapy in the context of ERT in *Gaa*$^{-/-}$ mice.

2. Materials and methods

2.1. Mice

Male and female, 4–6 week old *Gaa*$^{-/-}$ 129SVE (Taconic, Hudson, NY, USA) and 4 month old male B6.*Rag-1*$^{-/-}$ mice (Jackson, Bar Harbor, ME, USA), were handled in accordance with the guidelines set by the University of Florida Institutional Animal Care and Use Committee.

2.2. ELISA and FACS

Anti-GAA IgG1 ELISA was performed as previously described [9]. 96-well plates (Thermo-Scientific: 3855) were coated with rhGAA (1 μg/mL) for experimental samples or a standard curve of IgG1κ (Sigma: M9269; 4000 ng/mL–62.5 ng/mL) and incubated overnight at 4 °C. Samples were diluted 1:50 and incubated for 2 h at 37 °C. HRP-conjugated rat anti-mouse IgG1 heavy chain secondary detection antibody (AbD Serotec: MCA336P) was incubated for 2 h at 37 °C. Plates were developed with Sigmafast OPD tablets (Sigma: P9187) and read using a μQuant microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA) at 450 nm. Mouse BAFF immunoassay was performed using manufacturer's protocol (R&D Systems: MBLYS0). Spleens were filtered through a 40 μm nylon mesh filter (Fisher: 22363547) to obtain a single cell suspension for FACS. Cells were blocked with Fc block (clone: 2.4G2; BD Biosciences, San Jose, CA, USA) for 30 min at 4 °C prior to labeling. Cells were labeled for 30 min at 4 °C with the following antibodies: FITC-CD21/CD35 (clone: 4E3) and APC-IgM (clone: II/41) from eBioscience (San Diego, CA, USA), and Pacific Blue-B220 (clone: RA3-6B2) from Biolegend (San Diego, CA, USA). FACS was performed on a LSRII (BD Biosciences, San Jose, CA, USA) and analyzed using FCS Express 4 (De Novo Software, Glendale, CA, USA).

2.3. BAFF-directed immunotherapy and rhGAA administration

Experimental mice (group sizes are indicated in figure legends) received two, 1 mg/kg or 5 mg/kg intraperitoneal (IP) injections of BAFF-neutralizing antibody (10 F4; GlaxoSmithKline, Middlesex, UK) at a volume of 100 μL in sterile PBS five days apart. Recombinant human GAA (rhGAA; Myozyme®; Genzyme Corp., Cambridge, MA, USA) was injected at 20 mg/kg in a volume of 100 μL in sterile PBS via tail vein (IV) at the indicated time points.

2.4. Pulse oximetry and GAA activity assay

Pulse oximetry was performed using a cardiopulmonary data recorder (Starr Lifescience Corp., Oakmont, PA, USA) as previously described [9]. GAA activity assay was performed as described previously [25].

2.5. ELISpot

ELISpot plates (Millipore: MAHAS4510) were coated with rhGAA or a standard IgG capture reagent, goat anti-mouse IgG (Abcam: ab6708), overnight at 4 °C. The plates were blocked with RPMI media supplemented with 5% FBS and 0.1% β-mercaptoethanol (cRPMI) for 1 h at room temperature. Bone marrow cells, aspirated from both femurs, and spleens were filtered through a 40 μm nylon mesh filter (Fisher: 22363547) to obtain a single cell suspension. Cells were resuspended in cRPMI at a concentration of 1×10^7 cells/mL. Cells were plated at 2×10^6 cells per well and serially diluted 2-fold and incubated overnight (37 °C; 5% CO_2). Cells were washed and rat anti-mouse IgG1 HRP (AbD Serotec: MCA336P) or rabbit anti-goat IgG HRP (Abcam: ab6741) was diluted in cRPMI and incubated for 1 h at room temperature. After washing, spots were developed with AEC substrate (BD Biosciences: 551015) and the reaction was stopped with water. The membrane was dried and scanned using an ImmunoSpot Analyzer (Hightech Instruments, Edgemont, PA, USA).