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Enzyme replacement therapy reverses B lymphocyte and dendritic cell dysregulations in patients with Gaucher Disease

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ABSTRACT

Gaucher disease (GD) is caused by mutations in the *GBA* gene encoding lysosomal enzyme, β-glucocerebrosidase (GCase). GCase deficiency results in accumulation of its substrates in cells of macrophage lineage, affecting multiple organ systems. Enzyme replacement therapy (ERT) with recombinant human GCase is the standard of care to treat GD. In GD, it is well established that there are immune alterations, clinically presenting as lymphadenopathy, gammopathies, and predisposition to hematological cancers. We examined the effect of ERT on immune dysregulations in treatment-naïve GD patients longitudinally after the initiation of ERT. Immunophenotyping was performed in peripheral blood samples obtained before and after ERT. T and B lymphocyte subsets, NK, NKT and dendritic cells were evaluated. In all treatment naïve patients at baseline, transitional B cells, characterized by $CD21^{low}$ expression were markedly elevated. After establishment of stable-dose therapy, $CD21^{low}$ cells were significantly reduced and subsequent increase in $CD21^{Hi}$ B lymphocytes indicated improved B cell maturation. Class-switching and memory B cell defects which were noted prior to treatment were found to be normalized. An increase in dendritic cells also resulted after the treatment. Our data shows that GD affects across various immune cell types and ERT or its effects directly improve affected immunological parameters.

1. Introduction

Gaucher disease (GD, OMIM#230800) is a rare metabolic disorder caused by mutations in the *GBA* gene and inherited in autosomal recessive manner. Subsequent deficiency of the lysosomal enzyme β-glucocerebrosidase (or acid β-glucosidase, EC 3.2.1.45) results in accumulation of glycosphingolipids in cells of monocytes/macrophages lineage. Macrophage directed enzyme replacement therapy (ERT) is the standard form of treatment for GD. ERT involves periodic intravenous infusions of recombinant GCase enzyme to supplement low levels of endogenous GCase. Substrate reduction therapy (SRT) which decreases sphingolipid production is also available as an oral treatment option [1]. Partial or total splenectomy was once advocated as a treatment option for GD patients, but with advent of ERT and SRT, this is rarely considered. However, splenectomy is still continuing to be performed mostly as diagnostic in patients with unexplained splenomegaly/hypersplenism who were later diagnosed with GD. Splenectomy is the one of the most significant modifying factors in GD, after which there is usually rapid progression of skeletal disease with recurrent osteonecrosis leading to intractable pain and deformities. It has been recently shown that prior splenectomy can lead to persistent immune defects in GD patients even after long term ERT [2,3].

While the pathogenic *GBA* variants are diverse, even among the patients with similar genotypes, GD symptoms are heterogeneous, and may manifest at any age affecting across various organ systems. As a generalization though, GD is clinically divided into non-neuronopathic (type 1) or neuronopathic types (type 2 and 3), depending upon the involvement of the central nervous system. In type 1 GD, clinical features include organomegaly (hepato- and splenomegaly), cytopenias, excessive fatigue, skeletal involvement with Erlenmeyer flask deformities, osteonecrosis, bone pain and osteoporosis. Excess lipid storage in macrophages also results in elevated biomarkers in serum including chitotriosidase (CHITO), angiotensin conversion enzyme (ACE) and tartrate resistant acid phosphatase (TRAP) which are often used to monitor disease progression and treatment response [4].

GD patients are susceptible to frequent infections and are known to have higher incidence of poly and monoclonal gammopathies of unknown significance and myelomas indicating possible immune

Abbreviations: ACE, angiotensin conversion enzyme; CBC, complete blood count; CHITO, chitotriosidase; DC, dendritic cells; ERT, enzyme replacement therapy; GCase, Glucocerebrosidase; HC, healthy control; Hgb, hemoglobin; Ig, Immunoglobulin; MGUS, monoclonal gammopathy of undetermined significance; NK cells, natural killer cells; NKT cells, natural killer T cells; PBMCs, peripheral blood mononuclear cells; SRT, substrate reduction therapy; Tc, cytotoxic T cells; Th, T helper cells; TRAP, tartrate resistant acid phosphatase; IRB, internal review board; yr, years; WBC, whole blood count.

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dysregulations [5]. Dendritic cells (DCs), which play a prominent role in adaptive immune response, have been observed to be decreased in untreated GD patients [6,3]. ERT has been shown to restore altered innate function of plasmacytoid DCs [7]. Otherwise, data is insufficient on immune dysregulation spanning various immune subsets and whether the role of ERT is significant in reversing them. In present study, we investigated the affected immune cell types in untreated GD patients as well as the effect of ERT on the immune dysregulations. Immunephenotyping on peripheral blood mononuclear cells (PBMCs) was performed to follow three subjects who have not been treated for GD or whose treatment has been interrupted for considerable time. These patients were then administered ERT and follow-up analysis was performed several months after the initiation of ERT. Remarkable decrease in serum biomarkers, including CHITO, ACE and TRAP was also observed following ERT. Immunophenotyping revealed that untreated GD patients manifest B cell defects which were normalized after 12 months of ERT, even though the extent of which could be influenced by prior splenectomy status. Improvement in overall DCs was also seen but to a lesser extent in splenectomized patients.

2. Material and methods

2.1. Subjects

Three patients with confirmed GD diagnosis from an IRB approved clinical study (NCT02000310) were included in the study. The handling of tissue samples and patient data was approved by the internal review board (IRB, Copernicus Group Independent Review Board) including the procedure whereby all patients gave informed consent to participate in the study. Written informed consent was obtained using IRB approved informed consent form.

2.2. Isolation of peripheral blood monocuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll-Paque Premium (GE Healthcare) and Leucosep tubes (Greiner Bio-One) according to manufacturers' protocols. Briefly, whole blood was diluted two times using 2% (vol/vol) FBS/PBS, overlayed onto Ficoll-Paque medium in leucosep tubes and centrifuged for 15 min at 800 g without brakes. Enriched cell fraction (PBMCs) is washed once and resuspended in same volume of 2%FBS/PBS as that of the starting whole blood.

2.3. Immunophenotyping

Direct immunofluorescence with specific antibodies was performed either on peripheral blood or from isolated PBMCs as previously described [3,8]. Briefly, PBMCs (400,000 cells) in 2%FBS/PBS were stained with required cocktail of antibodies at 4 °C for 30 min, washed with PBS and resuspended in 1% paraformaldehyde. The samples were acquired on Accuri C6 flow cytometer (BD Bioscience, San Jose, CA) and analyzed using FCS express software (De Novo software, Glendale, CA). Gating strategies for lymphocyte subsets and DCs were similar to those described earlier. Individual fractions of PBMCs were compared to normal values calculated as average from ten non-GD controls. Reference ranges (95% confidence intervals) were generated in the same lab using 40 normal controls (data not shown), except for memory T cells and total DCs for which earlier published data has been used [9,10].

2.4. Statistical analysis

All statistical analysis was performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA). *P* values were calculated using Student's paired *t*-test between values from pre and post ERT treated samples.

3. Results and discussion

3.1. Clinical characteristics of patients

Three patients with confirmed type 1 GD (2 females, 1 male, ages 32–40 yr) were included in the study. Patient demographics and clinical features are summarized in Table 1. Patient 01 had splenectomy three months before initiation of ERT, while patient 03 had undergone splenectomy before 28 years at the age of 12 yr. Patient 01 had never been treated for GD, while patient 02 was earlier treated with imiglucerase for 14 years, but discontinued 7 years before the study. Patient 03 was administered imiglucerase a few times at sub-optimal doses (once every 5 weeks, due to shortage of the drug) and later discontinued. There was an interruption of therapy for atleast 6 months prior to enrollment. All three subjects showed organomegaly, thrombocytopenia and low hemoglobin levels while having no obvious bone crisis.

All three patients had decreased hemoglobin (Hgb) levels and platelet counts prior to therapy. However, after 12 months of administering optimal dose of ERT (imiglucerase for Patient 01 and velaglucerase for patients 02 and 03), both Hgb level and platelet counts improved in

Table 1
Patient characteristics and clinical features. Summary of patient details including the sex and age at enrollment, splenectomy status and the time lapse between splenectomy and initiation of ERT are shown. Clinical symptoms at the time of enrollment are also presented. Hemoglobin levels, platelet counts as well as the level of serum biomarkers, chitotriosidase (CHITO), angiotensin converting enzyme (ACE) and Tartrate resistant acid phosphatase (TRAP) are noted. The two values represent from the start of treatment and 12 months after ERT. Reference ranges are mentioned in parenthesis. NA: Not available.

Pt #	Age (yrs)	Sex	Splenectomy	Years between splenectomy and ERT	Clinical features at diagnosis	Hgb g/dL (11.1–15.9)	Platelets 10^3 /μl (150–450)	CHITO nmol/h/ml (<78.5)	ACE IU/L (25–106)	TRAP IU/L (3–10)
01	32	M	At age 32	0.25	Organomegaly thrombocytopenia osteopenia low hemoglobin presence of gaucher cells in bone marrow No bone pain.	10.8, 14.3	194, 359	1528, 374	NA	NA
02	32	F	No	NA	Organomegaly thrombocytopenia osteopenia low hemoglobin Fatigue No osteoporosis No bone pain.	10.4, 12.0	63, 123	2018, 204	222, 44	63, 12
03	40	F	At age 12	18	Organomegaly thrombocytopenia cholelithiasis low hemoglobin Anemia No bone pain	9.1, 12.2	65, 344	7366, 2175	322, 63	23, 9

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