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Persistent immune alterations and comorbidities in splenectomized patients with Gaucher disease

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ABSTRACT

Gaucher disease (GD) is an autosomal recessive disorder caused by mutations in the gene encoding acid-β-glucosidase, resulting in functional disruptions in degradation of glycosphingolipids and lysosomal accumulation of the substrates. The most frequent clinical presentations of GD are thrombocytopenia, splenomegaly and bone pain. Prior to advent of enzyme replacement therapy, splenectomy was performed for complications of hypersplenism such as severe thrombocytopenia and transfusion dependency. Though there is evidence about worsening bone disease after splenectomy, there is no systematic study to assess its effects on the immune system in GD patients. In order to investigate the long-term immunological effects of splenectomy, we used flow cytometry to compare the immunophenotypes of GD patients who had undergone splenectomy (SGD) to those with intact spleen. The results show that SGD patients have significantly fewer $CD27^+/IgM^+$ B-cells but more $CD4^+/CD45RO^+$ and $CD8^+/CD45RO^+$ T-cells. The most surprising finding was an almost complete absence of circulating dendritic cells in SGD patients. In addition, splenectomized subjects had comorbidities, the most common being monoclonal gammopathy of undetermined significance (MGUS). Taken together, these results highlight the persistence of multiple immune alterations and comorbidities coexisting in higher frequency in the SGD group and they are not affected by GD specific therapy.

1. Introduction

Gaucher disease (GD) is an autosomal recessive disorder that results from mutations in the gene encoding the lysosomal enzyme acid β-glucosidase (EC number 3.2.1.45). These "loss of function" mutations result in impaired degradation of glycosphingolipids, leading to accumulation of the immediate substrates, glucosylceramide and glucosylsphingosine in lysosomes [1]. The most frequent presentations of the disease are thrombocytopenia, splenomegaly and bone pain. There is no uniformity in the phenotypes, and for some patients with mild symptoms, it can be many years before they are diagnosed with GD. The current standard therapy for GD involves regular administration of enzyme replacement therapy (ERT) or substrate reduction therapy [2]. The accumulation of glucosylceramide and glucosylsphingosine is most pronounced in the phagocytic cells, and macrophages are assigned as the dominant disease effector cell type. Untreated GD results in an inflammatory response inducing changes in other immune cells including T-cells, B-cells, NK-cells, neutrophils, monocytes and dendritic cells [3].

Prior to the advent of ERT, therapeutic splenectomy was performed for symptomatic splenomegaly to treat the hematologic complications of hypersplenism, especially thrombocytopenia and intractable anemia. The availability of ERT reduced the incidence of splenectomy and bone disease but despite the access to enzymatic and molecular diagnostic methods, splenectomy is still occasionally employed as a diagnostic modality, especially to rule out or for staging of a suspected malignancy in a patient presenting with splenomegaly and thrombocytopenia [4].

The spleen is a major lymphatic organ and the lymphocyte composition changes immediately after splenectomy but then stabilizes to a large extent within a few months [5]. However, permanent changes in the immune system have been reported including a significant reduction in circulating splenic marginal zone B-cells ($CD19^+/CD27^+/IgM^+$) which might be connected to the increased incidence of sepsis in splenectomized patients [6–8]. In GD patients, splenectomy has been shown to worsen the disease in other organs and increase the risk of deterioration in bone disease [4,9,10], pulmonary hypertension [11], malignancy [12] and thrombocytosis [13]. Splenectomized

Abbreviations: CBC, complete blood count; COPD, chronic obstructive pulmonary disease; DC, dendritic cells; ERT, Enzyme replacement therapy; GD, Gaucher disease; HC, healthy control; Ig, Immunoglobulin; MGUS, Monoclonal gammopathy of undetermined significance; NK cells, Natural killer cells; NKT cells, Natural killer T cells; SC, splenectomized control; SGD, splenectomized Gaucher disease; IRB, internal review board; WBC, whole blood count.

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Gaucher disease (SGD) patients have also been reported to have increased leukocyte count compared to GD patients [14].

In this study, we compare the immune profile of SGD patients with that of non-splenectomized GD patients as well as healthy control (HC) reference group. Clinical evaluation of the patients including bone disease and other comorbidities and possible correlation with the immune findings are also elaborated. Both the clinical and immunophenotyping results from this study highlight the persistent immune alterations in SGD patients as compared to GD and HC especially in $CD45RO^+$ T-cells, $CD27^+/IgM^+$ B-cells and DC populations despite therapy.

2. Subjects and methods

2.1. Subjects

Patients with GD ($n = 19$) (age range 32–67 years) (5 males, 14 females) were recruited under IRB approved protocols (NCT01358188 and NCT02000310). Nine had undergone splenectomy, and ten GD patients with intact spleens were used as controls. For non-GD controls, samples from anonymized age and gender matched healthy donors ($n = 9$) and splenectomized subjects without GD ($n = 2$) were utilized. All patients with GD had been on a steady dose of ERT for at least six months or longer at enrollment. Additionally, SGD patients were all splenectomized at least one year prior to enrolling in the study. The two splenectomized control patients with hereditary spherocytosis (SC) had splenectomies respectively 30 years and 93 days prior to sample collection. For the GD and SGD group, blood samples were collected immediately before their routine ERT infusion. Blood samples were analyzed for complete blood count (CBC) analysis (Quest Diagnostics, Baltimore, MD) and quantitative immunoglobulin levels. The samples were also used for flow cytometry based immunophenotyping of different lymphocyte populations as well as dendritic cell population (DC) within 24 h of blood draw. Clinically, functional immunity was evaluated by assessing vaccine response in the splenectomized subjects [15,16].

2.2. Staining and flow cytometry

Immunophenotyping was performed as previously described [17], with some modifications using the following antibodies; anti-IgG1 FITC, anti-CD5-FITC, anti-CD8-FITC, anti-CD14-FITC, anti-CD22-FITC, anti-CD34-FITC, anti-IgG1-PE, anti-CD3-FITC/CD16 + CD56-PE, anti-CD11C-PE, anti-CD21-PE, anti-CD27-PE, anti-CD183-PE, anti-CD194-PE, anti-CD20-PerCP and anti-HLA-DR-PerCP (BD Bioscience, San Jose, CA). Anti-CD19-FITC, anti-IgA-FITC, anti-IgD-FITC, anti-CD8-PE, anti-CD19-PE, anti-IgG-PE, anti-IgG1-PerCP, anti-CD3-PerCP, anti-CD4-PerCP, anti-CD8-PerCP and anti-CD3-APC (Invitrogen, Carlsbad, CA). Anti-Lineage-FITC (anti CD3/CD14/CD16/CD19/CD20/CD56), anti-CD196-PerCP, anti-IgM-PerCP and anti-CD45-APC (Biolegend, San Diego, CA). Anti-CD4-FITC (eBioscience, San Diego, CA), anti-CD45RO-FITC (Abcam, Cambridge, MA) and anti-BDCA2-APC (Miltenyi Biotech, San Diego, CA).

Briefly, after washing the whole blood with PBS, 100 μl of blood was stained with the relevant cocktail of antibodies at 4 °C for 30 min followed by red blood cell lysis using BD FACS lysis solution (BD Bioscience, San Jose, CA). Samples were acquired on Accuri C6 flow cytometer (BD Bioscience, San Jose, CA) and analyzed using FCS express software (De Novo software, Glendale, CA). During acquisition, a lymphocyte gate was assigned and 10,000 events were collected for the T-cell and NK cells and NKT cells and 25,000 events for the B-cell analysis. For dendritic cells, a million ungated events were acquired.

2.3. Statistical analysis

Graphs are generated as scatter plots and statistical analysis was performed using GraphPad Prism software showing mean ± SD. All data comparisons were analyzed as two tailed, two sample unequal variance using the Student's t-test to determine significance. A p-value less than 0.05 is considered significant.

3. Results

3.1. Demographics and clinical characteristics

The demographics and clinical characteristics of GD patients are shown in Table 1. The β-glucosidase mutations, total WBC, lymphocyte and platelet count for each subject are also listed. The gender distribution and the average values for WBC, lymphocytes, age and platelet count for the three groups; GD, SGD and HC are summarized in Table 2. The CBC analysis showed a non-significant increase in both WBC and total lymphocyte count in SGD patients compared to both GD patients and HC. The most notable difference is in the platelet count where the SGD patients have significantly higher number of platelets than both GD patients ($p < 0.0001$) and HC ($p < 0.001$) which is in agreement with the published data [13,18]. The clinical details of bone involvement and other comorbidities are summarized in Table 3. Recurrent pulmonary infections were reported in two SGD patients (SGD-2 and SGD-7), who also had a deficient vaccine response. Both of these subjects had history of smoking and a clinical and radiologic diagnosis of COPD (chronic obstructive pulmonary disease). All SGD patients had bone disease, and six out nine required surgical intervention, as opposed to only one non-splenectomized GD patient that required surgery. Bone involvement in the non-splenectomized patients was limited to mostly marrow changes. On the other hand, in the splenectomized patients with GD, there were in addition, pronounced structural bone complications. Of note, all patients in the splenectomized group suffered from multiple comorbidities, the most common one was gammopathy, and two developed monoclonal gammopathy of undetermined significance (MGUS) (Table 3).

3.2. Alterations in lymphocyte subpopulations in patients with Gaucher disease undergone splenectomy

To investigate whether the increase in the overall lymphocyte count in SGD reflected a general increase in all lymphocyte subsets, or if specific cell types and subsets were affected, multiparametric flow cytometry analysis was performed on peripheral blood, which had been stained using fluorescently tagged specific antibody combinations to identify various lymphocyte subsets. The gating strategy for identifying T-cells, B-cells and NK-cells (TBNK) based on the expression of $CD3^+$, $CD20^+/CD19^+$ or $CD3^-/CD16^+$ or $CD56^+$ respectively is shown in supplemental Fig. S1. No significant changes in the TBNK composition between the three groups were observed (Fig. 1A–C). Thus, the increase in total lymphocytes in SGD patients is the result of a non-significant increase in the absolute number of T-cells and B-cells and a significant increase in NK-cells compared to GD patients (Fig. 1D–F). NKT cells, ($CD3^+/CD16^+$ or $CD56^+$) did not show any change between the groups (data not shown). Overall, this analysis demonstrates that there are only minor changes in the overall TBNK populations between GD and SGD patients.

3.3. Higher number of $CD8^+/CD45RO^+$ cells found in both SGD and GD patients

To assess the specific alterations in T cell subsets in SGD patients, first, $CD3^+$ T-cells were analyzed including the distribution of T-helper cells and cytotoxic T-cells, using CD4 and CD8 markers respectively. While there was no difference in the $CD4^+$ population among the three groups, an increase in the fraction of positive $CD8^+$ cells was observed in GD and SGD patients compared to HC. However, this increase was only significant for the GD group (Fig. 2A, B). Comparing the absolute number of $CD4^+$ and $CD8^+$ shows a significant difference in