Gaucher disease gene GBA functions in immune regulation

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Inherited deficiency of acid β-glucosidase (GCase) due to biallelic mutations in the GBA (glucosidase, β, acid) gene causes the classic manifestations of Gaucher disease (GD) involving the viscera, the skeleton, and the lungs. Clinical observations point to immune defects in GD beyond the accumulation of activated macrophages engorged with lysosomal glucosylceramide. Here, we show a plethora of immune cell aberrations in mice in which the GBA gene is deleted conditionally in hematopoietic stem cells (HSCs). The thymus exhibited the earliest and most striking alterations reminiscent of impaired T-cell maturation, aberrant B-cell recruitment, and enhanced mononuclear phagocyte presentation and the egress of mature thymocytes. These changes correlated strongly with disease severity. In contrast to the profound defects in the thymus, there were only limited cellular defects in peripheral lymphoid organs, mainly restricted to mice with severe disease. The cellular changes in GCase deficiency were accompanied by elevated Th1 and Th2 cytokines that also tracked with disease severity. Finally, the proliferation of GCase-deficient HSCs was inhibited significantly by both GL1 and Lyso-GL1, suggesting that the “supply” of early thymic progenitors from bone marrow may, in fact, be reduced in GBA deficiency. The results not only point to a fundamental role for GBA in immune regulation but also suggest that GBA mutations in GD may cause widespread immune dysregulation through the accumulation of substrates.

glucocerebrosidase | glucosylphosphoinositol | lysosomal storage disease

Gaucher disease (GD) is the most common lysosomal storage disorder in humans, which arises from defective acid β-glucosidase (lysosomal glucocerebrosidase; GCase) due to mutations in the GBA (glucosidase, β, acid) gene (1). Although GCase deficiency affects all cell types, overt lysosomal accumulation of the primary substrate glucocerebrosides (GL1) and a minor substrate, glucosylphosphoinositol (Lyso-GL1), is restricted to the cells of the mononuclear phagocyte lineage (1). Most classic manifestations of GD, notably hepatosplenomegaly, bone marrow infiltration, cytopenia, and other organ involvement, have, thus, been attributed to the accumulation of activated macrophages engorged with GL1 and Lyso-GL1. However, the neurodegenerative manifestations of the rare neuronopathic forms of GD are thought to arise from direct toxic effects of accumulated lipids in neurons (2).

Increasing clinical evidence suggests that the pathophysiology of classic GD is more complex and involves system-wide dysfunction of cell types other than macrophages (3–5). The involvement of immune cells has been implicated, but the underlying molecular defect is poorly understood (6–9). For example, GD has been associated with impaired host-defense against microbial infections (10, 11), up-regulation of T-helper (Th1) and Th2 cytokines (6, 12, 13), dysfunction of monocytes (14), and an increased risk for lymphoid malignancies, most strikingly for multiple myeloma (15–19). The reduced differentiation of MSCs resulted in impaired osteoblastogenesis and bone formation, causing severe osteoporosis. Here, we report the full characterization of dysfunctional immune cells in the lymphoid organs, altered serum cytokine levels, and the effects of GL1 and Lyso GL1 on HSC differentiation. Importantly, we show that disease severity in the GBA-deficient mouse correlates with the immune defect.

Results

Deletion of LuxP-flanked GBA gene in Mx1-Cre recombinase mice was accomplished by i.p. injection of polyI:polyC (3). GBA deletion resulted in reduced GCase activity in blood leukocytes (Fig. S1A). There was a ~40% reduction in Mx1-Cre+/−/GBA−/− mice (lacking the GBA gene on one allele, with the other allele floxed) compared with Mx1-Cre+/−/GBA+/− mice. However, >95% reduction was noted in Mx1-Cre+/−/GBA−/− and Mx1-Cre+/−/GBA−/− mice in which the gene was deleted, respectively, on a GBA floxed or heterozygote null background (hereafter referred to as KO mice).

The GBA KO mice displayed progressive cytopenia and organomegaly, with extensive infiltration of the lymphoid organs by lipid-laden macrophages (Gaucher cells) in the bone marrow and the thymus, as well as the spleen and the lymph nodes (3). Fig. S1D shows that, in addition to infiltration by Gaucher cells in the liver and the spleen, there was impressive extramedullary hematopoiesis indicated by presence of megakaryocytes and erythroid precursors. Interestingly, these sites of extramedullary hematopoiesis tended to form islands of lipid-laden Gaucher macrophages surrounded by erythroid precursors (Fig. S1D). In the thymus, fluid infiltration by lipid-laden Gaucher macrophages resulted in the disruption of normal architecture and poor demarcation between the cortex and the medulla (Fig. S1C).

Because the extent of splenomegaly was variable, we classified disease severity based on spleen weight normalized to body weight, expressed as a multiple of spleen weight in age-matched control [wild-type (WT)] mice (mean WT spleen weight, 0.29 ± 0.07% body weight). Disease was considered mild, moderate, or severe, respectively, when spleen weight was <3×, 3–6×, or >6× normal. Because of bone marrow infiltration, we examined whether spleen size correlated with the severity of anemia. Only a weak correlation was noted (r² = 0.24; P = 0.002) (Fig. S1E). This is interesting, although not unexpected, as in the mice, the spleen functions as the major site for stress erythropoiesis and, thus, likely compensates for the anemia caused by inflammation

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and marrow infiltration by Gaucher macrophages. In contrast, in humans, splenomegaly itself frequently leads to anemia attributable to excessive sequestration and clearing of RBCs.

We hypothesized that infiltration of primary and secondary lymphoid organs would lead to distinct immune dysregulation. We, therefore, analyzed immune cell subsets from thymus, spleens, and lymph nodes of 6-mo-old GBA KO mice by cell-surface flow cytometry. We have previously reported the striking changes in immune cell composition of the GBA KO thymus, which was confirmed in the current study (3). Namely, there was a significant increase of total cluster of differentiation (CD)8+ (cytotoxic T cells), B220+ (B cells), CD11b+ (macrophages), natural killer (NK)T+ [NK1.1+ T-cell receptor (TCR)β+] NK-T cells, and MHCI (1A/1E+) cells (dendritic cells and B cells). Moreover, an increase in thymic CD4+ CD44+ (P < 0.04) and 1A/1ECD11c+ cells (P = 0.03) (Fig. 1) suggests enhanced antigen presentation by T cells and dendritic cells, respectively. In contrast, CD8+ T cells bearing the early activation marker CD69 (CD8+CD69+) and regulatory T cells (CD4+CD25+) were significantly decreased (both, P < 0.01) (Fig. 1A).

Changes in immune cell composition in GBA KO mice were organ-specific. First, in contrast to the striking defects in thymic cell composition in GBA KO mice, there were only limited alterations in peripheral lymphoid organs. In mild and moderate disease, increases were restricted to CD11b+ cells in lymph nodes (P = 0.06) and CD44+ cells in spleen (P = 0.068) (Fig. 1B and C). Second, in contrast to the thymus, the splenic CD8+CD44+ ratio was diminished in KO mice (Fig. 1C). Although these changes may not be related, reduced naïve splenic CD8+ cells are known to arise from impaired thymic T-cell development. Third, there was a reciprocal change, notably increases and decreases, respectively, in B220+ cell populations in the thymus (3) and spleen of GBA KO mice (Fig. 1D). This could be attributable to a shift of B-cell maturation from the bone marrow and the spleen toward the thymus in GBA1 deficiency (below).

We compared the altered immunophenotype of mild vs. moderate or severe disease in GBA KO mice. In mild disease, cell alterations were confined primarily to the thymus, with minimal changes in the spleen, lymph nodes, or bone marrow. This suggests that the earliest stages of GD likely involve thymocyte alterations. Notably, thymic composition correlated with GD severity (Fig. 2). The CD11c+ and CD11b+ cell populations were increased in severe disease (Fig. 2A). However, the most striking and progressive changes with worsening GD severity occurred in MHCI+ (1A/1E+)−expressing and B220+ (CD45R+)−expressing thymocytes (Fig. 2B and C). Both subsets increased by >10-fold in severely affected vs. unaffected mice with intermediate effects in moderate disease. Interestingly, T-cell subsets defined by CD4 and CD8 expression shifted with increasing disease severity, favoring CD4+CD8− [(double-negative (DN)) and single-positive (SP) CD4+ T-cell populations over CD4+CD8− (double-positive (DP)), suggesting a significant defect in T-cell maturation (Fig. 2D).

Similarly, CD44+, an early thymocyte differentiation and activation marker, was highly expressed on thymocytes in severe GD (Fig. 2E). We further analyzed four major subgroups, namely CD4+CD8−, CD4−CD8+, CD4+CD8+, and CD4−CD8− for CD44 expression. This showed a near-complete loss of CD44+, DP CD4+CD8+, and single-positive (SP) CD4+CD8− with an increase in CD4+CD8−, DN CD4−CD8+, and SP CD4−CD8− cells (Fig. 2F). Of note is that the DN CD4−CD8− subset may also include B cells and other small accessory cells. Moreover, CD25+ expression on this population of DN T cells decreased with increasing disease severity (Fig. 2G). This gradual loss of CD25+ with severity, taken together with increased CD44+ expression, is consistent with a block at the “lineage-negative” stage of T-cell development. Furthermore, the up-regulation of CD80 (Fig. 2H) and 1A/1E+ (Fig. 2I) in severe disease is suggestive of enhanced antigenic stimulation in affected GBA KO mice.

As the disease progresses to yield severely sick mice immune dysregulation generalizes to peripheral organs paralleling changes noted early in the thymus. For example, although there was no substantial change in splenic B220+ cells, there was a marked expansion of B220+CD11b+CD11c+ cells (Fig. 3A). Considering that the T-cell ratio is decreased in severely sick spleens (Fig. 3B), the latter subgroup is unlikely to be of a T-cell origin. More interestingly, whereas splenic 1A/1E+ cells did not change significantly, there was an increase in 1A/1E− cells, this almost mirrors B220+ expression (cf., Fig. 3A and C). Whether or not these two subgroups are of the same lineage and share functions in the context of GD remains to be elucidated.

Also noted was a decrease in splenic CD4+ and CD8+ T cells in severely sick mice (Fig. 3D). This is consistent with decrements in CD4+ and CD8+ populations in GD patients (7). However, it
contrasts with the dramatic increase of this population in the thymus of severely sick GD mice (cf., Figs. 2 vs. 3D). Thus, in addition to inhibited T-cell maturation, the migration of mature thymocytes may also be blocked in severely sick mice. Another possibility is that these CD4^+ and CD8^+ T cells, although mature, may in fact be under-activated from the disease and may thus accumulate in the thymus. Finally, CD11b^+ macrophages and NK1.1^+γ/δTCR^+ (NKT) cells were increased and decreased, respectively, in severely sick mouse spleens (Fig. 3 E and F).

In contrast to the findings in the spleen, severe GD did not affect lymph node T-cell subsets (as determined by CD4 and CD8 expression) or the frequency of 1A/1E^+CD86^- cells (cf., Fig. 3 G and H vs. D and C, respectively). However, the CD4^+ and CD8^+ subpopulations showed early activation as determined by CD69 expression [Fig. 3 I and J; cf., Fig. 4 (thymus)]. Nonetheless, as in the spleen, there was a progressive decline in the TCR ratio as a function of disease severity (cf., Fig. 3 K vs. B). Finally, CD44^+ expression in lymph nodes showed no change in severe disease, contrasting the increases and decreases noted in the thymus and the spleen, respectively (Fig. 3L; cf., Fig. 1 A and C).

We have previously reported the cytokine profile of pooled GBA deficient mice (3). Fig. S2 shows plasma cytokine levels stratified by disease severity. Compared with mild disease, several cytokines were elevated in mice with moderate or severe disease: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-9, IL-10, IL-12 (p40), IL-13, IL-17, macrophage colony-stimulating factor (M-CSF), eotaxin, IFNγ, macrophage inflammatory protein (MIP)-1α, and MIP-1β (Fig. S2).

Finally, we sought to explore the mechanism underlying the profound immune cell and cytokine dysregulation in GBA KO mice. There was a weak, but significant correlation between spleen size and the accumulated major GCase substrate, namely glucosylceramide (GL1, r^2 = 0.22, P = 0.0139) but somewhat stronger correlation with the minor substrate, glucosylsphingosine (Lyso-GL1; r^2 = 0.48; P = 0.00004) (Fig. 4 A and B). As early lymphoid development takes place in the bone marrow, and early lymphoid progenitors populate thymus and spleen from the bone marrow, we sought to first identify the effects of GBA1 deficiency on hematopoietic stem and progenitor populations in spleen and bone marrow (Fig. S3 and Fig. S4). There was an increase and decrease in short-term HSC (ST-HSC) (lin^-kit^+sca^-CD150^-CD48^-) and multipotent progenitor (MPP) (lin^-kit^+sca^-CD150^-CD48^-) populations, respectively, in the spleens of GBA KO mice, without significant differences in the long-term HSC (LT-HSC) (lin^-kit^-sca^-CD150^-CD48^-) population (Fig. S3).

As bone marrow cells showed no differences in these progenitor populations prior to development of overt disease (Fig. S4), we chose to challenge cells with the two substrates GL1 and Lyso-GL1, and study proliferation ex vivo. Bone marrow from groups of five to six mice was pooled and treated ex vivo with GL1 (8 μM) or Lyso-GL1 (2 μM). Cell number was determined on days 0, 5, and 10. Compared with control mice that were relatively insensitive to GL1 and Lyso-GL1, both lipids profoundly inhibited, by >50%, the proliferation of HSC precursors from GBA4 KO mice (Fig. 4C). This suggests, but does not prove, that HSC and progenitor cell populations from bone marrow, although unaffected per se, are susceptible to inhibition by both accumulated GCase substrates, in particular Lyso-GL1. This would support our prior report of inhibitory effects of these substrates on MSC differentiation (3).

Discussion

GD is traditionally classified as a macrophage-specific sphingolipidosis (1). However, several clinical manifestations, such as gammopathies (15, 16), predisposition to infections (10, 11), and lymphoid malignancies (15–18), suggest a more diverse dysregulation of the immune system, surely beyond the macrophage. Notably, we have shown that the immune cell composition of the thymus is significantly altered in GBA4 KO mice (3). Major changes include elevated CD4^+ cells, relative depletion of CD4^+CD8^- precursors, and increased antigen presentation.
Here, we describe these changes in the context of the natural history of the disease through correlations with disease severity. Furthermore, we show that peripheral immune organs, such as the spleen, lymph nodes, and bone marrow, display milder, and often interestingly reciprocal, changes, suggesting that the thymus may be an early player in GD pathogenesis.

We speculate that dysregulated thymocyte maturation may arise from abnormal antigen presentation. This is suggested by significant increases in B220^+ and MHCII expression in GBA KO mice. Restricted to thymic epithelial and antigen-presenting cells (APCs), such as dendritic cells, B cells, and macrophages, MHCII plays a crucial role in positive and negative selection

![Fig. 3. Changes in immune cell composition in the spleen and lymph nodes are less profound and occur only in severe disease. Gated FACS analysis of immune cells from the spleen (A–F) and lymph nodes (superficial cervical, axillary, mesenteric, and inguinal nodes) (G–L) of GBA1-deficient (GD) vs. control mice, showing the effect of moderate and severe disease (as shown), classified on the basis of spleen weight (see Results). Analysis was performed using either double or single markers, as shown. At least six mice were used.](image-url)
CD4 expression of the early activation markers CD44 and CD25 on β before TCR an early developmental block, but also by the subsequent apo-
pisosis (27, 28). This suggests that thymic B220+ cells may also regulate
the thymus of patients with myasthenia gravis (26). However, T-cell apoptosis. If so, the dramatic loss of CD4+CD8+ DP cells
r 0.014; Lyso-GL1, marrow stem cells isolated from WT or GBA1-de
0.29 ± 0.07% body weight). Statistics were as follows: GL1, r2 = 0.22 and P = 0.014; Lyso-GL1, r2 = 0.48 and P = 0.00004. (C) Proliferation assay on bone
marrow stem cells isolated from WT or GBA1-deficient (Mx1-Cre+/−/GBA1−/+ (GD) mice (n = 5 mice; bone marrow pooled) and treated with either vehicle
(DMSO, red), GL1 (green), and Lyso-GL1 (blue). The resultant progenitor, LT-
HSCs, ST-HSCs, and MPP were cultured and cell numbers counted on days 0, 5, and 10 (see Materials and Methods).

During thymocyte maturation. Marked increases in thymocyte
MHCII expression in GBA KO mice might, thus, impact cell fate. MHCII may enable DP thymocyte depletion through pathological apoptosis, while stimulating positive selection during T-cell maturation (20, 21). The latter may underlie the accumu-
lation of mature CD4+ and CD8+ single-positive cells in GBA KO thymocytes in severe disease.

The increased thymic MHCII expression in GBA KO mice was accompanied by an up-regulation of B220+ cells. Although B220+MHCII+ cells represent activated B cells, it is unclear whether they migrate to the thymus from the bone marrow as active cells or as lymphoid precursors. Although we have not positively identified DP cells in the thymus, a parallel increase in the expression of the two markers is highly suggestive of B-cell activation. Activated B cells are thought to regulate thymic development either by acting as APCs (22–24) or by coordinating dendritic cell negative selection (25). Increased activated B cells are seen in the thymus of patients with myasthenia gravis (26). However, B220 expression is also increased on apoptotic immature T cells (27, 28). This suggests that thymic B220+ cells may also regulate T-cell apoptosis. If so, the dramatic loss of CD4+CD8+ DP cells in severely affected GBA KO mice can be explained not only by an early developmental block, but also by the subsequent apo-
pisosis of immature T cells. That this T-cell impairment begins before TCR β-chain rearrangement is testified by altered expression of the early activation markers CD44 and CD25 on CD4+CD8+ T cells.

Fig. 4. High levels of lipid substrates impair hematopoiesis. (A and B) Cor-
relation of splenomegaly with splenic glucosylceramide (GL1) (A) or glu-
cosylsphingosine (Lyso-GL1) (B) levels. Spleen weight expressed as a multiple (N) of spleen weight of age-matched control mice (control spleen weight = 0.29 ± 0.07% body weight). Statistics were as follows: GL1, r2 = 0.22 and P = 0.014; Lyso-GL1, r2 = 0.48 and P = 0.00004. (C) Proliferation assay on bone

NK1.1+TCRγδ+(NKT) cells exist within CD4+CD8−, CD4−CD8+, and DP thymocyte populations and are classically activated by lipid antigens via CD1d (29–31). Intriguingly, although the NKT cell population is increased in GBA KO mice, CD1d was barely detectable in the thymus and underwent no change in the lymph node or spleen. This calls for further studies on the significance of the elevated NKT cell population in GBA1 deficiency.

The altered immune cell composition of lymphoid organs in GBA KO mice was accompanied by elevated proinflammatory cytokines, some of which tracked with disease severity. This complex pattern, in which anti-inflammatory cytokines, such as IL-13, were elevated in parallel, suggested the activation of both innate and adaptive immune systems. IL-13, in particular, is one archetype of the Th2 response (32). Further underscoring a role of Th2 response in GD are the reported elevations in chitori-
sidosase (33) and alternatively activated macrophages (34), as well as the occurrence of fibrosis (35). Microarray studies con-
firm marked increases in chitinase-like molecules in spleens from GBA KO mice (3). Further studies should thus be focused on the relative importance of Th1 vs. Th2 responses in pathogenesis of GD (36).

How exactly thymic cell populations become the initial targets of GBA deficiency remains unclear. One hypothesis is that bone marrow dysregulation precedes changes in the thymus. The thymus, albeit critical for T-cell maturation, does not have the ca-
pacity to self-renew. It requires the recruitment of progenitors from bone marrow (37). Most notably, biochemical inhibition of acid β-glucosidase in vitro impairs marrow hematopoiesis (38). Here, we show that the two accumulated GCase substrates, GL1 and Lyso-GL1, inhibit proliferation of HSC derived by GBA KO mice, although it seems that the progenitor population within bone marrow is unchanged in early stages of the disease. We, thus, propose that the antiproliferative action of GL1 and Lyso-GL1 on HSCs as the disease progresses may reduce the “supply” of early thymic progenitors from bone marrow. Additionally, we provide evidence for impairment of T-cell maturation and egress of mature thymocytes in advanced GD. This prompts us to determine whether dense Gaucher cell infiltration in the thymus, as described hitherto, as well as in human GD (39), leads to elevated local concentrations of LysoGL1 and of sphingosine, thereof, via the action of neutral glucocerebrosidase, GBA2 (40, 41). In this sce-
nario, the abolition of sphingosine-1-phosphate gradient could be envisioned to block lymphocyte egress from the thymus (42).

In conclusion, we show that the conditional deletion of the GBA gene in hematopoietic cells leads to profound, widespread, and organ-specific dysfunction of immune cells. The most striking
dysregulation was noted in the thymus, with features of impaired T-cell maturation, aberrant B-cell recruitment, enhanced antigen presentation, and impaired egress of mature thymocytes. These effects can, at least in part, be attributed to the antiproliferative effects of the accumulated lipids (41). Similar immune phenotypes have been described in other inherited lipidosis (43, 44). Thus, on a broader note, the studies underscore the importance of defining the role of aberrant immunity and the interactions between lipids and immune cells in genetic lipidosis and reversal of immune
dysregulation as an additional therapeutic target (45).

Materials and Methods

Animals. The generation of conditional GBA KO mice has been described previously (3). Six-month-old mice were classified as having mild, moderate, or severe GD based on extent of splenomegaly (see Results). Thymi, spleens, and lymph nodes were removed following euthanasia from six GBA1 KO mice aged ~6 mo. A group of age-matched, uninduced littermates were used as controls. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale Medical School.

Immunophenotyping. Immune-cell profiling of the lymphoid organs (thymus, spleen, and lymph node) from GBA KO and control mice was performed using anti-mouse CD16/CD32 (Mouse BD Fc Block; BD Biosciences) and antibodies obtained from BD Bioscience, Accurate Chemical and Scientific, eBioscience, and Abcam. Dissected lymphoid organs were placed in a 70-μm
sterile cell strainer (Fisher Scientific) in a Petri dish containing 5 mL of cold 5% (vol/vol) FCS/PBS. Single-cell suspensions were prepared by grinding using a 1-ml BD syringe plunger. Following centrifugation, the cells were resuspended in 5 mL of RBC Lysis Buffer (Qiagen Scientific). After washing twice with 5% FCS/PBS, cells were counted to $1 \times 10^6$/mL and saved on ice.

FACS analysis was performed as described in SI Methods.

**Bone Marrow Stem Cell Isolation and Proliferation Assays.** Bone marrow was isolated and pooled from hind legs and spine from five or six GBA KO mice and littermate controls. The marrow was lineage depleted using a $\mu$MabTiotic Mouse Lineage Depletion Mixture (BD Biosciences). Lineage-depleted cells were stained with Mouse Lineage Cell Detection Mixture-Biotin (Miltenyi Biotin), streptavidin AEC-Fluor 780 (biotin), 7-aminoactinomycin D (7AAD) (eBioscience), PE anti-mouse Sca-1 (BioLegend), APC anti-mouse c-Kit (BioLegend), and FITC anti-mouse CD48 (BD Biosciences). Further analysis was performed as described in SI Methods.

**Assays.** Serum cytokine levels were measured using a Bio-Plex Mouse Cytokine 23-Plex Panel (Bio-Rad) per the instructions of the manufacturer. For acid sphingolipid activity assay, WBs were isolated from 100 $\mu$L of blood obtained via tail or retro-orbital bleeding. Enzyme activity was measured as described previously (3).

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FACS Analysis of Immune Cells. For FACS analysis, 100 μl (1 x 10^6 cells) was added to each well of a 96-well U-bottom plate followed by centrifugation and resuspension in 50 μl FACS buffer (PBS with 3% FCS and 0.05% sodium azide). The cells were then incubated (20 min at 4°C) with purified rat anti-mouse CD16/CD32 antibody (Mouse BD Fc Block, BD Biosciences) to prevent nonspecific Fc receptor binding. The cells were stained (30 min at 4°C) with appropriate combinations of fluorochrome-tagged rat-anti mouse antibodies. Following three washes with FACS buffer, stained cells were resuspended in 400 μl of fixing buffer (3% paraformaldehyde in PBS), and analysis was performed on a FACS Calibur flow cytometer (Beckton Dickinson). Typically, 40,000 total events were acquired for analysis with WinMDI 2.8 software.

Analysis of Bone Marrow Cells and Proliferation Assays. The stained cells were sorted on MoFlo (Beckman Coulter) for progenitors (Lin^−^Kit^+^Sca^−^), LT-HSCs (Lin^−^Kit^+^Sca^+^CD150^+^CD48^−^), ST-HSCs (Lin^−^Kit^+^Sca^+^CD150^−^CD48^+^), and multipotent progenitors (Lin^−^Kit^+^Sca^−^CD150^−^CD48^+^). Cells were plated in 48-wells in the complete medium supplemented with 10% FCS, antibiotics, 2 mM L-glutamine, 1 mM NEAA, supplemented with murine IL-3 (10 ng/ml), IL-6 (10 ng/ml), SCF (50 ng/ml), TPO (10 ng/ml), and human Flt3 L (50 ng/ml) (all from Peprotech). After 24 h of culture, stem and progenitor cells were aliquoted in equal numbers into three wells each and treated with GL1 (8 μM) or Lyso-GL1 (2 μM) (both from Matreya). Cell proliferation was assayed by trypan blue dead cell exclusion using a hemacytometer on d 0, 5, and 10.
Fig. S2. Cytokine levels in GBA1-deficient mice correlate with disease severity. Plasma cytokine levels in unaffected (control) GBA1-deficient (GD) mice and those with moderate or severe GD. Assay is described in Materials and Methods; data are presented as pg/mL (means ± SD). Statistical analysis was performed using Student’s t test with Bonferroni’s correction (n = 3–6 mice; *P < 0.05; **P < 0.01).

Fig. S3. Changes in HSC and erythroid progenitor populations in spleens of GBA1-deficient mice. Gated FACS analysis of precursor populations from the spleen of GBA1-deficient (GD) and control mice (see Results) on HSC and erythroid progenitor populations. LSK, Lin−Sca+kit+ cells; MPP, multipotent progenitors. Notable is that, whereas the LSK cells are unaffected, ST-HSC population drops significantly in GD mice. There also appears to be an increase in maturing erythroid lineage cells. Analysis was performed using either double or single markers, as shown. At least six mice were used.
Gated FACS analysis of bone marrow cell populations from GBA1-deficient (GD) versus control mice at 3 mo of age prior to development of disease. At least six mice were used. CFU-E, colony-forming unit-erythroid; C/GMP: common myeloid and granulocytic/monocyte progenitors; LSK, Lin−Sca1+kit+ cells; LT-HSC, long-term hematopoietic stem cells (HSC); MKP, megakaryocyte progenitors; MPP, multipotent progenitors; Pre-E, early erythroid progenitors; Pre-Meg-E, Megakaryocyte-erythroid precursor; ST-HSC, short-term HSC.

**Fig. S4.** Gated FACS analysis of bone marrow cell populations from GBA1-deficient (GD) versus control mice at 3 mo of age prior to development of disease. At least six mice were used. CFU-E, colony-forming unit-erythroid; C/GMP: common myeloid and granulocytic/monocyte progenitors; LSK, Lin−Sca1+kit+ cells; LT-HSC, long-term hematopoietic stem cells (HSC); MKP, megakaryocyte progenitors; MPP, multipotent progenitors; Pre-E, early erythroid progenitors; Pre-Meg-E, Megakaryocyte-erythroid precursor; ST-HSC, short-term HSC.