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Neutropenia in type Ib glycogen storage disease

Janice Y. Chou^{1,*}, Hyun Sik Jun¹, and Brian C. Mansfield^{1,2}

¹ Section on Cellular Differentiation, Program on Developmental Endocrinology and Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

² Correllogic Systems, Inc., Germantown, MD 20876

Abstract

Purpose of review—Glycogen storage disease type Ib (GSD-Ib), characterized by disturbed glucose homeostasis, neutropenia, and neutrophil dysfunction, is caused by a deficiency in a ubiquitously expressed glucose-6-phosphate transporter (G6PT). G6PT translocates glucose-6-phosphate (G6P) from the cytoplasm into the lumen of the endoplasmic reticulum, where it is hydrolyzed to glucose either by a liver/kidney/intestine-restricted glucose-6-phosphatase- α (G6Pase- α) or by a ubiquitously expressed G6Pase- β . The role of the G6PT/G6Pase- α complex is well established and readily explains why G6PT disruptions disturb interprandial blood glucose homeostasis. However, the basis for neutropenia and neutrophil dysfunction in GSD-Ib are poorly understood. Recent studies that are now starting to unveil the mechanisms are presented in this review.

Recent Findings—Characterization of G6Pase- β and generation of mice lacking either G6PT or G6Pase- β have shown that neutrophils express the G6PT/G6Pase- β complex capable of producing endogenous glucose. Loss of G6PT activity leads to enhanced ER stress, oxidative stress, and apoptosis that underlie neutropenia and neutrophil dysfunction in GSD-Ib.

Summary—Neutrophil function is intimately linked to the regulation of glucose and G6P metabolism by the G6PT/G6Pase- β complex. Understanding the molecular mechanisms that govern energy homeostasis in neutrophils has revealed a previously unrecognized pathway of intracellular G6P metabolism in neutrophils.

Keywords

Glycogen storage disease type I; glucose-6-phosphate transporter; glucose-6-phosphatase; neutropenia; apoptosis; endoplasmic reticulum

Introduction

Glycogen storage disease type Ib (GSD-Ib, MIM232220) is an autosomal recessive disorder with an incidence of 1 in 500,000 [1, 2]. Predominantly considered a metabolic disorder characterized by hypoglycemia, excessive glycogen accumulation in the liver and kidney, and abnormal metabolic serum profiles, GSD-Ib is also an immune disorder characterized by neutropenia and myeloid dysfunctions [1, 2]. While the genetic basis of the disease, mutations in the glucose-6-phosphate transporter (*G6PT* or *SLC37A4*) gene, is well established [3, 4], only the metabolic component of the disease is understood biochemically.

*Correspondence should be addressed to: Janice Y. Chou, Building 10, Room 9D42, NIH, 10 Center Drive, Bethesda, MD 20892-1830, Tel: 301-496-1094, Fax: 301-402-6035, chouja@mail.nih.gov.

Most cells derive their energy from glucose taken up across the cell membrane by diffusion facilitated by the glucose transporters [5]. Inside the cell, glucose is converted to the membrane-impermeable molecule glucose-6-phosphate (G6P) by either hexokinase or glucokinase, trapping the glucose inside the cell. In the tissues responsible for maintaining interprandial blood glucose homeostasis – primarily the liver and kidney – G6P derived from glycogenolysis or gluconeogenesis, is transported across the endoplasmic reticulum (ER) membrane by the G6PT [1, 2]. Compartmentalized in the ER lumen, G6P is hydrolyzed to glucose and released. There are two enzymatically active G6P hydrolases embedded in the ER membrane with their active sites facing into the lumen [6–8]. One, glucose-6-phosphatase- α (G6Pase- α or G6PC), is restricted to expression in the liver, kidney and intestine [1, 2]. The other, G6Pase- β (G6PC3), is expressed ubiquitously [9, 10]. Deficiencies in G6Pase- α cause GSD type Ia (GSD-Ia) [1]. In contrast, deficiencies in G6Pase- β do not cause GSD, but were recently shown to cause a severe congenital neutropenia syndrome [11] of unknown incidence.

Both G6PT and G6Pase- α must be functionally coupled to transport and hydrolyze G6P to glucose [4, 12]. A detrimental mutation in either protein prevents the other functioning efficiently [1, 2]. Surprisingly, despite the G6PT/G6Pase- α inter-dependence, GSD-Ia patients do not suffer from immune dysfunctions, while GSD-Ib patients do, suggesting the immune disorders in GSD-Ib reflect the role of G6PT in cells expressing G6Pase- β but not G6Pase- α [10, 13].

Recently it was shown that G6Pase- β can couple with the G6PT to hydrolyze G6P to glucose [10, 13]. Mouse lines deficient in G6PT [14] or G6Pase- β [13] have been generated to examine the biology of the G6PT/G6Pase- β complex, revealing that G6P translocation and metabolism in the ER are critical for normal neutrophil functions.

Clinical phenotype

Mutations in the *G6PT* gene of approximately 160 GSD-Ib patients have been studied [3]. All mutations decrease or completely abolish cytoplasmic G6P transport into the ER for hydrolysis by G6Pase- α or G6Pase- β , disrupting endogenous glucose production [1, 2]. In gluconeogenic organs that co-express G6PT and G6Pase- α , this disruption leads to the metabolic phenotype common to GSD-Ia and GSD-Ib characterized by hypoglycemia, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, lactic acidemia, and growth retardation [1, 2]. Long-term presentations include hepatic adenomas with risk for malignancy and renal failure. In non-gluconeogenic organs that co-express G6PT and G6Pase- β , the impacts of the mutations are less well understood. The most obvious impact is seen in neutrophils, where the disruption is manifested as neutropenia and neutrophil dysfunction [1, 2, 13, 14]. However, the picture of glucose metabolism that is emerging from these cells suggests there may be ramifications in other tissues that are not yet recognized.

Neutrophils from GSD-Ib patients exhibit impaired mobility, chemotaxis, and calcium mobilization, as well as diminished respiratory burst and phagocytotic activities [1, 2, 15, 16]. As a result, recurrent bacterial infections are commonly seen in GSD-Ib patients and up to 77% of patients manifesting neutropenia also develop inflammatory bowel disease (IBD), indistinguishable from idiopathic Crohn disease [17, 18]. Oral manifestations of GSD-Ib include dental caries, gingivitis, periodontal disease, delayed dental maturation and eruption, oral bleeding diathesis, and oral ulcers [19]. A study evaluating the hypothalamus-pituitary-thyroid axis of 7 GSD-Ib patients showed that 57% had thyroid autoimmunity and hypothyroidism [20]. Seronegative myasthenia gravis may be another complication [21].

In a Collaborative European Study of 57 GSD-Ib patient, 54 suffered from neutropenia. Of these, 64% were first neutropenic before age 1 year, and a further 18% between ages 6 to 9 years [17]. Neutrophil function was examined in 18 neutropenic patients and all were abnormal [17]. In a medical database of North American that consists of 36 GSD-Ib patients with a mean age of 13.7 years, it was found that IBD was diagnosed at an average age of 8.7 years [18]. All patients with IBD had neutropenia [17, 18].

Genetics

Human *G6PT* is a single copy gene consisting of 9 exons [4, 22] on chromosome 11q23 [23]. There are two alternately spliced transcripts, *G6PT* [3, 4] and the variant *G6PT* [*vG6PT*, 24, 25] which contains an additional exon-7 sequence [4, 22]. *G6PT* is ubiquitously expressed [26] while *vG6PT* is expressed primarily in the brain, heart, and skeletal muscle [25]. Seventy-nine separate *G6PT* mutations have been identified in ~ 160 GSD-Ib patients studied to date [3, <http://www.hgmd.cf.ac.uk/ac/gene.php?gene=SLC37A4>]. These include 32 missense, 11 nonsense, 19 insertion/deletion, and 17 splicing mutations, which appear scattered throughout the coding and exon-intron junction regions. These mutations show some ethnic variability. In Caucasian patients (216 alleles), c.1042_1043delCT and p.G339C are the prevalent mutations, representing 31% and 14%, respectively. In Japanese patients (38 alleles), p.W118R is the prevalent mutation, accounting for 37%. The number of known alleles in other ethnicities is too low to draw clear statistical conclusions.

Of the 160 patients studied to date, 7 patients carrying deleterious *G6PT* mutations are not reported to have manifested either neutropenia or frequent infections [27–30]. A genotype-phenotype study with a cohort of 22 GSD-Ib patients carrying 16 different *G6PT* mutations, including 9 patients homozygous or compound heterozygous for nonsense mutations failed to show any correlation between individual mutations and the presence of neutropenia, bacterial infections and systemic complications [28]. While these numbers are low, they do suggest there may be modifiers that can compensate for or stabilize low level expression *in vivo*.

Function of the G6PT protein

Human *G6PT* and *vG6PT* are hydrophobic proteins composed of 429 and 451 amino acids, respectively [3, 4, 24, 25]. Human *G6PT* is anchored in the ER by 10 transmembrane domains [31, 32] (Fig. 1). *G6P* transport across the ER membrane and *G6P* hydrolysis in the ER lumen are tightly coupled processes, with *G6Pase- α* activity required for efficient *G6P* transport [4, 12]. In the absence of *G6Pase- α* activity there is only a low basal level of *G6P* uptake activity [4]. A *G6P* transport activity assay has been developed based upon cells co-expressing both *G6PT* and *G6Pase- α* [4, 33, 34]. Using this assay, mutations identified in the *G6PT* gene of GSD-Ib patients were shown to decrease or completely abolish *G6P* transport activity, establishing the molecular basis of this disorder [4, 33, 34]. The *vG6PT* also functions as a *G6P* transporter [25], although little more is known of this transporter.

G6PT belongs to the organophosphate:phosphate antiporter family of the major facilitator superfamily [35]. Using reconstituted proteoliposomes expressing only *G6PT*, *G6PT* was shown to be a phosphate-linked antiporter transporting *G6P* into the ER lumen and phosphate out [36, 37].

Treatment and prognosis

The metabolic abnormalities of GSD-Ib can be adequately controlled with dietary therapies [38–40] that maintain normoglycemia and enable patients to attain near normal growth and pubertal development, with fewer complications as they age. However, dietary therapies fail

to improve myeloid functions. For patients exhibiting persistent, therapy-refractory hypoglycemic episodes, more invasive therapies have been investigated. In 7 GSD-Ib patients orthotopic liver transplantation (OLT) was assessed [41–46] and in 1 patient hepatocyte transplantation was performed [47]. Both transplantations improved the metabolic abnormalities for all patients. Of the 7 patients receiving OLT, 5 showed persistent neutropenia following transplantation [41, 42, 45, 46] but 3 patients reported less frequent infectious episodes [45, 46]. For the 2 patients in which OLT resolved neutropenia [43, 44], one exhibited improved myeloid function, evidenced by decreased infectious episodes and gastrointestinal symptoms, and normalized neutrophil function [44]. How the transplants corrected the immune functions is not clear. Secondary effects like “microchimerism”, where donor-derived leukocytes, present in the transplanted organ, migrate to the recipient’s bone marrow and assume their original function, can not be ruled out [48]. Indeed, Adachi *et al* detected 5% of chimerism in the recipient leukocytes [44]. The studies do suggest that a good metabolic control is beneficial for neutrophil function of GSD-Ib patients.

GSD-Ib patients manifesting neutropenia, and/or neutrophil dysfunctions that predispose to frequent infections and IBD are treated by granulocyte colony-stimulating factor (G-CSF) therapy which improves neutropenia and decreases the number and severity of infections [17, 18, 49]. The toxicity of G-CSF therapy, evaluated in a 3-year study consisting of 13 GSD-Ib patients with a mean age of 9.9 years, showed no significant short-term toxicity, although all did develop splenomegaly [50]. One major complication of patients with severe chronic neutropenia receiving G-CSF therapy is myelodysplasia/acute myeloid leukemia (AML) [51, 52]. The French Severe Chronic Neutropenia Registry collected data on 15 GSD-Ib patients, among them 14 patients received a median cumulative duration of 2.8 years of G-CSF therapy, and found no cases of AML [51]. However, three separate studies have reported the development of AML in GSD-Ib patients [53–55]. One did not receive G-CSF therapy [53] while the other two received G-CSF over a period of 6 [54] and 14 [55] years, respectively. The AML observed following 14 years of continuous G-CSF therapy is a classical monosomy 7 and translocation t(3;8)(q26;q24) [55]. This suggests that GSD-Ib patients receiving long-term G-CSF therapy can still develop AML and should receive regular bone marrow examinations.

A recent study has evaluated antioxidant supplementation for GSD-Ib patients using vitamin E [56]. While patients’ neutrophil function did not improve with this therapy, neutrophil counts increased and the frequency and severity of infections decreased, suggesting this might be a beneficial strategy.

The G6PT-deficient GSD-Ib (*G6pt*^{−/−}) mice mimic the full phenotype of human patients [14]. Studies using *G6pt*^{−/−} mice showed that wild-type mice harboring bone marrow (BM) transplants from *G6pt*^{−/−} mice manifest neutropenia and neutrophil dysfunctions [57]. These findings suggest that abnormal *G6PT* expression in BM and resident neutrophils leads to abnormal myeloid function and that BM transplantation may be considered for treating neutrophil complications in GSD-Ib. Recently, a GSD-Ib patient manifesting severe IBD and recurrent infections was treated by BM transplantation, which improved neutrophil function and the severity of IBD although a mild neutropenia persisted [58]. While an isolated case, the study suggests that myeloid abnormalities in GSD-Ib may improve following BM transplantation.

The feasibility of gene therapy for GSD-Ib has been evaluated using an adenoviral vector carrying human G6PT (Ad-hG6PT) to treat *G6pt*^{−/−} mice [59]. The study showed that Ad-hG6PT-infusion transiently corrects metabolic and myeloid abnormalities in *G6pt*^{−/−} mice.

While promising, longer term efficacy studies and extensive safety evaluations are required before proposing that gene therapy may be a viable future option for GSD-Ib patients

Neutrophil stress and apoptosis underlie myeloid dysfunction in GSD-Ib

In the past, the molecular basis of GSD-Ia and GSD-Ib has been framed by defects in the G6PT/G6Pase- α complex following the failure of either G6PT or G6Pase- α [1, 2]. Recently it has emerged that the framework should be in terms of the function of the G6PT/G6Pase- α complex in gluconeogenic tissues or the G6PT/G6Pase- β complex in the myeloid tissues. For decades, it was unclear whether the mechanism of immune deficiency in GSD-Ib is due to defects in G6P metabolism, or a completely different function of the “G6P” transporter. In 2003, a second G6P hydrolase activity, G6Pase- β , was identified [9, 10]. Both G6Pase- β and G6Pase- α have similar kinetic properties [10, 60] and topology, with their active sites localized on the luminal side of the ER membrane [6–8]. G6Pase- β also couples functionally with G6PT [10] to form an active complex that hydrolyses G6P to glucose (Fig. 1). More interestingly, G6Pase- β , like G6PT, is expressed ubiquitously [9]. Based on this, we hypothesized that the G6PT/G6Pase- β complex may produce endogenous glucose to some extent within the ER lumen in non-gluconeogenic cells and tissues, including neutrophils, and this endogenous glucose might play a role in neutrophil homeostasis and function [13, 61].

The lumen of the ER serves as a critical site for the maturation and assembly of proteins destined for the cell membrane or secretion [62]. When cells experience conditions that alter the ER environment such as glucose deprivation, protein folding can be dramatically affected leading to the accumulation of unfolded proteins in the ER. This in turn activates signal transduction cascades termed the unfolded protein response, characterized by the induced expression of ER chaperones [63–65]. If the stress is prolonged, apoptotic cell death occurs [63–65]. Therefore, if endogenous glucose production in the ER of neutrophils is critical, any deficiency in the G6PT/G6Pase- β complex should lead to ER stress and increased apoptosis. Two recent studies [13, 61] provide experimental evidence in support of the hypothesis that G6PT-deficient or G6Pase- β -deficient neutrophils, unable to produce endogenous glucose, would manifest enhanced ER stress and apoptosis which underlie neutropenia in these genetic disorders. One study showed that neutrophils from G6Pase- β -deficient (*G6pc3*^{−/−}) mice which are unable to hydrolyze endoluminal G6P to glucose (Fig. 1) manifested enhanced ER stress and apoptosis [13]. The second study delineated the underlying cause of GSD-Ib using G6PT-deficient neutrophils isolated from *G6pt*^{−/−} mice that are unable to translocate G6P from the cytoplasm into the lumen of the ER (Fig. 1). The study showed that *G6pt*^{−/−} neutrophils also exhibit ER stress, evidenced by increased production of the molecular chaperons, GRP78, GRP170, and protein disulfide isomerase [61]. Consistent with an earlier report, showing that human GSD-Ib neutrophils exhibited an increased rate of apoptosis [66], the *G6pt*^{−/−} neutrophils exhibited an increase in apoptotic cell numbers evidenced by increased Annexin V binding and caspase-3 activation [61].

In neutrophils, apoptosis is exquisitely regulated. Neutrophil apoptosis can be initiated by two main pathways, the extrinsic death receptor pathway that responds to extracellular stimuli and the intrinsic mitochondrial pathway that is controlled by the Bcl-2 family proteins [63]. The mitochondrial pathway is predominant during spontaneous neutrophil apoptosis [67]. Recent studies suggest that neutrophil apoptosis in *G6pt*^{−/−} neutrophils results from increased oxidative stress mediated, at least in part, by the intrinsic pathway [61]. In *G6pt*^{−/−} neutrophils, reactive oxygen species (ROS) production is stably elevated relative to the control neutrophils, despite the induction of Mn-superoxide dismutase [68] expression. Another contribution to ROS production in *G6pt*^{−/−} neutrophils may be via the oxidation and reduction of disulfide bonds in the ER [63, 69] as a result of upregulation of

protein disulfide isomerase that catalyzes thiol-disulfide exchange. The levels of Bax [70, 71] are elevated in the mitochondrial membrane of *G6pt*^{-/-} neutrophils over those seen in the control neutrophils. Consistent with this, membrane permeability increased, and correlated with the release of both Smac/Diablo and Omi/HtrA2 [72–74] into the cytosol of *G6pt*^{-/-} neutrophils. The activation of both caspase-3 and caspase-9 is also greater in the *G6pt*^{-/-} neutrophils. Therefore the intrinsic pathway is implicated strongly in the apoptosis seen in the *G6pt*^{-/-} neutrophils [61]. The similarities seen between *G6pc3*^{-/-} [13] and *G6pt*^{-/-} [61] neutrophils argue strongly that neutrophil dysfunction seen in GSD-Ib is not a result of a novel activity of G6PT in non-gluconeogenic organs, but rather a deficiency in endogenous glucose production caused by a defective G6PT/G6Pase-β complex (Fig 1).

The question remains why endogenous glucose production via the G6PT/G6Pase-β complex is vital for normal neutrophil functions. Clearly neutrophils lacking a functional G6PT are different from normal neutrophils. When neutrophils from normal subjects are incubated in the presence of extracellular glucose, their intracellular concentration of G6P increases 4.8-fold [75]. In contrast, neutrophils from GSD-Ib patients have a G6P level 73% that of control cells, and accumulate only 1.6-fold more when incubated in glucose [75]. As a result, G6P levels in neutrophils of GSD-Ib patients remained lower than those in neutrophils of normal subjects, being only 25% of the control level in the presence of glucose. Neutrophils require a constant supply of glucose for function/survival but are unable to produce glucose via gluconeogenesis [76]. Glucose in neutrophils is supplied primarily by uptake from the blood. Neutrophils from GSD-Ib patients have a decreased rate of glucose transport [77], which could explain the reduction in G6P accumulation in response to glucose but the role the glucose transporter has not been elucidated. Future studies should be directed towards the role of the G6PT/G6Pase-β-mediated ER uptake and hydrolysis of G6P in energy homeostasis and normal function of neutrophils.

Conclusion

GSD-Ib deficient in G6PT is an inherited genetic disorder that affects metabolic and myeloid functions. The immune deficiency in GSD-Ib is characterized by neutropenia, neutrophil dysfunction, and IBD. G6PT functions as an ER G6P transporter that transports cytoplasmic G6P into the lumen of the ER for hydrolysis to glucose either by the liver/kidney/intestine-restricted G6Pase-α or by a ubiquitously expressed G6Pase-β. There is emerging evidence that endogenous glucose production by the G6PT/G6Pase-β complex is essential for normal neutrophil functions. G6PT-deficient neutrophils unable to produce endogenous glucose experience enhanced ER stress, oxidative stress and apoptosis which are responsible for neutropenia manifested by GSD-Ib patients. GSD-Ib neutrophils are impaired in glucose uptake and have reduced intracellular levels of G6P, suggesting that energy metabolism may be impaired but the molecular mechanism by which G6PT deficiency affects neutrophil functions remains to be elucidated.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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Figure 1.

Proposed pathways for G6P metabolism in wild-type, *G6pt*^{-/-} and *G6pc3*^{-/-} neutrophils. Glucose transported into the cytoplasm via GLUT1 is metabolized by hexokinase to G6P which can participate in glycolysis, hexose monophosphate shunt (HMS), glycogen synthesis, or be translocated into the lumen of the ER by the G6PT. In wild-type neutrophils, G6P localized within the ER lumen can be hydrolyzed by G6Pase-β and the resulting glucose transported back into the cytoplasm to reenter any of the previously mentioned cytoplasmic pathways. In *G6pt*^{-/-} neutrophils which lack a functional G6PT, cytoplasmic G6P can not be translocated into the lumen of the ER and in *G6pc3*^{-/-} neutrophils, which lack a functional G6Pase-β, ER-localized G6P can not be recycled to the cytoplasm. The GLUT1 transporter, responsible for the transport of glucose in and out of the cell, is shown embedded in the plasma membrane. The G6PT transporter, responsible for the transport of G6P into the ER and G6Pase-β, responsible for hydrolyzing G6P to glucose and phosphate, are shown embedded in the ER membrane by 10 and 9 transmembrane helices, respectively.