Impaired Metabolic Function and Signaling Defects in Phagocytic Cells in Glycogen Storage Disease Type 1b

L. Kilpatrick, B.-Z. Garty, K. F. Lundquist, K. Hunter, C. A. Stanley, L. Baker, S. D. Douglas, and H. M. Korchak
Department of Pediatrics, University of Pennsylvania Medical School, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19104

Abstract

Patients with glycogen storage disease (GSD) type 1b (1b), in contrast to patients with GSD type 1a (1a), are susceptible to recurrent bacterial infections suggesting an impairment in their immune system. In this study, phagocytic cell (neutrophil and monocyte) respiratory burst activity, as measured by superoxide anion generation, oxygen consumption, and hexose monophosphate shunt activity, was markedly reduced in both neutrophils and monocytes from GSD 1b patients as compared with either GSD 1a patients or healthy adult control cells. Degranulation, unlike respiratory burst activity, was not significantly different in neutrophils from GSD 1b patients as compared with controls. Both neutrophils and monocytes from GSD 1b patients showed decreased ability to elevate cytosolic calcium in response to the chemotactic peptide f-Met-Leu-Phe. In addition, calcium mobilization in response to ionomycin was also attenuated suggesting decreased calcium stores. Thus, reduced phagocytic cell function in GSD 1b is associated with diminished calcium mobilization and defective calcium stores. Defective calcium signaling is associated with a selective defect in respiratory burst activity but not degranulation. (*J. Clin. Invest.* 1990. 86:196–202.) Key words: glycogen storage disease • respiratory burst • calcium mobilization

Introduction

In both glycogen storage disease (GSD)[1] type 1a (1a; 1, 2) and type 1b (1b; 3–5) inherited hepatic defects prevent the conversion of glucose-6-phosphate to glucose resulting in hypoglycemia during fasting. The clinical features of GSD 1b are indistinguishable from GSD 1a with the exception that patients with GSD 1b are often neutropenic and are prone to recurrent infections whereas patients with GSD 1a are not (6–11).

Critical to host defense against infections are circulating phagocyte cells (i.e., neutrophils and monocytes). There is increasing evidence of functional defect(s) in GSD 1b patients' phagocytic cells which limits their ability to destroy invading microorganisms. Defects in neutrophil chemotaxis and bactericidal activity have been described (8, 12–15). An important mechanism in phagocyte bactericidal activity is a process termed respiratory burst activity. The activation of phagocytes by a variety of stimuli can trigger the respiratory burst by activating the assembly of an active NADPH oxidase which catalyzes the reduction of oxygen to superoxide anion. NADPH serves as a source of reducing equivalents and is oxidized to NADP. Elevated levels of NADP allows a more rapid conversion of glucose-6-phosphate to CO_2 via the hexose monophosphate shunt (HMPS). Respiratory burst activity is greatly reduced in GSD 1b neutrophils (9, 10, 12, 16); however, the specific defect in neutrophils is unknown. Whether this defect is similar to that observed in the liver or is unique to neutrophils has not been determined. Glucose-6-phosphatase activity is dependent on the coupling of three integral microsomal membrane components: (*a*) a glucose-6-phosphate specific translocase, (*b*) the enzyme glucose-6-phosphatase, a nonspecific phosphohydrolase, and (*c*) a phosphate translocase which mediates the efflux of inorganic phosphate (17–19). It is not readily apparent how a defect in the glucose-6-phosphate translocase (GSD 1b) can produce alterations in phagocytic cell function, while the absence of the enzyme glucose-6-phosphatase (GSD 1a) does not, since the absence of either component will impair hydrolysis of glucose-6-phosphate.

The purpose of this study was to characterize respiratory burst activity in neutrophils and monocytes from three patients with GSD 1b in response to different stimuli. Respiratory burst activity was measured by three independent parameters (superoxide anion generation, oxygen consumption, and HMPS activity [glucose conversion to CO_2]). All three parameters were decreased in neutrophils from GSD 1b patients but not neutrophils from GSD 1a patients as compared with controls. Respiratory burst activity was also reduced in monocytes from GSD 1b patients as compared with either controls or GSD 1a patients, indicating that the abnormality in respiratory burst activity is not unique to these patients' neutrophils. In contrast to decreased respiratory burst activity, degranulation in neutrophils from GSD 1b patients was not significantly different from control neutrophils indicating that not all phagocyte cellular functions are altered in this disease. Finally, to investigate whether the signaling mechanisms involved in regulating respiratory burst activity were altered in this disease, calcium mobilization in response to the stimulus f-Met-Leu-Phe was assessed in phagocytic cells from GSD 1b patients and compared with those obtained from GSD1a patients and controls. Calcium mobilization was decreased in both neutrophils and monocytes from GSD 1b patients. In addition, calcium mobilization by a calcium ionophore was reduced in GSD 1b, indicating reduced intracellular stores. Thus, a selective defect

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Address correspondence to Dr. Kilpatrick, Division of Infectious Diseases and Immunology, Children's Hospital of Philadelphia, 34 St. and Civic Center Blvd., Philadelphia, PA 19104.

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1. *Abbreviations used in this paper:* GSD, glycogen storage disease; HMPS, hexose monophosphate shunt; 1a and 1b, types 1a and 1b of GSD.

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in respiratory burst activity is associated with a signaling defect in these patients' phagocytic cells.

Methods

Blood samples. The study population consisted of three unrelated GSD 1b patients: two females aged 3 and 8 yr and one male aged 22 yr; two of these patients have been previously reported (14). Also enrolled in the study were seven unrelated GSD 1a patients between the ages of 5 and 28 yr of which four were males and three were females. Written permission was obtained from parents of GSD 1a and GSD 1b patients for all blood samples in accordance with policies of the Institutional Review Board at the Children's Hospital of Philadelphia. Control blood samples were obtained from healthy adults routinely used as donors in our laboratory.

Neutrophil separation (20). Neutrophils were isolated from heparinized blood (10U/ml) by Ficoll-Hypaque centrifugation and dextran sedimentation, followed by hypotonic lysis to remove residual erythrocytes. Cells were washed twice and resuspended in phosphate buffered saline containing 5 mM glucose.

Monocyte separation (21). Monocytes were separated from neutrophils by the Ficoll-Hypaque centrifugation method described above and resuspended in Dulbecco's modified Eagle's medium (DME) with 4 mM glutamine and penicillin/streptomycin. The cell suspension was plated onto gelatin coated flasks and incubated at 37°C for 45 min in 5% CO_2-95% air. Nonadherent cells were removed by washing the flasks several times with DME. Adherent monocytes were detached by the addition of 5 mM EDTA in DME with 20% fetal bovine serum, washed, and resuspended in DME with 20% fetal bovine serum. These cell populations were 92–96% monocytes, based on latex ingestion and nonspecific esterase staining.

Lymphocyte separation. Lymphocytes were isolated by the Ficoll-Hypaque method described above. The nonadherent cells cited in the monocyte preparation contain lymphocytes and platelets. These cell suspensions were removed from the flasks after the 45-min incubation and washed several times in PBS to remove contaminating platelets.

Oxygen consumption. Aliquots of cell suspensions ($1–2 \times 10^6$ cells/ml) were transferred into a polarographic chamber and oxygen uptake was measured using a Clark-type oxygen electrode. Basal oxygen consumption was measured for 5 min before the addition of 0.5 μg of phorbol myristate acetate (PMA)/ml. Oxygen consumption after PMA stimulation was expressed as nanomoles of O_2 per minute per 10^6 cells.

HMPS activity. Flux through the HMPS was determined according to the method of Newburger et al. (22) by monitoring the rate of production of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose. Cell suspensions (1.0×10^6 cells/ml) were incubated for 30 min at 37°C in 25-ml erlenmeyer flasks in the absence (resting) or presence (stimulated) of PMA (0.5 μg/ml). The reaction was initiated by the addition of 2 mM glucose containing 0.25uCi/ml of [1-^{14}C]glucose or [6-^{14}C]glucose. HMPS activity was calculated as the difference between stimulated and resting CO_2 release from [1-^{14}C]glucose minus that from [6-^{14}C]glucose.

Superoxide anion production. Superoxide anion generation by neutrophils and monocytes was determined by following the reduction of cytochrome *c* by the continuous recording method as previously described (23).

Neutrophil degranulation. To monitor the release of both azurophil and specific granule contents, neutrophils were pretreated with cytochalasin B (5 μg/ml). β-Glucuronidase, an azurophil granule marker, was determined by incubation with phenolphthalein glucuronidate as substrate (24). Lysozyme, a marker for specific and azurophil granules, was determined as the rate of lysis of *Micrococcus lysodeikticus,* measured by the decrease in absorbance at 450 nm (25). Total enzyme activities were determined simultaneously in duplicate reaction mixtures containing the detergent Triton X-100 (0.2% final concentration).

Fura-2 fluorescence spectroscopy. Cytosolic free calcium concentrations were determined in fura-2–loaded neutrophils and monocytes according to the method of Korchak et al. (26). Cells were incubated in 10 μM fura-2 acetoxymethyl (AM) in Hepes buffer at 37°C for 5 min. This suspension was then diluted 10-fold with Hepes buffer at 37°C and incubated for a further 20 min. Suspensions were centrifuged (800 rpm, 10 min) and cells resuspended in buffer. This loading protocol optimizes fura-2AM uptake and its conversion to the free acid fura-2 and results in a cell-associated concentration of 0.5 μM fura-2. Immediately before use, aliquots of 0.5 ml were microfuged and the cells were resuspended in fresh buffer at a cell concentration of 2.5×10^6 cells/ml. Fluorescence changes were monitored in a stirred suspension of preloaded cells, at the dual excitation wavelengths of 340 and 380 nm and emission 505 nm, and cytosolic calcium concentrations were calculated by the ratio method of Grynkiewicz et al. (27).

Statistics. Values were expressed as the mean±standard deviation of the mean (SD). Student's *t* test was used for statistical analysis; a *P* value of < 0.01 was accepted as significant.

Results

Respiratory burst activity

Measurements of oxygen consumption, HMPS activity, and superoxide generation were made in order to determine whether phagocytic respiratory burst activity was altered in neutrophils and monocytes from GSD 1b patients as compared with either GSD 1a patients or controls.

Oxygen consumption. Table I shows the rate of oxygen consumption after stimulation with PMA in neutrophils and monocytes from patients with GSD 1b and GSD 1a and controls. The rate of stimulated oxygen consumption in neutrophils from GSD 1b patients was 1.4 nmol O_2/min per 10^6 cells; whereas oxygen consumption was 4.9 and 5.6 nmol O_2/min per 10^6 cells from controls and GSD 1a patients, respectively. Rates of oxygen consumption were also lower in neutrophils from GSD 1b patients than in neutrophils from controls when latex beads were used as stimuli (data not shown). As shown in Table I, monocytes from GSD 1b patients also showed a decreased response (1.1 nmol O_2/min per 10^6 cells) to PMA from that of monocytes from GSD 1a patients (2.2 nmol O_2/min per 10^6 cells) and controls (2.5 nmol O_2/min per 10^6 cells).

HMPS activity. As shown in Table II, after stimulation with PMA, HMPS activity was increased 9.8-fold in neutrophils from control and 7.4-fold in neutrophils from GSD 1a patients. In contrast, the addition of PMA to neutrophils from

Table I. Oxygen Consumption in Response to PMA (0.5 μg/ml)

	Neutrophils	Monocytes
	nmol O_2/min per 10^6 cells	
GSD 1b	1.40±0.34*	1.10±0.19*
	(n = 3)	(n = 3)
GSD 1a	5.57±0.67	2.18±0.15
	(n = 3)	(n = 3)
Control	4.91±0.87	2.54±0.22
	(n = 9)	(n = 6)

Values are means±SD for the number of individuals in parenthesis. * $P < 0.01$ for control vs. GSD 1b and GSD 1a vs. GSD 1b. Resting rates of oxygen consumption were < 0.1 nmol O_2/min per 10^6 cells in both neutrophils and monocytes and were similar in all three groups.

Table II. Phagocyte HMPS Activity in Response to PMA

	Activated/resting ratio	
	Neutrophils	Monocytes
GSD 1b	1.38±0.65* (n = 3)	2.60±1.38* (n = 3)
GSD 1a	7.41±1.56 (n = 7)	5.67±1.56 (n = 7)
Control	9.79±2.67 (n = 13)	6.37±1.61 (n = 10)

Activated/resting ratio was determined by the rates of glucose oxidation via the HMPS in the presence of PMA (activated) and in the absence of stimuli (resting). Values are mean±SD for the number of individuals in parenthesis.
* $P < 0.01$ for control vs. GSD 1b and GSD 1a vs. GSD 1b. Resting value of HMPS activity in control neutrophils and monocytes were 0.23±0.8 and 0.26±0.7 nmol $^{14}CO_2$/min per 10^6 cells. Resting rates in both cell types from GSD 1a and GSD 1b patients were not significantly different from control values.

GSD 1b patients produced only a 1.4-fold increase in HMPS, a minimal stimulation of the pathway as compared with controls or GSD 1a patients. Similarly, monocytes from GSD 1b patients also showed a blunted response (a 2.6-fold increase) to PMA stimulation of HMPS activity as compared to the 6.4- and 5.7-fold increase in monocytes from control and GSD 1a patients, respectively.

Superoxide anion generation. Activation of neutrophils and monocytes by a variety of stimuli elicits the assembly of an active NADPH oxidase which generates superoxide anion. As shown in Fig. 1, after an initial lag period of 50 s, the rate of cytochrome *c* reduction was linear for ~ 4 min in neutrophils from controls upon stimulation with PMA (0.5 μg/ml). In neutrophils from GSD 1b patients, however, the lag period between PMA stimulation and the onset of cytochrome c reduction was increased and the rate of superoxide anion generation (Fig. 1) was reduced to 30.9%±7.7 (n = 3, mean±SD, $P < 0.01$) of that in controls.

Superoxide anion generation in response to the chemotactic peptide f-Met-Leu-Phe was also examined in neutrophils from control, GSD 1a and GSD 1b patients as illustrated in Fig. 2. In control neutrophils, stimulated with f-Met-Leu-Phe (10^{-7} M), there was an initial lag period of 16 s which was followed by a linear rate of reduction of cytochrome *c* for ~ 3 min. In neutrophils from GSD 1a patients, the rate of superoxide anion generation was within the range of controls. In contrast, f-Met-Leu-Phe–stimulated superoxide anion generation in neutrophils from GSD 1b patients was only 16.8±13.9% (n = 3, mean±SD, $P < 0.01$) of that in controls. In addition, neutrophils from GSD 1b patients also demonstrated decreased superoxide anion generation as compared with controls in response to 10^{-7} M f-Met-Leu-Phe in the absence of extracellular glucose (Fig. 3). Similarly, monocytes from GSD 1b patients had an attenuated rate of superoxide anion generation when stimulated with 10^{-7} M f-Met-Leu-Phe equal to only 31.5%±2.4% (n = 3, mean±SD, $P < 0.01$) as compared to controls.

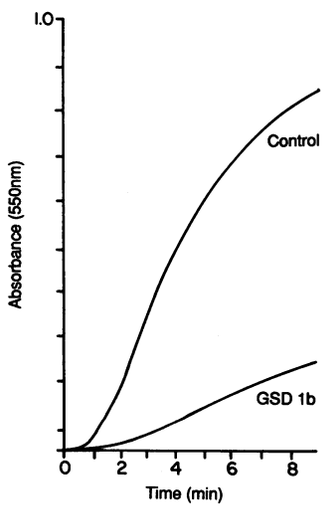

Figure 1. Superoxide anion generation in neutrophils (1 × 10^6 cells/ml) from control and GSD 1b patients stimulated with PMA (0.5 μg/ml). Superoxide anion generation was monitored continuously as cytochrome *c* reduction. Baseline cytochrome *c* was recorded for 1 min before the addition of stimuli.

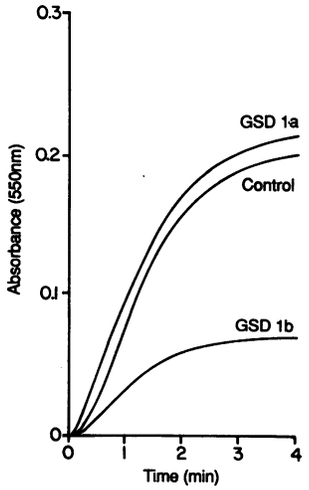

Figure 2. Superoxide anion generation in neutrophils (1 × 10^6 cells/ml) from control, GSD 1a, and GSD 1b patients stimulated with 10^{-7} M f-Met-Leu-Phe. Superoxide anion generation was monitored continuously as cytochrome *c* reduction. Baseline cytochrome c was recorded for 1 min before the addition of stimuli.

Degranulation

F-Met-Leu-Phe is a "complete" secretagogue and triggers the extracellular release of both azurophil and specific granule

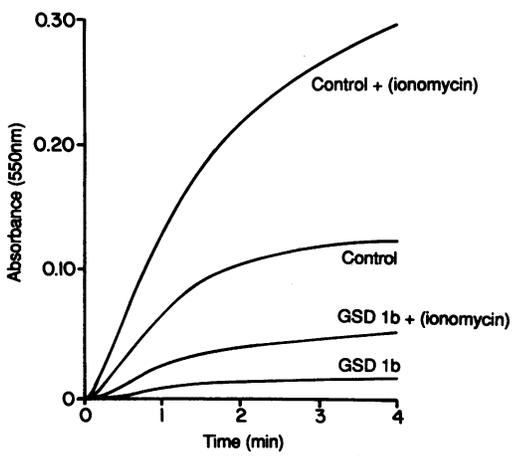

Figure 3. Effect of pretreatment with ionomycin on the time course of superoxide anion generation in neutrophils from control and GSD 1b patients stimulated with f-Met-Leu-Phe. Cells were suspended in glucose-free medium and preincubated with 200 nM ionomycin for 1 min at 37°C before the addition of 10^{-7} M f-Met-Leu-Phe (zero time). Superoxide anion generation was measured as described in Methods and Fig. 1.

Table III. Neutrophil Degranulation in Response to 10^{-7} M F-Met-Leu-Phe

	β-glucuronidase	Lysozyme
	% release	
GSD 1b	29.7±3.2 (n = 3)	42.3±4.8 (n = 3)
Control	29.5±2.7 (n = 6)	45.8±4.4 (n = 6)

Values are means±SEM for the number of experiments in parenthesis.

contents as well as superoxide anion generation in cytochalasin B–treated neutrophils. As shown in Table III, there were no significant differences in neutrophils from GSD 1b patients as compared to control neutrophils, in the release of either lysozyme (specific and azurophil granules) or β-glucuronidase (azurophil granules) in response to 10^{-7} M f-Met-Leu-Phe.

Calcium mobilization

Stimulation of control neutrophils with 10^{-9} M f-Met-Leu-Phe, a concentration which stimulates chemotaxis (28), triggered a prompt increase in cytosolic calcium (Fig. 4). Resting cytosolic calcium was 70 nM; in the presence of 10^{-9} M f-Met-Leu-Phe, cytosolic calcium rose to a peak concentration of 400 nM by 10 s, followed by a return toward resting levels. Restimulation of the same neutrophils with 10^{-7} M f-Met-Leu-Phe, a concentration which activates the respiratory burst, elicited a greater increase in cytosolic calcium with peak values reaching 750 nM.

In both neutrophils (Fig. 4) and monocytes (Fig. 5) obtained from GSD 1b patients, resting cytosolic free calcium was similar to controls. However, both cell types showed a dramatic reduction in elevation of cytosolic free calcium in response to 10^{-9} and 10^{-7} M f-Met-Leu-Phe. At the lower concentration of f-Met-Leu-Phe (10^{-9} M), there was little or no change in cytosolic free calcium from resting levels. The higher concentration of f-Met-Leu-Phe (10^{-7} M) stimulated calcium mobilization, however, the peak cytosolic free calcium was 38.4±15.2% (n = 3, mean±SD, $P < 0.01$) of that in controls. This attenuated calcium response to 10^{-7} M f-Met-Leu-Phe was also evident in monocytes from GSD 1b patients, peak cytosolic calcium was 56.7±10.2% (n = 3, mean±SD, $P < 0.01$) of that in controls.

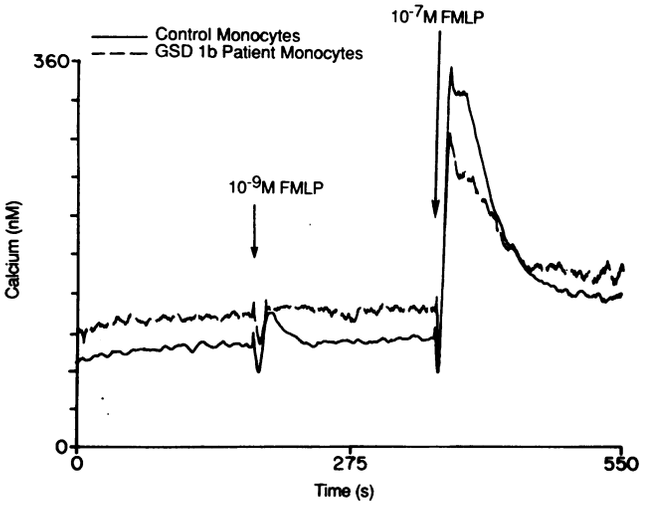

Figure 5. Time course of changes in cytosolic calcium in monocytes from control and GSD 1b patients in response to stimulation with 10^{-9} M and 10^{-7} M f-Met-Leu-Phe.

In contrast, neutrophils and monocytes obtained from GSD 1a patients showed normal mobilization of calcium and were able to attain peak levels of cytosolic calcium after stimulation with f-Met-Leu-Phe (10^{-7} M) that were comparable to controls. As illustrated in Fig. 6, peak cytosolic calcium levels of neutrophils from GSD 1a patients were 101.9±12.6% (n = 7, mean±SD) as compared with controls; whereas in monocytes from GSD 1a patients, peak cytosolic calcium levels were 107.0±25.8% (n = 5, mean±SD) as compared to controls.

Since monocytes as well as neutrophils isolated from GSD 1b patients have an abnormal respiratory burst activity which is associated with defective calcium mobilization, we determined whether this defect was specific to phagocytic cells or whether defective calcium mobilization was a more universal finding in these patients. Lymphocytes do not undergo a respiratory burst, however in response to the lectin phytohemagglutinin (PHA), they do mobilize calcium (29). Calcium mo-

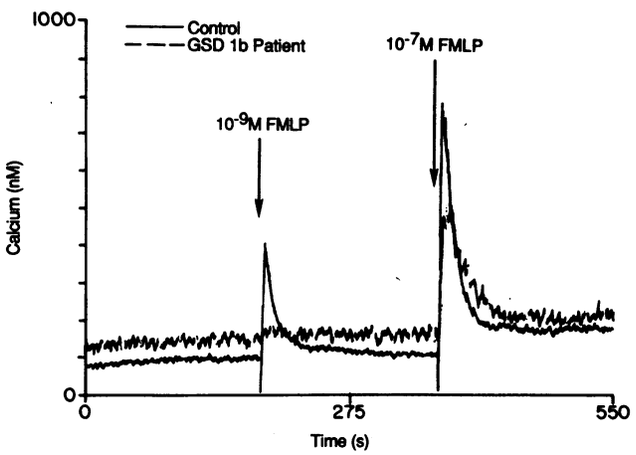

Figure 4. Time course of changes in cytosolic calcium in response to 10^{-9} M and 10^{-7} M f-Met-Leu-Phe in neutrophils from control and GSD 1b patients. Alterations in cytosolic calcium were monitored as changes in fluorescence of fura-2–loaded cells.

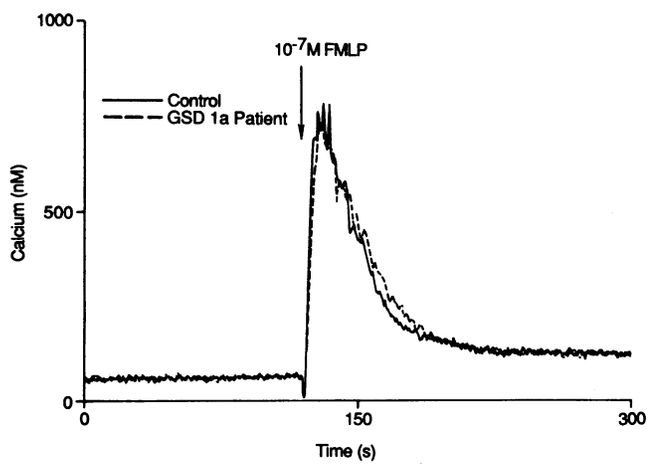

Figure 6. Time course of changes in cytosolic calcium in neutrophils from control and GSD 1a patients in response to stimulation with 10^{-7} M f-Met-Leu-Phe.

bilization in response to PHA (100 μg/ml) was not defective in circulating mixed lymphocytes from GSD 1b patients as compared to controls (data not shown).

Effects of ionomycin on superoxide anion generation. Since calcium mobilization was found to be defective in GSD 1b phagocytic cells, increasing cytosolic calcium with a calcium ionophore should correct the respiratory burst defect. Ionomycin (200 nM) does not itself trigger significant superoxide anion generation (results not shown and reference 30). However, addition of 200 nM ionomycin to control neutrophils, 1 min before f-Met-Leu-Phe (10^{-7} M) stimulation, increased the rate of superoxide anion generation by 121.3±35.0% (n = 3, mean±SD). In neutrophils from GSD 1b patients, the addition of ionomycin also increased the rate of superoxide anion production by 316.3±61.2% (n = 3, mean±SD, $P < 0.01$) as compared with their neutrophils stimulated with f-Met-Leu-Phe alone (Fig. 3). However, while pretreatment of neutrophils from GSD 1b patients with ionomycin before f-Met-Leu-Phe stimulation did increase the rate of superoxide anion, it was still below the rate of superoxide anion generation of control cells stimulated with either ionomycin plus f-Met-Leu-Phe (14.5±3.0%, n = 3, mean±SD, $P < 0.01$) or with f-Met-Leu-Phe alone (32.7±11.2%, n = 3, mean±SD, $P < 0.01$).

Effects of ionomycin on calcium mobilization. As shown in Fig. 7, the addition of 200 nM ionomycin to control neutrophils results in the rapid increase in cytosolic calcium to a level of ~ 800 nM. The addition of ionomycin to neutrophils from GSD 1b patients also produced a rapid rise in cytosolic calcium, however peak calcium was 300 nM which was significantly lower as compared to controls. Furthermore, measurements of cytosolic calcium triggered by ionomycin in the absence of extracellular calcium (preincubation with 5 mM EGTA) demonstrated a smaller response in neutrophils from GSD 1b patients, indicating, but not proving, that these cells may have defective intracellular calcium stores (data not shown).

Discussion

Functional defects in neutrophil activity from patients with GSD 1b have been reported by a number of laboratories (9, 10, 12, 16, 31), though most are single case studies due to the rarity of this disease (< 100 reported cases). Since various stimuli have markedly different effects on different parameters associated with respiratory burst activity (32), in this study we characterized oxidative metabolism of neutrophils from GSD 1b patients by three independent parameters (increased oxygen consumption, HMPS activity, and superoxide anion generation). In each of the GSD 1b patients studied, neutrophil respiratory burst activity was depressed as compared to either control or GSD 1a patients.

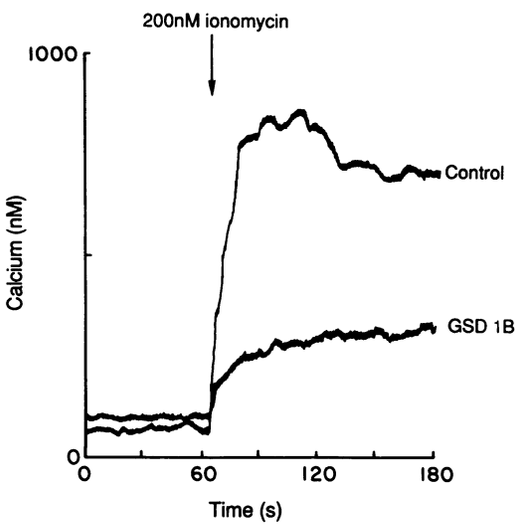

Figure 7. Time course of changes in cytosolic calcium in neutrophils from control and GSD 1b patients in response to stimulation with 200 nM ionomycin.

Although the numbers of circulating monocytes are not decreased in patients with GSD 1b, these phagocytic cells have many of the same characteristics as neutrophils, including the ability to generate respiratory burst activity in response to stimulation. Ueno et al. (13) studied a single GSD 1b patient's monocytes and reported normal chemotaxis but decreased chemiluminescence in response to stimuli, indicating that respiratory burst activity may also be altered in GSD 1b monocytes. The results of the present study demonstrate an impairment in respiratory burst activity in monocytes from GSD 1b patients as well as in their neutrophils. However, the magnitude of inhibition in monocytes is not as severe as in their neutrophils. The reason for this difference is not apparent, but may be due to different metabolic capabilities of the two cell types; neutrophils are primarily glycolytic cells while monocytes contain more mitochondria and are capable of producing ATP via oxidative phosphorylation.

Bashan and co-workers (16) have shown decreased glucose transport in GSD 1b neutrophils and postulated that this was the primary cell defect in phagocytic cells. In our studies, we have found that, when neutrophils are suspended in glucose free medium, defective respiratory burst activity remains. In the absence of glucose, neutrophils use internal glycogen stores as a carbon source (33), and it has been shown that GSD 1b neutrophils have normal glycogen stores and ATP levels similar to controls (16). Therefore, it is unlikely that the blunted respiratory burst activity in neutrophils from GSD 1b patients is solely a result of decreased glucose uptake.

Respiratory burst activity is dependent on both functional NADPH oxidase and HMPS pathways. There are several different sites in the pathways where a defect could occur that would alter respiratory burst activity; these include defects (*a*) in the NADPH oxidase enzyme complex, (*b*) in enzymes of the HMPS pathway, (*c*) in cofactors which regulate the HMPS pathway, or (*d*) in the activation of the oxidase.

Recently, two laboratories have demonstrated normal superoxide anion generation (10) and HMPS activity (16) in GSD 1b neutrophil cell lysates. Normal activities of the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase have also been shown in neutrophil lysates from GSD 1b patients (9, 16). In addition, HMPS activity in resting neutrophils was normal in GSD 1b (this study and reference 9). These results indicate that the enzymes in both the NADPH oxidase and the HMPS pathways are functional in these patient's neutrophils and suggest that the defect in respiratory burst activity may not be at the level of either pathway, rather it may be in the regulation of this cellular function. Decreased respiratory burst activity could then be a result of a defect in the signal transduction pathway which activates the respiratory burst.

Different ligand–receptor systems in the neutrophil can activate common physiological processes such as superoxide anion generation and degranulation (34). A defect at the level

of the receptor could result in decreased respiratory burst activity. This does not appear to be the case in GSD 1b. Our results demonstrating defective respiratory burst activity triggered by three different agonists (f-Met-Leu-Phe, PMA, and latex beads) argues against a specific receptor defect in this disease. In addition, neutrophils from GSD 1b patients showed normal ligand-stimulated degranulation, as measured by release of enzyme markers from either azurophil or specific granules, which supports the notion that there are intact and functional receptors on these patients' phagocytic cells.

Activation of the neutrophil by interaction of a ligand such as f-Met-Leu-Phe with its specific receptor elicits a rapid breakdown of phosphatidyl inositol 4,5-bisphosphate and generation of inositol 1,4,5 trisphosphate and diacylglycerol. This event is accompanied by a rapid elevation of cytosolic calcium and activation of protein kinase C. Calcium is an important second messenger and is known to be involved in glycogen breakdown, glucose transport, and respiratory burst activity in phagocytic cells (28, 30, 32, 35). A dual role for calcium and protein kinase C has been proposed in the generation of superoxide anion generation via the activation of the membrane associated NADPH oxidase (28, 30). The present study demonstrates that the respiratory burst abnormality in phagocytic cells from GSD 1b patients is associated with impaired calcium mobilization, whereas these processes are normal in GSD 1a patients. In GSD 1b phagocytic cells, the rapid transient rise in cytosolic free calcium in response to the chemotactic peptide f-Met-Leu-Phe was markedly reduced as compared to controls. This impairment in calcium mobilization is not merely a consequence of defective respiratory burst activity, since neutrophils from patients with chronic granulomatous disease show a blunted respiratory burst but normal calcium mobilization (unpublished observation and 36). Therefore, the alteration in calcium mobilization is an indication of a signaling defect in phagocytic cells from patients with GSD 1b.

The demonstration that superoxide anion generation is enhanced in GSD 1b phagocytic cells by raising cytosolic calcium through the addition of ionomycin supports the concept that a defect in calcium mobilization is at least in part responsible for the decreased respiratory burst activity. It is thought that the transient rise in cytosolic calcium in response to f-Met-Leu-Phe is a result of calcium release from the endoplasmic reticulum. The blunted rise in calcium in response to the calcium ionophore ionomycin in neutrophils from GSD 1b patients, in the presence and absence of EGTA, indicates that the decreased calcium mobilization in response to f-Met-Leu-Phe may be a consequence of decreased internal calcium stores. The defective ligand-induced rise in cytosolic calcium appears to be of critical significance to triggering of superoxide anion generation but not degranulation. These results are in accordance with the observation that, in control neutrophils, the buffering of intracellular calcium results in a greater diminution of superoxide anion generation than in degranulation (30, 37).

A role for glucose-6-phosphate in regulating calcium sequestration and release by the endoplasmic reticulum has been suggested by Wolf et al. (38) in the pancreatic islet and by Benedetti and co-workers (39, 40) in the liver. In light of these observations, it is possible that the absence of glucose-6-phosphate translocase activity in GSD 1b may be involved in the alteration in calcium sequestration in these patients' phagocytic cells. However, this hypothesis does not explain why respiratory burst activity is blunted when PMA is used as a stimulus. PMA is a direct activator of protein kinase C and causes the translocation of protein kinase C from the cell cytosol to the membrane and subsequent activation. It has been postulated that translocation of protein kinase C is required for the activation of NADPH oxidase (41, 42). Since PMA reduces the requirement for calcium (43–46) and there is still decreased respiratory burst activity, then multiple defects must be postulated. Numerous components which regulate or are involved with phagocytic cell respiratory burst activity are membrane associated and alterations in the capabilities of any of these components would compromise bactericidal activity. The fact that the hepatic metabolic abnormality in GSD 1b is associated with a membrane translocase may be indicative of a more fundamental membrane defect in this disease. However, the demonstration that calcium mobilization is normal in response to PHA in mixed lymphocyte populations from GSD 1b patients indicates that defective calcium mobilization is not a global finding in circulating cells of the immune system, but is specific to phagocytic cells. Therefore, altered phagocytic cell function in GSD 1b patients appears to be associated with diminished calcium mobilization and defective calcium stores. The defective calcium signaling is associated with a selective defect in respiratory burst activity but not degranulation. Further studies are required to establish the relationship between abnormal phagocytic cell function and the known hepatic defect in GSD 1b.

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