Impaired Chemotaxis and Neutrophil (Polymorphonuclear Leukocyte) Function in Glycogenosis Type IB¹

NORMAN L. KOVEN, MIMI M. CLARK, CAROLYN S. CODY, CHARLES A. STANLEY,
LESTER BAKER, AND STEVEN D. DOUGLAS

*Divisions of Allergy-Immunology-Pulmonology-Bone Marrow Transplantation and Endocrinology, The
Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Department of Pediatrics,
Philadelphia, Pennsylvania 19104*

ABSTRACT. Polymorphonuclear leukocyte (PMN) function was investigated in two patients with glycogen storage disease type IB and neutropenia. Glycogen storage disease type IB was documented by liver biopsy and a normal amount of latent glucose-6-phosphatase activity. Patient A had stomatitis, skin infections, and septicemia; patient B had respiratory infections, periodontitis, and oral candidiasis. Absolute neutrophil counts ranged from 114 to 2580/mm³. Diminished and delayed migration of PMN into a skin "window" occurred in B. Random and directed PMN migration under agarose toward f-Met-Leu-Phe, pepstatin A, and zymosan-activated serum were severely diminished in both patients. At 10⁻⁷ M f-Met-Leu-Phe, mean random and directed migration were 52 and 23% (A, *n* = 3) and 48 and 13% (B, *n* = 4) of controls. These results were independent of incubation time and chemoattractant concentration. Patients' PMN had diminished quantitative nitroblue tetrazolium reduction compared to controls. B had a significant defect in PMN bactericidal activity with *Escherichia coli* with <0.2 log killing at 2 h. These results further characterize the defect in PMN migration reported by Beaudet *et al.* (J Pediatr 97:906, 1980). The finding of other abnormalities of PMN function suggests a metabolic defect in the neutrophil which may be related to the microsomal membrane defect in hepatocytes in glycogen storage disease type IB. (*Pediatr Res* 20: 438-442, 1986)

Abbreviations

PMN, polymorphonuclear leukocyte
ANC, absolute neutrophil count
FMLP, N-formyl-methionyl-leucyl-phenylalanine (f-Met-Leu-Phe)
GSD, glycogen storage disease (glycogenosis)
G6P, glucose-6-phosphate
NBT, nitroblue tetrazolium reduction

GSD type IB is characterized by the clinical features of glucose-6-phosphatase deficiency (GSD type IA), including hepatomegaly, growth retardation, fasting hypoglycemia, and lactic acidosis, but normal latent enzymatic activity in the liver (1, 2). Biochemical studies have demonstrated a defect in the microsomal transport system for G6P in GSD type IB (2, 3). Recurrent infection occurs more prominently in type IB, and neutropenia is another distinguishing feature of this type (4, 5). Studies of PMN migration in a patient with GSD type IB and neutropenia indicated impaired PMN mobilization *in vivo* and impaired random and directed PMN migration *in vivo* (1). We have further investigated PMN function in two patients and demonstrated abnormalities in metabolic and bactericidal functions as well as motility.

CASE REPORTS

Case A. A 7-yr-old Caucasian female was the only child of healthy parents with no known consanguinity. Type I GSD was diagnosed in infancy with typical clinical and biochemical features and open liver biopsy which revealed increased glycogen and fat content. Glycogen content of the liver was 10.8% (normal 5%), and G6P activity was 1.6 μ mol/min/g (normal 2-10) in fresh liver tissue (intact microsomes), but rose to 7.9 μ mol/min/g in deoxycholate-treated tissue (disrupted microsomes). The difference represented 80% latency of activity, similar to that previously reported in patients with type IB GSD (1, 2).²

Neutropenia was first noted at age 2 yr and has since been consistently observed, ANC ranging from 114 to 1680/mm³. Notable infections have included recurrent stomatitis, oral candidiasis, and skin infections, in particular, furuncles on the fingers and hands. Septicemia with *Streptococcus pneumoniae* occurred postoperatively at age 2, a dental abscess occurred at age 4, and mastoiditis occurred at age 6. Studies of PMN function were performed during admissions to the Clinical Studies Unit for metabolic evaluation at age 2 and 3 yr.

Case B. An 18-yr-old Caucasian man was the third child of healthy parents who are first cousins. Two older siblings are normal. Type I GSD presented in infancy with seizures and hypoglycemia and laboratory findings of lactic acidosis, hyperuricemia, and lack of rise in blood glucose after epinephrine or glucagon administration. Open liver biopsies supported the diagnosis, revealing elevated glycogen content (10%), but normal G6P activity (8.0 μ mol/min/g in frozen tissue), findings which were reported as "pseudo type I" glycogen storage disease (6).

² G6P phosphohydrolase activity of liver homogenates from Patient A and controls was performed by Dr. Barbara Brown, Washington University School of Medicine, St. Louis, MO. The assays were for 10 min at 30° C after 30 min preincubation in ice with and without 0.2% deoxycholate. Latency is defined as $1 - \frac{\text{activity without deoxycholate}}{\text{activity with deoxycholate}} \times 100$.

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Address all correspondence to Steven D. Douglas, M.D., Director, Division of Allergy-Immunology-Pulmonology-Bone Marrow Transplantation, The Children's Hospital of Philadelphia, 34th Street and Civic Center Blvd., Philadelphia, PA 19104.

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The technique of latency determination was not available at that time.

Neutropenia has been generally observed, ANC ranging from 152 to 2580/mm³. Recurrent infections have included upper respiratory infections, otitis media, pneumonia, caries, periodontitis, and oral candidiasis. A superficial abscess of the leg occurred after trauma at age 12, treated with antibiotics. Studies of PMN function were performed during admission to the Clinical Studies Unit for metabolic evaluation at age 15 and 16 yr.

MATERIALS AND METHODS

Informed consent was obtained from the parents for all studies. Fresh liver tissue and patients' PMN were fixed in 1.5% glutaraldehyde for electron microscopy.

Leukocyte migration *in vivo* was studied by the dermal abrasion method of Rebeck and Crowley (7) and by epinephrine stimulation (8). Following subcutaneous injection of 0.3 ml epinephrine, leukocyte counts and differentials were obtained at 0, 5, 10, 15, 30, and 60 min.

In vitro studies. Venous blood was collected in syringes containing 20 U heparin per ml of blood, and PMN were isolated and collected by Dextran gravity sedimentation, as previously described (9). PMN migration *in vitro* was studied under agarose according to the method of Nelson *et al.* (10). In each migration assay, patients' PMN were run simultaneously with control PMN on the same agarose plate. A 10- μ l aliquot, containing 2.5×10^5 PMN, was placed in the center well of each three well series. Ten μ l of chemoattractant was placed in the outermost well, and 10 μ l of buffer (phosphate-buffered saline) in the inner well. Chemoattractants, including FMLP (Sigma), pepstatin A[†] (Sigma), and zymosan (Sigma) activated serum were utilized at maximally effective concentrations. Plates were incubated at 37° C in a humidified 5% CO₂ atmosphere for 2 h, unless otherwise specified, and examined under a B/L Trisimplex projection microscope at 2.5 $\times$ magnification. Results were expressed as total migration in cm from the well by the leading front. Each patients' migration was compared to his own control migration and expressed as percent of the control. When multiple assays at the same chemoattractant concentration were performed, Student's paired *t* test for dependent/independent variables was used as a test for statistical significance.

Spectrophotometric quantitation of NBT reduction was measured as previously described (12). PMN bactericidal activity was assessed by the method of Douglas *et al.* (13). PMN H₂O₂ generation was measured by a modification of the method of Root *et al.* (14).

RESULTS

In vivo studies. PMN morphology under light and electron microscopy was normal in both patients. Table 1 presents observations of *in vivo* granulocyte mobilization in both patients. A granulocytosis was seen with fever postoperatively on one occasion in patient A, ANC rising to >4000/mm³. On most other occasions, ANC remained low even during serious infections. In patient B, ANC increased from 490 to 1240/mm³ 10 min after epinephrine stimulation, indicating the presence of a normal marginating granulocyte pool that was not preferentially entrapped in the vasculature. Results of a Rebeck skin "window" experiment in patient B are presented in Table 2, revealing diminished and delayed migration of PMNs into the abrasion site.

In vitro studies. Results of PMN migration experiments are summarized in Table 3. Random and directed PMN migration under agarose toward FMLP, pepstatin A, and zymosan-activated serum were diminished in both patients. The mean random migration of PMN was 64% of control for patient A (*n* = 12) and 42% of control for patient B (*n* = 22). The "leading front" directed migration values for various chemoattractants ranged

Table 1. *Granulocyte mobilization in vivo*

Febrile response—patient A*			
Day	Temperature (° C)	White blood cell count/mm ³	ANC/mm ³
1	37.0	11,400	1140
2	39.5	7,000	4060
3	38.7	7,300	4161
4	37.5	7,700	2310
5	37.5	7,500	1650
6	37.0	5,400	756
Epinephrine stimulation—Patient B			
Time (min)	White blood cell count/mm ³		ANC/mm ³
0	3500		490
5	4600		920
10	6200		1240
15	5700		456
30	4800		672
60	3800		304

* Neutrophil release with fever in a patient (A) with glycogenosis IB and neutropenia.

† Serial leukocyte counts and smears were obtained in a patient (B) with glycogenosis IB and neutropenia following subcutaneous injection of 0.3 ml epinephrine.

from 15 to 57% of control for patient A and 4 to 57% of control for patient B. In contrast, random and directed migration in a patient with chronic neutropenia and a patient with GSD type III were normal (Table 3). Parents' PMN migration was normal (data not shown). No cell directed inhibitors or chemotactic factor inactivators were present in patients' sera, and zymosan activation effected normal control PMN migration (data not shown).

Figure 1 presents the results of dose-response experiments which revealed diminished random and directed PMN migration over a 2 log range of chemoattractant concentration. Patients' random and directed migration were similar to control random migration over the entire range of FMLP concentration studied. Figure 2 demonstrates the PMN migration in B was also severely impaired with prolonged chemoattractant incubation up to 6 h. These experiments demonstrate that the defect in PMN migration was independent of chemoattractant concentration and incubation time.

Table 4 represents the results of latex stimulated quantitative NBT reduction. The patients' PMN had diminished NBT reduction, with a Δ OD of 0.055 for patient A compared to 0.16 for control and a Δ OD of 0.11 for patient B compared to 0.17 for control. In contrast, a patient with chronic neutropenia and a patient with GSD type III had normal NBT reduction.

PMN bactericidal activity with *Escherichia coli* is presented in Figure 3. Patient B had a significant defect with <0.2 log killing at 2 h; patient A had a mild reduction with approximately 0.67 log killing at 2 h.

Parents' PMN had normal NBT reduction and bactericidal activity (data now shown).

H₂O₂ generation in PMNs (of 1.56×10^5 cells/reaction mixture) was evaluated using latex beads and phorbol 12-myristate 13-acetate as stimulants. The H₂O₂ was measured by the extinction of fluorescence of scopoletin during its oxidation by horseradish peroxidase in resting and stimulated cells. The results were expressed as the percent of relative fluorescence, and it was observed that patient B and control resting values were 93 and 98%, respectively. The phorbol 12-myristate 13-acetate-stimulated values for the patient was 78% which is diminished as compared to 49% for the control value. The latex stimulated cells of patient B did not show any difference when compared to control.

Table 2. *Rebuck skin window**

Time (h)	Patient B			Control		
	Cells/ 10^{-3} mm 2 †	% PMN	% MNP	Cells/ 10^{-3} mm 2	% PMN	% MNP
2	ND‡	ND	ND	237	97	2.5
4	70	21	79	435	100	0
6	124	10	90	354	77	23
8	82	5	95	407	89	11
12	227	8	92	ND	ND	ND
18	ND	ND	ND	238	25	75
24	ND	ND	ND	144	8	92

* Serial coverslips were applied and examined under 1000 $\times$ magnification for adherent PMN and mononuclear phagocytes (MNP) for 24 h after dermal abrasion of patient B.

† Total number of cells in 10 fields of a 10-mm grid.

‡ Not done.

Table 3. *Neutrophil chemotaxis*,†*

Chemoattractant	A	B
FMLP		
1×10^{-7} M	23 ($n = 3$) (range 16–36) (NS)	13 ($n = 4$) (range 7–27) ($p < 0.01$)‡
5×10^{-7} M	31 ($n = 3$) (range 19–39) ($p < 0.05$)	19 ($n = 5$) (range 5–32) ($p < 0.001$)
Pepstatin A		
1×10^{-6} M	23	ND
5×10^{-6} M	ND	13 ($n = 2$)
1×10^{-5} M	15	9
Control ZAS	57	12
Autologous ZAS	56	57

* PMN migration is expressed as the percent of control leading front migration for each study, either as the mean of n different experimental days or single determinations. PMN migration in a patient with chronic neutropenia was 80% of control at 5×10^{-7} M FMLP and 69% of control ($n = 2$) at 1×10^{-7} M FMLP. PMN migration in a patient with GSD type III was 89% of control at 5×10^{-7} M FMLP and 100% of control at 1×10^{-7} M FMLP. Control PMN migration under agarose with FMLP at 90 min was 1.17 ± 0.33 mm.

† ZAS, zymosan-activated serum; ND, not done.

‡ Paired t test for dependent/independent variables.

Fig. 1. Effect of chemoattractant concentration on PMN migration under agarose in two patients with GSD type IB and neutropenia. Leading front migration (y axis) as a function of FMLP concentration over the range of 1×10^{-8} M to 1×10^{-6} M (x axis). O, patient; Δ , control; —, total migration; ---, random migration.

PMN Migration Under Agarose

Fig. 2. Effect of incubation time on PMN migration under agarose in patient B with GSD type IB and neutropenia. O, patient; Δ , control; —, 5×10^{-7} M FMLP; ---, 1×10^{-7} M FMLP.

Table 4. *Quantitative NBT reduction**

	Resting	Latex stimulated	Δ OD
Patient A	0.01	0.065	0.055
Control A	0.00	0.16	0.16
Patient B	0.00	0.11	0.11
Control B	0.00	0.17	0.17
GSD type III	0.01	0.58	0.57
Control C	0.00	0.41	0.41
Chronic neutropenia	0.01	0.38	0.37
Control D	0.02	0.46	0.44
Normal range ($n = 24$ adults)	0–0.10	0.16–0.64	0.16–0.60

* Each value represents mean of three samples, expressed in units of OD at 515 nm. Sample size for patient A and control A equal to 1.5×10^6 PMN; all other samples equal to 2.5×10^6 PMN.

DISCUSSION

Our study demonstrates abnormal PMN function in two patients with GSD type IB, with defects in motility, NBT reduction, and bactericidal activity. These results suggest a basis for the association of GSD type IB with recurrent infections and neutropenia previously recognized (1, 15, 16), and further characterize the defect in PMN migration reported by other investigators (1,

Fig. 3. Neutrophil bactericidal activity with *E. coli* in two patients with GSD type IB and neutropenia. Number of viable bacteria in the suspension plotted as a function of incubation time; 8.0 bacteria/PMN in time 0 mixture. CT, control; PT, patient.

17). Beaudet *et al.* (1) and Anderson *et al.* (17) reported impaired random and directed PMN migration in one patient with GSD type IB and neutropenia who suffered from recurrent staphylococcal infections. Our dose-response and kinetic studies demonstrate that the impaired migration is independent of incubation time and chemoattractant concentration.

The observation of impaired random PMN migration indicates an intrinsic defect in PMN motility. This observation is consistent with that reported by Anderson *et al.* (17) who documented impaired neutrophil motility and impaired redistribution of surface adhesion sites. Our patients differ from their patient, however, in that the latter had normal intracellular killing of staphylococci *in vitro*, despite a history of severe recurrent staphylococcal infections, whereas our patients had diminished bactericidal activity with *E. coli*. This difference may reflect heterogeneity of PMN function among individual patients. The difference may also be related to technical factors in that isolated PMNs with *E. coli* at a bacteria/PMN ratio of 8:1 were used in our studies whereas Anderson *et al.* (17) used a whole blood assay for killing with *Staphylococcus aureus* at a bacteria/PMN ratio of 50:1. Since bactericidal activity and phagocytosis-associated chemiluminescence were normal, Anderson *et al.* (17) concluded that the neutrophil disorder in GSD type IB was restricted to motility and locomotion. In contrast, our results suggest a more generalized neutrophil defect, affecting intrinsic motility, chemotaxis, and perhaps phagocytosis.

G6P of liver and kidney is a multicomponent, membrane-bound enzyme system of the endoplasmic reticulum (2). This system involves transport of the substrate, G6P, from the cytoplasm into the cisternae of the endoplasmic reticulum, hydrolysis on the luminal surface of the membrane, and efflux of inorganic phosphate out of the cisternae (2, 3, 17). A deficiency of this enzyme system causes type I GSD. There are two forms of the disease: type IA, the more common form, in which little or no G6P activity is detected in liver, kidney, or small intestine; and type IB, or pseudotype I, in which patients with similar clinical findings appear to have normal G6P activity. Lange *et al.* (2) demonstrated that this so-called "functional deficiency" of the enzyme in type IB is due to a defect in the G6P transport

moiety of the enzyme. As a result of this defect in G6P transport, G6P activity in fresh, unfrozen liver tissue in GSD type IB is low. The activity is normal, however, if frozen liver tissue is used for the assay, as is ordinarily done with biopsy material. Freezing or detergent action disrupts the microsomal membrane such that G6P transport is not necessary. A defect of the G6P transport system of the microsomal membrane in hepatocytes is the biochemical defect in GSD type IB.

The mechanism responsible for abnormal PMN function in GSD type IB is not known. If the metabolic defect found in hepatocytes in GSD type IB is also present in analogous fashion in neutrophils, it would result in a block of G6P transport from the cytosol into the endoplasmic reticulum of the PMN. G6P concentration is important in PMN chemotaxis, the hexose monophosphate shunt, and phagocytosis. Stossel *et al.* (19) showed that there was a marked acceleration of glycogen breakdown in guinea pig peritoneal PMNs phagocytizing latex particles in the absence of glucose; there was increased utilization of G6P, without changes in glycogen phosphorylase or glycogen synthetase activity or in levels of ATP, ADP, AMP, inorganic phosphate, or cyclic AMP. Earlier work by Beck (20) suggested that primary control of the phosphogluconate pathway in leukocytes depends upon the G6P concentration. Furthermore, G6P concentration is very important in chemotaxis as a substrate in anaerobic glycolysis, which provides the major source of energy for PMN chemotaxis (21). If a block of G6P transport is present in neutrophils in GSD type IB, as it is in hepatocytes, then these PMN functions may be affected.

Other studies of GSD type IB patients support the concept of a metabolic defect of G6P transport in the neutrophil. Heyne and Gahr (22) showed that PMN from a patient with GSD type IB had reduced respiration during *Candida albicans*-stimulated phagocytosis, compared to type IA patients and controls. Bartram *et al.* (16) suggested that this metabolic defect is not only responsible for altered oxygen consumption during phagocytosis, but also for other hematological changes. They suggested that the variability in severity of neutropenia observed in GSD type IB patients is related to variability in the activity of the G6P translocase, and that the frequency and severity of bacterial infections parallels the neutrophil count. Narisawa *et al.* (3, 23) presented evidence in GSD type IB patients that supports this latter concept. Two siblings with persistent neutropenia, frequent infections, and severe hypoglycemic episodes had a complete defect in the G6P transport system as determined by enzyme latency (22). In contrast, a third patient, an adult with no history of neutropenia or severe hypoglycemia, had a partial deficiency of the G6P transport system (24). Finally, Seger *et al.* (25) demonstrated that impaired hexose monophosphate shunt activity and oxygen consumption in PMN from a patient with GSD IB was corrected by cell homogenization and the addition of exogenous NADPH, further supporting the concept of a defect of G6P transport in the neutrophil.

Our study confirms the previously recognized observation of abnormal PMN function in patients with GSD type IB and neutropenia, with defects in motility and chemotaxis. In addition, our results suggest a more generalized PMN defect, affecting motility and chemotaxis, NBT reduction, and bactericidal activity. The mechanism responsible for abnormal PMN function in GSD type IB is not known, but a defect of the G6P transport system of the microsomal membrane in hepatocytes is the biochemical defect in GSD type IB. If an analogous defect of G6P transport is present in neutrophils in GSD type IB, then PMN chemotaxis, hexose monophosphate shunt activity, and phagocytosis may be affected because these processes are dependent on G6P concentration. Other studies support this concept of a metabolic defect of G6P transport in the neutrophil in GSD type IB. The role of G6P transport in the neutrophil requires further investigation because it may be the basis for abnormal PMN function in GSD type IB.

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