Short Communication. Glycogenosis Ib: Neutrophil Microbicidal Defects Due to Impaired Hexose Monophosphate Shunt

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Summary

We studied neutrophil microbicidal function and oxidative metabolic activity in a patient with glycogenosis Ib. The intracellular killing defect and the respiratory burst abnormality in glycogenosis Ib neutrophils were confirmed. The impaired oxygen-dependent microbicidal activity was shown to result from impaired hexose monophosphate shunt activity (impaired endogenous NADPH synthesis) and could be corrected by homogenization of the cells, followed by the addition of exogenous NADPH. Our data are thus consistent with a possible role for glucose-6-phosphate transport in neutrophil microbicidal function. We recommend a continuous prophylaxis with co-trimoxazole in patients with glycogen storage disease Ib.

Abbreviations

GSD, glycogen storage disease
HMP, hexose monophosphate
NBT, nitroblue tetrazolium
PMN, polymorphonuclear neutrophils

Patients with GSD Ib suffer from recurrent infections whereas patients with GSD Ia do not. In GSD Ib, quantitative anomalies of PMN have recently been documented, such as neutropenia, diminished random and directed PMN migration (1, 2, 6), decreased phagocytosis-stimulated oxygen consumption (3), decreased NBT reduction (6), and a defect in PMN bactericidal activity (6). The relationship of these PMN anomalies to the primary enzyme defect and/or to the secondary metabolic consequences of the genetic defect remained at first unclear. A defective glucose-6-phosphate transport system as postulated by Narisawa *et al.* (8) for liver, and thus limited availability of this substrate for the hexose monophosphate shunt was then suggested by Heyne and Gahr (4). We now demonstrate defective HMP shunt activity in the PMN of a patient suffering from GSD Ib.

MATERIALS AND METHODS

PMN functions were investigated in one patient with GSD Ib, in healthy adults, and in contrast patients (GSD Ia). GSD was documented in biopsied liver: GSD Ib by a normal amount of latent, GSD Ia by absent glucose-6-phosphatase activity. The patient with GSD Ib, aged 13 yr, had suffered from numerous cutaneous infections. Neutrophil counts ranged from 0–6000/ μ l. His PMN were studied when counts were from 1650–2340/ μ l. PMN were prepared by dextran sedimentation followed by Fi-

coll-Hypaque centrifugation. This technique routinely resulted in cell populations containing >95% PMN. Oxygen consumption was measured polarographically with an oxygen-sensitive electrode. O_2^- generation was determined by measuring the superoxide dismutase inhibitable reduction of cytochrome c as described before (9). HMP shunt activity was measured by determining the amount of [$1-^{14}C$]glucose converted to [$^{14}CO_2$] as described by Newburger and co-workers (10). The preparation of a mixed membrane fraction (particulate fraction) of PMN was performed according to Suzuki and Lehrer (12).

RESULTS

Random and directed PMN migration under agarose toward zymosan-activated serum was diminished (mean, 73 and 61% of control, respectively) in the GSD Ib patient. His PMN had decreased oxygen consumption compared with controls and GSD Ia patients, using both a soluble (phorbol myristate, Fig. 1) and a particulate (opsonised zymosan; results not shown) stimulant. Although phagocytosis of opsonised baker's yeast proceeded normally, there was a significant defect in PMN bactericidal activity with *Staphylococcus aureus*, with <50% killing at 120 min (Fig. 1).

Experiments designed to find a cause for the respiratory burst anomaly and for the impaired bactericidal activity suggested deficient NADPH synthesis. Membrane activation as evidenced by depolarization of the resting membrane potential and by the lag time of O_2^- production was normal in PMN as was glucose-6-phosphate dehydrogenase activity in red cells. In contrast, HMP shunt activity after stimulation with methylene blue was decreased to 28% of controls in the GSD Ib patient but not in a patient with GSD Ia. Although O_2^- production with whole cells (with endogenous NADPH only) was clearly deficient in GSD Ib (26% of controls), it was normalized after homogenization of the cells, followed by the addition of 200 μ M exogenous NADPH (Table 1).

DISCUSSION

The decreased PMN bactericidal activity in this patient suffering from GSD Ib is caused by insufficient NADPH synthesis due to an impaired HMP shunt. This results in a diminished supply of reducing equivalents needed for the generation of microbicidal oxygen metabolites. HMP shunt activity could be impaired in GSD Ib if the substrate glucose-6-phosphate were unavailable for the enzymes of the shunt as it is for glucose-6-phosphatase in hepatocytes. Our findings thus bring into focus the association of GSD Ib, a defect of glucose-6-phosphate transport (7), im-

Fig. 1. Polymorphonuclear neutrophils (PMN) microbicidal functions in a patient with glycogen storage disease (GSD) Ib, patient(s) with GSD Ia and control(s). *Left hand panel*, oxygen consumption after stimulation with phorbol myristate acetate (1 μ g/ml). Results are expressed as $\bar{x} \pm 2$ SD in 10 controls, $\bar{x} + 1$ SD in four GSD Ia patients, and as individual values on three different experimental days in the GSD Ib patient. *Right hand panel*, killing of *Staphylococcus aureus* (strain SG511). The ratio of bacteria: PMN was 1, the concentrations of PMN and control serum 2.5×10^6 /ml and 20%, respectively. Results are expressed as mean of triplicates in a control, a GSD Ia and a GSD Ib patient, respectively.

Table 1. Hexose monophosphate shunt activity and O_2^- production in glycogenosis Ib and Ia*

	HMP shunt	O_2^- production	
	Whole cells†	Whole cells‡ (endogenous NADPH)	Particulate fraction§ (+200 μ M NADPH)
GSD Ib patient	0.21; 0.32; 0.45	2.44	3.90
GSD Ia patients	0.88 ($n = 1$)	9.21 $\pm$ 2.19 ($n = 4$)	3.20 ($n = 1$)
Controls	1.19 $\pm$ 0.38 ($n = 13$)	9.26 $\pm$ 2.46 ($n = 10$)	5.60 $\pm$ 1.17 ($n = 5$)

* Results are expressed as means of duplicates of different experimental days, or as mean ± 1 SD of n different patients or controls.

† In nmol glucose oxidised/30 min $\times 10^6$ PMN after methylene blue (1 mM) stimulation.

‡ In nmol O_2^- produced/min $\times 10^6$ PMN after phorbol myristate acetate (1 μ g/ml) stimulation.

§ In nmol O_2^- produced/min $\times$ mg protein after phorbol myristate acetate (1 μ g/ml) stimulation.

paired HMP shunt, and decreased O_2^- production. It also draws attention to a possible role for glucose-6-phosphate transport in neutrophil microbicidal function. Because causal therapy for the intracellular killing defect in GSD Ib is not yet available, we recommend a continuous prophylaxis with co-trimoxazole previously shown to penetrate PMN (11) and to suppress infections in patients with other intracellular microbicidal defects *e.g.*, chronic granulomatous disease (5).

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 13. This work was supported by grants from the Swiss National Science Foundation (3.884-0.81 and 3.853-0.81) and from the Schweizerische Krebsliga.
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 15. Received for publication November 9, 1982.
 16. Accepted for publication May 13, 1983.

0031-3998/84/1803-0299\$02.00/0

PEDIATRIC RESEARCH

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Vol. 18, No. 3, 1984

Printed in U.S.A.

Letter to the Editor

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In their recent paper, "Development of hepatic fatty acid oxidation and ketogenesis in the newborn guinea pig," Stanley *et al.* (1) suggest that the role of carnitine in the development of fatty acid oxidation is very different in the rat when compared with the newborn guinea pig. Our studies on fatty liver degeneration in hypoxic newborn rats support the suggestion that a unique feature of the newborn rat is its lipid metabolism (2, 3, 4, 5).

A high percentage of newborn rats, kept at a simulated altitude of about 4600 m since they were 1-d-old, died between 10–15 days of age with severe fatty liver degeneration as shown by chemical, light, and electron microscopic studies (6). Fetuses from female rats kept under hypoxic conditions during pregnancy did not show any abnormalities. Out of 18 newborn guinea pigs kept under the same hypoxic conditions, only three died from fatty liver. Newborn rabbits did not show any changes in their livers. Newborn mice showed some lipid liver increase, but

they survived without any apparent impairment to their health (Fig. 1). Hypoxic newborn rats sucked milk quantitatively and qualitatively similar to that of the controls. Perinatal rats did not show any abnormality in their lipid metabolism if made hypoxic

Fig. 1. Total lipids of the liver of normal and altitude suckling animals. Altitude animals were born or introduced into the altitude chamber when 1-d-old and remained there 14 d. N, normals at 1260 m and Alt, rats at a simulated altitude of approximately 4700 m. At the bottom of the bars, number of animals (from Ref. 3).

TABLE 1. Lipid content of liver, plasma, and carcass of hypoxic and normal control young rats fasted 16 hr

	No. of Animals	Body Wt, g	Hct, %	Total Lipids, % Liver Wet Wt	Triglycerides, % Liver Total Lipids	Plasma Total Lipids, mg/100 ml	Triglycerides, % Plasma Total Lipids	Plasma Free Fatty Acids, mEq/liter	Total Lipids, % Carcass Wt	Triglycerides, % Carcass Total Lipids
7 days old, 6 days at approx 4,700 m	6	12.2±0.1*	41.7±0.5	7.1±0.7		1,013±98		0.9±0.1		
6 days old, normals	6	11.4±0.5	38.5±1.0	5.1±0.2		848±64		1.1±0.1		
10 days old, 9 days at approx 4,700 m	7	10.9±0.3	49.5±1.1	9.1±0.4	30.9±1.4	973±55	29.1±3.1		7.3±0.7	83.5±11.9
11 days old, normals	9	20.1±0.7	40.2±0.5	4.7±0.2	16.5±1.5	736±43	8.5±1.0	0.8±0.1	5.8±0.8	79.5±5.1
14 days old, 13 days at approx 4,700 m	8	20.0±1.4	44.7±0.5	10.6±0.9	56.7±2.4	1,110±144	47.0±2.7	1.4±0.1	16.3±1.7	87.6±2.3
14 days old, normals	7	27.9±0.6	35.8±0.4	5.5±0.1	14.7±1.2	924±39	27.2±2.1	1.6±0.1	12.9±0.2	
17 days old, 16 days at approx 4,700 m	6	22.0±2.0	42.9±1.2			1,580±107	55.9±3.2	4.3±0.6		
18 days old, normals	5	19.9±1.1	39.8±0.4	4.2±0.1	17.6±1.8	966±36	11.9±2.4	0.9±0.1		

* Values are means ± standard error.

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