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Neutrophil adherence receptor deficiency regressing with granulocyte-colony stimulating factor therapy in a case of glycogen storage disease type Ib

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Abstract Neutrophils from patients suffering from glycogen storage disease type Ib (GSD-Ib) show marked functional deficiencies (chemotaxis, respiratory burst, and phagocytosis). Here we describe neutrophil adherence receptor (L-selectin CD62L and \(\beta_2\) integrins CD11b/CD18) deficiency in a patient with genotype of GSD-Ib, who presented with recurrent infections, diminished neutrophil count and impaired functions. Treatment with granulocyte-colony stimulating factor (G-CSF) had a beneficial effect on the infectious status, the enhancement of phagocytosis and the regression of the adherence receptor defect. Conclusion: this is the first observation of a patient with glycogen storage disease type Ib with a deficiency in leucocyte adherence receptor expression, which regressed with growth factor therapy. It underlines the potential role of these receptors in the genesis of recurrent infections which occur in patients with this disease.

Keywords \(\beta_2\) integrins · Glycogen storage disease · type Ib · Granulocyte-colony stimulating factor · Polymorphonuclear neutrophil · L-selectin

Abbreviations fMLP formyl-methionyl-leucyl-phenylalanine · G6P glucose-6-phosphate · G-CSF granulocyte-colony stimulating factor · GSD-Ib glycogen storage disease type Ib · MFI mean fluorescence intensity · MoAb monoclonal antibody · PMN polymorphonuclear neutrophil · ZAS zymosan activated serum

Introduction

Patients suffering from glycogen storage disease type Ib (GSD-Ib) have characteristic clinical features such as hepatomegaly, fasting hypoglycaemia, lactic acidosis, hyperlipidaemia, hyperuricaemia, growth retardation and renal enlargement [1]. GSD-Ib is caused by a defective glucose-6-phosphate (G6P) translocase, which transports G6P across the microsomal membrane [4, 21, 27, 36]. Most patients with GSD-Ib suffer from infectious complications [37] except in those rare cases suggesting an allelic variant [7, 19]. Both neutropenia and the impaired functions of polymorphonuclear neutrophils (PMN) and monocytes cause the increased susceptibility to bacterial infections. The lymphocyte functions are normal [14, 37]. These functional deficiencies include a decrease in in vivo mobilization and mobility, in vitro migration, phagocytosis rate and respiratory burst. It has recently been found that GSD-Ib is an inherited autosomal recessive disorder mapping to chromosome 11q23 [3, 35]. However, the relationship between neutrophil deficiency function and the metabolic defect is still unclear.

We describe a patient with GSD-Ib who presented with recurrent infections, diminished neutrophil functions and a neutrophil adherence receptor deficiency (L-selectin CD62L and \(\beta_2\) integrins CD11b/CD18). This receptor deficit regressed with granulocyte-colony stimulating factor (G-CSF) therapy. To our knowledge,
this observation constitutes the first case of GSD-Ib with a deficiency in neutrophil adherence receptor expression.

Case report

The propositus was born at term after an uneventful pregnancy from non-consanguineous parents. Six hours after birth, the newborn presented with convulsions due to a hypoglycaemic attack (blood glucose 1.4 mmol/l, normal range 3.9–5.5 mmol/l). Complementary investigation revealed lactic acidemia (blood pH 7.2, lactic acid 3.77 mmol/l, normal range 0.34–0.78 mmol/l), hyperuricaemia (uric acid 416 μmol/l, normal range 238–420 μmol/l), hypercholesterolaemia (cholesterol 8.9 mmol/l, normal range <5.16 mmol/l), hypertriglyceridaemia (triglycerides 7.5 mmol/l, normal range 0.46–1.48 mmol/l) and normal liver transaminases. Clinical examination revealed hepatomegaly. Administration of either glucose or glucagon resulted in no rise in blood glucose, but the lactate level rose significantly. A liver biopsy demonstrated distended hepatocytes containing large and prominent vacuoles of glycogen and fat. The diagnosis of type 1 GSD was strongly suspected and appropriate glucose supplementation was given in order to maintain normal blood glucose and lactic acid concentrations, which was followed by a good growth (Fig. 1). Neutropenia was first noted at the age of 7 years (PMN <0.8x10³/l), and has since been consistently observed (Fig. 2A). Recurrent infectious complications (2 to 6 times a year) included primarily skin infections, in particular furuncles on the face, buttocks, legs, and perianal abscesses, recurrent otitis and one episode of respiratory infection. These episodes were treated with systemic antibiotics and required numerous surgical operations. This patient also suffered from oral mucosal ulcerations, once to twice a month. Bone marrow smears were hypercellular without impairment of maturation. The additional findings of neutropenia associated with recurrent infections lead us to consider the diagnosis of GSD-Ib. A deficiency of glucose 6-phosphatase activity has never been demonstrated in liver from this patient, but a recent study has mapped the gene of G6P translocase to chromosome 11q23 [3]. Veiga-da Cunha et al. [35] have shown that this region contains mutations and included our case and his family in their study. Mutation analysis revealed 121I delICT/N for the propositus, 121I delICT/N for the father and 121I delICT/N for the mother. This molecular finding confirmed the diagnosis of GSD-Ib.

At age 18 years, the frequent occurrence of infections (1 to 3 times a month) justified treatment with G-CSF (Fig.2B). The administration of filgrastim (Neupogen, 5 μg/kg per day s.c.) was complicated by myositis of both lower limbs and the treatment was discontinued. Two months later, the patient felt faint when he was administrated one injection of molgramastim (Leucomax). Finally, he demonstrated a good clinical tolerance to the injection of lenograstim (Granocyte, 5 μg/kg per day, 3 times weekly, s.c.) and the PMN count increased above the threshold of 1.5x10³/l. The patient’s infectious status was improved since aplasia disappeared and frequency of skin infections decreased to once per year.

Materials and methods

The patient’s PMN functions were explored before and after 1 year of therapy with G-CSF. Each analysis of PMN was twice repeated (Fig. 2B). During treatment, blood samples were drawn before the following G-CSF injection (which was administrated 3 times weekly), so the time elapsed between growth factor injection and blood sampling was evaluated to a maximum of 24 h. PMN were obtained from heparinised venous blood by sedimentation on 2% dextran, followed by osmotic lysis of residual erythrocytes. PMN were then washed and resuspended in a PBS buffer. Chemotaxis was measured using the agarose technique of Nelson et al. [28]. PMN were allowed to migrate under agarose. Formyl-methionyl-leucyl-phenylalanine (fMLP, Sigma, 10⁻³ M) or zymosan (10⁻³ M) were used as chemoattractants. Oxidative metabolism was measured with two techniques. The luminol-amplified chemiluminescence used a LKB luminometer. Phorbol myristate acetate (Sigma, 100 μg/ml) and opsonised zymosan A of Saccharomyces cerevisiae (Sigma, 0.5 g/l) were used as stimuli. Superoxide anion generation was measured by the superoxide dismutase (SOD) method for the determination of cytochrome C according to De Chatellet et al. [10] using opsonised zymosan as the stimulus. Phagocytosis was measured by flow cytometry with the Phagostest Orpegen kit (Becton Dickinson). In brief, heparinised blood was incubated with fluorescent Escherichia coli. After washing and red cell lysis, the DNA was stained. Fluorescence-activated cell analyses were performed with a FACSscan (Becton Dickinson) instrument using the PC-Lysis software.

Quantification of cell surface molecules was assessed according to the quantitative direct immunofluorescence method using Quantum Simply Cellular (Dako), on EDTA K₂ anticoagulated whole blood. The monoclonal antibodies (MoAb) conjugated with fluorescein isothiocyanate, isotypic control, CD62L MoAb (FM4C6), CD11b MoAb (2LP1M19c), and CD18 MoAb (MMH23), all from Dako, were applied under saturating conditions. After lysis of erythrocytes (Ortho-Mune, Orthodiagnostic), cells were analysed by flow cytometry. Under saturating conditions, antibodies are likely to bind to surface antigens through monovalent interaction. The number of antigenic sites per bead is proportional to the mean fluorescence intensity (MFI) measured [31]. Thus, for each sample tested, corrected MFI (i.e., specific MFI minus that of the negative control analysed under the same conditions) could therefore be converted into antibody binding capacity per cell.

Results

Before undergoing any therapy with a growth factor, both chemotactic and spontaneous motility to zymosan activated serum (ZAS, 10⁻³ M) and fMLP (10⁻⁷ M) of the patient’s neutrophils were diminished compared to neutrophil migration of healthy controls (ZAS 1.20/0.50 mm, control 2.76/1.51 mm; fMLP 0.96/0.55 mm, control 2.60/1.58 mm). Oxidative metabolism was altered. Chemiluminescence of PMN was diminished against zymosan (2.74 mV/s per 10⁶ PMN; control 5.18 mV/s per 10⁶PMN) and most markedly impaired against phorbol myristate acetate (0.65 mV/s per 10⁶ PMN; control: 1.38 mV/s per 10⁶PMN). Production of superoxide anion was also altered (30.4; control: 54.0 nMol O₂/10⁶ PMN during 30 min). Phagocytosis was impaired since only 43% of PMN ingested bacteria (normal >80% of PMN phagocytosis). Treatment of the patient with G-CSF for 1 year resulted in a marked increase in phagocytosis (95.7% of PMN phagocytosis) with no change in both chemotaxis and oxidative metabolism, which remained low.

Cytometric evaluation of adherence receptors showed an abnormal bimodal distribution of fluorescence on neutrophils from the GSD-Ib patient (Fig. 3). These histograms revealed two populations of PMN. The first one, 30% of all analysed neutrophils, presented a low expression of both L-selectin and β₂ integrins (CD11b/CD18). The second (70% on the PMN), showed normal or moderately increased expression of these receptors in comparison with heal-