A New Glucose-6-Phosphate Dehydrogenase Deficient Variant in a Patient With Chediak-Higashi Syndrome

By Josef T. Prchal, William M. Crist, Ahmad Malluh, Alena Vitek, W. Newlon Tauxe, and Andrew J. Carroll

A description is given of enzymatic characteristics of a new glucose-6-phosphate dehydrogenase variant (G-6-PD) found in 2 male members of an inbred Pakistani family. Properties of this enzyme differ in respect to electrophoretic mobility, utilization of deamino NADP, and $k_v$ for both G-6-PD and NADP from the variant reported to be common in Pakistan. The propositus, in addition to G-6-PD deficiency, also had Chediak-Higashi syndrome. Some male members of this family are affected by two distinct clinical disorders. One disease is characterized by intermittent jaundice and anemia, often concurrent with infection, compatible with G-6-PD deficiency. The other is characterized by severe, frequent, and occasionally fatal infections that are not necessarily associated with jaundice, a disease pattern compatible with Chediak-Higashi syndrome. A possible additive relationship of G-6-PD deficiency with Chediak-Higashi syndrome in causing frequent infections was examined. Only moderately decreased activity of G-6-PD was found in nonerythroid cells of 2 male children having this G-6-PD deficient variant. Moreover, the study of the patient’s mother revealed no obvious survival disadvantage of her nonerythroid cell clones carrying the G-6-PD-deficient variant as compared to the clones carrying the normal G-6-PD isoenzyme. Ascorbic acid therapy for the brother affected by both of these diseases did not lead to increased hemolysis. No obvious clinical improvement of severity and infection frequency was noted after several months of ascorbic acid therapy.

G LUCOSE-6-PHOSPHATE DEHYDRO-GENASE is a polymorphic enzyme with over 100 variants described thus far. Some of the deficient variants appear to be particularly common within certain racial and ethnic groups. For example, about 2.5% of Pakistanis were found to have G-6-PD deficiency characterized by no definite ill effects; this variant was designated as G-6-PD Campbellpore. This report describes one inbred Pakistani family with a previously undescribed G-6-PD mutant associated with intermittent hemolytic anemia. One of the affected boys also had Chediak-Higashi syndrome, an autosomal recessive disorder characterized by partial albinism, granular giantism in many cell lines, frequent infections, and often neutropenia.

CASE REPORTS

An 8-mo-old Pakistani male, N.A. was admitted with fever to Children’s Hospital, University of Alabama in Birmingham. His height was in the 50th percentile and weight at the 7th percentile. He was pale and irritable. Multiple skin areas and hair were hypopigmented. His hemoglobin was 6 g/dl and the reticulocyte count varied between 0% and 7%, and his neutrophil count ranged between 90 and 4800/cumm. His lymphocyte and platelet counts were normal. The child was iron deficient with a serum ferritin of 5 ng/ml. He responded to iron therapy with normalization of the hematocrit and skin pigmentation. The parents were first cousins and consanguinity had also occurred in previous generations. The patient’s mother had aborted two female fetuses.

A.S. is 8 yr and 4 mo older than his brother, the propositus. He had a severe hemolytic crisis at the age of 1 yr that required multiple blood transfusions. Both boys were found to have severe G-6-PD deficiency, but the elder had no history of significant infections, nor had he had any morphological evidence of Chediak-Higashi syndrome.

MATERIALS AND METHODS

Biochemical Study

G-6-PD was partially purified and characterized by standard methods. The $K_v$ for NADPH was determined according to Yoshi-

Fig. 1. Pedigree of Pakistani family S. The high death rate observed in this kindred could possibly be due to presence of some other autosomal recessive condition present as a result of the high degree of in-breeding.
phoresis on cellulose acetate was performed, as described previously. Reduced (GSH) and oxidized (GSSG) glutathione levels, as well as erythrocyte and leukocyte glutathione reductase, were determined as described by Beutler.

**Table 1. Kinetic Data of Described G-6-PD Variant**

<table>
<thead>
<tr>
<th></th>
<th>Red Cell Enzyme Activity (% of Normal)</th>
<th>Electro-horesic Mobility (% of Normal)</th>
<th>$K_m$ G-6-P (µM)</th>
<th>$K_m$ NADP (µM)</th>
<th>2-deoxy G-6-P NADP (µM)</th>
<th>Deamino NADP (% of NADP)</th>
<th>Heat Stability</th>
<th>pH Optimum</th>
<th>$K_m$ NADPH (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-6-PD-Birmingham</td>
<td>&lt;5%</td>
<td>Phosphate 122%</td>
<td>12</td>
<td>22</td>
<td>&lt;4</td>
<td>256%</td>
<td>Normal</td>
<td>6.5-9</td>
<td>10.91</td>
</tr>
<tr>
<td>Normal control</td>
<td>7.69 U/g Hb</td>
<td>Phosphate 41%</td>
<td>5</td>
<td>&lt;4</td>
<td>61%</td>
<td>Normal</td>
<td>100%</td>
<td>8.5-9</td>
<td>11.1</td>
</tr>
<tr>
<td>Reported range of normals (Glock and McClean method)</td>
<td>100%</td>
<td>Phosphate 40-70%</td>
<td>3-6</td>
<td>&lt;4</td>
<td>55%-60%</td>
<td>Normal</td>
<td>9</td>
<td>9.1 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

Red Cell Survival

Jand6 demonstrated that the chromium released from destroyed labeled red cells is not utilized for red cell binding, but is excreted in the urine at a relatively standard daily rate. Shih et al. 7 studied the kinetics of excretion of chromium-51 into the urine and determined that the chromium-51 is quantitatively excreted into the urine after red cell destruction. More recently, these authors 8 have determined the red cell survival as based on urinary excretion of chromium calculated from 3 to 4 24-hr urine collections. We have modified this procedure to study erythrocyte destruction rates in this case. Erythrocytes from approximately 10 ml of whole blood were labeled with chromium and washed 3 times in sterile physiologic saline. An aliquot of the dose was retained as a standard. Thereafter, the urines were collected at 4-24-hr intervals. Each urinary sample was counted for radioactivity along with the dose aliquot, and the creatinine excretion was determined to eliminate possible errors of collection and varying glomerular filtration rate. The radioactivity of each urine sample was expressed as a percentage of the total injected radioactivity; this value was subtracted from 100% radioactivity in sequential fashion; and from the declining slope of the resulting curve, the red cell survival was determined.

**RESULTS**

Characterization of G-6-PD Variant

The enzymatic properties of the as-yet-undescribed G-6-PD variant are summarized in Table 1. Repeated testing of both brothers' purified erythrocytes revealed activity less than 5% of normal, whereas the mother's activity was 64% of the normal, a level compatible with heterozygosity. The enzyme was electrophoretically fast on starch gel, using phosphate buffer (Fig. 2). With EBT and Tris buffers, electrophoretic mobility was normal. The mobility of this variant on a cellulose-acetate electrophoretic system was also identical to that of a normal "B" enzyme. The $K_m$ for NADP was increased (22 µmole), but the $K_m$ for G-6-P was decreased (12 µmoles). The rate of utilization was obtained prior to the study.

![Fig. 2. Starch gel G-6-PD electrophoresis in phosphate buffer, indicating faster migration of the mutant enzyme (S) as compared to migration of normal G-6-PD type B in a control subject (C).](image-url)
with "Cr and washed before injection. No ascorbic acid was added prior to the injection. Giant granules were present and melanin was absent. The fibroblasts of the elder brother, A.S., were normal. Table 2 depicts the activity of G-6-PD in these cells, the decrease of G-6-PD was significantly increased at 256%. Heat stability was normal, and the pH activity curve was biphasic with peaks at pH 6.5 and pH 9. The Kₐ for NADPH appeared to be at the upper limits of normal. Comparison of these characteristics with other published G-6-PD variants were indicative of an as-yet-undescribed G-6-PD variant. We have designated this variant G-6-PD-Birmingham.

Studies of Nonerythroid Cells of G-6-PD Deficient Boys and Heterozygous Mother

Fibroblasts, derived from skin biopsy, were examined by light microscopy. The fibroblasts of the propositus, N.S., were typical of Chediak-Higashi disease. Giant granules were present and melanin absent. The fibroblasts of the elder brother, A.S., were normal. Table 2 depicts the activity of G-6-PD in the granulocytes, lymphocytes, platelets, and fibroblasts of both A.S. and N.S. The data reveal that in these cells, the decrease of G-6-PD activity is only mild when compared with erythrocyte G-6-PD activity. Electrophoresis was performed on cell lysates of purified granulocytes, lymphocytes, and platelets of the mother of these G-6-PD-deficient boys (obligate heterozygote). Only slightly decreased intensity of the fast band (G-6-PD-Birmingham) compared to the slower band (G-6-PD type B), using starch gel with phosphate buffer system were found in all cell lysates.

Evaluation of Glutathione Metabolism

Table 3 shows levels of GSH, GSSG, and glutathione reductase in neutrophils and lymphocytes of the propositus N.S. and in a normal control. Normal glutathione reductase activity of granulocytes, when there is no evidence to indicate defective glutathione synthesis, rule out an impaired glutathione metabolism as a cause of infection in N.S.¹⁴,¹⁵

The Red Cell Survival of the Propositus and Influence of Ascorbic Acid on his Red Cell Survival

The survival of red cells before and after ascorbic acid challenge was estimated by labeling N.S.'s erythrocytes with chromium-51 followed by sequential detection of radioactive chromium in the urine. The results, demonstrated in Fig. 3, reveal that the lifespan of the red cells in the steady state is only slightly decreased. Furthermore, the challenge by 1 mg of oral ascorbic acid produced no significant hemolysis. The study was repeated 1 yr later, carried for longer period of time, and the red cell survival was also performed by more conventional method based on determining blood radioactivity. The repeated study showed similar results (see Fig. 4). Results of identical experiments carried out in two normal controls is shown in Fig. 5. No change of disappearance slopes were observed after challenge with 4 g of ascorbic acid.

DISCUSSION

G-6-PD-Birmingham is a G-6-PD mutant, characterized by normal hemoglobin concentration in the

<p>| Table 2. G-6-PD Activity in Nonerythroid Cells |
| Granulocytes | Lymphocytes | Platelets | Fibroblasts |</p>
<table>
<thead>
<tr>
<th>(in IU/10⁹ Cells)</th>
<th>(in IU/10⁹ Cells)</th>
<th>(in IU/10¹² Cells)</th>
<th>(in IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.S.</td>
<td>1.24</td>
<td>42.97</td>
<td>6.44</td>
</tr>
<tr>
<td>A.S.</td>
<td>1.41</td>
<td>29.69</td>
<td>12.84</td>
</tr>
<tr>
<td>Normal control</td>
<td>3.46</td>
<td>99.74</td>
<td>16.03</td>
</tr>
</tbody>
</table>

Satisfactory fibroblast culture of N.S. was not established because of bacterial contamination. The enzyme activity expressed in IU (μmole of the product generated in 1 ml of solution in 1 min at 37°C).

Table 3. Some Parameters of Glutathione Metabolism of Erythrocytes and Leukocytes in N.S. and Normal Control

<table>
<thead>
<tr>
<th>Erythrocyte GR (IU/g Hb)</th>
<th>Erythrocyte GSH (μmole GSH/g Hb)</th>
<th>Erythrocyte GSSG (μmole GSSH/g Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without FAD</td>
<td>With FAD</td>
<td></td>
</tr>
<tr>
<td>N.S.</td>
<td>7.63</td>
<td>0.0717</td>
</tr>
<tr>
<td>Normal control</td>
<td>7.64</td>
<td>0.012</td>
</tr>
<tr>
<td>Normal range</td>
<td>6.57 ± 1.04</td>
<td>0.0123 ± 0.0045</td>
</tr>
<tr>
<td>Granulocyte GR (IU/g Hb)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without FAD</td>
<td>With FAD</td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>66.99</td>
<td>131.98</td>
</tr>
<tr>
<td>Normal range</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4. Red cell survival of the patient whose first study is shown in Fig. 3, repeated 1 yr later. Blood radioactivity is depicted as triangles, urine radioactivity as full circles. Chromium T½ prior to ascorbic acid administration was 39.7 days with a coefficient of correlation of 0.9995. These evaluations were based on the urinary chromium excretion. Variability of blood radioactivity did not permit detection of minor changes of red cell survival. The dose of ascorbic acid was proportionately increased to 1.2 g corresponding to patient’s increased weight. The data based on urinary radioactivity excretion were corrected by creatinine excretion, necessitated because of incomplete emptying of urinary bladder in a 3-yr-old child. The blood was labeled by 19Cr without any addition of ascorbic acid.

steady state and intermittent hemolytic episodes associated with infections. The enzymatic characteristics of this variant demonstrate that it has not been previously described. Specifically, it appears to be distinct from G-6-PD-Campbellpore, which has been reported to be very prevalent among the Pakistanis.

A significant G-6-PD deficiency in leukocytes would result in a defective pentose shunt; this would impair the generation of NADPH, which is necessary not only to protect the host against bacteria but also to protect phagocytic cells from the oxidative radicals produced during the process of disposal of infectious agents. This defect could be expected to impair further the antibacterial defense already reduced by Chediak-Higashi disease. G-6-PD deficiency is generally not complicated by infections, and this metabolic defect is detrimental only to the red cells; however, increased susceptibility to infection has been described in a patient with severe leukocytic deficiency of G-6-PD. Our data would indicate that nonerythroid cells in two studied subjects having the 0-6-PD-defect are not complicated by infections, and this metabolic defect is analogous to patient’s increased weight. The data based on urinary radioactivity excretion were corrected by creatinine excretion, necessitated because of incomplete emptying of urinary bladder in a 3-yr-old child. The blood was labeled by 19Cr without any addition of ascorbic acid.

Fig. 5. The effect of ascorbic acid administration on the urinary chromium excretion in 2 healthy subjects. Subject 1 (open circles) had red cells washed prior to injection. Subject 2 (filled circles) had the blood injected after labeling without washing. Ascorbic acid was not used after labeling.

G-6-PD Birmingham/Chediak Higashi Syndrome is further suggested by the analysis of the family pedigree, which reveals that the male rather than the female members of this family are afflicted by frequent infections. However, two aborted female fetuses might have developed Chediak-Higashi disease. The in vivo influence of this G-6-PD mutant on the neutrophil lifespan could perhaps be best tested by examining the proportions of normal and G-6-PD-Birmingham neutrophils in a heterozygous female. Thus, the neutrophils with the mutant enzyme might be at a disadvantage in comparison with neutrophils that exhibited normal G-6-PD activity, and the latter would then be expected to predominate. In a somewhat analogous situation, suppression of leukocytes carrying a sex-linked defect of purine metabolism (Lesch-Nyhan syndrome) has indeed been described in a female who was also heterozygous for G-6-PD isoenzymes A and B, as well as for the Lesch-Nyhan syndrome. In this instance, both the erythrocytes and leukocytes displayed a single G-6-PD isoenzyme and normal hypoxanthine guanine phosphoribosyl transferase activity. Presumably, those cells expressing the opposite G-6-PD isoenzyme, as well as Lesch-Nyhan defect, were at a survival disadvantage. The mother of our two deficient boys exhibited a similar proportion of both G-6-PD isoenzymes (G-6-PD-B and G-6-PD-Birmingham) in her neutrophils, which suggested that the neutrophils that expressed the deficient mutant enzyme and the normal neutrophils would have relatively equal chances of survival.

Ascorbic acid has been reported to ameliorate the clinical course of patients with Chediak-Higashi disease. On the other hand, ascorbic acid has been reported to lead to a decrease of erythrocyte GSH
level in G-6-PD-deficient erythrocytes,\(^2\) to decrease the red cell survival of in vitro treated human G-6-PD-deficient erythrocytes in an animal model,\(^3\) to produce mild hemolysis in G-6-PD deficient subjects,\(^4\) and to lead to severe intravascular hemolysis after intravenous administration in massive doses of 80 g for 2 consecutive days.\(^5\) However, Winterbourn proposed that ascorbate might aid in alleviating G-6-PD deficiency.\(^6\) Challenge with doses of ascorbic acid considered to be therapeutic caused no measurable decrease in life span of red cells in this particular G-6-PD variant. In point of fact, the red cell survival appeared to improve following an ascorbic acid challenge based on the urinary chromium excretion method; however, we did not observe any significant change of red cell survival when measured by more conventional method based on following of blood radioactivity, possibly due to greater scatter of the points. Moreover, the apparent change of the slope of urinary radioactivity may have been caused by more than one red cell population present in the patients with red cell metabolic defect. This possibility is further supported by the fact that the putative beneficial influence of a single dose of ascorbic acid would be expected to be transient following a single dose of ascorbic acid, however, as shown in Fig. 4, there was no change of the slope for 120 hr after ascorbic acid administration. We have not had an opportunity to repeat the study again on this patient without ascorbic acid administration to clarify the effect of the ascorbic acid on this patient's red cell survival. Unfortunately, after receiving 200 mg of ascorbic acid twice daily for 8 mo, the patient has not displayed any obvious clinical benefits with respect to infections similar to experience of others.\(^7\)

We conclude that the frequency and severity of infection in our patient with Chediak-Higashi syndrome cannot be clearly attributed to the possible additive interaction from the concomitant presence of the newly described G-6-PD-deficient variant.

REFERENCES

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