Hemolytic Anemia, Recurrent Metabolic Acidosis, and Incomplete Albinism Associated With Glutathione Synthetase Deficiency

By J. T. Prchal, W. M. Crist, M. Roper, and V. P. Wellner

The clinical and laboratory features of a 3-mo-old black male infant with glutathione (GSH) synthetase deficiency of the generalized type was evaluated. Partial albinism, brisk hemolytic anemia, recurrent febrile episodes, and mental retardation were noted. Also, severe recurrent metabolic acidosis and marked oxoprolinemia and oxoprolinuria were found in the proband but not in his first-degree relatives. The relationship of these disease manifestations to the underlying metabolic defect is discussed.

GLUTATHIONE (GSH) is present in high concentrations in erythrocytes and is thought to play a central role in protection of erythrocytes against oxidative damage. While a modest decrease of erythrocyte glutathione may be associated with hemolytic anemia and may be found in association with glucose-6-phosphate dehydrogenase (G6PD) deficiency and GSH reductase deficiency, an extreme decrease of GSH is found in gammaglutamyl synthetase deficiency and GSH synthetase deficiency. Glutathione synthetase deficiency, a rare genetic defect leading to red blood cell (RBC) glutathione deficiency, has been described in eight previous families. There are two distinct forms of GSH synthetase deficiency. In the first, the defect is confined to RBCs and hemolysis may be observed; in the other, the deficiency of the enzyme is present in all cells and metabolic acidosis may be present.

We recently evaluated a black infant with incomplete albinism and GSH synthetase deficiency extending to nonerythroid cells, who has chronic severe hemolytic anemia, metabolic acidosis, and frequent infections. A description of the clinical and laboratory features and results of metabolic studies in this patient form the basis for this report.

CASE REPORT

A 3-mo-old black male infant was referred to the Children's Hospital for metabolic acidosis, seizures, and anemia. Past history revealed an uncomplicated term pregnancy. His neonatal period was complicated by hyperbilirubinemia (19.4 mg/dl) requiring exchange transfusions. Partial albinism was noted at birth. His skin has a golden yellow coloration and his hair is fine and lightly brown. By 3 mo of age, he developed fever and grunting respirations; an arterial blood pH was 7.05 and bicarbonate 4.3 meq/liter. Increasing doses of NaHCO₃ failed to correct the acidosis, and he was transferred to the Children's Hospital in Birmingham. On the day of transfer, he developed tonic-clonic movements in the left upper and lower extremities. Physical examination revealed an 8 lb. 14 oz. infant with respirations of 60/min, pulse of 160/min, and an axillary temperature of 100.1°F. Poor head control was noted. His complexion was so light skinned as to appear Caucasian. Severe hyperbilirubinemia (19.4 mg/dl) requiring exchange transfusions necessitating hospitalization and intravenous fluid and antibiotic therapy. His hemoglobin was 7.8 g/dl, hematocrit 25%, and reticulocyte count 18%. The white cell and platelet counts were normal. Serum chloride was 98-110 meq/liter and serum potassium 4.6-5.9 meq/liter. The anion gap and glomerular filtration rate were normal, as was renal bicarbonate reabsorption.

During this hospitalization, the infant was found to have persistent hemolysis (hemoglobin 7-9 g/dl; reticulocyte count 11%-30%) that did not improve with resolution of the acidosis. Target cells were found on the peripheral blood smear. The RBC indices were: mean corpuscular volume (MCV) 88/dl, mean corpuscular hemoglobin (MCH) 26 pg, and mean corpuscular hemoglobin concentration (MCHC) 31%. Direct and indirect Coombs tests were negative. The hemoglobin electrophoresis and isopropanol tests for unstable hemoglobin were normal. A Heinz body preparation after acetylphenylhydrazine challenge was positive.

The child is now 3 1/2 yr old and requires daily bicarbonate replacement to control his acidosis. He has had at least 8 febrile episodes requiring medical attention. Also, he has had an episode of staphylococcal toxic epidermal necrolysis necessitating hospitalization and intravenous fluid and antibiotic therapy. His hemoglobin remains low (7-9 g/dl) with continued brisk hemolysis (reticulocyte count 8%-13%). His skin remains pale. The patient has severe motor and mental retardation, functioning at the developmental level of a 3-mo-old. The family pedigree is depicted in Fig. 2.

MATERIALS AND METHODS

GSH and glutathione disulfide (GSSG) and selected RBC enzymes were assayed by standard techniques. Gamma-glutamyl cysteine synthetase and GSH synthetase were measured as described by Minnich, using l-[3H]glutamic acid, L-cysteine-HCl, l-gamma-glutamyl-L-cysteine, and [14C]-glycine. The erythrocytes and granulocytes were purified and skin fibroblasts cultured as previously described.
Albinism

Albinism Proven GSH Deficiency

Died at Infancy, Jaundiced Some Dark Pigment

Propositus Not Tested

GSH SYNTHETASE DEFICIENCY

El-Heterozygosity for GSH Synthetase Deficiency

Fig. 2. Family pedigree. The proband's brother is depicted to be heterozygous for GSH synthetase deficiency; however, homozygosity cannot be completely excluded.

Fig. 1. Photograph of the propositus and his mother.

RESULTS

GSH, GSSG, and RBC Enzyme Levels

The GSH, GSSG level, and activities of RBC enzymes relevant to glutathione metabolism are shown in Table 1. The mild decrease of erythrocyte GSH level of the father is likely due to his coincidental glucose-6-phosphate dehydrogenase (G6PD) deficiency (0.8 IU/g Hb at 37°C, electrophoretic mobility compatible with A-variant). None of the relatives studied has anemia nor any evidence of hemolysis. The increased activity of G6PD found in the propositus is likely due to the increased proportions of young red cells that were present in this subject with hemolysis. The young red cells are known to have increased G6PD activity. When other RBC enzymes were measured in the above subjects (erythrocyte hexokinase, pyruvate kinase, glucose phosphate isomerase), no decrease of activity nor kinetic abnormalities were detected, except for an increased activity of hexokinase and a somewhat milder increase of pyruvate kinase activities in the propositus, as expected during hemolysis.

GSH Synthetase Activity in Nonerythroid Cells, Inhibitor, and Enzyme Stability Study

The GSH synthetase activity of separated granulocytes and cultured fibroblasts is shown in Table 2. Again, the propositus had a significant decrease of GSH synthetase activity in both granulocytes and fibroblasts, comparable to his RBC GSH synthetase deficiency. The activity of this enzyme in the fibroblasts of both parents was intermediate between that found in a control and that of the propositus. No inhibition of GSH activity was evident when the hemolysate of the propositus and of a control was coincubated; GSH synthetase activity of the mixture was additive. Also, incubation of the patient's hemolysate at 56°C revealed no material difference in enzyme activity decline at 20, 40, and 60 min of incubation.

Table 1. Glutathione Levels and Activities of Some Enzymes of the Glutathione Cycle of the Propositus and Family Members

<table>
<thead>
<tr>
<th>Glutathione Reductase (IU/g Hb)</th>
<th>GSH Peroxidase (IU/g Hb)</th>
<th>G6PD (umole/g Hb/min)</th>
<th>GSH (umole/g Hb)</th>
<th>GSSG (umole/g Hb)</th>
<th>GSH Synthetase (umole GSH/g Hb/min)</th>
<th>GC Synthetase (umole GC/g Hb/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without FAD/With FAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propositus</td>
<td>7.1/9.9</td>
<td>31.4</td>
<td>19.6</td>
<td>0.2; 0.57</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Brother</td>
<td>27.2</td>
<td>5.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>31.9</td>
<td>5.5</td>
<td>6.6</td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Father</td>
<td>28.9</td>
<td>0.5</td>
<td>4.6; 4.3</td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Control</td>
<td>6.5/8.3</td>
<td>31.9</td>
<td>8.8</td>
<td>6.4</td>
<td>0.01</td>
<td>0.18</td>
</tr>
<tr>
<td>Normal range</td>
<td>5.4 ± 1.52/8 ± 1.63</td>
<td>32 ± 3.8</td>
<td>8.3 ± 1.6</td>
<td>6.6 ± 1.04</td>
<td>0.0123 ± 0.0045</td>
<td>0.19 ± 0.03</td>
</tr>
</tbody>
</table>

FAD, flavin adenine dinucleotide; IU, international unit; SC, γ-glutamyl cysteine.
60 min, the GSH synthetase activity of both the propositus and the normal control declined by about 50%, as compared to that observed at time 0.

**Plasma Amino Acid Analysis**

The plasma amino acids were generally in the normal range, except for 5-oxoproline, which was 5.04 mM (normal, 0.8 mM). The urine 5-oxoproline was increased (Table 3).

**Other Studies**

Slit lamp examination revealed lack of lens opacities. A skin biopsy showed decreased melanin pigmentation and normal melanocytes. Because of the marked discrepancy of skin pigmentation of the propositus and his parents, the HLA haplotypes of the family were determined and a probability of paternity of greater than 92% established.

**DISCUSSION**

Our family with GSH synthetase deficiency demonstrates a number of interesting clinical and biochemical abnormalities. This patient has partial albinism, suffers from severe hemolysis, and his course has been characterized by severe chronic metabolic acidosis requiring frequent bicarbonate treatment. He also demonstrates an unusually high plasma–urine 5-oxoproline ratio, striking propensity to recurrent febrile episodes, and marked neurologic impairment with recurrent seizures. The deficient enzyme from our patient is characterized by normal enzymatic stability. Also, the enzyme deficiency is present in nonerythroid as well as erythroid cells, including granulocytes and fibroblasts. It appears to be inherited as an autosomal recessive, since both parents and one sibling have intermediate levels between that observed in the patient and that obtained from a normal control. Since the proband’s parents are first cousins, it is more likely that he is homozygous for a single mutant gene and not a compound heterozygote. The asymptomatic brother has a more severe decrease of GSH synthetase than his parents; however, his erythrocyte GSH is normal. Since he is not available for repeated enzyme studies or for oxoproline measurements, we assume that he is heterozygote, although homozygosity of this enzymatic deficiency cannot be completely ruled out. Only six other described patients have had this type of generalized defect.

GSH synthetase deficiency is a biochemical defect expressed in all cell types or alternatively present only in the erythroid cells. In the latter case, the enzyme is unstable and therefore cannot be resynthesized in the mature enucleate erythroid cells that lack the necessary apparatus to synthesize proteins. The resultant lack of glutathione leaves the mature red cells vulnerable to oxidant stress. The brisk rate of hemolysis seen in our patient is difficult to explain, since his GSH synthetase level and RBC GSH are higher than that seen in most previously reported patients. Other reported patients have had mild or even compensated hemolysis. It is possible that the generalized nature of this patient’s enzymatic defect, with its attendant severe recurrent acidosis and frequent infections, results in exacerbated RBC stress with attendant accelerated hemolysis. One might speculate that the impaired activity of hexokinase and phosphofructokinase in an acid environment may play a contributory role in the observed hemolysis. Alternatively, the GSH synthetase present in our patient could be associated with kinetic abnormalities associated with lower in vivo activity than that detected under artificial in vitro conditions. However, the relatively “high” RBC GSH level makes this possibility less likely.

The generalized defect seen in our patient is also responsible for a marked build up of 5-oxoproline, since GSH synthetase is needed to convert gamma-glutamyl cysteine to glutathione. The abnormally high level of gamma-glutamyl cysteine is converted to 5-oxoproline and glutamate by the action of gamma-glutamyl cyclotransferase. The build up of this substrate is also responsible for the severe chronic metabolic acidosis observed.

Low levels of GSH have been associated with cataract formation, neutrophil dysfunction, and neurologic disease, and GSH is involved in melanin metabolism as well. Interestingly, no cataracts were seen in our patient or in a child with congenital deficiency of glutathione reductase and a low RBC GSH level. However, it is possible that lens opacities will eventually develop. Also, GSH recently has been reported to

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Table 2. GSH Synthetase in Nucleated Cells

<table>
<thead>
<tr>
<th></th>
<th>Fibroblasts (umole GSH/g protein/min)</th>
<th>Granulocytes (umole GSH/g protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propositus</td>
<td>0.0485</td>
<td>0.025</td>
</tr>
<tr>
<td>Mother</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>0.169</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.489</td>
<td>0.603</td>
</tr>
</tbody>
</table>

Table 3. 5-Oxoproline in Urine and Plasma of Propositus, Parents, and Control

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Urine (mM)</th>
<th>Plasma (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propositus</td>
<td>1</td>
<td>56.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>27.1</td>
<td>5.04</td>
</tr>
<tr>
<td>Mother</td>
<td></td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td></td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>
play a role in granulocyte-mediated defense against infections; however, GSH also converts neutrophil leukotriene B₄, a potent stimulator of neutrophil chemotaxis and adherence, to leukotriene C₄, which does not have the above functions. Glutathione also serves as a substrate for the glutathione–peroxidase system, which dispenses toxic peroxides and thus prevents autooxidative damage to phagocytic cells. Indeed, a patient with GSH synthetase deficiency suffering from frequent infections and transient neutropenias has been reported. Neutropenia has not been observed during any febrile episodes. Recently, a Norwegian deficiency was causative of the neurologic syndromes and transient neutropenias. Selective atrophy of the granule cell layer of the cerebellum and focal lesions of the cortex and thalamus were found. It was uncertain whether the GSH synthetase deficiency was causative of the neurologic impairment. Our patient suffered cardiac respiratory arrest at birth, which alone or in conjunction with posticteric encephalopathy caused by neonatal hyperbilirubinemia, could have caused the motor and intellectual retardation. The postmortem examination of the Norwegian patient, however, was said to rule out posticteric encephalopathy. Our patient’s striking hypopigmentation is of interest, since glutathione is involved in melanin metabolism. The exact relationship of glutathione deficiency to decreased pigment formation is unclear. Also, it should be pointed out that incomplete albinism is not infrequent among blacks.

In order to determine whether our patient’s albinism was inherited along with or independently of GSH synthetase deficiency, it would be desirable to evaluate GSH synthetase levels in incomplete albino relatives of the proband. Unfortunately, these subjects were not available for the study.

GSH SYNTHETASE DEFICIENCY

REFERENCES

Hemolytic anemia, recurrent metabolic acidosis, and incomplete albinism associated with glutathione synthetase deficiency

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