Special Article

Hereditary Hemolytic Anemia with Special Reference to Erythroenzymopathies**

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I. Introduction

Hereditary hemolytic anemia is a syndrome caused by inborn errors of metabolism, the major cause of which are either membrane defects, hemoglobinopathies, decreased or lack of one of the two globin chain production, erythroenzymopathies (red cell enzyme anomalies) or abnormal porphyrin metabolism. In this article, the results of an epidemiological survey in Japan is presented first, followed by a brief review on hemolytic anemias due to membrane defects, hemoglobinopathies and thalassemias, and then the main part of it is devoted to erythroenzymopathies associated with hereditary hemolytic anemia on which I have been working hard during these 24 years.

II. Epidemiological survey of hereditary hemolytic anemia in Japan

The study was made in 1974 by us supported by the grant-in-aid from the Ministry of Health and Welfare. The results revealed that the incidence of hereditary hemolytic anemia among Japanese was 5.7–20.3 per million people, 70% of which was hereditary spherocytosis. In contrast, hemoglobinopathies constituted 1.5%, thalassemias 3.6% and erythroenzymopathies 6.4%. It has been said that the incidence of hereditary spherocytosis among Caucasian is 200–300 per million1. Hence, the incidence of this disorder in Japanese appears to be 1/7–1/10 of that in Caucasians. Other hereditary hemolytic anemias such as sickle cell anemia (one of the hemoglobinopathies), thalassemia and glucose 6-phosphate dehydrogenase deficiency (one of the erythroenzymopathies) are frequently seen among certain ethnic groups. Hence, it can be concluded that the incidence of hereditary hemolytic anemia in Japanese is, in general, definitely less than that in the people of other races.

III. Hereditary hemolytic anemias due to membrane defects

Very rapid progress in clarifying the pathogenesis of these disorders had been made in the past 2–3 years. In the 1970s, a great deal of knowledge was gained about the red cell membrane cytoskeleton. In 1978, Greenquist et al.2) found a spectrin deficiency in hereditary spherocytosis of mouse. In human, 20–30% of hereditary spherocytosis and all the cases of pyropoikilocytosis examined up to the present, were found to be due to defective spectrin dimer-dimer interaction (α-chain variant) whereas 10–30% of hereditary elliptocytosis was due to defective spectrin-band 4.1 interaction. Other defects clarified include spectrin deficiency in rare autosomal recessive hereditary spherocytosis, band 4.1 deficiency in rare cases of hereditary spherocytosis, defective spectrin dimer-dimer interaction (β-chain variant) in rare cases of hereditary spherocytosis and defective ankyrin-band 3 interaction in hereditary elliptocytosis3).

However, in many cases of hereditary spherocytosis and hereditary elliptocytosis, the pathogeneses still remains to be proven.

IV. Hemoglobinopathies associated with hemolytic anemia

Although the results of a population survey of hemoglobinopathies in several parts of Japan

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**Presented at the 81st Annual Meeting of the Japanese Society of Internal Medicine, held on April 2, 1984, in Fukuoka.
Hereditary Hemolytic Anemia

revealed that the incidence was around 1/3000, most of them were asymptomatic.

Sickle cell anemia has not been found in Japanese. Hence, unstable hemoglobin is the most important hemoglobinopathy in Japan which causes hereditary hemolytic anemia. It is inherited as an autosomal dominant trait.

Usually, an amino acid substitution or deletion around the heme pocket of a globin chain causes instability of the hemoglobin molecule, resulting in denaturation of hemoglobin and formation of Heinz bodies. Heinz-body containing red cells cannot pass through the narrow space of endothelial cells of the splenic sinus and hemolysis occurs by both intravascular and extravascular mechanisms. Before splenectomy, Heinz bodies are not marked but after splenectomy, they are easily detectable because of the lack of splenic pitting function. In general, splenectomy is effective, but the degree of effectiveness varies from case to case.

Unstable hemoglobin hemolytic anemia as well as erythronzymopathies associated with hemolytic anemia belong to so-called congenital nonspherocytic hemolytic anemia. For these years, many blood specimens from patients with congenital nonspherocytic hemolytic anemia have been sent to our laboratory from many hospitals in all parts of Japan asking us to determine red cell enzyme activities. Recently, we began to check unstable hemoglobin by isopropanol test. In collaboration with the Central Clinical Laboratory of Yamaguchi University Hospital, suspected blood samples were tested for unstable hemoglobins⁴. Fourteen families were found to have unstable hemoglobins and in 11 families the primary structures of abnormal globin chains were determined. They are Hb Tochigi, Hb Genova, Hb Abraham Lincoln, Hb Tottori, Hb Bristol, Hb Yokohama, Mb Miyashiro, Hb Köln (2 families), Hb Saitama and Hb Christchurch. Hbs Tochigi, Tottori, Yokohama, Miyashiro and Saitama are new unstable hemoglobins.

V. Thalassemias

Recently, thalassemias have attracted the attention of many investigators in the fields of medicine, biology, biochemistry and human genetics because their pathogenesis can now be analysed precisely at the gene level, and in addition, in some forms of thalassemia, prenatal diagnosis can be made⁵. In Japan, thalassemias are not common. The incidence of heterozygotes appears to be around 1/5000. The β-thalassemia heterozygote is predominant in Japan.

Regarding α-thalassemia, most cases result from deletion of the α-globin gene. In contrast, most cases of β-thalassemia appear not to be due to gene deletion but to a single base change resulting in a nonsense codon, defective splicing and so forth.

VI. Erythronzymopathies associated with hereditary hemolytic anemia

Mature red cells depend mainly on Embden-Meyerhof glycolytic pathway for energy production. Recently, ATP production from adenosine via adenosine kinase and adenylate kinase has appreciated as being another important pathway, because it was found that adenosine deaminase overproduction, a recently described disorder, deprives the red cell of adenosine, a substrate of adenosine kinase, and causes hereditary hemolytic anemia⁶. The pentose cycle is important for the production of NADPH which is required to protect hemoglobin and membrane from oxidative stress caused by certain drugs. NADPH is necessary for the glutathione reductase reaction, which reduces oxidized glutathione to reduced glutathione. A pyrimidine specific nucleotidase, pyrimidine 5′-nucleotidase was found to be important for red cells in the maturation process of reticulocytes. Pyrimidine 5′-nucleotidase acts on pyrimidine nucleotides, constituents of RNA in ribosomes, and produces pyrimidine nucleosides, which can diffuse out of the red cell. This fact was clarified recently as a result of the discovery of this enzyme deficiency associated with hemolytic anemia, an experiment of nature⁷. The Rapoport-Luebering cycle is also an important bypass for the red cell, because 2,3-diphosphoglycerate (DPG) is formed in this pathway and DPG plays an important role in the regulation of hemoglobin-oxygen dissociation.

Up to the present, 16 kinds of red cell enzyme anomalies have been known to cause congenital nonspherocytic hemolytic anemia⁸. A patient
with H-subunit of lactate dehydrogenase deficiency was found in Japan but was asymptomatic, probably because both pyruvate and lactate can diffuse out of the cell and pyruvate accumulation does not occur. Regarding 2,3-diphosphoglyceromutase deficiency, there are case reports of hereditary hemolytic anemia in one hand and report of polycythemia in the other hand. Beutler claims that there often are secondary glutathione reductase deficiency due to riboflavin deficiency and also secondary glutathione peroxidase deficiency due mainly to selenium deficiency and have to be careful to make a diagnosis of hereditary enzyme deficiency without careful family studies.

Numbers of cases of erythroenzymopathies associated with hereditary hemolytic anemia found in our laboratory, in Japan as well as in the world are shown in Table 1. In Japan, 11 out of 16 kinds were found and in total, 129 cases in 103 families have been reported. Among these cases, 82 families and 92 patients with 10 kinds of erythroenzymopathies have been reported from my laboratory, which constitute over 70% of the cases found in Japan.

Apart from pyruvate kinase in which the isozymes between red cells and other organs are different, many enzymes listed in Table 1 stem from the same structural genes and do not have isozymes. However, the symptom is mainly limited to hemolytic anemia, and only in a few enzyme anomalies, other organ manifestations occur. What are the reasons for this? Mature red cells lack ribosomes and cannot synthesize enzymes. In addition, normal mature red cells can survive rather long period, 120 days. Many abnormal enzymes which cause hemolytic anemia have unstable characteristic. Because of this, enzyme activity decreases rapidly in a patient’s red cells, while the other organs have ribosomes and can produce abnormal enzyme molecules and can compensate the metabolic function even if they are defective.

1) Pyruvate kinase (PK) deficiency
PK deficiency was discovered by Valentine, Tanaka and Miwa in 1961 as the first enzyme

Table 1. Numbers of families and Cases of Erythroenzymopathies Associated with Hereditary Hemolytic Anemia

<table>
<thead>
<tr>
<th>Name of Abnormal Enzymes</th>
<th>Found in Our Laboratory</th>
<th>Found in Japan</th>
<th>Found in the World</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Families</td>
<td>Cases</td>
<td>Families</td>
</tr>
<tr>
<td>Embden-Meyerhof Glycolytic Pathway</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexokinase</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Glucosephosphate isomerase</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>4</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Aldolase</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Triosephosphate isomerase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>44</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>Rapoport-Luebering Cycle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,3-Diphosphoglyceromutase</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pentose Cycle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>16 (45)</td>
<td>20 (54)</td>
<td>26 (67)</td>
</tr>
<tr>
<td>Glutathione metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>1</td>
<td>1*</td>
<td>3</td>
</tr>
<tr>
<td>Glutathione synthetase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ß-Glutamylcysteine synthetase</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nucleotide metabolism</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenylate kinase</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pyrimidine 5'-nucleotidase</td>
<td>6</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Adenosine deaminase (overproduction)</td>
<td>2</td>
<td>2 (4)</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>83 (112)</td>
<td>92 (128)</td>
<td>101 (144)</td>
</tr>
</tbody>
</table>
deficiency of the Embden-Meyerhof pathway which causes congenital non-spherocytic hemolytic anemia. Family studies showed autosomal recessive inheritance. It was also noticed at that time that the patients' leukocytes had normal PK activity. Later, it became clear that there are 3 isozymes, namely, M1, M2 and L in humans as a result of electrophoretic and kinetic studies by Imamura et al.

In collaboration with Dr. Tanaka's laboratory, we were able to demonstrate for the first time the existence of electrophoretically slow-moving abnormal PK in a patient with PK deficiency. Although, several laboratories had demonstrated that red cell PKs of PK deficient patients showed abnormal kinetics, they did not succeed to demonstrate electrophoretically distinct abnormal PKs.

Around 1975, the investigators of PK deficiency considered it necessary to standardize the procedures for the characterization of red cell PK variants in order to compare the results internationally. A subcommittee of the Expert Panel for the Red Cell Enzymes was established in the International Committee for Standardization in Haematology (ICSH), in which I played a role as a chairman. The procedures were determined and issued in 1979. Characteristics of 7 PK variants were determined by us by the ICSH methods. They have in general, high Km for phosphoenolpyruvate, thermal instability and often low Ki for ATP.

As red cells, leukocytes and platelets differentiate from the same multipotential hematopoietic stem cell and as leukocytes and platelets both have M2-type PK, M2-type should be present at the early stage of maturation of erythroblasts. The mature red cell has L-type PK. Using immunofluorescent antibody techniques, conversion of PK isozymes during the maturation of erythroblast was studied. In normal subjects, M2-type PK was clearly seen at the proerythroblast stage, then markedly declined with cell maturation, whereas L-type PK continued to increase. A similar phenomenon was seen in K-562 human leukemia cell line by induction of differentiation with hemin, too. Hemoglobin synthesis and L-type PK production appear to be interrelated during the differentiation and maturation of erythroblasts. In a classical PK deficiency case, M2-type PK was still clearly seen in orthochromatic erythroblasts, whereas L-type PK was scarcely detected during maturation. In other 3 PK deficiency cases, change of L-type PK showed a similar pattern to that of normal while M2-type PK was still clearly seen at the later stage of maturation as in the classical type probably by compensatory mechanism.

2) Phosphoglycerate kinase (PGK) deficiency

The primary structure of human red cell PGK was determined in 1980 by Yoshida's laboratory. A tertiary structure became almost certain. In four PGK variants, single amino acid substitutions were determined. For PGK, it has become possible to discuss the relationship between structure and function (degree of hemolysis) at the molecular level like that of hemoglobinopathies.

3) Pyrimidine 5'-nucleotidase (P5N) deficiency

P5N deficiency was initially described in 1974. Basophilic stippling is the hallmark of the disease. We collected 10 cases and they showed different kinetic and electrophoretic characteristics. P5N was strongly inhibited by lead, and it has become clear that the basophilic stippling and hemolytic anemia seen in acute lead poisoning was caused by P5N inhibition by lead. We experienced a case of acute lead poisoning with decreased P5N activity. In addition to competitive inhibition of pyrimidine nucleotides against purine nucleotides, Matsumoto et al. showed that intravascular hemolysis also occurred during the passage of red cells in the spleen. The latter mechanism is similar to that of unstable hemoglobin hemolytic anemia.

4) Adenosine deaminase (ADA) overproduction

ADA overproduction (45- to 70-fold) associated with hemolytic anemia was initially described by Valentine et al. in 1977. We found 2 cases in 2 families. Red cell ADAs were purified from normal subjects and a patient and compared. Kinetic studies, amino acid composition as well as peptide mapping all showed no difference between the two, almost certainly indicating that the disease was caused by overproduction of a normal enzyme.

Jap J Med Vol 24, No 1 (February 1985)
REFERENCES