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Brief Notes on Selected Hemoglobinopathies Experienced in Yamguchi University

Yuzo Ohba

Ehime Rosai Hospital
13-27 Minami-Komatsubara-cho Niihama-shi, Ehime-ken 792-8550, Japan.
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Abstract Hemoglobin (Hb) variants were found in Japanese for the first time in 1959, ten years after the discovery of sickle cell Hb (Hb S) in USA. Hb Ms as a cause of hereditary nigremia marked the first stage of hemoglobinopathy research in Japan, and a variety of unstable hemoglobins causing hemolytic disease the second stage. Although the prevalence of varints of Hb A (α or β) among Japanese was estimated to be only 1 : thousands, they were rich in variety. Virtually all types of abnormal Hbs were found among Japanese: hereditary nigremia, unstable Hb hemolytic disease, hyperunstable Hb disease mimicking a relatively severe form of thalassemia syndrom syndrome, hereditary erythremia due to high oxygen affinity Hbs, and so on. Most Hb variants were clinically silent, but some of them showed unique properties of interest.

Thalassemia (thal) traits were more frequent than the structural variants of Hb A (1 : 1000 for β-thal, more for α-thal). Like Hb variants, mutations leading to β-thal were rich in variety, but several of them comprized over a half of all cases. Two most prevalent β-thal mutations, a base substitution producing terminator at codon 90 (with β^0-thal expression) and that at -31 within the ATA box (β^+-thal), seemed unique to Japanese.

Key Words: hemoglobinopathy, abnormal hemoglobin, thalassemia

Introduction

Survey, identification, and clinical evaluation of Japanese hemoglobinopathy constituted a major research activity at the Department of Clinical Laboratory Science, Yamaguchi University School of Medicine. The data harvested there include most cases of clinical significance, and are representative of hemoglobinopathy in Japan. Abnomral hemoglobins (Hb) and thalassemia (thal) mutations identified in this laboratory before 1990 were listed in a previous paper[1]. This review recollects some remarkable hemoglobinopathies encountered there. To save space, references are limited to appropriate review articles and newer originals.

1. Half a century after the discovery of sickle cell hemoglobin, fourty years after the start of hemoglobinopathy survey in Japan

The separation of sickle cell hemoglobin or Hb S [β6(A3)Glu→Val] by moving-boundary electrophoresis and subsequent proposal of the new disease concept of 'molecular disease' in 1949[2,3] opened the era of worldwide surveys of variant Hbs which lasted some 25 years. Hb S and Hb C [β6(A3)Glu→Lys] were frequent among negroes, and Hb D [β121(GH4)Glu→Gln] in some populations in Pakistan and the Mideast. During 1950's, Hb

E [β26(B8)Glu→Lys] and thal syndromes were found to be endemic in the Southeast Asia.

Population surveys for variant Hbs by zone electrophoresis was started at Kyushu University in November, 1957, soon followed by Yamaguchi University and other institutions. No Hb variant was found by April, 1959.

Before giving up the surveys in Yamaguchi University, literatures were searched for probable cases of hemoglobinopathy. Of the two candidates thus selected, one was happened to be acatalasemia, but the other was 'hereditary nigremia' which later became popular as Hb M-Iwate [α87(F8)His→Tyr] disease. It was lucky that agar gel electrophoresis at pH 7 had just been adopted there in addition to the usual alkaline gel electrophoresis. The dark brown-colored Hb M-Iwate easily separated from the brilliant red, normal hemoglobin (Hb A) on electrophoresis at pH 7.

By the end of 1962, Hb Ms, unstable Hbs causing hemolytic disease, β-thal traits and hereditary persistence of fetal Hb (HPFH) were found in Japanese. The quantitative increase of a normal minor Hb ('F-like Hb', which was later identified as Hb A_{1c}) in diabetic petients was already known to some Japanese investgators in the field, five years before the first report from USA. A few university hospitals, notably Kyushu, Yamaguchi, Gifu and Nagoya, remained as major sites of hemoglobinopathy survey during the 1960's ; Kawasaki Medical School stayed as the largest center during the 1970's and on.

2. Hb Ms marked the early stage of hemoglobinopathy research in Japan

Hereditary cyanosis due to abnormal Hb (Hb M) with unique absorbance spectrum of aquomet-Hb (Hi), was reported in 1948[4], a year before the discovery of Hb S. In Japan, a large family segregating "hereditary nigremia" was known since 1936, but it took almost a quater century before the discovery of Hb M-Iwate in a patient's blood. With one exception (Hb M-Milwaukee-I [β67(E11)Val→Glu]), Hb Ms are those abnormal Hbs with substitution of tyrosine for histidine either at the proxymal (F8) or distal (E7) site, where the phenolic side chain of the newly introduced tyrosyl residue provides an internal ligand to the oxidized heme iron.

All four such substitutions in Hb A[1] and one of the fetal Hb Ms[5),6)] were found in Japanese. Studies in physical, chemical, and functional aspects of various Hb Ms characterised the research activities on hemoglobinopathy in Japan during the 1960's and 1970's.

Hb Bristol-Alesha (β67[E11]Val→Met→Asp)[7] provoked a problem on the definition of Hb M. A significant proportion of this abnormal Hb appeared to be stabilized as Hi. The chromatographically purified Hb Bristol-Alesha gave essentially normal absorbance spectrum upon oxidation to Hi. However, electron paramagnetic resonance (EPR) spectrum of the whole blood disclosed the existence of an unique, internally liganded form of Hi which conformed to the definition of Hb M[5]. Although this abnormal Hb tended to exist in a Hi form, the carriers never expressed cyanosis because the Hb was quite unstable and comprised but a small proportion of the total Hb. Thus, Hb Bristol-Alesha was excluded from the list of Hb M by most authors, even though it would actually be a Hb M from physicists' viewpoint.

3. All abnormal hemoglobins expressing cyanosis were not Hb M

Two groups of abnormal Hbs were clinically indistinguishable from Hb M because of cyanosis as the cardinal sign. Hemichromes are unstable derivatives of oxidized Hb which are intermediates in the process of denaturation from reversible Hi to irrevesible products. A few abnormal Hbs were rapidly oxidized, but stabilized at the 'reversible hemichrome' stage. A representative case was Hb Higashitochigi[deletion of Glyβ24 (B6) or 25(B7)] we found in a Japanese boy[8].

Hemichrome formation under oxidative stress was a feature of many unstable Hbs, but their clinical expression was hemolysis rather than cyanosis. Hb Tochigi [deletion of Gly-Asn-Pro-Lys β56(D7)-59(E3)] was an example. Hb Iwata [α87(F8)His→Arg] was clinically silent because it was a very unstable α-globin mutant comprising but a few percent of the total Hb in the carriers. Stereochemistry of hemichrome derivatives

abnormal Hbs have been poorly elucidated.

Abnormal Hbs with extremely low oxygen affinity remains only partially oxygenated at the partial oxygen pressure (P_{O2}) of arterial blood, leading to cyanosis due to a high proportion of deoxy-Hb. Hb Kansas [β102 (G4) Asn→Thr] as the prototype of this group was also found in two probably unrelated Japanese. The stereochemical basis for its very low oxygen affinity has been elucidated by X-ray crystallography[9]. Although it was a cause of congenital cyanosis, its oxygen delivering function was even more efficient than normal Hb A.

4. Abnormal hemoglobins were the cause of congenital Heinz body hemolytic disease

The first case report of unstable Hb disease (congenital Heinz body hemolytic anemia) appeared in 1953. However, no abnormal Hb was detectable in the patient's blood by electrophoresis of red cell lysate. It took some ten years before the invention of heat denaturation procedure, an universal test for the detection of unstable Hb, and establishment of the general concept of unstable Hb (hemolytic) disease. After critical experience on Hb Hammersmith [β42 (CD1) Phe→Ser ; cause of severe hemolytic anemia ; the abnormal Hb not detectable by electrophoresis], the world first case was revisited. This time the abnormal Hb (Hb Bristol) was selectively precipitated from red cell lysate by the isopropanol procedure, and the amino acid substitution [β67 (E11) Val→Asp] was demonstrated.

This did not finish the story of Hb Bristol, however. Some twenty-five years later (thirty-eight years after the case report), it turned out that the true substitution was Val →Met (*G*TG→*A*TG : Hb Alesha), but this particular methionyl residue was rapidly modified into aspartyl by a post-translational event, and the abnormal Hb was renamed as Hb Bristol-Alesha as cited above[7].

5. Unstable Hb Ube-1 was as interesting as Hb M-Iwate

A case report of an 11-year-old boy with severe hemolytic anemia (Hb 2.5-5 g/dl, MCH 39-46 pg, Hb F 3.1%), growth retardation, lip cyanosis, hepato-splenomegaly, and 'hair standing on' figure on cranial X-ray film, which appeared in 1959[10], might be the first report of unstable Hb disease in Japan. An abnormal Hb was detected by electrophoresis in a blood sample of another case of hemolytic anemia in 1960[11]. However, these cases were lost without establishment of the diagnosis.

An abnormal Hb (Hb Ube-1) with curious electrophoretic behavior was discovered in 1961, from a girl patient who had underwent splenectomy because of a chronic hemolytic anemia. This led to the first well documented case report of congenital Heinz body hemolytic anemia in Japan. However, the final identification of the abnormal Hb as Hb Köln [β98 (FG5) Val → Met] took twelve years. Red cell lysate and even the 'purified Hb Köln', revealed complex, non-reproducible patterns in electrophoresis and ion exchange chromatography; there were up to four isoforms in various proportions and smearing between the bands or peaks[12]. Hb Köln with all four hemes/tetramer appeared as a sharp, dark colored band within that of Hb A in isoelectrofocusing (IEF) and the molecules with two hemes/tetramer (no heme on the abnormal β-subunits) located along the anodic edge of Hb A_2. In the early days, however, only the ill defined hybrids of the two forms, with three hemes/tetramer and an intermediate mobility, were recognized as the abnormal Hb. Thus the roughly purified abnormal Hb was largely lost as 'Hb A_2' in the second electrophoresis for further purification. The fourth isoform was occasionally emerged from a diethylaminoethyl (DEAE) column, just in front of the mixed peak of Hb A and Hb Köln with four hemes/tetramer.

It gradually turned out that Hb Köln was the most popular unstable Hb worldwide. The reason for the high frequency of this particular mutation (*G*TG→*A*TG at codon 98 of the β-globin gene) is unknown. Twelve sporadic cases were identified in Yamaguchi University, well over a hundred in the world.

In my experience, selective precipitation of the abnormal subunit from red cell lysate by treatment with parachloromercuribenzoate (PCMB) was quite effective for the preparation of the material for chemical analysis of unstable Hbs[13]. In case of Hb Köln, the

precipitate thus formed was the almost white, heme depleted abnormal β-globin.

6. Unstable hemoglobins often have abnormal affinity for oxygen

Hb Köln has high affinity for oxygen, and blood Hb concentration has to be set at a higher level in its carrier in order to compensate for the inefficient oxygen delivering property. This exemplifies the notion that blood Hb concentration in unstable Hb disease depends not only on the severity of hemolysis but also on oxygen affinity of the blood. Even post-splenectomy erythremia was reported for Hb Köln.

Hb Toyoake [β142(H20) Ala→Pro] and Hb Nagoya [β97(FG4) His→Pro] were among further examples of unstable Hb with high oxygen affinity, causing a 'compensated' hemolytic disease without apparent anemia.

7. Unstable Hb diseases were quite heterogenous

The number of unstable Hbs causing hemolytic disease in the world amounted to some seventy a decade ago[14]. The clinically insignificant abnormal Hbs which gave positive results on instability tests *in vitro* outnumbered this. The lists of unstable Hbs are to be supplemented from the latest syllabi of variant Hbs[15,16]. Unstable Hbs causing very severe hemolysis in the heterozygotes may be incompatible with reproductive life because all cases appeared to be the results of *de novo* mutation. Unstable Hbs and cyanotic Hbs whose clinical expressions were dominant provided human geneticists with good chances to observe *de novo* mutation in man.

Over twenty different unstable Hbs found in some fourty unrelated families were identified in Yamaguchi University as the cause of hemolytic disease. Those Japanese cases with such a severe hemolysis as cited above included Hb Hammersmith, Hb Bristol -Alesha (two cases), Hb Mizuho [β68(E12) Leu→Pro], Hb Koriyama [β91-95(F7-FG2) Leu-His-Cys-Asp-Lys tandem repeat], and Hb Nottingham [β98(FG5) Val→Gly] (two cases). *De novo* mutation and subsequent propagation was observed in a number of abnormal Hbs expressing either cyanosis or milder hemolytic disease.

8. High oxygen affinity hemoglobins were a cause of erythremia

The association of abnormal Hb with familial erythremia was first reported for Hb Chesapeake [α92(FG4) Arg→Leu] in 1966[17]. This prompted to find the co-segregation of erythremia with Hb Hiroshima [β146(HC3) His→Asp], one of the two Hbs found in the surveys of the atomic bomb survivers in Hiroshima. About fifty abnormal Hbs are known to cause erythremia[1,15]. All have increased affinity for oxygen; the inefficient oxygen delivery is compensated by increased red cell mass. Thanks to the X-ray crystallographic data at atomic level, the mechanisms for the high oxygen affinity have been proved or explained[18]. The amino acid substitution in Hb Hiroshima was initially reported as β143(HC1) His→Asp. However, the difficulty in stereochemical explanation for the high oxygen affinity led to direct crystallographic study in Cambridge, England, which corrected the location of amino acid substitution and elucidated the mechanism for the abnormal functional properties.

Hb Chesapeake have also been found in Japan by chance during quantification of Hb A_{1c} by cation exchange high pressure (performance) liquid chromatography (HPLC) as a routine test for diabetes mellitus. Erythremia was so slight in its carriers that it would not be recognized unless detailed family study was undertaken. On the other hand, a large number of high oxygen affinity Hbs causing remarkable erythremia were difficult to detect by electrophoresis or chromatography. Hb San Diego [β109(G11) Val→Met] and Hb Miyano [α41(C6) Thr→Ser] were examples we experienced.

9. Hemoglobin variants with low oxygen affinity could cause a mild 'anemia'

Carriers of an abnormal Hb with low oxygen affinity are expected to have an innocent 'anemia' as a reflection of the improved oxygen release. Mild 'anemia' itself is seldom a problem to be studied in detail, unless a within-normal Hb level is critical for some non-clinical reason, e.g. enrolment to special occupation or training. Hb Yuda [α130 (H13) Ala→Asp][19] could be such an example, although it was found by chance and

subjected to detailed studies only because of academic interest.

10. Only one abnormal hemoglobin showed enhanced β N-terminal glycation

Almost all variants of Hb A appeared to be glycated at normal rate at the N-terminal α-amino group of the β-subunits to produce Hb A_{1c}. The only exception known to date is Hb Himeji [β140(H18)Ala→Asp], which were found in two probably unrelated Japanese and two Portuguese famielis. Proportion of the N-terminal glycated fraction (Hb X_{1c}) was much higher than the normal counterpart (Hb A_{1c}) in all heterozygous carriers[1,20]. Stereochemical interpretation for the increased affinity of this Hb for glucose has been suggested.

11. The solvent conditions of the cation exchange chromarography for quantification of Hb A_{1c} could cause dissociation of some abnormal hemoglobins into the α and β subunits

Scrutiny of a routine chromatogram for quantification of Hb A_{1c} led to the discovery and identification of Hb Tonosho [α110(G17)Thr→Ala][21], where the only abnormality was a tiny notch at the descending slope of the 'rest' (Hb A + Hb A_2) peak. Hb molecules exist as an equilibrium mixture of tetramers [$(\alpha\beta)_2$] and dimers ($\alpha\beta$) in a dilute solution *in vitro*. Hb Tonosho further dissociated into the α- and β-subunits under the rather drastic conditions of the eluent buffer solutions. Clinically silent Hb variants are being discovered by chance because of a qualitatively or quantitatively abnormal peak of Hb A_{1c} in automated cation exchange HPLC[22]. Hb Tonosho was exceptional in this regard ; it did not interfere with the Hb A_{1c} peak, yet it was detectable to a very alert medical technician.

12. Some abnormal hemoglobins did not satisfy the requirements for substrate of the methionyl aminopeptidase

Translation of globin messenger RNA (mRNA) starts with methionine at codon number zero, and the N-terminal Met is enzymatically removed from the nacent peptide. In several Hb variants the amino acid substitution at or near the next N-terminal residue Val violated the requirements for the specificity of the aminopeptidase. Hb Niigata [β1(NA1)Val→Leu] was a good example[23], where Metβ0 remained uncleaved. Moreover, the N-terminal Met was a substrate of N-acetyltransferase, and the abnormal β-globin was acetylated up to about one-third.

13. Some variant hemoglobins were under non-enzymatic modification *in vivo*

A new, specific post-translational modification from methionyl to aspartyl residue was found in Hb Bristol-Alesha as cited above. Deamidation of asparaginyl to aspartyl at specific sites were observed in a few variant Hbs. Hb Providence [β82(EF6)Lys→Asn→Asp] was an example. The Lys (EF6) is one of the 2,3-diphosphoglycerate (2,3-DPG, which is important for stabilization of deoxy conformation of the tetrameric Hb molecule and hence lowering oxygen affinity of erythrocytes *in vivo*) binding sites, which was lost by the substitution. Partial deamidation of Asn to Asp further reduced the 2,3-DPG affinity by electrostatic repulsion. The glutaminyl residue which substituted for the same lysyl in Hb Tsurumai[24] was never deamidated.

14. Hb F-Yamaguchi, the first example of mutation in the $^{A}\gamma^{T}$ subunit of Hb F

Like α-globin genes, the presence of at least two γ-globin loci per haploid was suggested by relatively small proportion of an abnormal Hb F in total Hb F. This was confirmed by amino acid analysis of a tryptic peptide γT-13 in which values for glycine and alanine relative to other amino acids significantly deviated from integer, while sum of the two did not. Actually there are two γ-globins which differ only at residue number 136, Gly ($^{G}\gamma$) or Ala ($^{A}\gamma$). Another source of heterogeneity came from Hb F-Sardinia [γ75(E19)Ile→Thr] which was found at a polymorphic level (frquency of about 10-20% in most populations throughout the world).

Hb F-Yamaguchi [$^{A}\gamma^{T}$80(EF4)Asp→Asn] was found during a survey of cord blood. The identification of this Hb indicated for the first time that the Ile/Thr polymorphism at γ75

resided in the $^{A}\gamma$-globin. The abnormal Hb comprised one-third of the total Hb F in the heterozygotes which was much higher than expected for an $^{A}\gamma$-variant. DNA analysis disclosed that the chromosome carrying the mutation had lost one γ-globin gene by a crossover $^{G}\gamma \rightarrow {}^{A}\gamma$. Several abnormal Hb Fs which were discovered later[15] confirmed the assignment of Thr75 to the $^{A}\gamma$-globin. According to the results of screenig of neonates in Osaka, Hb F-Yamaguchi was frequent among Japanese; all appeared to be linked to the γ-thal deletion cited above.

15. β-Thalassemia mutations were rich in variety

Thalassemia (thal) is defined as quantitative deficit of a specific subunit or subunits of Hb. Just as Hb S was in a mixed equilibrium in natural selction from malaria in the tropical Africa, various thal mutations were the case in the 'thalassemia belt' which covered from the Mediterranean Basin to Southeast Asia.

Quantitative deficit of the β-subunits results in decreased amount of Hb per erythrocyte (microcytosis) and relative excess of the α-subunits. The free α-subunits undergo rapid denaturation *in vivo* and the products of denaturation cause premature destruction of red cells and their precursors in the bone marrow (ineffective erythropoiesis). Approach to β-thal from protein chemistry had been limited to quantification of Hb A_2/Hb A ratio which was almost always increased, and of β/α globin biosynthesis ratio (incorporation of radioactive leucine) which must be decreased by the definition of β-thal. Two types of β-thal mutations were recognized : β^0-thal with no β-globin synthesis *in cis* to the mutant allele and β^+-thal with decreased output. Homozygosity of β^0-thal expresses microcytic anemia with extreme ineffective erythropoiesis, while heterozygosity simply presents a mild, asymptomatic microcytic anemia. Information on thal mutations at DNA level accumulated at an explosive speed, and mechanisms for the specific deficit of the β-subunits were largely elucidated during the 1980's.

Over two hundreds of different β-thal mutations have been identified[16,25] ; most of them are point mutations (substitutions of a single base, deletion or insertion of one to several bases). Some β-thal mutations are predominant in some particular populations especially in the areas where β-thal is endemic. For Japanese, frequency of β-thal traits was estimated to be about 1 : 1000, and homozygotes are quite rare. Thirty-five different mutations from 262 families were identified in Fukuoka, Kyushu, and Yamaguchi universities, and in Kawasaki Medical School by 1996[26].

16. Two β-thalassemia mutations were unique to Japanese and most frequent among Japanese

Two β-thal mutations appeared to be prevalent only in Japanese: codon (CD) 90 $CAG \rightarrow TAG$ (β^0-thal phenotype) and -31A→G (β^+-thal). A number of homozygotes of this β^+-thal mutation were found, whose clinical expression was nearly equivalent to heterozygotes of the β^0-thal, although proportions of Hb A_2 and Hb F were somewhat higher. The only well documented homozygote of the β^0-thal was transfusion dependent.

17. Aggravating factors have remained to be discovered in some cases of β-thalassemia heterozygotes

There were several cases of β-thal heterozygotes manifesting unusually severe hemolysis, the aggravating factor(s) seemed independent of the thal mutations and remained to be found.

18. Thalassemia genes are being imported

Some thal mutations found in Japanese were highly prevalent in the Southeast Asia including China, e.g. deletion of four nucleotides from CD41-42 and substitution IVS-II-654C→T. Hb E [β26(B6)Glu→Lys or CD26GAG→GTG] is a β^+-thal mutant which is even milder than the -31A→G ; its heterozygotes are clinically silent and homozygotes are somewhat milder than β^0-thal heterozygotes. However, its double heterozygotes with β-thal could express microcytic anemia with severe ineffective erythropoiesis (β-thal intermedia～major) ; the reason has poorly been understood ; instability of Hb E

poorly been understood ; instability of Hb E under oxidants might be a factor. Hb E is endemic in the Southeast Asia, but it is quite rare among Japanese. All homozygotes and double heterozygotes identified in Japan were immigrants and travelers from the Southeast Asia.

19. $\delta\beta$-Thalassemias are quite rare in Japan

$(\delta\beta)^0$-thal is much rarer than β-thal. Despite a large deletion including both β- and δ-globin genes, clinical expression of $(\delta\beta)^0$-thal mutations are much milder than β-thal because of better compensation with the increased production of the γ-subunits. One Japanese homozygote with no Hb A or Hb A_2 (100% Hb F) was well documented.

One heterozygote of Hb Lepore-Washington-Boston (unequal $\delta \rightarrow \beta$ crossover within CD87 and IVS-II-7) was found in a Japanese recently with mild expression of $(\delta\beta)^+$-thal trait[27].

20. α-Thalassemia traits are most frequent but clinically too mild to be detected

The β-subunits (Hb H $=\beta_4$) are only mildly unstable compared to the free α-subunits. They remain in red cells of α-thal (trace in α-thal-1 heterozygotes or α-thal-2 homozygotes; about 10% in their double heterozygotes) and behave like an unstable Hb. Because of the presence of two very homologous α-globin genes ($\alpha 2$ and $\alpha 1$) in tandem and *Alu* repeats near-by, α-globin genes are subject to deletion or duplication by unequal crossover. Loss of one α-globin gene results in the α-thal-2 (α^+-thal : clinically silent in heterozygotes) and loss of both α-globin genes α-thal-1 (α^0-thal : slightly milder than β^0-thal in the heterozygotes). Homozygotes of the α-thal-1 are incompatible with life. Double heterozygotes of the two types of α-thal express Hb H disease (α-thal intermedia) which is more severe than the β^0-thal hetrozygotes but milder than homozygotes (classical β-thal major). The α-thal mutations are more frequent than the β-thal and a number of cases with Hb H disease were found in Japanese.

21. Merging concepts of unstable Hb disease and thalassemia

Many β-thal mutations code a grossly abnormal β-globins, e.g. truncated, with frameshift, etc. However, their clinical expression was almost always typical β^0-thal in which the expected products were never detectable. In Hb Showa-Yakushiji [β110 (G12) Leu → Pro ; C*T*G → C*C*G], even a single amino acid substitution without truncation or frameshift expressed typical β^0-thal phenotype. Presumably, the products of these β-thal mutations would be extremely unstable and completely hydrolyzed almost at the same time as they are produced. In a number of frameshift mutations near the C-terminal of the β-globin, however, clinical expression was much more severe with remarkable ineffective erythropoiesis and hemolysis. Precipitation of irreversibly denatured Hb (Heinz bodies) could be an indirect evidence for the production and rapid denaturation of the abnormal Hb, but the expected β-globin was never identified in the precipitate. Such a special group of β-thal is called 'dominant type β-thal intermedia' or 'Heinz body β-thal'. Hb β-Makabe (frameshift at codon 123) and an unpublished case (flameshift at codon 125) were good examples found in Japanese.

The boundary between β-thal trait (quantitative deficit of the β-subunits leading to mild microcytic anemia) and unstable Hb disease (qualitative abnormality of the β-subunits leading to hemolysis) is not clearcut, 'hyperunstable Hbs' bridging between the two conceptually different types of hemoglobinopathy. Hb Koriyama (cited above) causing a most severe unstable Hb disease was barely detectable in the peripheral blood of its heter ozygote. Hemolytic manifestation of abnormal Hbs increased with increase in their instability. However, after a certain point of high instability, hemolysis decreased with extreme increase in instablity of abnormal Hb, only expressing microcytosis of thal traits without overt hemolysis.

22. Inconstant clinical expression of hyperunstable α-globin abnormal hemoglobin

Clinical manifestation of hyperunstable α-globin variants was more difficult to explain. Of three heterozygotes of Hb Toyama [α136 (H19) Leu→Arg] in a family, two had

stable Hb, while the third only showed slight microcytosis without enhanced hemolysis. The abnormal Hb amounted to less than 0.1% of total Hb in the three individuals.

Proportion of the abnormal Hb in the peripheral blood differed greatly (less than 0.1% to over 10%) among its heterozygotes in five families with Hb Hirosaki [α43(CE1) Phe→Leu], Hemolysis apeared to be proportional to the amount of the abnormal Hb among members of two families[1,28]. It was presumed without evidence that proteolytic activity in the red cell precursors to hydrolyze 'unwanted' protein would be a factor for the harmful, hemolytic effect of hyperunstable Hbs.

Conclusion

The era of mere observation of nature's (god's) experiments on mutation and natural selection in man almost came to the end a half century after the discovery of Hb S. Now variant Hbs with particular mutations can be produced artificially.

How to cure the severe forms of hemoglobinopathy (sickle cell diseases, some unstable Hb diseases) and thalassemia (β-thal major) is the next, very difficult problem. Switching back of β-globin production (adult Hb production) to the γ- (fetal Hb production) appears to be one direction to this goal, one of the oldest problem which became accessible to real research only recently. Basic research on this line would also provide some keys to open the mystery of differentiation and maturation of cells.

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