ABNORMALITY IN A SPECIFIC PROTEIN OF THE ERYTHROCYTE MEMBRANE IN HEREDITARY SPHEROCYTOSIS

S. Hayashi, R. Koomoto*, A. Yano*, S. Ishigami*, G. Tsujino**, S. Saeki, and T. Tanaka

Received March 13,1974

Department of Nutrition and Physiological Chemistry, Osaka University Medical School; *Department of Clinical Research, Research Institute for Microbial Diseases, Osaka University; and **Second Department of Medicine, Children's Medical Center of Osaka City; Osaka, Japan

SUMMARY

Erythrocyte membrane proteins from fifteen patients with hereditary spherocytosis were analyzed by polyacrylamide disc gel electrophoresis in the presence of 0.1% SDS. Almost complete deficiency was found in a protein component, IVb, in four cases. A small but significant decrease in this component was noted in most of the other cases.

Hereditary spherocytosis (HS)*** is one of the commonest forms of congenital hemolytic anemia, and is characterized by spheroidal red blood cells which show increased osmotic fragility. Although many lines of evidence indicate that the primary defect resides in the erythrocyte membrane (1), the exact nature of the defect has not yet been clarified. We examined the membrane proteins of HS erythrocytes using the analytical method of SDS-polyacrylamide gel electrophoresis recently established by Fairbanks et al. (2). The study revealed almost complete deficiency in a specific membrane protein component of the erythrocytes in four of fifteen cases of HS, and small but significant decrease in the amount of this protein in the erythrocytes of most of the other cases.

*** Abbreviations: HS, hereditary spherocytosis; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

HS cases. Fifteen patients with HS from ten different families were studied. All the patients showed at least three characteristic signs for HS; namely 1) microspherocytes, 2) increased osmotic fragility of incubated erythrocytes, and 3) accelerated autohemolysis, which could be prevented by glucose. The possibility of autoimmune hemolytic anemia was excluded by negative Coombs tests.

Preparation of erythrocyte ghosts. Ghosts were prepared from 10 ml of freshly drawn blood by the method of Steck et al. (3). Red cells were washed three times with 0.15 M NaCl in 5 mM sodium phosphate, pH 8, with careful aspiration of the buffy coat to remove leucocytes and platelets. The ghosts were prepared by hypotonic lysis in 5 mM sodium phosphate, pH 8, and washed several times until no further hemoglobin could be removed.

Polyacrylamide disc gel electrophoresis in SDS. The procedure used was essentially that of Fairbanks et al. (2), except for use of 5% instead of 5.6% polyacrylamide gel and 0.1% rather than 1% SDS. These modifications allowed faster electrophoresis and better separation of bands IVa and IVb. Suspensions of ghosts were dissolved in 1% SDS and incubated with 40 mM dithiothreitol. Aliquots of the solution, usually containing 25 µg of protein, were subjected to electrophoresis at room temperature in a Mitsumi disc electrophoresis apparatus, using 0.04 M Tris, pH 7.4, containing 0.02 M sodium acetate, 2 mM EDTA and 0.1% SDS as the electrophoresis buffer. Protein bands were stained with coomassie blue.

Gel densitometry was done using a Gilford spectrophotometer with a Model 2410 linear transport accessary, scanning through a 0.05 mm slit at a wave-length of 550 nm. The resulting curves were analyzed on a DuPont Model 310 curve resolver.

RESULTS AND DISCUSSION

The electrophoretic patterns of HS and normal erythrocyte membranes were compared. In HS erythrocyte membranes a specific abnormality was

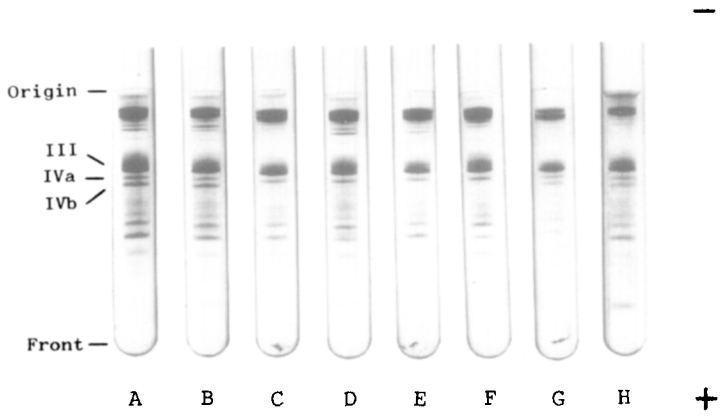

Fig. 1. Electrophorograms of erythrocyte membranes from six HS patients and two normal subjects. A and B, normals; C, HS case 1; D, HS case 2; E, HS case 3; F, HS case 4; G and H, other cases of HS. Cases 2, 3 and 4 were siblings. The experimental procedure is described in MATERIALS AND METHODS.

detected in a protein component, temporarily designated as IVb, with a molecular weight of about 75,000. No other consistent abnormalities were found in the electrophoretic pattern for HS membranes. As shown in Figs. 1 and 2, nearly complete deficiency in the IVb band was found in four patients (cases 1 to 4), three of which were siblings. The electrophoretic patterns were generally quite reproducible, and when the four cases were studied on different occasions their IVb band was constantly found to be nearly missing. Fairbanks et al. (2) reported that component IV appeared as a single band in the presence of 1% SDS but split into two bands in 0.1 or 0.2% SDS. In contrast to their results, even in 1% SDS we obtained two bands from normal controls. The reason for this discrepancy is not clear. To ascertain that the deficient band was IVb and not the neighboring IVa, calcium-dependent loss of the IVa component, a phenomenon reported by Triplett et al. (4), was examined using erythrocytes from case 1. As shown in Fig. 3, addition of calcium caused disappearance of IVa in the preparation from patient cells as well as in a preparation from normal cells, thus confirming that the IVb component was defective in the patient cells.

The IVb band was present in electrophorograms for the other eleven HS

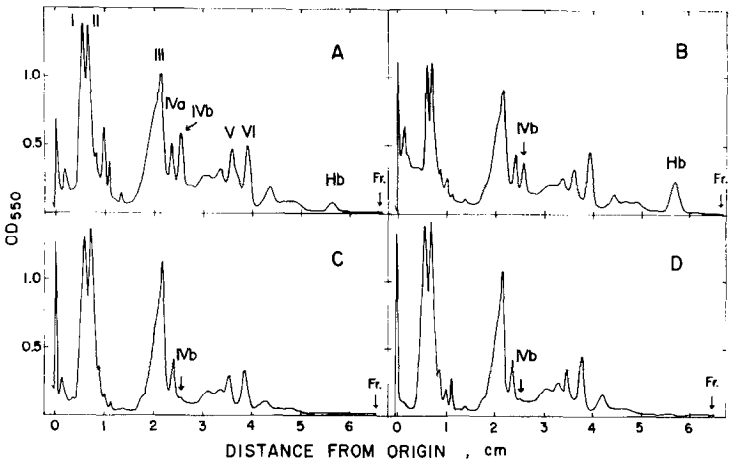

Fig. 2. Comparison of electrophorograms of erythrocyte membranes of normal subjects and cases of the two types of HS. A, normal; B, HS other than that of the IVb-deficient type; C, HS case 1 (IVb-deficient type); D, HS case 2 (IVb-deficient type). The experimental procedure is described in MATERIALS AND METHODS.

cases but appeared to be less dense than in normal electrophorograms. To examine this more quantitatively, the gels were subjected to densitometry and the heights of the seven major peaks were measured. Taking peak III as an internal standard, a statistically significant decrease in the IVb component was found in the ghosts of the HS cases. Comparison of the intensity of IVb with that of the neighboring band, IVa, seemed to be the easiest way to detect a decrease in IVb. Peak IVb was smaller than IVa (IVb/IVa < 1) in all but two of the HS cases, but was larger than IVa (IVb/IVa > 1) in all twenty-nine normal subjects examined (Fig. 4). However, the degree of decrease in the IVb component had no apparent correlation with the severity of the disease in the fifteen patients.

No obvious change in the IVb band was found in electrophorograms of erythrocyte membrane preparations from cases of other hematological disorders, namely, one case of hereditary elliptocytosis, one case of paroxysmal nocturnal hemoglobinuria, one case of non-spherocytic hemolytic anemia of the pyruvate kinase-deficient type, and three cases of iron-deficiency anemia.

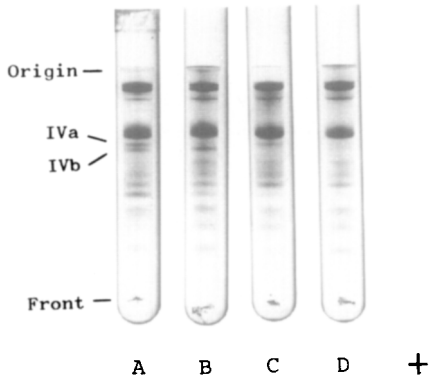

Fig. 3. Loss of the IVa component on calcium treatment of erythrocytes of a normal subject (A and B) and HS case 1 (C and D). Cells were hemolyzed for ten minutes at 4° in 10 mM Tris, pH 7.4, with (B and D) or without (A and C) 3 mM $CaCl_2$. The hemolysates were adjusted to isotonic conditions by addition of 3 M NaCl and incubated at 37° for thirty minutes. The resealed ghosts were hemolyzed again and the membranes were centrifuged at 35000 x g for ten minutes and washed twice with 10 mM Tris. Electrophoresis and staining of gels were performed as described in MATERIALS AND METHODS.

Abnormality in erythrocyte membrane protein has long been suspected as the primary cause of HS (5). Recently, some abnormalities in HS membrane proteins were found by two groups of workers using different methods, which gave different electrophoretic patterns (6,7). However, both groups regarded these abnormalities as a secondary, rather than the primary cause of HS, either because the results were variable or because the abnormality was not specific to HS.

Heterogeneity in the clinical entity of HS has been postulated (8) but has not been generally accepted. The present study suggests that there are at least two different types in HS. In the first type, including about one-quarter of our fifteen patients, the disease is characterized, and probably caused, by a specific and nearly complete deficiency in the membrane component IVb. In the second type, the IVb component tends to decrease but this may be secondary to an unknown primary defect. The primary cause of HS of this type may be the defective microfilaments as recently suggested by Jacob

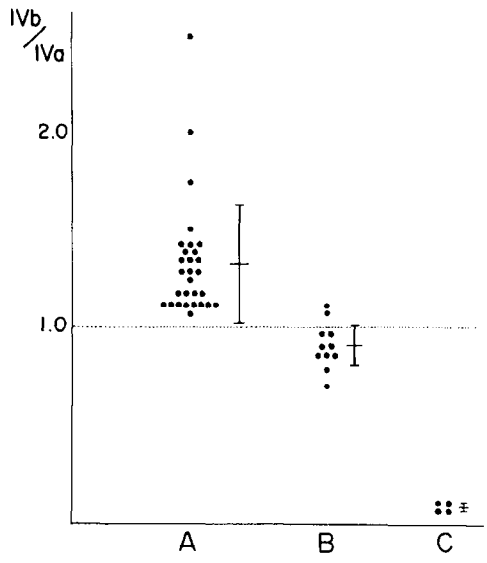

Fig. 4. Comparison of the IVb/IVa ratios of twenty-nine normal subjects (A), eleven patients with HS other than that of the IVb-deficient type (B), and four patients with HS of the IVb-deficient type (C). Means and standard deviations are also shown. The heights of IVa and IVb were measured on densitograms from the top to the level of the lowest point on the right side of the IVb peak. By analyzing the densitograms on a DuPont Model 310 curve resolver, the amount of the component IVb in HS of the IVb-deficient type was estimated to be 10 to 15 percent of normal.

(9). It is also possible that in cases of HS of either type the IVb component differs functionally from normal. Efforts are being made to extract and purify the IVb protein from erythrocyte membranes.

The hereditary nature of HS of the IVb-deficient type is obvious from the fact that cases 2, 3 and 4 were siblings. Inheritance of HS is generally regarded as of the Mendelian autosomal dominant type. Survey of family members, however, suggested that HS of the IVb-deficient type might be inherited in a recessive manner like other hereditary diseases of a deficient type. Further studies are required on the inheritance of HS of this type.

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