JCI The Journal of Clinical Investigation

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G Tchernia, … , N Mohandas, S B Shohet

J Clin Invest. 1981;68(2):454-460. https://doi.org/10.1172/JCI110275.

Research Article

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Deficiency of Skeletal Membrane Protein Band 4.1 in Homozygous Hereditary Elliptocytosis

IMPLICATIONS FOR ERYTHROCYTE MEMBRANE STABILITY

G. TCHERNIA, NARLA MOHANDAS, and S. B. SHOHET, *The Cancer Research Institute and Departments of Medicine and Laboratory Medicine, University of California, San Francisco, California 94143; Laboratoire Central d'Hematologie, Hopital A. Beclerc, 92140, Clamart, France*

ABSTRACT Erythrocytes from three patients with severe hemolytic anemia, marked erythrocyte fragmentation, and elliptocytic poikilocytosis, were studied in terms of both their membrane protein composition and their mechanical characteristics. Erythrocytes from the patients' parents and one minimally affected and one normal sibling were also studied. Morphologic observations implied that the severely affected patients suffered from homozygous hereditary elliptocytosis because erythrocytes of both parents and the one minimally affected sibling showed moderate elliptocytosis on smear, whereas those of an unaffected sibling had normal morphology. The parallel findings of markedly reduced levels of band 4.1 in the erythrocyte membrane proteins of the patients and an intermediate reduction in the cells of the parents and the putative heterozygous sibling, suggested that the elliptocytic shape of the cells was related to the reduced levels of band 4.1. Additional studies showed marked abnormalities in cellular deformability and membrane fragility in the erythrocytes from the homozygous patients. Importantly, these changes were also closely proportional to the reduced levels of band 4.1, suggesting a central role for this protein in the maintenance of normal membrane stability and normal cell shape. It seems likely that this role for band 4.1 is intimately related to its known biochemical connection to the "membrane skeleton" through its linkage with spectrin and actin.

INTRODUCTION

Protein bands 1 and 2, or spectrin, the major extrinsic protein of the erythrocyte membrane, was first described by Marchesi and Steers in 1968 (1). It is thought to play a major role in the regulation of erythrocyte shape and deformability (2, 3). Elegant biochemical studies during the past few years have shown that three other extrinsic proteins, band 5 (actin), bands 2.1 (ankyrin), and band 4.1, are intimately associated with spectrin and form a skeletal network that underlies the membrane lipid bilayer (4–9). Band 2.1 has been shown to be the polypeptide responsible for linkage of this "membrane skeleton" to the bilayer through its association with the integral transmembrane protein, band 3 (10). Band 4.1 is known to bind to both spectrin and actin and is thought to be involved in the modulation of spectrin-actin interactions (7–9). Taken together, the membrane skeletal protein complex appears to be responsible for the remarkable mechanical stability and membrane flexibility of erythrocytes. To date, the only clear example of deficiency of one of these erythrocyte membrane skeletal proteins is in murine hemolytic anemias (3, 11). Erythrocytes from four mutants of the common house mouse (*Mus musculus*), with recessively inherited hemolytic anemias, have been shown to have varying degrees of spectrin deficiency, and the extent of spectrin deficiency has correlated closely with the severity of anemia. Moreover, in one of these conditions, replacement of spectrin by exchange hemolysis reduced the abnormal cell fragmentation and fusion and modulated the markedly aberrant osmotic behavior of those erythrocytes (12).

Homozygous hereditary elliptocytosis, a transfusion-dependent hemolytic anemia with erythrocyte fragmentation, poikilocytosis, and spherocytosis, has been described by several investigators (13–16). In initial studies of poikilocytic erythrocytes from a patient referred to the first author, Dr. Claude Feo and Dr. Sigmund Fischer found that the cell membranes were deficient in the membrane skeletal protein, band 4.1

Received for publication 23 December 1980 and in revised form 26 March 1981.

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/81/08/0454/07* $1.00
Volume 68 August 1981 454–460

(17). Subsequent detailed cytologic and biochemical studies on seven members of this family in our laboratories have shown that this patient as well as two other affected siblings have homozygous elliptocytosis and marked deficiency in band 4.1. In addition, the three heterozygous family members were found to have reduced quantities of band 4.1, whereas the clinically normal family member was found to have normal amounts of this protein. Moreover, the band 4.1-deficient erythrocytes showed a major reduction in mechanical stability and a marked tendency toward fragment formation. We propose that the deficiency of band 4.1 results in a weakening of spectrin-actin interactions that is responsible for the observed in vivo and in vitro instability of the erythrocyte membrane.

METHODS

Blood cell morphology. Whole blood was collected into acid citrate dextrose and shipped from Algeria to San Francisco. The delay from the time the blood was drawn to the time of analysis varied from 24 to 36 h. Portions were fixed in glutaraldehyde (1% in isotonic buffered saline) for subsequent morphologic examination by phase-contrast and scanning electron microscopy. Samples for scanning electron microscopy were prepared by the method described by Bessis and Weed (18).

Preparation of erythrocyte membranes and Triton shells. Whole blood, anticoagulated with acid citrate dextrose, was washed once with isotonic phosphate-buffered saline (0.01 M, pH 7.4) and passed over a microcrystalline cellulose column to remove leukocytes. The filtered cells were then washed again with phosphate-buffered saline, and membranes were isolated by hypotonic hemolysis and two additional washings with 0.01 M Tris (pH 7.4) and 1 mM EDTA. All procedures were carried out at 4°C and in the presence of 1 mM phenylmethylsulfonylfluoride, a protease inhibitor, to minimize proteolysis. In the last series of experiments on all affected patients, 1 mM diisopropylphosphorofluoride was also added as a protease inhibitor, and no differences in the gel patterns were observed.

Triton shells were prepared from the isolated membranes by washing once with 56 mM sodium borate, pH 8.4, and by subsequently mixing one part of a packed ghost suspension with five parts of 56 mM sodium borate containing 1% Triton X-100. The mixture was incubated for 20 min on ice and then centrifuged at 15,000 rpm in a Sorvall 2B angle-head centrifuge (Sorvall Instruments, New Haven, Conn.) to collect the triton shell pellet. Again, all procedures were at 4°C and in the presence of the protease inhibitor.

Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed on isolated membranes and Triton shells using 4, 5, and 6% polyacrylamide cylindrical gels, as well as a 4–15% gradient Laemmli slab-gel system (19). The staining intensity of various bands was determined by scanning the gels in a Joyce-Loebl gel scanner. The quantification of staining intensities of various protein bands was done by two methods. First, the area under each band was obtained using the integrator on the gel scanner. Second, the tracings of these different bands were cut separately and weighed in an analytical balance. The protein content determinations were based on scanning of at least 12 gels from each individual.

Deformability measurements. Whole cell deformability was measured by a laser diffraction method employing the ektacytometer with the image analysis system previously described (20). In this instrument, distortion of a laser diffraction pattern of cells suspended in a shear field provides a measure of cell deformation. The instrument also provides a record of a deformability index (DI)[1] as a continuous function of applied shear stress. In simplest terms, the DI is a measure of the extent of cell ellipticity induced by stress. It has previously been shown that the DI at maximum shear stress (DI_{max}) is a reliable measure of whole cell deformability. In addition, measurement of DI_{max} under varying osmotic conditions permits estimation of the relative importance of erythrocyte surface-to-volume ratio and internal viscosity in whole cell deformability (20).

Fragility measurements. In order to obtain a measure of the mechanical fragility of the erythrocytes, the cells were maximally deformed in the ektacytometer, and the time-course of fragmentation at constant applied shear stress was monitored by following changes in the DI with time. When the cells were maximally deformed into prolate ellipsoids (600 dyn/cm^2 shear stress), they produced a narrow elliptical pattern with a DI value approaching 0.95. With time, as the cells fragmented in the shear field, a circular diffraction pattern produced by the cell fragments was superimposed on the elliptical pattern resulting in decreasing values of DI. By examination of timed portions this decrease was shown to be proportional to the reduction in the number of intact discocytes remaining in the suspension and was highly reproducible for 20 normal samples. The reduction in DI with time thus provides an estimate of the mechanical fragility of the erythrocytes. Because erythrocyte deformation in the ektacytometer varies with internal viscosity and hence MCHC, this determination was performed on cells with the same mean corpuscular hemoglobin concentration values. This was accomplished by separating various erythrocyte populations on discontinuous stractan gradients (20) and isolating cell populations with identical cell densities, and hence constant mean corpuscular hemoglobin concentration, before the measurements.

Family study. The Algerian family we have studied is composed of two parents and their five daughters. The parents were first cousins and appear to be hematologically normal except for modest elliptocytosis on smear and a minimal increase in the osmotic fragility of their erythrocytes (father: hemoglobin 14.4 g/100 ml, mean cell volume 80 μm^3, mean cell hemoglobin concentration 35.5/100 ml, reticulocyte count 53,000/mm^3; mother: hemoglobin 12.4 g/100 ml, mean cell volume 82 μm^3, mean cell hemoglobin concentration 35.3 g/100 ml, reticulocyte count 114,000 mm^3). The proband, who is the third daughter (age 8 yr), had a hematocrit of 14% 1 mo after birth and required blood transfusions. At 4 mo of age, splenomegaly was noted. Based upon normal hemoglobin electrophoresis, normal hemoglobin stability determinations, normal glycolytic enzyme levels, the slight increase in osmotic fragility of the parents' erythrocytes, and the marked increase in osmotic fragility of the patient's erythrocytes, an initial working diagnosis of hereditary spherocytosis was made. In an effort to avoid increased susceptibility to infection, splenectomy was delayed until 3 yr of age. Reasonable hemoglobin levels were maintained by repeated transfusions (35 in 3 yr). Subsequent to splenectomy, the patient has required no further transfusions and has had normal growth and development. However, her peripheral blood smear shows marked elliptocytosis and cell fragmentation. Her erythrocyte indices at age 8 are: hemoglobin 13.1 g/100 ml, mean cell volume 68 μm^3, mean cell hemoglobin concentration 33.5 g/100 ml,

reticulocyte count 125,000/mm³. The first daughter (age 14) has normal hematologic indices although her peripheral blood smear shows modest elliptocytosis. The second daughter, age 13, is also hematologically normal but in contrast her peripheral blood smear shows no elliptocytosis. The fourth daughter (age 3) had a hemoglobin of 4.8 g/100 ml 15 d after birth and required transfusion at that time. Splenomegaly was noted at 6 mo. She received 15 transfusions during her 1st yr of life and was subsequently transfused every 5 wk. She has recently been splenectomized and has not required transfusion in the 4 mo after surgery. Her peripheral blood smear shows marked elliptocytosis and cell fragmentation. The fifth daughter (age 7 mo) also had neonatal anemia and required two transfusions during the first 2 mo of life. Her hematologic status has not been fully evaluated at this time.

RESULTS

The pedigree of the family and the Rh blood group types are illustrated in Fig. 1. Appreciable numbers of elliptocytes were seen in the parents and in the first daughter both in unfixed blood samples examined by phase-contrast microscopy and in fixed samples examined by scanning electron microscopy (Fig. 2A–C). No elliptocytes were seen in the peripheral blood of the second daughter, and discocytes were predominant (Fig. 2D). The blood films of the third (proband) and fourth daughter, as well as the fifth daughter, when not transfused, were markedly abnormal (Fig. 2E). Along with elliptocytes, a large number of fragmented cells and an increased number of spherocytes, as well as other poikilocytes, were seen. Because the abnormal morphology of erythrocytes in the three youngest daughters was similar to that seen in patients with pyropoikilocytes, their thermal sensitivity was examined as previously described (21). The completion of fragmentation was defined by total loss of original morphology and replacement by uniformly spherical cells and fragments. Fragmentation of erythrocytes from the parents, as well as the first daughter and the proband was complete at 50°C. This was not different from normal cells, and appreciably different from pyropoikilocytosis cells (46°C).

The extent of the abnormality in the DI curves in isotonic media of various family members is shown in Fig. 3 and appears to correspond to that in the peripheral blood morphology. The shape of the DI curve and the DI_{max} value of the second unaffected daughter with discocytic erythrocytes were within the normal range, whereas the DI_{max} values of the two parents and the first daughter without hemolysis, but with circulating elliptocytes, were reduced to intermediate levels. The DI curves of the proband and the other two severely affected sisters with hemolytic anemia and large numbers of circulating spherocytes and fragments were markedly abnormal. In all three of the patients the DI_{max} of their erythrocytes was disproportionally further reduced in hypotonic media (230 and 180 mosM), indicating that the reduced surface-to-volume ratio of the erythrocytes was the dominant factor limiting the deformability of these cells, and not increased internal viscosity, which would have produced increased deformability with lowered osmolality (22). When the deformability of various subpopulations of erythrocytes separated on discontinuous stractan gradients (20) was examined, all subpopulations from the severely affected siblings had abnormally reduced deformability. This contrasted with the erythrocytes from the father in whom the lower density subpopulations exhibited normal deformability. Morphologic examination of the various density fractions from the proband showed appreciable enrichment of elliptocytes in the lighter density gradient. The number of elliptocytes decreased with increasing density while the number of fragmented cells increased.

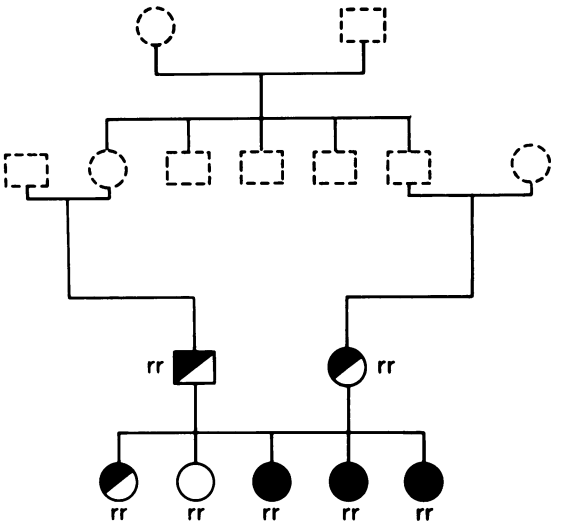

FIGURE 1 The pedigree of the family and the Rh blood groups of various family members. Because both parents had the same Rh subgroups it was not possible to obtain any evidence for linkage of the gene for elliptocytosis with any of the Rh genes, as has been suggested in some families with elliptocytosis.

The SDS polyacrylamide gel electrophoresis patterns of normal erythrocytes and cells of various family members are shown in Fig. 4. The striking feature of the gel pattern is the marked deficiency of band 4.1 in the proband that is illustrated here. No band 4.1 could be seen in this patient when simple 4, 5, or 6% cylindrical acrylamide gel electrophoresis systems were used. When a high resolution Laemlli 4–15% gradient gel system was used to resolve 4.1 into two bands (4.1a and 4.1b) (23), band 4.1a was totally absent even in heavily loaded gels. Trace amounts of stained material were found in the 4.1b region. At the present time, without sufficiently active 4.1 antibodies we cannot be sure of the nature of this material. The

[1] *Abbreviations used in this paper*: DI, deformability index; DI_{max}, deformability index at maximum shear stress.

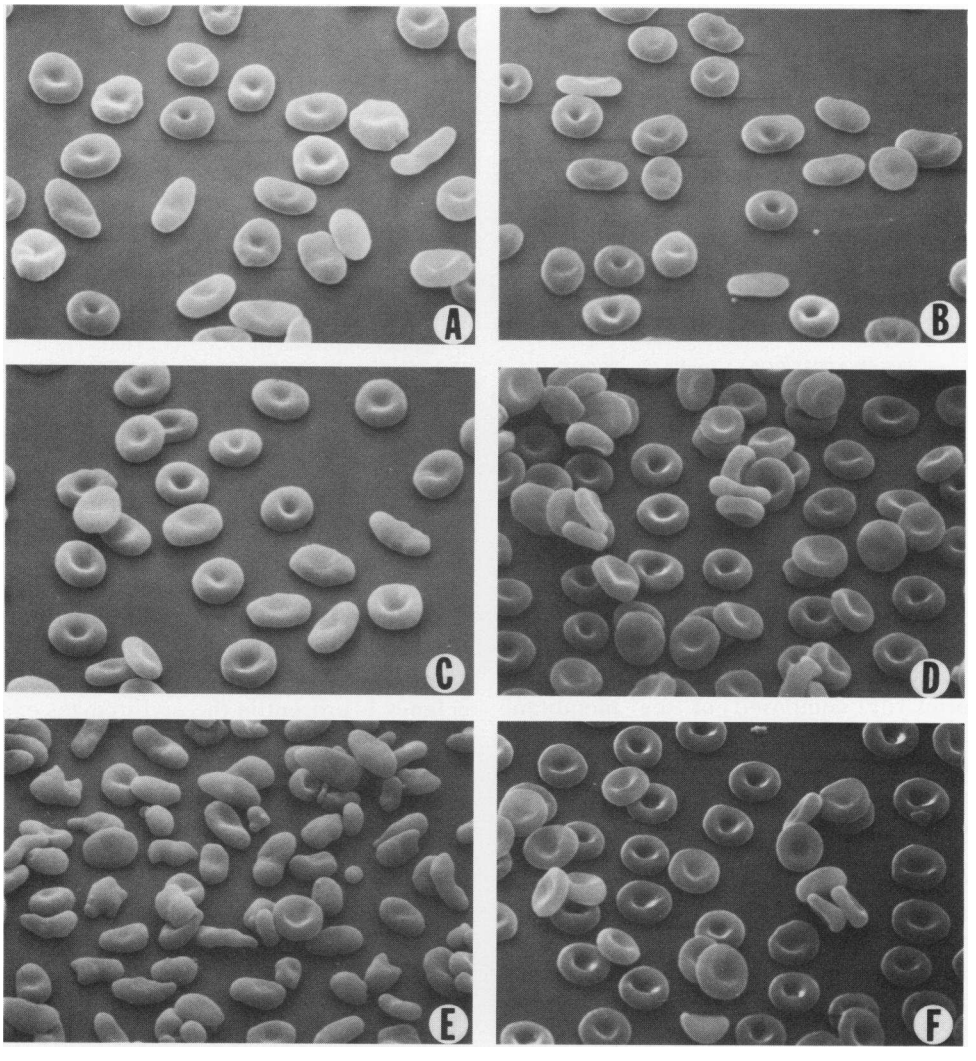

FIGURE 2 Morphology of erythrocytes from various family members and a normal individual. Elliptocytic erythrocytes were apparent in both parents (A and B) and the first daughter (C). Discocytic erythrocytes were the dominant feature in the second daughter (D) and the normal individual (F). The proband (daughter 3) had elliptocytic erythrocytes along with a number of fragmented cells (E).

two additionally seriously affected siblings gave identical gel patterns as the proband showing marked deficiency in band 4.1 (data not shown). Parallel quantification in the cylindrical gel system of the staining intensity of band 4.1 and band 4.2 (another apparently unrelated peripheral membrane protein) showed that all three seriously affected patients have no band 4.1 and normal amounts of band 4.2 when compared with band 3 (Table I). In addition, it was found that both parents and the first daughter had decreased levels of band 4.1 (approximately half the normal value) whereas the second daughter with discocytic erythrocytes had normal amounts. The constancy of the immediately adjacent band 4.2 in all cases where we found a reduction of band 4.1 indicated that the resolution of our electrophoresis system was adequate to detect changes in band 4.1 without ambiguity. The deficiency of band 4.1 we have found here appears to be specific for these patients with elliptocytoses. It has not been found in the blood of six hereditary spherocytosis or four hereditary pyropoikilocytosis patients we have studied to date.[2] In addition to normal amounts of band 4.2, erythrocytes from all family members including the seriously af-

[2] During the preparation of this manuscript we became aware of another patient of Doctors T. Mueller and M. Morrison of St. Jude's Hospital in Memphis, Tenn., with a profound deficiency of band 4.1 in the erythrocyte membrane proteins. Unfortunately, no family studies could be performed.

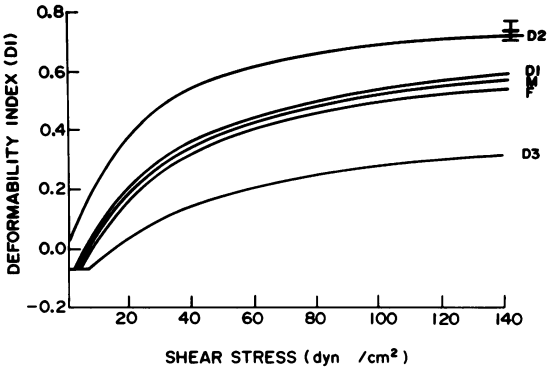

FIGURE 3 DI vs. shear stress for erythrocytes from father (F), mother (M) and the three daughters (D1, D2, D3). The mean and standard deviation for DI_{max} values in 50 hematologically normal individuals is also shown.

fected daughter had normal amounts of band 2.1 and actin.

The marked deficiency of band 4.1 in the patients was further confirmed in the gel patterns of Triton shells made from isolated membranes (Fig. 5). Triton shells are primarily composed of the membrane skeletal proteins, with only minor amounts of the major intrinsic protein band 3. Analysis of such preparations permits better visualization of band 4.1, which is somewhat overshadowed by band 3 in gel patterns of intact membranes. Band 4.1a was totally absent in two of the three seriously affected patients who had not been transfused, with only trace amounts of band 4.1b present. The Triton shells of the last affected daughter contained trace amounts of band 4.1a, presumably from the residual transfused normal cells. The parents and the first daughter had reduced amounts of 4.1 in these Triton shell preparations, just as in the whole membrane preparations, and the second daughter with discocytic cells had normal amounts of band 4.1.

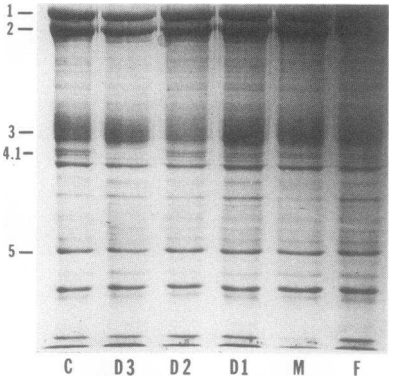

FIGURE 4 SDS polyacrylamide gel electrophoretic patterns using the 4–15% Laemmli gradient system of erythrocyte membranes from the parents (F and M), the three daughters (D1, D2, D3) and a normal control (C).

TABLE I
Comparison of Bands 4.1 and 4.2 Levels in the Various Members of the Family with Elliptocytosis

	Band 4.1/band 3	Band 4.2/band 3
	%	
Controls (13)	15.2±3.2	12.1±3.4
Father	7.6±2.1	12.8±3.8
Mother	6.3±2.0	12.1±2.8
Daughter 1	8.2±2.1	10.4±2.8
Daughter 2	14.0±2.5	11.1±3.2
Daughter 3 (proband)	0.0	12.0±3.9
Daughter 4	0.0	10.9±2.8
Daughter 5	0.0	12.9±3.5

The values for the controls are the mean and standard deviation for 13 hematologically normal individuals. The values for the various family members represent the mean and standard duration from scans of 12 gels from two separate phlebotomies.

The marked deficiency of band 4.1 in the hemolytic patients was apparent in all subpopulations of density gradient-separated cells. This implied that the deficiency is present in the erythrocytes of all ages. This also suggested that band 4.1 deficiency is probably the result of reduced or deficient synthesis in the marrow and not the result of its proteolytic digestion during erythrocyte maturation.

Recognizing that proteolysis can be a significant problem in the analysis of the protein composition of isolated membranes, we took precautions during the analysis. First, leukocyte contamination, a major cause of proteolysis, was minimized by passing blood through microcrystalline cellulose. Second, EDTA and the protease inhibitors phenylmethylsulfonylfluoride and diisopropylphosphorofluoride were used in all the lysing and washing buffers to minimize further poten-

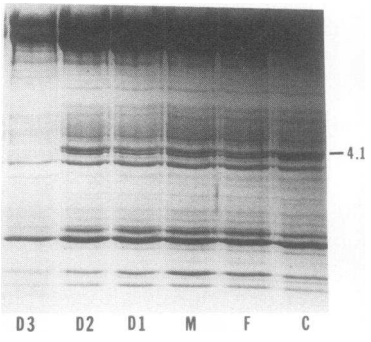

FIGURE 5 SDS-polyacrylamide gel electrophoretic patterns using the 4–15% Laemmli gradient system of Triton shells prepared from erythrocyte ghosts of a normal control (C), two seriously affected daughters (D3, D4), and a heterozygote (D1). Extraction with Triton removes a major portion of band 3 from the membranes, enabling clear visualization of band 4.1, which is adjacent to this integral protein.

tial proteolysis. Finally, all buffers were prechilled, and the procedures were carried out at 4°C. In order to confirm further the absence of significant proteolysis, intact normal erythrocytes and the proband's erythrocytes were mixed on the basis of total hemoglobin in ratios of 1:3, 1:1, and 3:1. Isolated membranes were then prepared from these premixed intact cell samples and their band 4.1 content analyzed. When the normal erythrocyte band 4.1 content was defined as 100%, the predicted and experimentally determined percentages were respectively as follows: 100–100%; 75–84%; 50–53%; 25–15%; 0–0%.

The mechanical stability of the erythrocyte membranes with band 4.1 deficiency is illustrated in Fig. 6. The rate of decline of DI with time is a measure of erythrocyte fragmentation. It can be seen that the fragmentation kinetics of erythrocytes from the second daughter with normal discocytic morphology is similar to that of controls. The erythrocytes from the parents and the first daughter without hemolysis but with reduced 4.1 levels, fragmented at a rate faster than those of controls. The erythrocytes of the three seriously affected sisters fragmented at a rate that was much faster than that of any of the controls, the parents, or the clinically unaffected sister.

DISCUSSION

The morphologic and biochemical observations in this family imply that the proband and two of her sisters suffer from homozygous hereditary elliptocytosis. Both parents have elliptocytic erythrocyte morphology; the three affected patients have increased elliptocytes, fragments, and spherocytes on smear, as well as a severe hemolytic disorder that can be ameliorated by splenectomy. Recent biochemical evidence has suggested that band 4.1 plays a crucial role in the modulation of spectrin-actin interactions. Ungewickell et al. (4) reported that band 4.1 is required for the formation of the spectrin-actin complex. Fowler and Taylor (7) showed that band 4.1, spectrin, and actin are all required for the optimal formation of skeletal supramolecular structures. In addition, Wolfe et al. (24) found that band 4.1 cooperatively augments the interaction of spectrin and actin at physiologic ionic strengths. Cohen and Foley (8) suggested that band 4.1 may play an important role in promoting the anchoring of actin filaments to the cytoplasmic surface of the erythrocyte by spectrin. Mueller and Morrison (25) have found evidence suggesting that the sialoglycoprotein PAS-2 may provide an integral membrane binding site for band 4.1. The relationship between the marked decrease in mechanical stability of the erythrocyte membrane and the deficiency of band 4.1 in erythrocyte membranes observed in the current study now provides direct evidence for an important physiologic role for band 4.1 in maintaining the structural stability of the erythrocyte.

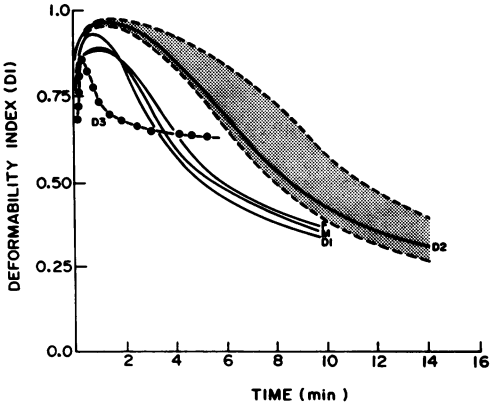

FIGURE 6 Mechanical fragility curves of erythrocytes from various family members. The decrease of DI with time is a measure of erythrocyte fragmentation. As the DI decreased, hemolysis increased and, simultaneously, fragmentation occurred. The DI of the proband (D3) decreased abruptly but achieved a plateau value higher than that of controls. This can be explained by the fact that the patient's cells were initially fragmented, and a lesser amount of surface area was available for subsequent fragmentation during assay. The fragmentation curves of the two other seriously affected daughters (D4, D5) were identical with the proband. The shaded area represents the range of variation of erythrocytes from 20 normal individuals.

The mechanical instability of the erythrocyte membrane may well account for the clinical picture seen in these patients. It seems reasonable to postulate that, because of the marked deficiency in band 4.1, the cytoskeleton is structurally inadequate and that the erythrocytes fragment soon after entering the circulation when subjected to the increased fluid stresses of the microcirculation. These fragmented cells, now with reduced deformability consequent to reduced surface area-to-volume ratio, are then trapped in the spleen, resulting in reduced erythrocyte life span and hemolytic anemia. The marked mechanical fragility, the sensitivity to osmotic stress, the large numbers of fragmented cells in the peripheral blood, and the improvement after splenectomy, all support this hypothesis. Moreover, the elliptocytic shape of the cells before fragmentation may well reflect a functional failure of the 4.1-depleted cytoskeleton to recover from the distortion of circulatory stresses.

Although deficiency of band 4.1 appears to be responsible for the elliptocytic morphology and membrane instability of erythrocytes in this family, alterations in other skeletal proteins may account for similar morphologic changes. Liu and Palek (26) recently reported defective dimer-tetramer association of spectrin in certain families with elliptocytosis and suggested that spectrin abnormalities may be responsible for decreased membrane stability. It seems

likely that various alterations in the interactions among the several skeletal proteins may eventually be found to be responsible for the heterogeneity seen in hereditary elliptocytosis.

From the current and previous studies, band 4.1 appears to be particularly important for maintaining membrane strength and integrity through its ability to integrate major components of the membrane skeleton.

ACKNOWLEDGMENTS

We would like to thank Professor M. Banabadji of the Centre National de Transfusion Sanguine, Algiers, for arranging the procurement of blood samples, and the Bradai family for their cooperation. We would also like to acknowledge the assistance of Dr. Louise Springer in the preparation of the scanning electron micrographs, and the help of Dr. Marc Shuman for a critical reading of the manuscript.

This work was supported in part by U. S. Public Health Service grants AM 16095 and AM 26263, and the MacMillan-Cargill Hematology Research Laboratory, University of California, San Francisco, Calif.

REFERENCES

1. Marchesi, V. T., and E. Steers. 1968. Selective solubilization of a protein component of the red cell membrane. *Science (Wash. D. C.).* **159:** 203–204.
2. Palek, J., and S. C. Liu. Dependence of spectrin organization in red blood cell membranes on cell metabolism: implications for control of red cell shape, deformability, and surface area. *Semin. Hematol.* **16:** 75–93.
3. Lux, S. E. 1979. Spectrin-actin membrane skeleton of normal and abnormal red cells. *Semin. Hematol.* **16:** 21–51.
4. Ungewickell, E., P. M. Bennet, R. Calvert, V. Ohanian, and W. B. Gratzer. 1979. In vitro formation of a complex between cytoskeletal proteins of the human erythrocyte. *Nature (Lond.).* **280:** 811–814.
5. Bennett, V., and P. J. Stenbuck. 1979. Identification and partial purification of ankyrin, the high affinity membrane attachment site for human erythrocyte spectrin. *J. Biol. Chem.* **254:** 2533–2546.
6. Tyler, J. M., B. N. Reinhardt, and D. Branton. 1980. Association of erythrocyte membrane proteins. Binding of purified bands 2.1 and 4.1 to spectrin. *J. Biol. Chem.* **255:** 7034–7039.
7. Fowler, V., and D. L. Taylor. 1980. Spectrin plus band 4.1 cross-link actin. Regulation by micromolar calcium. *J. Cell Biol.* **85:** 361–376.
8. Cohen, C. M., and S. F. Foley. 1980. Spectrin-dependent and -independent association of F-actin with the erythrocyte membrane. *J. Cell Biol.* **86:** 694–698.
9. Cohen, C. M., J. M. Tyler, and D. Branton. 1980. Spectrin-actin associations studied by electron microscopy of shadowed preparations. *Cell.* **21:** 875–883.
10. Bennett, V., and P. J. Stenbuck. 1979. The membrane attachment protein is associated with band 3 in human erythrocyte membranes. *Nature (Lond.).* **280:** 468–473.
11. Greenquist, A. C., S. B. Shohet, and S. E. Bernstein. 1978. Marked reduction of spectrin in hereditary spherocytosis in the common house mouse. *Blood.* **51:** 1149–1155.
12. Shohet, S. B. 1979. Reconstitution of spectrin deficient mouse erythrocyte membranes. *J. Clin. Invest.* **64:** 483–493.
13. Lipton, E. L. 1955. Elliptocytosis with hemolytic anemia: the effects of splenectomy. *Pediatrics.* **15:** 67–83.
14. Grech, J. L., E. A. Cachia, F. Calleja, and F. Pullicino. 1961. Hereditary elliptocytosis in two maltese families. *J. Clin. Pathol. (Lond.).* **14:** 365–373.
15. Pryor, D. S., and W. R. Pitney. 1967. Hereditary elliptocytosis. A report of two families from New Guinea. *Br. J. Haematol.* **13:** 126–134.
16. Nielson, J. A., and K. W. Strunk. 1968. Homozygous hereditary elliptocytosis as the cause of haemolytic anemia in infancy. *Scand. J. Haematol.* **5:** 486–496.
17. Feo, C., S. Fischer, J-P. Piau, M. J. Grange, and G. Tchernia. 1980. Premierè observation de l'absence d'une protéine de la membrane érythrocytaire (bande 4.1) dans un cas anemie elliptocytaire familiale. *Nouv. Rev. Fr. Hematol.* **22:** 315–325.
18. Bessis, M., and R. I. Weed. 1973. The structure of normal and pathologic erythrocytes. *Adv. Biol. Med. Phys.* **13:** 35–91.
19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* **227:** 680–682.
20. Clark, M. R., R. C. Unger, and S. B. Shohet. 1978. Monovalent cation composition and ATP and lipid content of irreversibly sickled cells. *Blood.* **51:** 1169–1178.
21. Zarkowsky, H. S., N. Mohandas, C. B. Speaker, and S. B. Shohet. 1975. A congenital haemolytic anaemia with thermal sensitivity of the erythrocyte membrane. *Br. J. Haematol.* **29:** 537–543.
22. Mohandas, N., M. R. Clark, M. S. Jacobs, and S. B. Shohet. 1980. Analysis of factors regulating erythrocyte deformability. *J. Clin. Invest.* **66:** 563–573.
23. King, L. E., Jr., and M. Morrison. 1977. Calcium effect on human erythrocyte membrane proteins. *Biochim. Biophys. Acta.* **471:** 162–168.
24. Wolfe, L. C., S. E. Lux, and V. Ohanian. 1980. Regulation of spectrin-actin binding by protein 4.1 and polyphosphates. *J. Cell Biol.* **87:** 203a. (Abstr.)
25. Mueller, T. J., and M. Morrison. 1980. Glycoconnectin (PAS 2), a component of the cytoskeleton of the human erythrocyte membrane. *J. Cell Biol.* **87:** 202a. (Abstr.)
26. Liu, S. C., and J. Palek. 1980. Decreased spectrin tetramer-dimer ratio and mechanical instability of membrane skeletons in hereditary elliptocytosis. *Clin. Res.* **28:** 318a. (Abstr.)