Hereditary Elliptocytosis With Protein Band 4.1 Deficiency in the Dog

By Joseph E. Smith, Kateri Moore, Marlys Arens, G.A. Rinderknecht, and Arlo Ledet

A dog with persistent elliptocytosis was studied. The dog had membrane protein band 4.1 deficiency, microcytosis, shortened erythrocyte lifespan, increased osmotic sensitivity, and a mild glutathione deficiency. Erythrocyte deformability and membrane stability were adversely affected.

The erythrocyte contains a cytoskeletal network under the membrane that modulates several membrane properties. Erythrocyte shape, membrane flexibility, structural integrity, and deformability seem to be the consequence of this cytoskeleton. It is formed from the following membrane proteins: spectrin (bands 1 and 2), actin (band 5), ankyrin (band 2.1), and protein band 4.1.2 The two polypeptides of spectrin associate side-to-side as heterodimers. The spectrin dimers, which are long worm-like molecules, join head-to-head with other spectrin dimers to form a tetramer.4,5 One end of the spectrin tetramer is bound through ankyrin to an integral membrane protein, band 3.6 That links the membrane skeleton to the lipid bilayer. The other end of the spectrin is bound to actin.7 Protein band 4.1 modulates the spectrin–actin interaction.8,9

Several abnormalities of the cytoskeleton occur in man. Because the erythrocyte has a limited repertoire of possible shapes, a particular shape may result from several primary defects. Hereditary elliptocytosis can result from protein band 4.1 deficiency, or abnormal spectrin structure.10,11 Some cases of hereditary spherocytosis result from an inadequate amount of spectrin;12 others from an abnormal spectrin that does not bind protein band 4.1.13 Some patients with hemolytic poikilocytic anemia have erythrocytes with an abnormal binding site for ankyrin;15 others with hereditary pyropoikilocytosis have an altered spectrin dimer–dimer association.16

Relatively few inherited abnormal erythrocyte shapes occur in animals. A spectrin deficiency in the common house mouse causes a recessively inherited hemolytic anemia.17 Four mutants with varying degrees of spectrin deficiency have anemias that correlate with the amount of residual spectrin.18 In this report, we describe a dog with elliptocytosis due to abnormality in protein band 4.1. This animal should provide another animal model for disorders involving the erythrocyte membrane cytoskeleton.

MATERIALS AND METHODS

Erythrocyte numbers, PCV, and hemoglobin were measured with a Coulter Counter, Model S, Sr (Coulter Electronics, Hialeah, Fla.).

The dog's parents had decreased band 4.1, decreased stability, and some elliptocytosis. This disorder in dogs closely resembles human patients with band 4.1 deficiency and should provide a valuable animal model to study the erythrocyte membrane cytoskeleton.
centrifuged, the supernatant removed, and plasma added. Blood smears from the plasma-heated erythrocyte suspension were examined by light microscopy.3'

CASE REPORT

The dog is a crossbred male and the offspring from a father–daughter mating (Fig. 1). He was seen initially because of emaciation secondary to malnutrition and chronic bacterial dermatitis. The first hemogram revealed an anemia (PCV, 32%) and elliptocytosis. He responded dramatically to adequate nutrition and antibiotic therapy, but elliptocytosis persisted. Only limited studies could be made on the parents.

RESULTS

The proband has a well compensated hemolytic disease. The PCV, erythrocyte number, and hemoglobin became normal, but the reticulocyte number remained abnormally high (Table I). The 51Cr-labeled erythrocyte lifespan (16.4 days) is shorter than that of normal dogs (24.6 ± 0.8). Serum iron, total iron-binding capacity, and erythrocyte Na+, K+, and water were normal. Erythrocyte enzyme activity reflected the shortened mean cell age. Hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, phosphofructokinase, and phosphoglycerate kinase were increased, but adenylate kinase, enolase, lactate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, aldolase, diphosphoglyceromutase, monophosphoglyceromutase, glutathione reductase, glutathione peroxidase, glucose phosphate isomerase, and 6-phosphogluconate dehydrogenase were not different from normal canine erythrocytes. Erythrocyte glutathione (GSH), which usually is increased with shortened erythrocyte life span, was unexpectedly lower than normal (Table 1).

When blood smears from the proband were examined by light microscopy or scanning electron microscope, numerous elliptocytes were seen (Fig. 2). Fragmented erythrocytes, budding cells, and spherocytes were also seen. The mean cell volume (MCV) was smaller than normal (Table 1). An increased sensitivity to hypotonic lysis (Fig. 3) reflected an altered surface-to-volume ratio.

The proband’s elliptocytes had normal thermostability. Fragmentation began at 51°C for both proband and control cells.

SDS-PAGE patterns of the proband, parents, and normal canine erythrocyte membrane are shown in Fig. 4. Band 4.1 was absent in membranes from the proband. The parents had decreased levels of band 4.1 when compared to normal erythrocytes, with band 3 as a reference (Table 2).

The deformability index of the proband was 84% of that observed in normal erythrocytes. His membranes fragmented at a faster rate than normal membranes when they were subjected to 600 dyne/sq cm shear stress (Fig. 5). Fragmentation time for 4 normal canine ghosts was 132 sec (SD = 20.5) and for elliptocytic ghosts was 31 sec.

DISCUSSION

Canine elliptocytosis with protein band 4.1 deficiency is similar to the human counterpart. Both are inherited as autosomal recessive traits with heterozygotes having about 50% band 4.1. Both have altered erythrocyte morphology, including elliptocytosis, membrane fragmentation, and poikilocytosis. Neither has increased the thermal fragmentation characteristic

Table 1. Hematologic Investigations

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Unit</th>
<th>Control Means ± 1 SD</th>
<th>Sire</th>
<th>Dam</th>
<th>Proband</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>x 10⁶/μl</td>
<td>6.91 ± 0.74</td>
<td>6.80</td>
<td>7.14</td>
<td>8.13</td>
</tr>
<tr>
<td>PCV</td>
<td>%</td>
<td>44.6 ± 4.33</td>
<td>42.0</td>
<td>46.8</td>
<td>42.7</td>
</tr>
<tr>
<td>Hb</td>
<td>g/dl</td>
<td>16.0 ± 1.68</td>
<td>15.2</td>
<td>16.6</td>
<td>15.4</td>
</tr>
<tr>
<td>MCV</td>
<td>f/l</td>
<td>65.3 ± 2.16</td>
<td>62.0</td>
<td>66.0</td>
<td>53*</td>
</tr>
<tr>
<td>MCH</td>
<td>%</td>
<td>35.5 ± 0.85</td>
<td>35.9</td>
<td>35.1</td>
<td>35.7</td>
</tr>
<tr>
<td>MCH</td>
<td>pg</td>
<td>23.4 ± 1.16</td>
<td>22.5</td>
<td>23.4</td>
<td>19.0*</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>%</td>
<td>1.3 ± 0.34</td>
<td>1.3</td>
<td>0.7</td>
<td>3.0*</td>
</tr>
<tr>
<td>GSH</td>
<td>μmole/g Hb</td>
<td>7.89 ± 0.550</td>
<td>7.88</td>
<td>7.49</td>
<td>6.38*</td>
</tr>
</tbody>
</table>

*Significant difference from normal (p < 0.05).
Fig. 2. Scanning electron micrographs of erythrocytes from a control (A), the proband with canine hereditary elliptocytosis (B), his dam (C), and his sire (D). Bar is equal to 5 μm.

Fig. 3. Osmotic fragility of the proband and control (n = 4) erythrocytes.

Fig. 4. Electrophoretic patterns of erythrocyte membranes from a control (C), hereditary elliptocytosis (HE), his dam (D), and his sire (S).
Mechanical instability of erythrocyte membrane, seen in both species, emphasizes the importance of band 4.1 in maintaining erythrocyte integrity. Loss of band 4.1's modulating influence on the spectrin–actin interaction must be important in resisting the fluid stresses of microcirculation.

Band 4.1 deficiency in the dog differs in some respects from the human disorder. In human patients the anemia is severe before splenectomy. Patients require multiple transfusions because their erythrocyte lifespan is too short to be adequately compensated by increased erythropoiesis. After splenectomy, their anemia becomes well compensated, with hemoglobin concentrations nearly normal. Although we do not know the degree of anemia during the growing period in canine elliptocytosis, it must not have been life-threatening.

The erythrocyte morphology of heterozygotes is less striking than that seen in the human counterpart. The mechanism for that difference is unexplained.

The marginally low glutathione level is probably secondary. Dogs with shortened erythrocyte lifespan and reticulocytosis usually increase the erythrocyte glutathione. Explanation of the low glutathione is not apparent.

### REFERENCES

13. Agre P, Orringer EP, Bennett V: Deficient red-cell spectrin in
spherocytosis of man. Altered binding of cytoskeletal components to
15. Agre P, Orringer EP, Chui DHK, Bennett V: A molecular
defect in two families with hemolytic poikilocytic anemia. Reduction
of high affinity membrane binding sites for ankyrin. J Clin Invest
68:1566–1576, 1981
dimer–dimer association and instability of erythrocyte membrane
skeletons in hereditary pyropoikilocytosis. J Clin Invest 68:597–605,
1981
17. Greenquist AC, Shohet SB, Bernstein SE: Marked reduction
of spectrin in hereditary spherocytosis in the common house mouse.
Blood 51:1149–1155, 1978
18. Lux SE, Pease B, Tomaselli, MB, John KM, Bernstein SE:
Hemolytic anemias associated with deficient or dysfunctional spectrin,
in Lux SE, Marchesi VT (eds): Normal and Abnormal Red Cell Membranes. New York, Liss, 1979, p 463
20. Report by the ICSH panel on diagnostic applications of
radioisotopes in haematology: Recommended methods for radioiso-
Philadelphia, Lea & Febiger, 1975, pp 76–77
W: International committee for standardization in haematology:
Recommended methods for red cell enzyme analysis. Br J Haematol
35:331–340, 1977
23. Smith JE, Moore K, Schoneweis D: Coulometric technique
24. Beutler E, Duron O, Kelly BM: Improved method for the
determination of blood glutathione. J Lab Clin Med 61:882–888,
1963
25. Dodge JT, Mitchell C, Hanahan DJ: The preparation and
chemical characteristics of hemoglobin free ghosts of human ery-
26. Beutler E, West C, Blume KG: The removal of leukocytes and
27. Fairbanks G, Stock TL, Wallach DFH: Electrophoretic analy-
sis of the major polypeptides of the human erythrocyte membrane.
Biochemistry 10:2602–2617, 1971
28. Mohandas N, Clark MR, Jacobs MS, Shohet SB: Analysis of
573, 1980
29. Mohandas N, Clark MR, Health BR, Rossi M, Wolfe LC,
Lux SE, Shohet SB: A technique to detect reduced mechanical
stability of red cell membranes: Relevance to elliptocytic disorders.
Blood 59:768–774, 1982
30. Bessis M, Weed RF: The structure of normal and pathologic
31. Mohandas N, Greenquist A, Shohet SB: Effects of heat and
metabolic depletion on erythrocyte deformability, spectrin extracta-
32. Vaicha J: Critical comparative review of the life span of red
Hereditary elliptocytosis with protein band 4.1 deficiency in the dog

JE Smith, K Moore, M Arens, GA Rinderknecht and A Ledet