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 deficiency10,11 or abnormal spectrin structure.12 Some cases of hereditary spherocytosis result from an inadequate amount of spectrin;13 others from an abnormal spectrin that does not bind protein band 4.1.14 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 exam-
ined by light microscopy.

**CASE REPORT**

The dog is a crossbred male and the offspring from a father-
daughter mating (Fig. 1). He was seen initially because of emacia-
tion 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 hemoglo-
in became normal, but the reticulocyte number
remained abnormally high (Table 1). The $^{51}$Cr-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, phospho-
ructokinase, and phosphoglycerate kinase were
increased, but adenylate kinase, enolase, lactate dehy-
drogenase, glyceraldehyde-3-phosphate dehydro-
genase, aldolase, diphosphoglyceromutase, monophos-
phoglyceromutase, glutathione reductase, glutathione
peroxidase, glucose phosphate isomerase, and 6-phos-
phogluconate 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 exam-
ined by light microscopy or scanning electron micro-
scope, numerous elliptocytes were seen (Fig. 2). Frag-
mented erythrocytes, budding cells, and spherocytes
were also seen. The mean cell volume (MCV) was
smaller than normal (Table 1). An increased sensitiv-
ity to hypotonic lysis (Fig. 3) reflected an altered
surface-to-volume ratio.

The proband's elliptocytes had normal thermosta-
tility. 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 defi-
ciency is similar to the human counterpart. Both are
inherited as autosomal recessive traits with heterozy-
gotes having about 50% band 4.1. Both have altered
erthrocyte 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 Mean ± 1 SD</th>
<th>Sire</th>
<th>Dam</th>
<th>Proband</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>x 10^6/μl</td>
<td>6.91 ± .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>fl</td>
<td>65.3 ± 2.16</td>
<td>62.0</td>
<td>66.0</td>
<td>53*</td>
</tr>
<tr>
<td>MCHC</td>
<td>%</td>
<td>35.5 ± .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 ± .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 5μm.

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).
Table 2. Comparison of Erythrocyte Membrane Protein Band 4.1 Levels

<table>
<thead>
<tr>
<th>Band 4.1/Band 3</th>
<th>Proband</th>
<th>Sire</th>
<th>Dam</th>
<th>Controls (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>0</td>
<td>8.7%</td>
<td>11.2%</td>
<td>21.3% ± 3.43% (SD)</td>
</tr>
</tbody>
</table>

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.

Canine elliptocytosis with protein band 4.1 deficiency provides another animal model for studying the relationship of erythrocyte membrane proteins to cellular integrity.

ACKNOWLEDGMENT

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