Coinheritance of α- and β-Spectrin Gene Mutations in a Case of Hereditary Elliptocytosis

To the Editor:

Many mutations in the α- and β-spectrin genes are known to be associated with hereditary elliptocytosis (HE). Spectrin abnormalities are detected as abnormal peptides after limited trypsin digestion of spectrin (Sp).1 We had an opportunity to study a Filipino family living in Kuwait. The proband first presented with hemolysis at the age of 4 months and he was transfusion dependent until he was 12 months old. This family was referred to us when the proband was 16 months old. The parents are unrelated and there is no hemolytic history on either side (Table 1). The proband’s blood film showed obvious elliptocytes with few poikilocytes. His mother’s red blood cells were elliptocytic whereas the father’s red blood cell morphology was apparently normal. In osmotic gradient ektacytometry, red blood cells from all three members produced trapezoidal deformability curves, which are typical of HE with reduced DIiso values (Table 1). Spectrin analysis showed that all three individuals are heterozygous for the HE SpαC474 variant and have defective Sp dimer self-association process (shown as increased percent of spectrin dimer content in the cold low-ionic-strength membrane extracts) (Table 1). The SpV14 peptide polymorphism was also identified in the proband and his mother. This polymorphism is associated with the low-expressed α-SpLely allele. The high percentage of the αV41 peptide in the mother is related to homozygosity for α-SpLely allele (Table 1).

Direct sequencing of exon 2 in α-Sp gene and exon 30 in β-Sp gene3 has shown that the mother is heterozygous for a previously described mutation in codon 28 of α-Sp gene (CGT → TGT; R28C).4 The father is heterozygous for a new β-Sp gene mutation in codon 2018 (GCC → GAC; A2018D), which we designate as β-SpKuwait. Another point mutation previously reported in this codon was β-SpCagliari (GCC → GGC; A2018G).5 The proband was found to be heterozygous for both mutations (α-Sp R28C and β-Sp A2018D). The homozygosity for α-SpLely allele in the mother has nullified the expected reduced expression of SpαC474 variant when this HE allele is inherited in cis to an α-SpLely allele. In analogy to another α-Sp HE mutation,6 the low percentage of the αV41 peptide in the proband’s tryptic digest can be accounted for by the cis inheritance of the α-SpLely variant in respect to the deleterious α-Sp allele.

The appearance of HE SpαC474 variants can be due to impairment of either spectrin genes, and more precisely exon 2 in α-Sp gene and exon 30 in β-Sp gene.1,10 Although the two point mutations detected in the heterozygous HE parents involve the codons of the Sp genes already known to be mutated in other HE cases, the present study is the first report describing an HE patient who has co-inherited both α- and β-gene mutations. The molecular combinations of these two mutations, including the presence of the α-spectrin mutation (R28C) on a low-expressed α-SpLely, have produced in the proband a hematological and biochemical picture of an apparently heterozygous HE phenotype, which is similar to that of his heterozygous HE parents, without any Sp deficiency (Table 1). The absence of typical elliptocytic cells in the father, despite his typical HE trapezoidal deformability curve, has already been observed in another HE family with a point mutation in β-spectrin.11 Because no more transfusions were needed 1 year after birth, the severity of the proband’s disease in the first year of life appears consistent with a transient poikilocytosis in infancy.10 However, such diagnosis has to be confirmed by an accurate clinical follow-up of the proband, especially as the percentage of αI/74 peptide being incorporated into his red blood cell membranes was found to be slightly higher than those observed in both parents (Table 1).

REFERENCES


<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Hb (g/dL)</th>
<th>Reticulocyte Counts (μL)</th>
<th>DIiso</th>
<th>SpBand 3 Ratio</th>
<th>% of SpD in 4°C Extracts</th>
<th>% of SpAIV4 Variant</th>
<th>% of SpαI74 Peptide</th>
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<td>Father</td>
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<td>Proband</td>
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<td>45.8</td>
<td>56.5</td>
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<tr>
<td>Normal</td>
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<td>0.38 to 0.52</td>
<td>0.98 ± 0.06 (n = 40)</td>
<td>21.1 ± 1.8 (n = 7)</td>
<td>2.3 ± 0.4 (n = 10)*</td>
<td>4.1 ± 1.6 (n = 4)†</td>
</tr>
</tbody>
</table>

DIiso, deformability index in isotonicity (osmotic gradient ektacytometry studies).

*Values observed in normal control subjects heterozygous for the SpαI74 allele.
† Values observed in normal control subjects homozygous for the SpαI74 allele.

Table 1. Hematological and Biochemical Data

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### Nerve Growth Factor Released by CD40 Ligand-Transfected L Cells: Implications for Functional and Phenotypic Studies on CD40+ Cells

*To the Editor:

Several studies on the differentiation, function, and phenotype of activated B lymphocytes are currently using an in vitro system in which CD40 stimulation of B cells is induced by CD40 ligand (CD40L) expressed on the surface of fibroblasts, usually mouse L cells transfected with the CD40L gene. 1 The ligation of CD40 on B cells by the CD40L, which is expressed under physiological conditions on activated y6, CD4+ B cells, or on mast cells and basophils, plays a crucial role in the induction and regulation of a T-cell–dependent humoral immune response. The CD40/CD40L interaction costimulates B-cell proliferation and differentiation into memory B cells. 2,3 Production of IgGs and isotype class switch are also regulated by T-B cell interaction through the CD40/CD40L pathway. In addition, the role of CD40 ligatin in B-cell terminal differentiation is also being actively studied.

CD40 is detected on other professional antigen-presenting cells (APC) such as thymic epithelial cells, activated macrophages, dendritic cells (DC), follicular dendritic cells (FDC), and also on activated fibroblasts, vascular epithelial, or glia cells. 4 Ligation of CD40 on macrophages, DCs, or epithelial cells induces activation, proliferation, and the production of various cytokines including interleukin-12 (IL-12), which is responsible for the development of Th1 responses. 5,6 CD40 stimulation of monocytes by fibroblastoid murine L cells or monkey CV-1/EBNA kidney cells expressing CD40L resulted in the induction of different sets of cytokines. 7,8 These differences were attributed to (1) the variable expression of CD40L, (2) the distinct costimulatory molecules on the cell membrane, and/or (3) the secretion of various costimulatory cytokines by these cells. 4 Experiments monitoring the expression of adhesion molecules on Langerhans cells upon activation by soluble CD40L or by CD40L expressed on the surface of L cells also gave rise to contradictory data. 7

The CD40 molecule belongs to the family of the nerve growth factor (NGF)/tumor necrosis factor (TNF) receptors, including CD27, Fas/APO-1, and the p75 low-affinity receptor for NGF. These molecules are involved in regulation of cell proliferation and programmed cell death, mainly in immune cells. Indeed, in addition to its neurotrophic activity, NGF has also been suggested to play a regulatory role on the cells of the immune system, and many studies have been focused on the activity of NGF on B lymphocytes. 8 The NGF has been found to be an autocrine survival factor for memory B cells and to be active in regulating Ig production by B cells. 9–11 Helper T lymphocytes of both the Th1 and Th2 type were shown to produce NGF, 9,11,12 suggesting a role for NGF in modulating immune responses.

We have tested the cell culture supernatant of irradiated L cells and CD40L-transfected L cells for the presence of soluble NGF by a sensitive quantitative enzyme-linked immunosassay (ELISA). The cells were cultured in Iscove's Modified Dulbecco's medium (IMDM) supplemented with 10% fetal calf serum and antibiotics. After irradiation (150 Gy) the cells were cultured in serum-free AIM-V medium at 4 × 10^5 cells/mL and supernatants were obtained after 24 hours. The ELISA for NGF detection was performed according to the manufacturer's instructions as follows: polystyrene 96-well immunoplates (Nunc, Roskilde, Denmark) were coated for 2 hours at 37°C with 100 µL of mouse monoclonal antibody (MoAb) 27/21 specific for NGF (Boehringer Mannheim, Mannheim, Germany) diluted in 0.05 mol/L carbonate buffer (pH 9.6). The plates were further incubated with the blocking solution (0.5% bovine serum albumin in coating buffer) for 30 minutes at 37°C. Cell culture supernatants and the NGF standard curve diluted in the same culture medium were incubated overnight at 4°C. The presence of bound NGF was detected by 4-hour incubation at 37°C with 4 µIU/well of anti-NGF antibody 27/21 conjugated with β-galactosidase (Boehringer Mannheim). The plates were then incubated overnight at 37°C with 200 µL of chloroform-nitroblue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) substrate buffer and the optical density was monitored at 575 nm using an Elisa Processor II (Behringwerke AG, Marburg, Germany). All the samples and the NGF standard curve were run in triplicate wells. As summarized in Table 1, we found that

| Table 1. Levels of NGF Detected in Culture Supernatants of Three Fibroblastic Cell Lines |
|---------------------------------|-------------------|
| Cell Line                      | NGF (pg/mL)       |
| L cells                        | 1.880 ± 23        |
| CD40L-L cells                  | 1.220 ± 46        |
| A9 cells                       | 810 ± 31          |
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