To the Editor:

Hereditary stomatocytosis is a rare chronic hemolytic anemia due to defective cell volume regulation. Affected red blood cells (RBCs) exhibit an increased Na⁺ and K⁺ permeability, normal Na⁺, K-ATPase activity, and increased water content of undetermined aetiology, but an integral membrane protein migrating to the band 7 region on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was found to be clearly decreased. Further investigations of RBC membrane proteins from several patients by two-dimensional gel electrophoresis indicated that the band 7 region on one-dimensional gels could be resolved into four distinct proteins (2 integral and 2 peripheral), among which only the basic integral membrane protein called 7.2b was completely absent.

Protein 7.2b is a recently purified 31-Kd protein that is phosphorylated and palmitoylated. During these investigations it was noted that protein 7.2b and the blood group Rh polypeptides exhibited common features, including solubility properties, molecular weight (Mr), and palmitoylation. Also, the absence or severe reduction of Rh antigens from RBCs of Rh-deficient individuals
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Fig 1. Immunostaining analysis of Rh and 7.2b proteins on human RBC membranes from Rh variants and hereditary stomatocytosis individuals. Membrane proteins from the Rh variants and the hereditary stomatocytosis patient (W.D.) were prepared and analyzed by Western blot analysis as previously described, using a detection system of goat antirabbit IgG conjugated to phosphatase alkaline. (A) Immunostaining with antiprotein 7.2b antiserum (no. R4407, 1:5,000 dilution). (B) Immunostaining with two polyclonal anti-Rh proteins, MPC1 (1:2,000 dilution) and MPC8 (1:4,000 dilution), raised against the N-ter (res. 34-46) and C-ter (res. 400-417) of Rh proteins, respectively. Arrows indicate the migration positions of the 7.2b and Rh proteins.

(Rhnull and Rhmod) is associated with a chronic hemolytic anemia of varying severity characterized by stomatocytosis, reduced osmotic fragility, and increased cation permeability.

Although Rh antigens have been found normally expressed in one patient with hereditary stomatocytosis, it is not known whether the Rh polypeptides are normal in these cells and, conversely, whether protein 7.2b is present or absent in Rh-deficient erythrocytes. In the present study, we report on the characterization of Rh proteins and protein 7.2b in erythrocytes from Rh-deficient and hereditary stomatocytosis patients using specific polyclonal antisera raised against these proteins.

Accordingly, RBC membrane proteins from individuals of known common and rare Rh phenotypes, including those from Rh-deficient patients (Rhnull and Rhmod) of both the regulatory and amorphic types were prepared, separated by SDS-PAGE, transferred to nitrocellulose sheets, and analyzed by immunoblotting with the antiprotein 7.2b antisemum, as shown in Fig 1A. Membranes from a hereditary stomatocytosis patient (W.D.) were simultaneously investigated as control. We found that protein 7.2b (apparent Mr, 31,000) was normally present in all membranes from the Rh variants, including those of 4 unrelated Rh-deficient cells, whereas this protein was, as expected, undetectable in membrane from patient W.D. In parallel experiments, we found that the Rh proteins (apparent Mr, 31,000) detected with the polyclonal antiserum MPC1 and MPC8 (specific for the N-ter and C-ter domains of the Rh proteins, respectively) could be identified on RBCs from RhD-positive and RhD-negative individuals, as well as those from patient W.D., but were absent, as expected, from Rh-deficient samples (Fig 1B). Together, these results indicate that, although RBCs from hereditary stomatocytosis and Rh-deficient patients exhibit similar features, these syndromes are associated with the molecular defect of distinct integral membrane proteins. This finding is in agreement with the known heterogeneity of disorders associated with stomatocytosis of peripheral blood cells, but how such defects are correlated with morphologic and functional abnormalities needs further investigations.

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REFERENCES

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Hereditary stomatocytosis and Rh-deficient patients exhibit distinct molecular defects [letter]

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