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Hemolytic Anemia Associated with Impaired Erythrocyte Shape Control

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Impaired human red blood cell (RBC) shape recovery capability after in vitro morphological perturbations has been described in a hemolytic condition with complete glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and in some cytoskeletal defects, such as the lack of glycophorin C. In all these conditions, a hemolytic state occurs [1,2].

We observed another case of hemolytic anemia associated with impaired shape recovery mechanisms.

The proposita is a pale and jaundiced 39-year-old woman, with spleen and liver enlargement. Her brother had been splenectomized for hemolytic anemia (not further defined because of his death). Laboratory findings showed signs of hemolytic anemia (Hb 8 g/dl, PCV 0.23, MCV 105 fl, reticulocytes 12.5%, total serum bilirubin 2.2 mg/dl, direct bilirubin 0.3 mg/dl); the blood smear examination revealed appropriate macrocytosis with extreme poikilocytosis but no microspherocytes; osmotic fragility was within the normal range; direct antiglobulin test and Ham-Dacie test were negative on different occasions. No hemoglobin abnormalities were found by cellulose acetate electrophoresis. Erythrocyte G-6-PD and pyruvate-kinase levels were found to be normal. Blood group determination did not show any uncommon phenotype. ATP and 2,3-DPG erythrocytic levels were found to be normal. RBC shape recovery capability was studied, as described elsewhere [1], by evaluating the percentage of RBC that recovered their normal discoid shape 10 and 60 min after complete echinocytic transformation induced by the crenating agent 2,4-dinitrophenol (DNP). The test was also performed after a supplementary incubation with the chloromethyl ketone peptide tos-phe-CH₂Cl (TPCK) which enhances the shape recovery process [1]. The percentage of patient RBC shape recovery was lower than that of controls (2 vs. 21.1 ± 4% and 14 vs. 38.1 ± 3.9%, respectively, 10 and 60 min after crenation); preincubation with TPCK resulted in a lesser increase of patient RBC recovery rate than in controls (7 vs. 48.8 ± 6% and 23 vs.

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a b b' a'

Fig. 1. PAS (lanes a', b') and Coomassie (lanes a, b) stained membrane proteins from a healthy control (lanes a', a) and the patient (lanes b', b).

$68.1 \pm 4.7\%$, respectively, 10 and 60 min after crenation). Erythrocyte membrane preparation and SDS-PAGE were carried out as described elsewhere [4]; gels were stained with periodic acid-Schiff (PAS) or Coomassie brilliant blue and quantitation of membrane proteins was performed by laser densitometry (2202-UIltrosan, LKB, Sweden). No quantitative abnormalities in PAS (fig. 1, lane b') and Coomassie (fig. 1, lane b) stained membrane proteins from the proposita were detectable (lanes a' and a in figure 1 represent PAS- and Coomassie-stained membrane proteins from a healthy control).

Hemolytic Anemia Associated with Impaired Erythrocyte Shape Control

107

This patient presents an apparently unique defect revealed by impaired RBC shape recovery capability after chemical crenation. Unlike previous reported cases, she presents a normal electrophoretic pattern of PAS- and Coomassie-stained membrane proteins and normal G-6-PD levels. Therefore, a qualitative rather than quantitative alteration in membrane sialoglycoproteins might be involved; this hypothesis is consistent with a recent report by Rybicki et al. [4] of a mild hemolytic condition in which either a mutant α -glycophorin chain or a hybrid of α - δ -chains has been characterized. An alternative explanation might be a lack or a defect in one of the enzymes (other than G-6-PD) involved in the HMP shunt. Further studies are required to determine the exact cellular lesion in our patient; however, this report should prompt closer attention to impaired RBC shapes in human hemolytic diseases.

References

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