Brief report

Hereditary spherocytosis and hereditary elliptocytosis: aberrant protein sorting during erythroblast enucleation

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Introduction

During erythroblast enucleation, membrane proteins distribute between extruded nuclei and reticulocytes. In hereditary spherocytosis (HS) and hereditary elliptocytosis (HE), deficiencies of membrane proteins, in addition to those encoded by the mutant gene, occur. Elliptocytes, resulting from protein 4.1R gene mutations, lack not only 4.1R but also glycophorin C, which links the cytoskeleton and bilayer. In HS resulting from ankyrin-1 mutations, band 3, Rh-associated antigen, and glycophorin A are deficient. The current study was undertaken to explore whether aberrant protein sorting, during enucleation, creates these membrane-spanning protein deficiencies. We found that although glycophorin C sorts to reticulocytes normally, it distributes to nuclei in 4.1R-deficient HE cells. Further, glycophorin A and Rh-associated antigen, which normally partition predominantly to reticulocytes, distribute to both nuclei and reticulocytes in an ankyrin-1–deficient murine model of HS. We conclude that aberrant protein sorting is one mechanistic basis for protein deficiencies in HE and HS. (Blood. 2010;116(2):267-269)

Methods

Antibodies

Rabbit antibodies specific for mouse GPC, band 3, and RhAG were generated in our laboratory. Anti-GPC was labeled with Alexa Fluor 555 and Alexa Fluor 488. Other antibodies were obtained from commercial sources detailed in “Immunofluorescence microscopy.”

Mice

Ankyrin-1–deficient nb/nb mice,10 provided by Dr Luanne Peters (The Jackson Laboratory), and protein 4.1R knockout mice7 were maintained in The New York Blood Center animal facility. The Institutional Animal Care and Use Committee of The New York Blood Center approved all protocols.

Immunofluorescence microscopy

Freshly harvested 4.1R-null and wild-type (WT) bone marrow cells were suspended in RPMI with 20% fetal calf serum (Invitrogen) and stained with Syto-17 (1 μM; Invitrogen–Molecular Probes) according to the manufacturer’s instructions. Other antibodies were obtained from commercial sources detailed in “Immunofluorescence microscopy.”

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anti–mouse band 3, rabbit anti-GPC, or rabbit anti-RhAG (1:100). After washing with 0.1% albumin–phosphate–buffered saline, the cells were labeled with Alexa Fluor 594–conjugated goat anti–rabbit IgG (1:100; Invitrogen) and Alexa Fluor 488–conjugated donkey anti–rabbit IgG (1:500; Invitrogen) for 1 hour at room temperature. After washing, the slides were mounted with VectaShield (Vector Laboratories).

**Results and discussion**

To explore whether aberrant protein sorting might be responsible for deficiencies of membrane proteins in HE, we examined sorting of GPC during enucleation of normal and protein 4.1R-null erythroblasts. Protein 4.1R knockout mice have fragmented red cells, which lack GPC, thus phenotypically mimicking human HE. By using immunofluorescent microscopy, we first analyzed GPC sorting in enucleating erythroblasts from WT bone marrow. We found that GPC partitioned almost exclusively to nascent reticulocytes, with little or no GPC observed in plasma membranes of extruding nuclei (Figure 1). Strikingly, in 4.1R-null erythroblasts, GPC distributed exclusively to nuclei (Figure 1). These data unequivocally establish that GPC deficiency in 4.1R-null erythroblasts, band 3, GPA, and RhAG sorted to both expelled nuclei and reticulocytes (Figure 2B–C). These data clearly demonstrate that one mechanism producing deficiencies of band 3, RhAG, and GPC in mature nb/nb red cells is their abnormal sorting during nuclear extrusion.

Although the deficiency of GPC in HE red cells secondary to 4.1R mutations and the deficiencies of band 3, GPA, and RhAG in HS red cells secondary to ankyrin-1 mutations are well documented, the concept that these deficiencies might be caused by mechanisms active at the time of enucleation had not been entertained. Our findings focus attention, for the first time, on this stage of erythropoiesis in HS and HE. Further, they raise the possibility that reticulocytes in HS and HE may differ from normal reticulocytes in their biophysical properties of membrane cohesion or membrane deformability because these 2 crucial properties are regulated by vertical linkages between specific membrane-spanning proteins embedded in the lipid bilayer and cytoskeletal proteins, such as the interactions of band 3, ankyrin-1, and spectrin or interactions of GPC, 4.1R, actin, and spectrin (recently reviewed in Mohandas and Gallagher15). If HS and HE reticulocytes are abnormal, then membrane loss of surface area may be exacerbated at this stage rather than occurring only in mature cells. Support for such a thesis can be inferred from an earlier study showing that surface area loss is a distinct feature of HS reticulocytes.16 Future studies will determine the relative contributions of sorting abnormalities and other mechanisms for the observed protein deficiencies in HS and HE.

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References


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Figure 2. Analysis of band 3, RhAG, and GPA sorting during enucleation of WT and nb/nb erythroblasts. DIC and immunofluorescent micrographs of WT and nb/nb-enucleating erythroblasts, including nascent reticulocyte (R) and extruding nucleus (N); probed with rabbit anti–mouse GPC and Alexa Fluor 594–labeled goat anti–rabbit; IgG (red; A); probed with rat anti–mouse TER 119 and Alexa Fluor 488–conjugated donkey anti–rat IgG (green) or rabbit anti–mouse band 3 and Alexa Fluor 594–labeled goat anti–rabbit IgG (red; B); or probed with rat anti–mouse TER 119 and Alexa 488–conjugated donkey anti–rat IgG (green) or rabbit anti–mouse RhAG and Alexa Fluor 594–labeled goat anti–rabbit IgG (red; C). Nuclei were identified by 4’6-diamidino-2-phenylindole staining (DAPI; blue). Dashed lines outline the spherical portion of extruding nuclei in the red and green images in which there is no fluorescent labeling of the nucleus. The number of enucleating erythroblasts examined under each staining condition was 6 or more. Of note, during extrusion the nucleus transiently deforms, and a portion of it is visualized within the nascent reticulocyte, as evidenced in these images.
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