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A hypothesis of target cell formation in sickle cell disease

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ABSTRACT

A fraction of erythrocytes appear as target cells in stained blood smears in sickle cell disease, due to a inheritance of the hemoglobin variant Hb S, polymerizing upon deoxygenation. These cells appear in a three dimension as thin cups. A process of their formation in this disease is proposed based on a band 3-based mechanism of the erythrocyte shape control, able to explain the erythrocyte echinocytosis by glucose depletion. It indicates that their formation is due to a stomatocytogenic slow outward transport of the dibasic form of endogenous P_i with an H^+ by band 3, promoted by the decrease of the Donnan ratio, which decreases cell pH and volume, attributed by a decrease of cell KCl concentration by the higher efflux of K^+Cl^- cotransport and Ca^{2+} activation of the Gardos channel. Its implications are briefly discussed with respect to target cells *per se*, target cell formation in other hemoglobinopathies, acquired and inherited disorders of the lipid metabolism and dehydrated hereditary stomatocytosis as well as a stomatocyte presence in a double heterozygote of Hb S and Hb C and of an involvement of the process of target cell formation in acanthocytosis in acquired and inherited disorders.

Introduction

Target cells in stained blood smears refer to erythrocytes having a central hemoglobin dot surrounded by a pale hemoglobin ring which is delimited by a hemoglobin ring. They appear as thin cups in a three-dimension, as can be viewed by scanning electron microscopy, also referred to as codocytes [1]. Target cells can be observed in a number of acquired and inherited disorders, including hemoglobinopathies (e.g. Hb S, thalassemia), impaired hemoglobin synthesis (e.g. iron deficiency), biliary obstruction, lecithin cholesterol acetyltransferase (LCAT) deficiency, inherited alterations of Na^+ and K^+ permeabilities [1–3]. They can also be observed following exposures to environmental toxins [4,5]. Target cells are more resistant to osmotic hemolysis, due to an increase of the cell surface area and volume ratio either due to a decrease of the cell volume or to an increase of the cell membrane surface area. Target cells can be induced in normal blood by increasing the tonicity of the plasma by adding potassium oxalate or sodium chloride which decreased cell volume by 15–18%, however, without altering the osmotic hemolysis resistance [2]. To our knowledge, target cells have not been reported induced in erythrocytes in buffered or unbuffered isotonic NaCl by increasing NaCl concentration. This may be attributed that anions Cl^- and HCO_3^-, rapidly exchanged by erythrocyte membrane band 3, are intrinsically echinocytogenic and stomatocytogenic, respectively (see later). However, leptocytes, which can be observed in some disorders (e.g. iron deficiency) [1], were observed in erythrocytes isolated in Hanks' buffered saline solution or zwitterionic N-2-hydroxye thylpiperazine-N′-2-ethanesulfonic acid buffered isotonic saline after decreasing cell volume by decreasing cell KCl saline with the K^+-ionophore valinomycin [6,7]. They were also observed in erythrocytes isolated in a phosphate buffered isotonic saline after increasing the membrane surface area with cholesterol with cholesterol rich liposomes [8]. Leptocytes are thin like target cells, but flat [1]. The basis of target cell formation remains still unknown. Our purpose is to propose an explanation of their formation in sickle cell disease based on a band 3-based mechanism of control of the erythrocyte shape [9], able to explain the erythrocyte echinocytosis by glucose depletion [10]. The explanation would indicate that target cell formation in this disease would be basically the result of a decrease of the Donnan ratio, which would decrease cell pH and volume, as a result of the decrease of cell KCl concentration. It would also indicate that target cells are dehydrated stomatocytes. Moreover, it would be guiding to explain target cell formation in other hemoglobinopathies, some acquired and inherited disorders of lipid metabolism and dehydrated hereditary stomatocytosis, also referred to as hereditary xerocytosis [3]. It would also explain a stomatocyte presence in the double heterozygote of hemoglobin Hb S and Hb C and would suggest an

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involvement of target cell formation in acanthocytosis in acquired and inherited disorders.

Models

The major features of the band 3-based mechanism of control of the erythrocyte shape are the following. Band 3 is 90–100 kDa membrane glycoprotein which rapidly exchanges monovalent anions Cl^- and HCO_3^-. Its cytoplasmic domain binds to protein ankyrin R which is bound in the mid-region of the flexible filamentous tetrameric anionic spectrin, the major protein component of the skeleton. Spectrin forms at its two ends ternary complexes with band 4.1 R and actin, other protein components of the skeleton. Actin forms a protofilament to which 5–6 molecules of band 4.1 R is bound. The latter is bound to the transmembrane glycoprotein C. The obligatory alternative recruitment of its inward-facing (band 3_i) and outward-facing (band 3_o) conformations in exchanges of Cl^- and HCO_3^- fold and unfold spectrin (or contract and relax the anionic skeleton) which promotes the echinocytosis and stomatocytosis of the erythrocyte, respectively. Band 3 also transports endogenous inorganic phosphate (P_i) (its dibasic form is co-transported with a H^+) and a wide variety of other anionic compounds including bulky ones, but at a slow rate [11]. The band 3_o/band 3_i equilibrium ratio increases with the increase of the Donnan equilibrium ratio of anions Cl^- and HCO_3^- and H^+ ($r = Cl_i^-/Cl_o^- = HCO_3^-/HCO_3^- = H_o^+/H_i^+$), which is determined by hemoglobin and the major organic phosphate intermediate 2,3-bisphophoglycerate (2,3-BPG). Substrates slowly transported by band 3, preferentially inwardly and outwardly are echinocytogenic and stomatocytogenic, respectively. However, an important aspect of this mechanism is Cl^- to be intrinsically echinocytogenic since far more Cl^- binds to band 3_i than to band 3_o whereas HCO_3^- is intrinsically stomatocytogenic since far more HCO_3^- binds to band 3_o than to band 3_i [12]. This aspect of this mechanism is supported by the echinocytosis by washing erythrocytes in saline [13]. It should be indicated that 20% of P_i is transported with Na^+ by another transporter [14]. However, the outward transport of P_i by band 3 would be favored with a decrease of a cell pH decrease. A variety of observations can be cited supporting the band 3-based mechanism [9,10,13,15–18].

The features of the proposed process of the echinocytosis by glucose depletion based on the band 3-based mechanism are the following. In the absence of glucose, the major organic phosphate 2,3-BPG is catabolized to lactate by the 2,3-BPG pathway (Rapoport–Luebering pathway) and by the glycolytic pathway (Embden–Meyerhof pathway) which forms the products inorganic phosphate (P_i), 3-phosphoglycerate and adenosine triphosphate (ATP). The last two products are reversibly transformed into adenosine diphosphate and 1,3-bisphosphoglycerate (1,3-BPG) by the glycolytic enzyme phosphoglycerate kinase. The product 1,3-BPG is transformed in 2,3-BPG by 2,3-BPG synthase/phosphatase, the unique enzyme of the 2,3-BPG pathway. The first product P_i is slowly outwardly transported by band 3 which would oppose to echinocytosis by increasing the band 3_o/band 3_i ratio. Eventually, the slow inward transport of P_i by band 3 would become predominant, thereby causing echinocytosis. A variety of observations on the erythrocyte glucose metabolism are compatible with the proposed process of the echinocytosis by glucose depletion [10].

Hypothesis

Sickle cell disease is the result of an inheritance of the hemoglobin variant Hb S, which polymerizes following deoxygenation. Its polymerization leads to sickle cell formation and vaso-occlusion crisis. Hemoglobin is a water soluble globular protein present at a concentration of 5 mM in the erythrocyte cytosol, which influences the Donnan ratio. Hemoglobin is a tetramer formed by two pairs of two types of polypeptide chains referred to as α and β. The inheritance of Hb S is due to a mutation of a base of the gene encoding the β polypeptide chain leading to a substitution of the polar glutamic acid at the position no 6 of this polypeptide chain by a hydrophobic valine (β6 Glu → Val). It was previously shown that Hb S binds to band 3 with a greater affinity than normal hemoglobin, presumably due to its lower number of negative charges [19,20]. It would be expected that Hb S to favor stomatocytosis according to the band 3-based mechanism since its lower number of negative charges would increase the band 3_o/band 3_i ratio by increasing the Donnan ratio. However, it promotes the efflux of K^+Cl^- cotransport at a significantly higher rate than normal erythrocytes which would decrease cell KCl concentration, pH and volume [19,20]. This cell pH decrease would promote the stomatocytogenic slow outward transport of endogenous P_i with an H^+ by band 3, which with the decrease of cell volume would lead to the formation of target cells. However, the Gardos channel which decreases cell KCl concentration by its activation by Ca^{2+} also contributes to target cell formation since of an abnormal Ca^{2+} homeostasis in sickle cell disease [21,22] and that there is an inhibition of the decrease of cell KCl concentration in this disease by the antifungal agent clotrimazole, an effective inhibitor of this channel [23]. The endogenous P_i would derive from the faster 2,3-BPG catabolism leading to ATP formation by pyruvate kinase, the penultimate enzyme of the glycolytic pathway, an increase of ATP hydrolysis by the membrane Na^+, K^+-ATPase in response to the perturbation of the normal cell NaCl and KCl concentrations [24] and ATP hydrolysis by the highly active membrane Ca^{2+}-Mg^{2+}-ATPase which minimizes the Ca^{2+} activating Gardos channel activity by extruding Ca^{2+} ions coupled with uptakes of H^+ ions, further decreasing cell pH [21]. Fig. 1 illustrates the process of target cell formation in sickle cell disease.

The explanation of target cell formation in sickle cell disease would indicate that target cells are dehydrated stomatocytes. It is likely that target cell formations in other hemoglobinopathies are due to a decrease of the Donnan ratio by a decreasing of cell KCl concentration because of a significantly higher efflux of K^+-Cl^- cotransport rate with some hemoglobin variants having a lower number of negative charges (e.g. Hb C $^{\beta 6\ Glu\rightarrow\ Lys}$, Hb O_{Arab} $^{\beta 121\ Glu\rightarrow\ Lys}$) [19,20], of an impaired hemoglobin synthesis which is associated with a microcytosis (e.g. iron deficiency, thalassemia, Hb E $^{\beta 26\ Glu\rightarrow\ Lys}$) [3,25–28] or of an abnormal Ca^{2+} homeostasis (e.g. Hb C $^{\beta 6\ \ Glu\rightarrow\ \ Lys}$, β-thalassemia) [29]. However, in a double heterozygote of Hb S $^{\beta 6\ Glu\rightarrow\ Val}$ and Hb C $^{\beta 6\ Glu\rightarrow\ Lys}$, dehydrated stomatocytes and other shapes instead of target cells were observed in erythrocyte fractions having a high cell density or a high hemoglobin concentration owing to a very low cell KCl concentration after a centrifugation of erythrocytes in a Percoll-Statcan continuous density gradient [30,31]. These stomatocytes can be explained by a cell pH significantly lower than that occurring in target cell formation as a consequence of a very low cell KCl concentration, which would significantly increase the amount of endogenous P_i slowly outwardly transported with an H^+ by band 3. The sources of endogenous of P_i in this double heterozygote would be the same as that Hb S in target cell formation since Ca^{2+} homeostasis is also abnormal in Hb C [29].

Target cells in dehydrated hereditary stomatocytosis [32–38] can be explained by the decrease of cell total NaCl + KCl concentrations which would promote the stomatocytogenic outward transport of P_i with an H^+ by band 3. The stomatocytosis in this disorder would be basically attributed to the same reason of the stomatocytosis in the double heterozygote of Hb S $^{\beta 6\ Glu\rightarrow\ Val}$ and Hb C $^{\beta 6\ Glu\rightarrow\ Lys}$. The sources of endogenous P_i in the dehydrated hereditary stomatocytosis are the 2,3-BPG catabolism and ATP hydrolysis by the membrane Na^+-K^+-ATPase without excluding