spectrin-dimer and thereby shifts the dimer-tetramer equilibrium in favor of the dimer, preventing the effective reformation of tetramers dissociated by shear.

In spite of the progress toward an understanding of hereditary elliptocytosis, several questions remain. Why and how do alterations in spectrin tetramer dynamics result in elliptocytic morphology? Are repeated passages of the cell in the microcirculatory bed necessary for the acquisition of the elliptocytic shape, and, if this is the case, does the ellipticity of the red cells increase with their time in circulation? To what extent does decreased proportion of tetramers contribute to altered tank treading behavior of the cell, and do these changes alter the effectiveness of oxygen delivery?

What are the implications of the findings of Harper and colleagues? One is that they provide new insight into how conformational changes induced by mutations far from the contact sites can alter spectrin tetramer–dimer balance, which reveals promoting the closed dimer conformation as a previously unsuspected mechanism for destabilization of the red cell membrane in hereditary elliptocytosis. In addition, the development of a mini-spectrin recombinant protein, affords a new means of studying of spectrin dimer–tetramer dynamics is a significant technical achievement, likely to prove valuable insights into furthering our understanding of red cell membrane properties. Importantly, the findings also reinforce the concept that red cell membrane protein interactions are dynamic and subject to perturbation by hydrodynamic forces that the cells encounter in the circulation. But in the broader context, the conclusions may also bear on the assembly and the structure-function relationships of other spectrin-like polymers.

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The liver-derived hormone hepcidin controls plasma iron concentrations and thus the availability of iron to erythrocyte precursors. Hepcidin acts by blocking the major flows of iron into plasma: absorption of iron in the gut, release of recycled iron from macrophages, and mobilization of iron from hepatic stores (see figure). A rise in hepcidin levels causes a decrease in plasma iron concentrations, which restricts the availability of iron for hemoglobin synthesis and can eventually lead to anemia. This mechanism is manifested in its purest form in patients with iron-refractory iron-deficiency anemia, caused by mutations in a negative regulator of hepcidin.

Inappropriately elevated hepcidin and the resulting hypoferremia are also thought to play a pathogenic role in the development of the common forms of iron-restricted anemia, those associated with infections and inflammatory disorders including autoimmune diseases and some cancers. In these conditions, multiple cytokines are implicated in increasing hepcidin synthesis, including interleukin (IL)-6, IL-1, IL-22, and several members of the transforming growth factor β superfamily. Inflammatory regulation of hepcidin probably evolved as a host defense mechanism to limit iron availability to microbes, but the same process may be maladaptive in noninfectious inflammatory disorders where it causes anemia. In chronic kidney disease, hepcidin levels are thought to be increased not only because of inflammation but also because of decreased renal clearance, the main route of hepcidin elimination from the body.

Current treatment options for iron-restricted anemias include ESAs and/or intravenous iron. However, increased hepcidin is thought to limit the effectiveness of these treatments. In order for ESAs to stimulate RBC production, concomitant availability of iron for hemoglobin synthesis is required. Therefore, hepcidin-mediated iron restriction may contribute to the hyporesponsiveness to ESA therapy that is frequently observed in inflammation and chronic kidney disease and has led to the usage of high doses of ESAs. Intravenous iron preparations are formulated as iron-carbohydrate complexes and must be processed by macrophages to make iron available for erythropoiesis. As macrophage iron export is regulated by hepcidin, increased levels of hepcidin limit the utilization of iron from intravenous iron preparations, and high doses of parenteral iron are required to overcome the hepcidin-mediated block. Concerns have already arisen about the side effects of high ESA doses, and the long-term effects of high intravenous iron doses are not known. Safer dosing of these drugs may be possible if they are used in combination with hepcidin antagonists. Even monotherapy with hepcidin antagonists may be effective in iron-restricted anemias. The proof of principle of the effectiveness of hepcidin ablation was demonstrated in mouse models of anemia of inflammation when hepcidin knockout mice developed milder anemia with faster recovery compared with wild-type mice.

Several approaches to antagonize hepcidin are currently under development. These include targeting the pathways known to regulate hepcidin production and neutralizing hepcidin peptide by anti-hepcidin antibodies or by engineered hepcidin binders such as anticlins and RNA-based spiegelmers. Cooke et al. describe the effectiveness of a fully human anti-hepcidin antibody in animal models. They developed humanized mice in which endogenous mouse hepcidin was replaced by human hepcidin and used them in a model of anemia of inflammation caused by heat-killed Brucella abortus. This model displays the major features of severe inflammatory anemia in humans, with multifactorial pathogenesis including iron restriction, shortened erythrocyte life span, and impaired production of erythroid precursors. Whereas an injection of ESA failed to increase hemoglobin in inflamed mice, a single injection of the anti-hepcidin antibody by itself increased hemoglobin by ~1.5 g/dL within 1 week. The most effective method was the combination of ESA and anti-hepcidin antibody, which increased hemoglobin by ~3 g/dL after 1 week compared with inflamed mice injected with a control antibody. The improvement in hemoglobin resulted from increased serum iron levels and better hemoglobinization of erythroid precursors (see figure), without affecting inflammatory responses in these mice.

In healthy cynomolgus monkeys, whose hepcidin peptide sequence is nearly identical to that of humans, a single injection of the fully human anti-hepcidin antibody (50 mg/kg) increased serum iron for at least 2 days, until the antibody became saturated with hepcidin and serum iron decreased to normal levels. This illustrates a potential hurdle to any therapy aimed at antagonizing hepcidin by binding the hormone. Hepcidin is produced at a high rate (estimated at several milligrams per day in an adult human and potentially much higher in inflammation) and is cleared from plasma within minutes. Hepcidin binders could thus be expected to (1) be rapidly saturated by hepcidin and (2) slow down the clearance of the antibody-bound hepcidin, resulting in hepcidin accumulation in plasma. However, as shown
in Figure 6 in the article by Cooke et al, chronic administration of the anti-hepcidin antibodies in cynomolgus monkeys demonstrated that antibody-mediated neutralization of hepcidin peptide is a feasible therapeutic approach. Once-a-week administration of a high-dose of anti-hepcidin antibody (300 mg/kg) for 4 weeks resulted in continuously elevated serum iron levels. Lower doses of the antibody allowed intermittent increase in serum iron, which by itself may be sufficient to improve erythropoiesis and correct anemia.

The efficacy of hepcidin antagonists in treating iron-restricted anemia in humans remains to be tested in clinical trials, several of which are in progress. Additional therapeutic options may soon become available for patients with anemias associated with kidney disease, cancer, and other inflammatory conditions.

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Anti-hepcidin therapy for iron-restricted anemias

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