Commentary

Hepcidin: A putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease

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Disorders of iron homeostasis, resulting in iron deficiency or overload, are very common worldwide (1). Normal iron homeostasis depends on a close link between dietary iron absorption and body iron needs (2). The paper by Nicolas *et al.* in this issue of PNAS (3) presents the exciting possibility that a central player in the communication of body iron stores to the intestinal absorptive cells may have been identified. This unlikely player, originally identified as a circulating antimicrobial peptide, is the hepatic protein hepcidin. Nicolas *et al.* found absence of hepcidin expression in mice exhibiting iron overload consequent to targeted disruption of the gene encoding the transcription factor Upstream Stimulatory Factor 2 (USF2).

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A brief review of normal iron metabolism is useful in understanding the proposed role for hepcidin. Dietary free iron, on reduction from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state on the luminal surface of the proximal small intestine (4), is transported into the enterocytes by the apical transporter DMT1 (also known as DCT1, Nramp2) (5). Dietary heme iron is taken up by an as-yet-unidentified transporter and released from the heme molecule within the enterocyte. The iron may be stored within the enterocyte as ferritin (and lost with the senescent enterocyte) or transferred across the basolateral membrane to the plasma by the transport protein Ireg1 (6) [other names are ferroportin1 (7) and MTP1 (8)]. This latter process requires oxidation of Fe^{2+} to Fe^{3+} by hephaestin (9). Once iron has entered the circulation, there are no significant physiologic mechanisms for iron loss other than menstruation.

Absorbed iron is bound to circulating transferrin and passes initially through the portal system of the liver, which is the major site of iron storage. Hepatocytes take up transferrin-bound iron via the classical transferrin receptor (TfR1) but likely in greater amounts by the recently identified homologous protein, TfR2 (10, 11). The major site of iron utilization is the bone marrow, where iron is taken up via TfRs on erythrocyte precursors for use in heme synthesis. Heme iron is recycled on ingestion of senescent erythrocytes by reticuloendothelial (RE) macrophages. Macrophages also express surface TfRs and take up iron from the circulation directly. Macrophage iron is either retained (stored as ferritin) or released into the plasma, where it is oxidized by ceruloplasmin and transported via transferrin for reutilization. The liver and the RE system thus represent the major sites of mobilizable iron stores.

Tight linkage of dietary iron absorption with body stores occurs in the proximal small intestine. Here duodenal crypt cells, the precursor cells for the absorptive enterocytes, sense the iron needs of the body and are "programmed" as they mature into absorptive enterocytes (12) to express appropriate levels of the previously described iron transport proteins (13). The crypt cells obtain information about body iron needs from two postulated regulators (2), the "stores regulator," which responds to body iron stores, and the "erythropoietic regulator," which responds to the body's requirement for erythropoiesis. The capacity of the stores regulator to change iron absorption is low relative to the erythropoietic regulator. Nonetheless, it plays an essential role in meeting increased iron needs and in preventing excess (14).

The importance of the stores regulator is highlighted by findings in hereditary hemochromatosis (HH). Patients with HH absorb excessive dietary iron relative to body stores, suggesting that the set-point for the stores regulator is altered. The excess iron accumulates over time, leading to tissue damage and organ failure (15). *HFE,* the gene defective in HH, encodes a major histocompatibility complex class I integral membrane protein found in a physical complex with β_2-microglobulin (β_2M) (16). This association with β_2M is necessary for transport of HFE to the cell surface (17). In the duodenum, the HFE/β_2M complex is confined to the crypt cells, where it is physically associated with TfR1 (18). These observations strongly suggest that HFE modulates the uptake (or release) of plasma-derived iron in these cells. Surprisingly, the duodenal mucosa in HH manifests features more characteristic of iron depletion than overload (6, 13, 19), suggesting that functional loss of HFE in the crypt cells leads to decreased iron uptake (or retention) of plasma iron, which programs the daughter enterocytes for increased dietary iron absorption.

A situation reciprocal in many regards to HH occurs in another common disorder of iron homeostasis, the anemia of infection or chronic disease. In HH, circulating iron levels are high, RE stores are low, and intestinal iron absorption is excessive. By contrast, in anemia of chronic disease, circulating iron levels are low, RE stores are high, and intestinal iron absorption is decreased (20, 21). Both disorders affect communication between the sites of iron storage (hepatocytes and the RE system) and uptake (duodenum). However, the means by which these sites communicate has been a mystery.

A clue to solving this mystery may lie in the unexpected phenotype of *USF2* knockout mice. Why unexpected? The investigators generated these mice to understand the role of USF2 in the glucose responsiveness

See companion article on page 8780.

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www.pnas.org/cgi/doi/10.1073/pnas.161296298

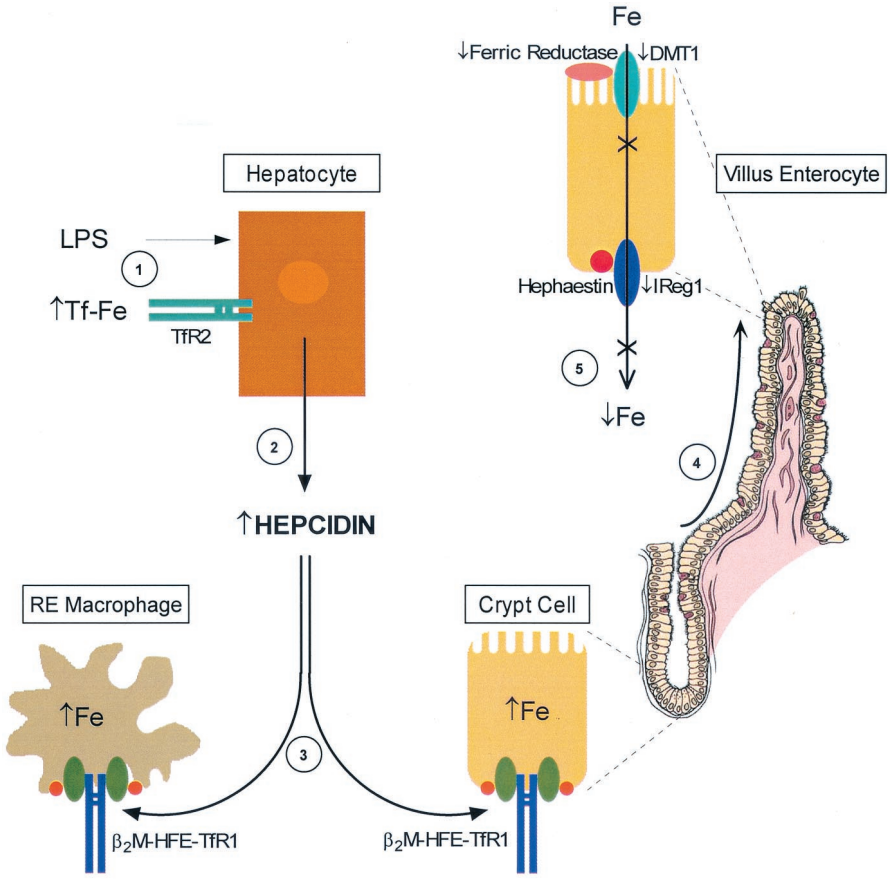

Fig. 1. Proposed steps in hepcidin regulation of iron homeostasis. (1) Increased hepatocellular uptake of transferrin-bound iron by TfR2 (or exposure to lipopolysaccharide) leads to (2) increased production and secretion of hepcidin, which (3) interacts with the β_2M-HFE-TfR1 complex and increases iron uptake or retention by RE macrophage duodenal crypt cells. (4) Crypt cells differentiate into daughter enterocytes programmed to have decreased expression of iron transport proteins, leading to (5) decreased dietary iron absorption.

of certain genes. Although useful for this purpose (22), the mice had the quite unanticipated finding of hepatic iron overload. Moreover, the character of this iron overload resembled that of human HH. Specifically, the hepatic iron distribution was predominantly periportal, whereas the RE system was relatively spared from iron loading. In attempting to explain these findings, the investigators discovered that *USF2* knockout mice fail to express either copy of the duplicated hepcidin genes immediately downstream of *USF2*, leading them to attribute the iron loading to absence of hepcidin.

A link between hepcidin and iron homeostasis had been previously made by Pigeon *et al.*, who independently cloned the orthologous murine cDNA by differential screening for hepatic mRNAs overexpressed with iron overload (23). Northern blots confirmed that hepcidin mRNA is increased with dietary iron loading and also increased in *β_2M* knockout mice. No iron-responsive elements were identified in the hepcidin transcript, so the mechanism for the change in mRNA content with change in iron status was unclear.

What's the infection connection? Hepcidin was independently discovered by two groups searching for novel antimicrobial peptides. Krause *et al.* purified the peptide from human blood [designating it Liver Expressed Antimicrobial Peptide (LEAP-1)] (24). Park *et al.* isolated it from human urine, designating it hepcidin (hepatic bactericidal protein) (25). Expression of hepcidin mRNA was nearly confined to the liver. The transcript encodes a precursor protein of 84 amino acids, including a putative 24-aa leader peptide. The circulating form consists of only the C-terminal 25 amino acids. Both groups demonstrated significant antibacterial and antifungal activities for the C-terminal peptide and classified hepcidin as a member of the cysteine-rich, cationic, antimicrobial peptides, including the thionins and defensins (26). As shown for other members of this class of proteins, hepcidin expression was increased in mice administered lipopolysaccharide (a classical inducer of acute-phase proteins involved in response to infection or inflammation) (23). This observation, coupled with the phenotype of the *USF2* knockout mice, leads us to suggest a possible role for hepcidin in the changes in iron homeostasis observed during inflammation. Because loss of hepcidin in *USF2* knockout mice is associated with increased circulating iron, decreased RE iron, and apparently increased intestinal iron absorption, we conjecture that the reverse situation (namely a decrease in circulating iron, increased RE iron, and decreased intestinal iron absorption) might result from increased hepcidin expression. If so, inflammation-induced increases in hepcidin could explain these very findings in the anemia of chronic disease.

Deficiencies of four different gene products have now been demonstrated to cause an HH-like phenotype—HFE (16, 27), β_2M (28), TfR2 (29, 30), and hepcidin (3)—implicating each of them as a participant in the iron stores regulator. TfR1 binds HFE in crypt cells and may also be involved (18). How do these interrelate? Nicolas *et al.* (3) speculated that TfR2 mediates iron uptake by hepatocytes, which, in turn, modulates expression of hepcidin, which, in turn, interacts with HFE, β_2M, and TfR in the duodenal crypt cell to regulate dietary iron absorption. Details of this speculated model are presented in Fig. 1.

Several testable predictions follow from this model. Individuals with mutations in TfR2 would be expected to produce lower than normal levels of hepcidin (for a given level of stored iron). Iron deficiency would be expected to result in decreased hepcidin expression as well. On the other hand, patients with HH caused by mutations in HFE would be expected to have higher levels. Administration of exogenous hepcidin would be predicted to result in increased RE iron retention and decreased intestinal iron uptake and perhaps produce findings similar to those seen in the anemia of chronic disease. These predictions can readily be tested in murine model systems as well as in the human disease states mentioned above.

There is an important caveat, as Nicolas *et al.* (3) point out. It has not yet been proven that the loss of hepcidin itself produces the HH phenotype found in the *USF2* knockout mice. It is possible that changes in expression of another gene on disruption of *USF2* led to the observed phenotype. Regardless of which gene is proven to be responsible, the *USF2* knockout mice pave the way for identification of a key component of the long-postulated iron stores regulator. This in turn could provide a potential drug or drug target to modulate iron absorption. That makes it a potentially "precious" metal regulator indeed, given the worldwide importance of disorders of iron metabolism.

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